Official Title:	**Multi-center, Double-blind, Randomized, Placebo-controlled, Parallel-group Efficacy and Safety Study of LRG-002 Hard Capsules (Lek d.d., Slovenia) Used in the Prophylaxis of Antibiotic-associated Diarrhea in Adults**
NCT Number:	NCT04321460
Document Date:	Clinical Study Protocol Version 3.0: 23 June 2020

SANDOZ A Novartis Division

CLINICAL STUDY PROTOCOL

Study title: Multicentre, double-blind, randomized, placebo-controlled, parallel-group efficacy and safety study of LRG-002 hard capsules (Lek d.d., Slovenia) used in the prophylaxis of antibiotic-associated diarrhea in adults.

Protocol No. CT_002_LRG_CAP

Protocol version: 3.0 dated June 23, 2020

Investigational Medicinal Product: LRG-002

International Nonproprietary Name: Lactobacillus rhamnosus GG 1 x 10^{10} CFU

Dosage form and strength: capsules

Phase of the Clinical Study: III

Study Sponsor: ZAO Sandoz.

Address: 72 Leningradsky prospect, bld. 3, Moscow, 125315, the Russian Federation

The Protocol Approval List by the Study Sponsor:

The Protocol Title: Multicentre, double-blind, randomized, placebo-controlled, parallel-group efficacy and safety study of LRG-002 hard capsules (Lek d.d., Slovenia) used in the prophylaxis of antibiotic-associated diarrhea in adults.

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Sponsor's representative:

ZAO Sandoz, The Russian Federation.

Address: Leningradsky prospect 72, bld. 3, Moscow, 125317, The Russian Federation

Tel. [redacted] - Fax [redacted]

_____[redacted]___ (*Employee's name*)

[redacted] (*Function*)

Tel.: [redacted] (Ext. [redacted])

E-mail: [redacted]

_____ "____"_____2020

Signature Date

Investigator Statement of Compliance with the Study Protocol

I confirm having read and understood the present Protocol, the Investigator's Brochure, including potential risks and side effects of the medicinal product and other product and study information provided by the Sponsor. I agree to perform the present Study in compliance with the Protocol requirements as well as to protect rights, safety, confidentiality and well-being of patients in accordance with ethic requirements set out in the Helsinki Declaration of the World Medical Association (WMA), in the Federal Law dated April 12, 2010, No. 61-ФЗ (FZ) "On Circulation of Medicines"; requirements of the RF Ministry of Health dated April 1, 2016, No. 200n "On Good Clinical Practice Approval"; principles of the National Standard of the Russian Federation GOST R (ГОСТ Р) 52379-2005 "Good Clinical Practice" (GLP) and other regulatory requirements of the Russian Federation. I agree to introduce any modifications to the Protocol only after notification of the Sponsor, unless it is necessary for safety, protection of rights and well-being of patients. I completely understand that any modifications provided by the Investigator(s) without prior discussion with the Sponsor's representative shall constitute Protocol violation (apart from procedures necessary for patients' health maintenance). I agree to conduct or manage the described study by myself. I agree to inform patients that medications are used for research purposes; I will ensure compliance with the requirements for obtaining the Informed Consent after the Ethics Committee's and the Independent Ethics Committee's approval and in accordance with the principles of Good Clinical Practice (GCP). In accordance with the principles of GCP, I agree to inform the Sponsor about any adverse events developed during the study. I agree to ensure that all employees, colleagues, and individuals involved in the study are informed of their obligations to comply with the above agreements. I agree to keep adequate and precise recording as well as to submit these records for analyses in accordance with the principles of GCP. I'll ensure that local IEC acting in accordance with GCP requirements, is responsible for the performance of Ethic review as well as for the approval of the Study. I also agree to report all changes in research activities and all unexpected issues, including any patients' risks and other aspects to the local IEC as soon as possible. In addition, I will not introduce any modifications to the Study without an approval by the Ethics Board/local IEC, unless there is a need for the management of any obvious unexpected life- and health-threatening cases in patients. I'm ready to provide the direct access to source medical records and agree for a review that will be performed by auditors and Sponsor's representatives and supervising agencies. I guarantee that the study drug provided by the Sponsor will only be used, as described in the present Protocol. I agree to comply with all other requirements regarding the responsibilities of clinical investigators, as well as all other important requirements of Good Clinical Practice.

Investigator:

Signature: _____

Date: "____"_____ 20__

Full name:

Function:

Facility:

Address:

LIST OF ABBREVIATIONS

AAD	antibiotic-associated diarrhea
AB	antibiotic
AB therapy	antibacterial therapy
ALD	alcoholic liver disease
BP	blood pressure
GAC	gastric adenocarcinoma
ALT	alanine aminotransferase
AO	join stock company
ASH	alcoholic steatohepatitis
AST	aspartate aminotransferase
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
ATC	Anatomical Therapeutic Chemical (classification)
ROS	reactive oxygen species
DS	dietary supplement
BSFS	Bristol Stool Form Scale
i.m.	intramuscular(ly)
i.v.	intravenous(ly)
ULN	upper limit of normal
HIV	Human immunodeficiency virus
IBD	inflammatory bowel disease
WMA	World Medical Association
IUS	intrauterine system
IUD	intrauterine device

RSC	Russian Society of Cardiologists
WHO	World Health Organization
HPLC	High performance liquid chromatography
g	gram
GOST (ГОСТ)	State Standard
GC	gas chromatography
d.d.	“d.d.” or “delniška družba”, Slovenian, public limited company
DBP	diastolic blood pressure
CI	confidence interval
DMH	dimethyl hydrazine
DNA	deoxyribonucleic acid
TD	traveler's diarrhea
PDE	permitted daily exposure
GIT	gastrointestinal tract
ZAO	Closed Join Stock Company
EU	European Union
IAAD	idiopathic antibiotic-associated diarrhea
RF	Record Form
IL	interleukin
PI	prescribing information
FPS	forename, patronym, surname
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
IFN	interferon

IFN-γ	interferon-gamma
CRO	contract research organization
kg	kilogramm(s)
NSD	number of sick days
cfu	colony forming units
CPS	capsular polysaccharides
CRC	colorectal cancer
L	liter(s)
LRGG	Lactobacillus rhamnosus GG
LD-50	half-lethal dose
LPS	lipopolysaccharides
CF	cystic fibrosis
mg	milligram(s)
MDA	malonic dialdehyde
MH	Ministry of Health
min	minutes
ICD	international classification of diseases
µg (mcg)	micrograms
mL	milliliters
mm	millimeters
PBMC	peripheral blood mononuclear cells
INN	international non-proprietory name
MNNG	methyl-nitro-nitrosoguanidine
MIC	minimum inhibitory concentration
PCRBC	Quantity of polychromatophilic red blood cells

NAFLD	Non-alcoholic fatty liver disease
e.g.:	for example
IEC	necrotizing enterocolitis
AR	adverse reaction
IEC	Independent Ethics Committee
AE	adverse event
AWD	acute watery diarrhea
AGE	acute gastroenteritis
DCS	depleted cultural supernatant
LLC	Limited Liability Company
RR	relative risk
ARD	acute respiratory disease
ICU	intensive care unit
FEV	forced expiration volume
OR	odds ratio
PVC	polyvinyl chloride
TJ	tight junctions
PCTFE	polytrifluorochloroethylene
PCA	pyrrole-2-carboxylic acid
PCR	polymerase chain reaction
QD	once a day
WP	working party
RIBT	radiation-induced bowel toxicity
RCTs	randomized clinical trials
BC	breast cancer

RMOAG (РМОАГ)	Society of arterial hypertension of the Russian Federation
RNA	ribonucleic acid
ORS	oral rehydration solution
RD	risk difference
MA	Marketing authorization
RF	Russian Federation
SBP	systolic blood pressure
SAR	serious adverse reaction
SAE	serious adverse event
et al.	contributors
SOP	standard operating procedure
ESR	erythrocyte sedimentation rate
MD	mean difference
IBS	irritated bowel syndrome
d	day
i.e.	that is (id est)
TCID	tissue culture infective dose
TER	transepithelial electric resistance
FAP	functional abdominal pain
FD	functional dyspepsia
FL	Federal Law
SFP	surname, patronym, forename
TNF	tumor necrosis factor
FSH	follicle stimulating hormone
CU	carbohydrate unit

CIBT	chemically-induced bowel toxicity
h	hours
NNT	number of patients in need of treatment
RR	respiration rate
HR	heart rate
ECG	electrocardiography, electrocardiogram
EPS	exopolysaccharides
AFB	Acid Fast Bacilli
AGRICOLA	AGRICultural On Line Access - bibliographic data base of the United States National Agricultural Library
AMED	Allied and Complementary Medicine Database - the specialized bibliographic database of the British Library on natural sciences and allied disciplines
ATCC	ATCC 53103 - L. rhamnosus strain
AUC	Area under the curve
$AUC_{0-\infty}$	Area under the curve from time zero to infinity
BALB	laboratory line of albino domestic mice being commonly used in clinical trials
BG2FO	one of Lactobacillus spp strains
C	degree Celsius
C. difficile	*Clostridium difficile*
C57BJ	an inbred line of laboratory mice being commonly used as human disease models
C57BL	an inbred line of laboratory mice being commonly used as human disease models
CaH	calcium hydride

CBA	an inbred line of laboratory mice
CCL C-C	Motif Chemokine Ligand 11 -chemokine 11 comprising CC motif
CD	cluster of differentiation – T-helper surface protein
CD62p	activated platelet markers
CdAD	Clostridium difficile associated diarrhea
CdD	Clostridium difficile associated diarrhea
CHCC	one of Lactobacillus spp strains
CHO	Chinese hamster ovary - cell line generated from Chinese hamster ovaries
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CMPG 5153	L. rhamnosus GG mutant strain
CP	Phosphoorganic pesticides parathion and chlorpyrifos
CTM	clinical trial manager
CXCL	interleukin-8
CYP	cytochrome P450
CYP2E	cytochrome P450
GCP	Good Clinical Practice
DN-114001	one of Lactobacillus casei strains
DOI	digital object (paper) identificator
E. cloacae	*Escherichia cloacae*
E. coli	*Escherichia coli*
EFSA	European Food Safety Authority
EMBASE	Database for Biomedical Research
EPEC	enteropathogenic *Escherichia coli*

ERK	protein kinase signal pathway
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology and Nutrition
et al.	et alii and others
ETEC	enterotoxigenic E.coli
F_0F_1-	structural ATP synthase complex
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GR	one of L. Rhamnosus strains
GRAS	Generally Regarded As Safe – FDA sttate
GSH	glutathione
H.pylori	Helicobacter pylori
HCl	hydrochloric acid
HIF	Hypoxia-inducible factors
HIV	human immunodeficiency virus
HNOO 1-DR20	one of Lactobacillus rhamnosus probiotics
HN001	one of L. Rhamnosus strains
HN019	one of L. Rhamnosus strains
HNOO	one of L. Rhamnosus strains
HNOl	one of L. Rhamnosus strains
ICH	International Conference for Harmonization
ITT	intention-to-treat, population dependent on treatment prescribed
IgA	Immunoglobulin A

IgG	Immunoglobulin G
IgM	Immunoglobulin M
IkB	inhibitory protein
JAMA	Journal of the American Medical Association
L.	Lactobacteria
LCR	Lactobacillus casei subsp. rhamnosus
LGG	one of L. Rhamnosus strains
LGR	one of L. Rhamnosus strains
LPPV	local person for pharmacovigilance
LPXTG	binding protein
MANTIS	Manual, Alternative and Natural Therapy Index System (the database for chiropractice, osteopathy and manual medicine)
MAMP	microbial molecular patterns
MAPK	mitogen activated kinase
MedDRA	medical dictionary for regulatory affairs
MRS	Liquid medium for Lactobacilli isolation according to the receipt recommended by Man, Rogosa and Sharpe as well as ISO
MTg	mouse thyroglobulin
NNT	number needed to treat
OTA	Ochratoxin A
PECD62p	activated platelet marker
PJS	Propionibacterium freudenreichii ssp. shermanii JS – probiotic strain
PP	per-protocol, population according to the Protocol
PROSAFE	probiotic and human strain collection

PRR	pattern-recognizing receptors
QPS	Qualified Presumption of Safety
RC	one of L. Rhamnosus strains
RR	risk ratio
S. boulardii	Saccharomyces boulardii
SF68	Enterococcus faecium strain SF68
Spp.	Species
Subsp	Subspecies
SUSAR	suspected unexpected serious adverse reaction
TGF	Transforming growth factor
TLR	Toll-like receptor
TOXLINE	Toxicology Literature Online
TY21A	*Salmonella typhimurium* strain
ToxFILE	bibliographic database for biochemical, pharmacological, physiological and toxicological effects of drugs and other chemical substances
VRE	vancomycin-resistant enterococci
ZO-1	zona occludens-1 – peripheric membrane protein

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1. Synopsis

Study Title	Multi-center, double-blind, randomized, placebo-controlled, parallel-group study of the efficacy and safety of LRG-002 hard capsules (Lek d.d., Slovenia) in the prophylaxis of antibiotic-associated diarrhea in adults.
Study Code	CT_002_LRG_CAP
Sponsor	Sandoz CJSC
Investigational Medicinal Product	LRG-002 = hard capsules with Lactobacillus rhamnosus GG not less than 1 x 10^{10} CFU per capsule (Lek d.d., Slovenia)
Reference product	Matching placebo, capsules
Background Antibacterial Therapy	Oral beta-lactam antibiotics, e.g. amoxicillin, Augmentin (amoxicillin clavulanate), cefixime, etc. (7-day course). The start of antibiotic therapy coincides with the first day of the study. Diagnosis of the underlying disease and the prescription of antibiotic therapy are not part of the study.
Phase of the Clinical Study	III
Study Population	It is planned to include (randomize) 520 patients (260 patients in each treatment group) taking into account a patient withdrawal rate of 20% or less. The minimal number of patients needed for statistical analysis is 416 patients with 208 patients of the per-protocol population in each treatment group.
Planned Study Duration	Maximum 17 days: Day 1: Screening/randomization/study treatment initiation Day 7 ± 2: Intermediate Follow-up Visit Day 15 ± 2: Treatment Completion / Study Termination Visit (after completion of LRG-002/Placebo regimen) The overall enrollment period is about 8 months.
Planned Number of Investigational Sites	Up to 40 sites in the RF

Study Goal	To demonstrate the efficacy and safety of the investigational medicinal product LRG-002 as compared with placebo, as an adjunct treatment for prophylaxis of antibiotic-associated diarrhea (AAD) in patients with acute respiratory diseases (ARDs) receiving a standard antimicrobial regimen.
Study Objectives	Main objective: To evaluate the incidence of AAD while taking LRG-002, hard capsules (Lek d.d., Slovenia) as compared to placebo in antibiotic-treated patients with ARDs. Secondary objective: To evaluate the safety of LRG-002, hard capsules (Lek d.d, Slovenia), as compared with placebo in antibiotic-treated patients with ARDs.
Study Methodology	**Study Design** A prospective, multi-center, double-blind, randomized, parallel-group clinical study. The study will comprise the following periods: Study Periods I. Screening + randomization + treatment initiation: Visit 1 (Day 1) II. Blinded treatment with LRG-002 in antibiotic-treated patients. **Or** Blinded treatment with matching placebo capsules in antibiotic-treated patients. Note: Duration of LRG-002 / Placebo administration should cover AB treatment period + 7 days after the AB treatment is completed = a total of 14 days. Visit 2 (Day 7 ± 2): Intermediate Follow-up Visit Visit 3 (Day 15 ± 2): Treatment Completion / Study Termination Visit.

	Domiciliary Visits by a doctor and a nurse at the patient's home are allowed. **<u>Visit 1 (Day 1)</u>** Patients start participating in the study after signing the Informed Consent Form. Procedures at Visit 1 for patients in each study group: • Collection of demographic data (year of birth, sex, age); • Body weight and height measurements; • Medical history; • Reporting concomitant treatment (including medications, medical devices and food supplements); • Complaints reporting; • Physical examination; • Vital signs assessment (heart rate [HR], blood pressure [BP]), body temperature [BT], respiratory rate [RR]); • Complete blood count; • Blood chemistry profile; • COVID-19 Antibody (IgM / IgG) combined express-test; • Urine pregnancy test; • A 12-lead electrocardiogram; • Evaluation of the inclusion/non-inclusion criteria; • Randomization; • Dispensing of the investigational medicinal product (IMP) / placebo; • Dispensing of patient diary, mercury thermometer and respective training; • Dispensing of laboratory kit with the necessary supplies to collect stool samples; • Adverse event assessment. Having completed the screening tests, eligible patients will be randomized on the same day to receive either LRG-002 or matching placebo capsules, each as an adjunct to the prescribed antibiotic (see inclusion criteria). The treatment group will be determined by randomization at a 1:1 ratio. Study subjects will be supplied with the

	patient diary and blinded investigational product (investigational medicinal product or placebo) depending on the assigned treatment group. Patients will be able to start study treatment only after receiving appropriate training. On Day 1, the study will be started in both groups from randomization and administration of the first dose of the investigational product depending on the assigned group after randomization: **Group T, treatment with LRG-002:** a single dose of 1 oral hard capsule taken during meals with some water; the product is to be taken orally 2 times per day for 14 days. **Group R, treatment with placebo:** a single dose of 1 oral hard capsule taken during meals with some water; the product is to be taken orally 2 times per day for 14 days. If the appointment is received in the afternoon, the administration of one capsule is allowed on the first day. The interval between taking the oral antibiotic and IMP/Placebo should be at least 3 hours in both groups. IMP/Placebo capsules must not be taken concomitantly with alcohol, fruit juices or hot beverages. In both groups, patients will be receiving an oral beta-lactam antibiotic (for instance, amoxicillin, amoxicillin + clavulanic acid, cefixime, etc.) (see Background Antibacterial Therapy). Patients will be able to start documentation in the patient diary only after receiving appropriate training. In the diary, the subjects should daily document: • Occurrence of bowel movements, specifying date/time for each bowel movement; • Stool consistency (Bristol Stool Form Scale, BSFS) for each bowel movement; • Intake of blinded IP (IMP/placebo); • Intake of antibiotic;

	• Occurrence of gastrointestinal complaints (assessment of the severity of symptoms using a 5-point verbal scale [0 to 4]); • Occurrence of pyrexia; • Use of any drugs, other than the prescribed antibiotic therapy (indicating the name, strength and time of administration). **Visit 2 (Day 7 ± 2)** Intermediate Follow-up Visit Procedures at Visit 2 for patients in each study group: • Complaints reporting, medical history update; • Reporting concomitant medications; • Body weight measurements; • Physical examination; • Vital signs assessment; • Complete blood count; • Blood chemistry profile; • Adverse event assessment; • Evaluation of exclusion criteria; • Review of patient diary; • Dispensing of laboratory kit with the necessary supplies to collect stool samples (if necessary); • Assessment of the adherence to study procedures (compliance with diary completion and study treatment); • Assessment of diarrhea, if documented in the diary regarding its classification as AAD or non-AAD (according to investigator's judgment). If the results of stool samples analysis are not available during visit, the assessment will be done after the investigator receives the results from the laboratory. **Visit 3 (Day 15 ± 2)**

	Visit 3 is scheduled at Day 15±2 when a patient is to come to the study site bringing along the completed diary, unused laboratory kit, unused product and empty packages. Procedures at Visit 3 for patients in each study group: • Complaints reporting, medical history update; • Body weight measurements; • Reporting concomitant medications; • Physical examination; • Vital signs assessment; • Adverse event assessment; • Evaluation of exclusion criteria; • Assessment of the adherence to study procedures (compliance with diary completion and study treatment); • Complete blood count; • Blood chemistry profile; • A 12-lead electrocardiogram; • Collection of unused laboratory kit; • Collection of unused medication; • Review and collection of patient diary; • Assessment of diarrhea, if documented in the diary regarding its classification as AAD or not (according to investigator's judgment). If the results of stool samples analysis are not available during visit, the assessment will be done after the investigator receives the results from the laboratory. **Collection of stool samples in case of diarrhea** **Visit 1** • Dispensing of laboratory kit with the necessary supplies to collect stool samples: **Stool sample collection in case of diarrhea (between V1 and V2):** • 1 container – stool sample for presence of common diarrheal pathogens (rotavirus, adenovirus, norovirus, *Campylobacter spp, Salmonella spp*, *Shigella* spp,*)*;

	• 1 container – stool sample for presence of *C. difficile* toxins A and B; • 1 container – stool sample for culture for opportunistic flora: *Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis, Candida spp, E. Coli* and for culture *Yersinia spp* (special container). **Visit 2** • Dispensing of laboratory kit with the necessary supplies to collect stool samples (if necessary): **Stool sample collection in case of diarrhea (between V2 and V3):** • 1 container – stool sample for presence of common diarrheal pathogens (rotavirus, adenovirus, norovirus, *Campylobacter spp, Salmonella spp, Shigella spp*,); • 1 container – stool sample for presence of C. *difficile* toxins A and B; • 1 container – stool sample for culture for opportunistic flora: *Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis, Candida spp, E. Coli* and for culture *Yersinia spp* (special container). **Visit 3** • Collection of unused laboratory kit (in case there was no diarrhea between V1 and V3). In case of diarrhea between visits, the patient must contact the site and collect stool samples in three containers (containers are received at V1 and V2). The site employee arrives at the patient to collect containers in thermo package and transport them to the laboratory. In the case of acute diarrhea (loose, watery stool [BSFS Type 6, 7] with an occurrence of 3 or more times within 24 hours), the patient should contact the investigator for advice on therapy. **Unscheduled Visits** The unscheduled visits will be conducted when necessary, e.g., in case of the index disease deterioration, AEs, or intolerability of IMPs (LRG-002 or placebo).

	Every unscheduled visit, irrespective of its cause, must include the procedures listed below with completion of the relevant eCRF pages (Unscheduled Visit): • Physical examination; • Measurement of vital signs (BP, HR, RR, BT); • A 12-lead ECG; • Evaluation of adverse events; • Evaluation of the concomitant therapy; • If indicated, any of the study procedures may be additionally performed upon the decision of the Principal Investigator. Taking into account frequency of visits, unscheduled visits may be conducted on the same days as the scheduled ones but at a different time (e.g., in case of worsening the patient may visit the site in the afternoon). Procedures for planned clinical study visits are the same in each treatment group during each study period.
Major Efficacy and Safety Parameters	**<u>Primary efficacy parameter</u>** To evaluate the incidence of AAD while taking LRG-002, hard capsules (Lek d.d., Slovenia) as compared to placebo in antibiotic-treated patients with ARDs; *AAD is defined as diarrhea associated with the AB use caused by C. difficile or of otherwise not identified etiology, upon analysis of stool samples and differential diagnostics according to investigator's judgment.* *Diarrhea is defined as loose or watery stool (BSFS Type 5-7) three times a day (frequent bowel movements with formed stool is not considered diarrhea) in accordance with WHO criteria; based on the diary data (BSFS) and confirmation of AAD by the investigator.* **<u>Secondary efficacy parameter</u>** • The occurrence of bowel movements per day (according to the diary data) in the treatment group compared with the placebo group

	• The incidence of any diarrhea in the treatment group compared with the placebo group • The incidence of *C. Difficile*-associated AAD in the treatment group compared with the placebo group; • The incidence of non-*C. Difficile*-associated AAD in the treatment group compared with the placebo group; • The duration of AAD (the time from the onset of AAD to the normalization of stool form according to BSFS (types 1, 2, 3 and 4) and the presence of normal stool within 48 hours) in the treatment group as compared to the placebo group; • The duration of any diarrhea (the time from the onset of diarrhea to the normalization of stool form according to BSFS (types 1, 2, 3 and 4) and the presence of normal stool within 48 hours) in the treatment group as compared to the placebo group; • Occurrence of changes in stool consistency (BSFS) (according to the diary data) in the treatment group as compared to the placebo group • The occurrence and severity of gastrointestinal symptoms, including nausea, vomiting, flatulence, abdominal pain and decreased appetite (according to the diary data) in the treatment group as compared to the placebo group; • Change in body weight at Visit 3 compared with Visit 1 in the placebo group as compared to the treatment group; • Hospitalization rate in the treatment group compared with the placebo group • The number of days of using standard symptomatic therapy (as "rescue medication") to relieve symptoms of acute diarrhea in the treatment group as compared to the placebo group; • The number of patients using standard symptomatic therapy (as "rescue medication") to relieve symptoms of acute diarrhea in the treatment group as compared to the placebo group. **Safety assessment parameters**

	Safety evaluation is based on the following parameters (between V1 and V3) compared between therapy and placebo groups. Frequency of adverse events (AEs) and/or serious adverse events (SAEs) in the treatment groups, including: - Overall AE rate as compared to placebo; - Frequency of AEs according to the results of physical examinations during each visit compared with placebo; - AE incidence according to the results of the vital signs assessment (HR, BP, BT, RR) at each visit in comparison with placebo; - AE incidence according to the ECG results on Day 15 (± 2) in comparison with placebo; - Frequency of AEs incidence according to the results of laboratory examinations on Days 7(± 2) and 15(± 2) compared with placebo; - Frequency of AEs according to the patients' records in the diary Diary records will be reviewed by the Investigator and for those records presumed to meet criteria of an AE, the patient will be questioned. If the investigator judges that the AE criteria have been met, all information relevant for an AE (including an evaluation of causality to the investigational product) will be documented. The AEs will be described according to the scheme given below: - AE description; - AE severity; - AE duration; - Causal relationship with the investigational product; - Outcome. All AEs will be coded according to MedDRA terms.
Inclusion Criteria	Patients will be considered eligible if they meet the following inclusion criteria:

	1. An Informed Consent Form for study participation voluntarily signed by a patient; 2. Male and female subjects, 18 to 65 years of age inclusive; 3. Antibacterial treatment for active ARD started/to be started on the first day of the study (7-day course of oral beta-lactam antibiotic). Only one AB should be used per subject, in an outpatient setting. Diagnosis of acute respiratory infections and the prescription of antibiotic therapy should be completed before signing of the Informed Consent Form. 4. Female patients are eligible for the study if they are: • **Unable to become pregnant** (i.e. postmenopausal women or those after surgical sterilization). Surgically sterile women are defined as patients with documented hysterectomy, and/or bilateral oophorectomy, and/or tubal ligation. Postmenopausal women are defined as women with amenorrhea for more than 1 year with the relevant clinical profile (e.g., age > 45 years, and absence of hormone replacement therapy). However, in controversial cases, it is recommended to confirm menopause by drawing a blood sample which would show an FSH level of > 40 IU/mL and estradiol level of < 40 pg/mL (< 140 pmol/L). • **OR** **Capable of childbirth**, but with negative pregnancy test at the screening visit, and the patient agrees to continuously and properly use one of the following suitable methods of contraception (i.e., in accordance with the approved Package Insert for the medicinal product and physician instructions) during the participation period: a. Complete abstinence; b. Oral contraceptives (combination drugs containing progestogen, or progestogen alone); c. Injectable progestogen; d. Levonorgestrel implants; e. Estrogen-containing vaginal ring; f. Transdermal contraceptive patches;

	g. Intrauterine device or intrauterine system; h. A male partner has been sterile (vasectomy with documented azoospermia) prior to inclusion of a woman, provided that he is the only partner of the female patient. For the purpose of this definition, "documented" is related to the result of a subject's medical examination performed by Investigator/responsible person or a subject's past medical history review for assessment of eligibility for enrollment, obtained during the interview with a subject or from his/her medical records; i. Double barrier method: a condom or an occlusive cap (diaphragm or cervical/vault caps) with a spermicide (foam/gel/film/cream/suppository); 5. Male participants are obliged to use appropriate contraception during the entire study period starting from signing of the Informed Consent Form and until the study end, and for 30 days after study completion; 6. The ability to understand the information about the clinical study, readiness to comply with the study protocol requirements, ability to take IP and evaluate symptoms on his (or her) own using diary/questionnaires as per protocol; 7. Ability to maintain the habitual lifestyle throughout the study, including diet. 8. Willing not to consume any products containing probiotics during participation in the clinical trial. 9. Willing not to take part in any other study during the present study.
Non-inclusion Criteria	Patients will not be eligible for study enrollment if they have one or more of the following criteria: 1. Any treatment (including medications, medical devices and food supplements) that can influence the stool consistency according to the Investigator's opinion should not be used within 14 days prior to Visit 1. 2. Use of anti-rejection medication after stem cell or solid organ transplant;

	3. Use of glucocorticosteroids within the last 8 weeks prior to Visit 1; 4. Use of proton pump inhibitors within 3 months prior to Visit 1; 5. Chemotherapy or radiation; 6. History of recurrent diarrhea; 7. Diarrhea or watery stool in the last 2 days prior to Visit 1; 8. Severe ARD expected to require additional antibiotics or to require antibiotics therapy more than 7 days; 9. Use of antimicrobials in the last 3 months prior to the study start; 10. Use of yeast/probiotic/fermented products within the last 2 weeks prior to V1; 11. Known allergy/sensitivity to the IMP; 12. Immunocomopromised patients; 13. Known digestion/absorption disorders of the gastrointestinal tract (e.g. inflammatory bowel disease, coeliac disease, pancreatitis, motility disorders etc.) and/or gastrointestinal surgery (with the exception of appendectomy more than 3 months before Visit 1); 14. Known irritable bowel syndrome; 15. Known small intestinal bacterial overgrowth; 16. Pyrexia > 38 °C; 17. Pregnant and/or breastfeeding women. 18. Participation in other clinical trials of medicinal products or medical devices at the screening Visit or for 30 days before the screening Visit. 19. Surgical intervention for 30 days before the screening visit or planned surgical treatment during the trial (before a follow-up visit is completed), including diagnostic procedures or hospital stay. 20. Known or suspected alcohol and/or drug addiction; 21. A suspected low compliance or incapability of the patient to perform the procedures and comply with restrictions according to the trial protocol (e.g., due to mental disorders); 22. Potential for translocation of probiotic across bowel wall (Presence of an active bowel leak, acute abdomen, active

	intestinal disease including colitis, or significant bowel dysfunction; presence of neutropenia or anticipation of neutropenia after chemotherapy; radiation therapy); 23. Any disorders of cardiovascular, renal, hepatic, gastrointestinal, endocrine, or nervous disorders, and other conditions/diseases which, in the Investigator's opinion, may render study participation unsafe for a patient or may affect a test result. 24. Positive result of express-test for detection of IgM antibodies for COVID-19.
Exclusion Criteria	Subject participation will be stopped in case any of the following reasons occur: 1. Disease progression (including pyrexia above 38 °C for 3 days or longer); 2. Positive culture or PCR results for *Shigella* and *Salmonella spp*, typhoid and paratyphoid species, pathogenic *E. coli*, and other pathogen species (including *C. difficile*), if supplementary antibacterial treatment is required during the study; 3. Need to change tactics of antibiotic therapy (extension course of antibiotic therapy or change of antibiotic group); 4. The Ethics Committee, regulatory authorities, or Sponsor terminate the trial or participation of the specific trial site for any reason; 5. The Investigator’s decision to withdraw the patient from the trial in the interests of the patient; 6. Withdrawal of the informed consent (unwillingness of the patient to continue his/her participation in the study); 7. Individual intolerance of Investigational products (according to Investigator’s judgment); 8. Clinically significant adverse event or serious adverse event; 9. Patient's non-compliance; 10. Error enrollment (e.g., the patient was included with violation of the inclusion/non-inclusion criteria); 11. The patient complies with the non-inclusion criteria during the study;

	12. The patient receives or requires an additional therapy which might influence the study results or patient's safety (see Prohibited concomitant therapy section); 13. Other conditions or events which, in the Investigator's opinion, require the patient's exclusion from the study.
Prohibited Concomitant Treatments	1. Systemic glucocorticosteroids within 8 weeks prior to and during the study; 2. Use of any antibacterials in the last 3 months prior to the study or of any antibacterials (except the AB per inclusion criterion 3) during the study; 3. Use of any proton pump inhibitor within 3 months prior to the study; 4. Other probiotics (bacteria or yeast, medications, food supplements, fermented products) and Broncho-munal® within 2 weeks prior to and during the study; 5. Any treatment (including medications, medical devices and food supplements) that can influence the stool consistency according to the Investigator's opinion within 14 days prior to and during the study (other than the drugs specified in items 3 and 4 of the Allowed Concomitant Treatments). See the list of prohibited drugs in Appendix No. 1.
Allowed Concomitant Treatments	1. Oral beta-lactam antibiotic (for instance, amoxicillin, amoxicillin + clavulanic acid, cefixime, etc.) (see Background Antibacterial Therapy) as per inclusion criterion 3, in the same dosage regimen as at study start. 2. Continuation of the drug administration started prior to the inclusion into the study for treatment of concomitant diseases not covered by any of the exclusion criteria. Continuation of oral contraceptive administration by female participants during the study. 3. Standard symptomatic treatment agents used to treat acute respiratory infections in case of medical need, including anilides, triazole derivatives, with the exception of drugs that can have a laxative effect (for example, throat lozenges with sugar substitutes)/

	4. Agents for relieving symptoms of acute diarrhea, in case of medical need, including electrolyte solutions, with the exception of sorbents. Prescribing therapy for acute diarrhea and acute respiratory infections is possible only by the decision of the investigator.
Statistical Analysis	The aim of the statistical analysis will be to prove the efficacy and safety of LRG-002 (Lactobacillus rhamnosus GG not less than 2 x 10^{10} CFU) to be superior to placebo in antibiotic-treated patients with acute respiratory diseases, as an adjunct treatment for prophylaxis of antibiotic-associated diarrhea (AAD). The analysis will be performed for comparison of: - LRG-002 and matching placebo. The primary efficacy endpoint will be analyzed with a logistic regression model with fixed factors for treatment group and age group. The primary efficacy endpoint will be considered achieved if the odds ratio for the occurrence of AAD between placebo and LRG-002 is significantly higher than 1 (i.e. the occurrence of AAD is lower in the LRG-002 group). The secondary parameters will be compared with the following method: - continuous variables will be compared by Mann—Whitney-Wilcoxon test. Binary and ordinal variables will be compared by chi- square test or Fisher's exact test (depending on the number of expected observations per cell: <5 or ≥5). The analysis of study subjects with AE (SAE) will be performed with chi-square test or Fisher's exact test. Poisson regression will be used for analysis of AE (SAE) frequency. The significance level is assumed as $p < \alpha$, with $\alpha = 0.05$. **Sample size calculation** Primary endpoint is the frequency of occurrence of AAD from the

	first to the last day of LRG-002 administration compared with placebo in antibiotic-treated patients with ARDs. A test based on odd ratio was used to calculate sample size. The following parameters were obtained from Figure 2 in a meta-analysis [1] using only data for AAD: Using only data for AAD studies in adults from the cited publication, the rate for the control group is 0.24 and for the treatment group 0.14. Using only data for LGG studies in adults and children, the rate for the control group is 0.26 and for the treatment group 0.15. In meta-analysis [14] figures from Table 1 demonstrate a rate of 0.196 for a treatment group and 0.29 for a placebo group (only trials for adults with LGG were taken into consideration). In meta-analysis [15] figures from Table 2 demonstrate a rate of 0.188 for a treatment group and 0.269 for a placebo group (only trials for adults with LGG were taken into consideration). Consideration of AAD studies for LGG from [16], concerning infections at adults (including *H. pylori*), results in 0.14 for a probiotic group and 0.22 for a control group. Combining all the strains, we obtain an incidence of 0.26 for placebo and an incidence of 0.15 for treatment. The following indicators were used for calculations: 1. Difference of absolute risks of AAD in placebo group and in treatment group is expected to be positive. 2. Incidence of AAD 0.26 is assumed for a placebo and 0.15 for the treatment. 3. Probability of type I error: 0.05; 4. Power of the study is 80%, which corresponds to the type II error: 0.20. 5. Statistical hypothesis is the evidence of superior efficacy: H0: OR=1 H1: OR≠1,

where OR is the odds ratio between both groups.

The ratio between the study and control group sizes is 1:1.

The number of patients in each group could be calculated using the following formula [2]:

$$n_T = n_T = \left(\frac{Z_{1-\alpha/2} + Z_\beta}{p_T - p_R} \right)^2 \left[p_T (1 - p_T) + p_R (1 - p_R) \right] = 208$$

Thus, the minimum number of patients required for statistical analysis is 416 (208 patients in each group). Taking into account a 20% withdrawal rate, one needs to randomize 520 subjects (260 patients in each group).

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Interim Analysis	Interim data analysis is not planned.
Blinding, Randomization	It will be a double-blind, randomized study. Patients will be included by applying stratified randomization in two strata: • Age < 50 years; • Age ≥ 50 years. Each treatment group will be balanced at a ratio of 1:1 within each stratum. Stratified randomization is a two-stage procedure in which patients who enter a clinical trial are first grouped into strata. Within each stratum, patients are then assigned to a treatment according to separate randomization schedules. Randomization module of EDC system assigns patient unique randomization code base on group of treatment and stratum. Randomization schedules will be hidden from medical personnel and all other persons involved in the study (monitors, data management, biometry, sponsor etc.), except the specialists responsible for labeling of the IMP and emergency unblinding.

2. General information

2.1 Protocol title, identification number and date

Title: Multicentre, double-blind, randomized, placebo-controlled, parallel-group efficacy and safety study of LRG-002 hard capsules (Lek d.d., Slovenia) used in the prophylaxis of antibiotic-associated diarrhea in adults.

Identification number: CT_002_LRG_CAP

Protocol version: 3.0

Date: June 23, 2020

2.2 Study Sponsor

Study Sponsor:

Name of organization:	ZAO "Sandoz", Russia
Address:	72, Leningradsky prospect, bld. 3, Moscow, 125317, Russia.
Telephone:	[redacted]
E-mail:	sandoz.russia@sandoz.com

Sponsor's representative:

[redacted]

Name: [redacted]

Tel.: +[redacted] Fax: [redacted]

[redacted]

E-mail: [redacted]

[redacted]:

[redacted]

[redacted] Novartis Pharma

[redacted]

Name: [redacted] Address: 72 Leningradsky prospect, bld.3, Moscow, Russia 125315 Tel.: [redacted] Cell number: [redacted] (24 h) Fax: [redacted] 60 E-mail: [redacted]

Sponsor Representative for medical issues:

Name:	[redacted]
Function:	[redacted]
Name of organization:	ZAO “Sandoz”, Russia
Address:	72, Leningradsky prospect, bld. 3, Moscow, 125317, Russia
Telephone:	Tel: [redacted] Fax: [redacted]
E-mail:	[redacted]

Alternate of Sponsor Representative for medical issues:

Name:	[redacted]
Function:	[redacted]
Name of organization:	ZAO “Sandoz”, Russia
Address:	72, Leningradsky prospect, bld. 3, Moscow, 125317, Russia
Telephone:	Tel.: [redacted] ext. [redacted] Fax: [redacted] Cell number: [redacted]
E-mail:	[redacted]

2.3 **Study Monitor**

Contract research organization (CRO)/organization responsible for the Study

[redacted]

Legal address: [redacted]

Tel.: [redacted], Fax: [redacted]

E-mail: [redacted]

2.4 Clinical laboratories and other relevant organizations of the Study

Name of organization: [redacted]

Address: [redacted]

[redacted] Moscow

Telephone: [redacted]

E-mail: [redacted]

3. Rationale of the study and the program of clinical trials of the medicinal product

3.1 Program of clinical trials of the medicinal product

In accordance with the clinical development program of the LRG-002 medicinal product, capsules (Lek. d.d., Slovenia), Sandoz is planning to perform the clinical trial on the territory of the Russian Federation:

Multicentre, double-blind, randomized, placebo-controlled, parallel-group efficacy and safety study of LRG-002, capsules (Lek d.d., Slovenia) used in the prophylaxis of antibiotic-associated diarrhea in adults. According to Federal Law No. 61-ФЗ (FZ) as of April 12, 2010 On Circulation of Medicines (as amended), the clinical trial with participation of Russia is required for medicinal product authorization on the territory of the RF. Therefore, this clinical trial is designed to investigate LRG-002 tested medicinal product efficacy and safety, capsules as compared with LRG-002 placebo, for prophylaxis of antibiotic-associated diarrhea (AAD) in patients with acute respiratory diseases (ARDs) receiving a standard antimicrobial therapy.

According to the results of the present clinical trial Sandoz is planning to have the LRG-002, capsules medicinal product, authorized.

3.1.1 Investigational drug

Name of the medicinal product: LRG-002, capsules.

Manufacturer: Lek d.d., Verovskova 57, 1526 Ljubljana, Slovenia.

Dosage form, dosage: capsules

Description: Each capsule contains live lyophilized probiotic bacteria of Lactobacillus genus.

Composition per 1 capsule: each capsule contains at least 1 x 10^{10} CFU of Lactobacillus rhamnosus GG as a lyophilizate.
Excipients: sucrose, maltodextrin, silicon dioxide, magnesium stearate, sodium ascorbate

Capsule shell: hypromellose, titanium dioxide (E171), gellan gum, water.

Pharmacotherapeutic group: anti-diarrhea microorganisms, lactic acid producing microorganisms.

ATC code: A07FA01

Pharmacodynamic properties

Lactobacillus rhamnosus GG comprised in LRG-002 is a well-known probiotic strain and a well-established probiotic bacterium which reconstitutes and maintains the reconciliation of gut microbiota in adults and children.

Along with other bacteria, Lactobacteria (benefit bacteria) are commonly present in human gut microbiota. These bacteria maintain the structure of intestinal cells and their contacts, healthy acid-alkaline reconciliation necessary for normal functioning of digestive enzymes, prevent harmful bacteria overgrowth, influence the intestinal motility (peristalsis) and stimulate the immune system.

Disturbance of the gut microbiota reconciliation (e. g. in viral and bacterial gastrointestinal infections, in therapy with wide spectrum antibiotics and chemotherapy drugs, in irradiation of abdominal and pelvic organs, and when travelling abroad) may cause digestion disorders (such as flatulence, diarrhea and constipation).

The use of Lactobacillus rhamnosus GG positively influences the reconstitution and control of physiologic balance of intestinal microbiota. A wide range of research literature and clinical trials of Lactobacillus rhamnosus GG indicate that this probiotic strain can positively affect gastrointestinal and immune functions. Furthermore, its safety and good tolerability have been demonstrated.

Pharmacokinetic properties

Probiotic bacteria as well as Lactobacillus rhamnosus GG provide a local effect on the gastrointestinal tract. There is no systemic absorption with oral administration. Routine pharmacokinetic studies are thus not applicable. The resistance of Lactobacillus rhamnosus GG to gastric acid and bile allows most of these bacteria to survive during the passage of stomach and duodenum. Bacteria adhere to the intestinal mucous membrane. Like all other microorganisms contained in the gastrointestinal tract, they are gradually excreted by peristalsis and defecation.

Intended indications

The LRG-002 medicinal product may be used as a part of complex therapy of antibiotic-associated diarrhea in adults.

Contraindications

Drug active substance or excipients hypersensitivity.

Special warnings and precautions for use

Before starting the therapy, the patient should consult a doctor when:

- the body temperature is above 38 °C,
- presence of blood or mucus in feces,
- diarrhea duration of more than two days,
- severe pattern of diarrhea accompanied by dehydration and body weight loss,
- diarrhea is accompanied with severe abdominal pain,
- the presence of other chronic diseases (e. g., diabetes mellitus, cardiovascular diseases) or immune deficit (e. g., HIV infection).
- a patient has diarrhea and other serious health problems (severe disease, cardiac diseases, central venous catheter installed),
- a patient has immune deficit (e. g., HIV infection or immunosupressive therapy),
- a patient has an intestinal barrier function disordered (e. g., short gut syndrome).

It's very important to compensate for water and electrolyte loss in diarrhea.

The product contains glucose and sucrose. Persons with rare hereditary pathologies, such as fructose intolerance, glucose-galactose malabsorption or sucrase-isomaltase insufficiency should not take this medicinal product.

This product contains less than 1 mmol of sodium (23 mg) per capsule, i.e. it is essentially sodium-free.

Pediatric population

Diarrheas in children younger than six years should be treated under medical supervision.

Use in gestation and breast feeding period

The particular caution is required in acute diarrhea during pregnancy and breastfeeding to prevent any occurrence of water and electrolyte deficit and other adverse reactions which would be dangerous for a child or a pregnant woman. The use in diarrhea during pregnancy and breastfeeding should be under medical supervision.

Posology and method of administration

Adults and children: one capsule once or twice a day (dependent on severity of symptoms).

The LRG-002 medicinal product capsules may be taken until symptoms disappeared. If diarrhea lasts for more than two days it's necessary to seek medical attention.

It's very important to compensate for water and electrolyte loss in diarrhea treatment.

Method of administration

To provide the maximum efficacy, LRG-002 capsules should be taken with meals.

LRG-002 capsules must not be taken concomitantly with alcohol, fruit juices or hot beverages.

For those who are not able or cannot swallow a capsule it should be opened, and its content should be poured out into a spoon and mix with some liquid. It is recommended to take LRG-002 capsules not earlier than 3 hours after the antibiotic intake.

Adverse Reactions

Adverse reactions are extremely rare in use of probiotic products.

Mild gastrointestinal disorders such as nausea, abdominal cramping, flatulence or abdominal distension have been reported.

Overdose

No case of overdose has been reported.

Interaction with other medicinal products and other forms of interaction

Drug-to-drug interaction studies have not been performed.

As other bacteria, the probiotic bacteria are sensitive to antibiotics. For this reason, it is recommended to take the LRG-002 product not earlier than 3 hours after the antibiotic intake.

Special warnings

Do not store above 25 °C.

Store in the original package in order to protect from moisture.

Effects on the ability to drive or use machines

Studies of the drug effect on the ability to drive vehicles and work with mechanisms have not been performed

3.1.2 Comparator product

LRG-002 placebo capsules.

3.2 Summary of relevant results of non-clinical and clinical studies for this study

3.2.1 Summary of non-clinical results

The Lactobacillus rhamnosus «GG» strain was first isolated in 1983 and well-studied for pharmacokinetics, pharmacodynamics, safety and toxicity. The capability of this LGG strain to adhere to intestinal mucous membrane, to inhibit different pathogenic bacterial growth, to reconstitute the integrity of intestinal epithelium and to regulate the immune response by means of the effect on pro-inflammatory cytokine release was demonstrated in in vitro and in vivo studies. All listed characteristics positively influence the normal intestinal microflora reconstitution and the gastrointestinal functional state usually affected in infection and antibiotic-associated diarrhea.

Furthermore, benefits of the LGG strain are not limited by the intestine. Thus, in many studies the possibility to use the LGG strain as an adjunctive therapy of different diseases including cancer had been proposed.

It had been shown in safety studies that the LGG strain did not cause any adverse reactions in animals despite administration in extremely high dosages.

Studies for Lactobacilles microbiological properties evaluation including antibiotic sensitivity and the potential for uptake and transferring of antibiotic-resistance genes were also performed. In these studies, it was established that despite of natural resistance of many lactobacilli to various antibiotics (including vancomycin) the possibility to transfer antibiotic-resistance genes to other species is minimal.

So, LGG is a non-pathogenic strain for both healthy and immunocompromised animals. After administration at high dosages this strain can’t induce any systemic infections in rodents. Further more, no undesirable metabolic activity was established. As for antibiotic resistance, the L. rhamnosus strain may be considered to be genetically stable. The information from non-clinical studies performed for the LGG strain is provided below.

3.2.2 Main pharmacological properties

3.2.2.1 In vitro investigations

Forrestier et al. (2001) performed the investigation evaluating the probiotic activity of the human isolate of the*Lactobacillus casei* subsp. *rhamnosus* (Lcr35).strain. The presence of adhesive properties of this strain had been demonstrated In the in vitro model based on the Caco-2 cell line. The inhibitory effect of Lcr35 on three pathogenic species, particularly enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and *Klebsiella pneumoniae*, was demonstrated. The reduction in number of adhesive pathogens as a result of pre-incubation, post-incubation and co-incubation with the Lcr35 strain was established. In addition, the Lcr35 cell-free supernatant antibacterial activity was studied for nine different human pathogenic bacteria: ETEC, EPEC, *Klebsiella pneumoniae*, *Shigella flexneri*, *Salmonella typhimurium*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Clostridium difficile*. According to the results of the assessment of viable bacterial cells dynamics, growth inhibition of all strains' was determined, however no bactericidal activity was found in this in vitro test. Based on the results of the observations, it can be assumed that the studied probiotic strain can be used to prevent colonization of the gastrointestinal tract by various pathogens.

Jacobsen et al. (1999) carried out a study evaluating the probiotic potential of 47 strains of Lactobacillus spp. Selected strains were studied for resistance to pH 2.5 and 0.3% bovine bile, adhesion to Caco-2 cells, and antimicrobial activity against pathogenic intestinal bacteria in model systems. 29 of the 44 strains remained viable after 4 hours of incubation in a medium with pH 2.5, but none of them retained the ability to replicate. A significant variability of the characteristics of the studied bacterial strains was determined. 299, 299v, DSM 12246, LGG, 18911-2, 19020-10, 19070-2, CHCC 2329, CHCC 3137 and BG2FO4 strains showed high adhesive ability, unlike other strains with moderate or low adhesive ability. The strains also differed in antimicrobial activity. The LGG strain caused severe growth inhibition of *Bacillus cereus*, moderate growth inhibition of *Listeria monocytogenes*, *Escherichia coli*, *Shigella flexneri*, *Yersinia enterocolitica*, and did not inhibit the growth of *Staphylococcus aureus* and *Salmonella typhimurium*.

The authors also evaluated the antimicrobial activity of 8 individual strains (including LGG) in relation to the resident microflora of the gastrointestinal tract. A moderate inhibitory effect of LGG strain on *Klebsiella oxytoca* and *Enterobacter cloacae* was determined, but it did not affect other commensal bacteria, including other lactobacilli. These results indicate a favorable effect of the LGG strain on the intestinal microflora, the inhibitory effect of this strain on the growth of many pathogenic bacteria and its very limited inhibitory effect on the growth of normal microflora.

Nemcova et al. (1993) also studied 14 strains of lactobacilli in order to determine their sensitivity to antimicrobial dietary supplements, acid resistance, adhesion to porcine intestinal epithelial cells and antimicrobial activity. Four strains were identified as *Lactobacillus casei* subsp. casei, two strains — as *Lactobacillus rhamnosus* and *Lactobacillus reuteri*, and three strains — as *Lactobacillus salivarius*. In antibiotic sensitivity tests, the sensitivity of lactobacilli to many of the antimicrobial supplements commonly used for pigs was established. A large numbers of strains resistant to ciadox and nourseothricin were found. The studied strains of lactobacilli showed acid resistance in a medium with pH 3. A high adhesion ability to pig intestinal epithelial cells was established for 13 lactobacilli strains, while one strain showed weak adhesion ability. All lactobacilli inhibited test bacteria in the presence of glucose. The main end products of glucose fermentation in all strains were lactate and acetate.

Zhang et al. (2005) studied the effects of *L.*GG strain, viable and non-viable, on TNF-α-mediated induction of the interleukin response. Caco-2 cell line was exposed to viable or thermally inactivated bacteria of the LGG strain at doses of 10^4 to 10^{10} CFU/L in a medium with additives of antibiotics and TNF-α or without additives. The LGG strain regulated TNF-α-induced production of IL-8 by Caco-2 cells in all three conditions. However, influence of viable bacteria of the LGG strain at higher doses in a medium without TNF-α with or without the addition of antibiotics in vitro induced the synthesis of IL-8 ($P<0.001$). The thermally inactivated bacteria of the LGG strain also reduced TNF-induced synthesis of IL-8 ($P = 0.01$), but at higher doses did not increase the synthesis of IL-8 to the same high level as viable bacteria ($P = 0.05$). Bacteria of the LGG strain reduced TNFα-induced NFB translocation to the nucleus and decreased the degree of IB reduction in the cytoplasm ($P = 0,05$). Bacteria of the LGG strain reduced TNF-α-induced synthesis of IL-8 by affecting the NFB/IB signaling pathway in Caco-2 cell line. The viable bacteria of the LGG strain at high doses significantly increased IL-8 synthesis, however, thermally inactivated bacteria of the LGG strain caused only a slight increase in IL-8 synthesis. The authors did not explain why exposure to bacteria of the LGG strain at very high doses (10^{10} CFU/ml) led to an increase in IL-8 synthesis. This indicates the potential for induction of inflammation in the intestine when taking very high doses of probiotics due to excessive bacterial growth. However, the results presented are observations taking from in vitro study, that is why they must be reproduced in vivo before making any reliable conclusions based on them. It is important to note that a potential of LGG strain to reduce the inflammatory response was also demonstrated in this study even against the background of antibiotic therapy.

Lebeer et al. (2007) studied the ability of bacteria of the *L*GG strain to form biofilms on abiotic surfaces compared with other *lactobacilli*, studied under the same conditions. According to the results of the analysis of biofilms on a microtiter plate, the ability of LGG strain bacteria to form biofilms in vitro is highly regulated by nutrient factors and conditions in the gastrointestinal tract, including low pH, high osmolarity and the presence of bile, mucins and indigestible polysaccharides. In addition, the phenotypic test of exopolysaccharides indicated their relative importance in the formation of biofilms. Assessing the formation of biofilms in different media is an additional approach to a better understanding of the mechanisms by which bacteria adapt to stressful environment and colonize different niches. Based on the results obtained, important characteristics of LGG strains were determined, increasing their higher adhesive ability and stability in the gastrointestinal tract, compared with other strains of lactobacilli.

By Donato et al. assumption (2010), probiotics support barrier function by influencing the signaling pathways mediated by pro-inflammatory cytokines. Caco-2 cells were inoculated in Transwell chambers to produce polarized monolayers with intercellular tight junctions (TJ). The bacteria of LGG probiotic strain were inoculated onto the apical side of the monolayers, and after 3 hours IFN-C (100 ng/ml) was added on the basolateral side and the culture was left overnight. Then the monolayers were transferred onto freshly prepared basal medium with TNF-α additive (10 ng/ml) or without the additive, and transepithelial electrical resistance (TER) was measured during the period of stimulation by TNF-α. In order to supplement TER data, the cells were prepared for immunofluorescence staining with zona occludens-1 (ZO-1). In order to measure the downstream signaling pathway, TNF-α cells were exposed to immunofluorescent staining for the NF-kB p65 subunit and then quantitative analysis of CXCL-8 mRNA was performed by qRT-PCR method. Basal culture medium was collected after overnight incubation with TNF-α to measure the level of chemokine secretion, including CXCL-8 (interleukin 8) and CCL-11 (eotaxin). After inoculation of the LGG strain bacteria, priming with IFN-C and 24-hour stimulation with TNF-α, epithelial cells retained TER and the distribution profile of ZO-1. The LGG strain bacteria reduced p65 nuclear translocation, which was demonstrated by immunofluorescence assay and evaluation of CXCL-8 mRNA expression level. After inoculation of LGG strain bacteria and cytokine load, a decrease in the level of CXCL-8 and CCL-11 proteins in the cells was determined. Signalling interactions between LGG strain bacteria and the epithelium of the model are mediated by two mechanisms. Firstly, LGG strain bacteria inhibit TNF-α-induced activation of NF-kB, as evidenced by the preservation of the epithelial barrier integrity for pro-inflammatory cytokines. Secondly, the effects of this probiotic are mediated, at least in part, by the response from ERK-

1/2, upon inhibition of which the protective effect of LGG on the integrity of the polarized epithelial monolayer disappears.

These observations indicate that the LGG strain bacteria facilitate the manifestation of the effects of pro-inflammatory cytokines on the integrity of the epithelial barrier and the inflammatory response, which are mediated, at least in part, by inhibition of NF-kB signaling pathway.

De Keersmaecker et al. conducted a survey study of antimicrobial substances synthesized by L. rhamnosus GG strain (2006). It was found that the bacteria of Lactobacillus rhamnosus GG strain synthesize a low molecular weight thermostable non-protein bactericidal substance, which is active against various species of bacteria in an acidic environment. The spent culture supernatant (SCS) of LGG strain bacteria cultured in MRS medium contained five substances that corresponded to the above description, if present in a given concentration. According to the results of HPLC analysis, these five components were identified in the antimicrobial fraction of LGG SCS as the following substances: unidentified substance with a retention time of 5.7, acetic acid, pyrrole-2-carboxylic acid (PCA), formic acid and lactic acid. Since the first three components were also present in sterile MRS medium, and formic acid did not show bactericidal activity against salmonella at the concentration in which it is present in LGG-ACS, lactic acid was identified as the main antimicrobial substance produced by LGG strain bacteria. Based on the results of various experimental approaches, it was concluded that under the studied culture conditions the high antimicrobial activity of LGG strain bacteria against salmonella was mediated by lactic acid.

Kankainen et al. (2009) compared the genomic sequence of the studied strain with a length of 3.0 MB and a similar genomic sequence of L. rhamnosus LC705 strain, an additional starter culture with a lower degree of binding to the mucous membrane. A high identity and sequence synteny were established for these genomes. However, the genome collinearity of both strains was interrupted by genomic islets, five for GG strain and four for LC705 strain. A significant number of strain-specific genes was assumed for these islets (80 for GG strain and 72 for LC705 strain). Islets specific for GG strain included genes encoding bacteriophage components, sugar metabolism and transport, and exopolysaccharide biosynthesis. Only one genomic islet of L. rhamnosus GG strain contained genes for 3 secreted LPXTG-like pilins (spaCBA) and pilin-specific sortase. The presence of fimbriae on the cell wall was determined by immunoblotting assay with antibodies to SpaC. Electron microscopy analysis with immunogold staining showed that the pilin of SpaC type is located at the top of the fimbriae, but is also sometimes determined along their entire length. Moreover, adhesion of GG strain to intestinal mucus in humans was blocked by serum to SpaC and did not occur in the mutant with inactivated SpaC gene. By analogy, the ability to bind to intestinal mucus was demonstrated for purified SpaC protein. According to

the authors, the presence of SpaC is necessary for the interaction of LGG strain with intestinal mucus and can explain the ability of this strain to stay longer in the human gastrointestinal tract as part of an intervention study, compared with LC705 strain. The presence of fimbriae that bind mucus on the surface of a non-pathogenic gram-positive bacterial strain explains the previously unclear mechanism of interaction of individual probiotic lactobacilli with the tissues of the host body.

3.2.2.2 Investigations in vivo

Lebeer et al. (2011) conducted in vivo study in mice to evaluate the role of long-chain exopolysaccharides with high galactose content (EPS) of the prototypic probiotic strain of Lactobacillus rhamnosus GG (LGG). Survival and stability of EPS-deficient LGG mutant in GIT of mice were studied as part of the competition assessment. A mixture of wild-type LGG strain (control) and CMPG5351 strain (EPS-deficient mutant) with 1:1 ratio was administered by oral probe to five female BALB/c mice for three consecutive days. From 6 to 72 hours after the first administration by probe, fecal samples were collected. A pronounced proportional decrease in the mutant population with a competitiveness index (CI) of less than 1 was determined in all fecal samples collected. To collect more detailed information on the relative contribution of adhesion to the external characteristics of LGG strains in feces, mice were sacrificed in 24 hours after the last probe administration and tissue samples (mucous membrane scrapings) were collected from different sections of the gastrointestinal tract (stomach, duodenum, cecum and colon). According to replica plating analysis, the relative percentage of the EPS-deficient mutant CMPG5351 strain was especially reduced in the mucus and mucous layer of cecum and colon. The observations obtained indicate an important protective function of long EPS molecules with a high galactose content for bacteria of LGG strain in GIT. Thus, EPS are a factor in the adaptation of LGG strain bacteria, which contributes to their survival and stability in the host organism. It should be noted that similar biological functions of EPS and CPS were described in pathogenic and symbiotic bacteria.

Lin et al. (2009) carried out a study in immature mice that were administered with LGG strain using a probe. The test animals were sacrificed and the intestinal mucosa was analyzed. An increase in the level of GSH oxidation and cullin-1 deneddylation was determined in intestinal tissue samples, which reflects the local formation of reactive oxygen species (ROS) and the final inactivation with the participation of Ubc12, respectively. Moreover, preliminary administration of LGG strain bacteria prevented TNF-α-induced activation of NF-κB in the intestine. These studies suggest that LGG strain bacteria can reduce the level of inflammatory signaling in the immature intestine by inducing local ROS formation, which may underlie the mechanism of the

preventive action of probiotic bacteria against necrotizing enterocolitis (NEC) in premature infants and reducing the severity of inflammatory bowel disease (IBD) in children.

Ritze et al. (2014) studied the possible protective effect of LGG strain bacteria against experimental non-alcoholic fatty liver disease (NAFLD) in a mouse model. Experimental NAFLD was induced by administering high fructose feed for eight weeks to C57BL/J6 mice. Fructose was administered with drinking water containing 30% fructose solution with LGG supplement at a concentration of 5.6×10^7 CFU/g bm or without the supplement. Mice were studied for changes in the microflora of the small intestine, intestinal barrier function, concentration of lipopolysaccharides (LPS) in the portal vein, inflammatory changes in the liver, and accumulation of fat in the liver. The administration of LGG strain bacteria was accompanied by the growth of beneficial bacteria in the distal small intestine. Moreover, LGG strain bacteria reduced the level of IkB protein in the duodenum and restored the concentration of proteins at the level of tight junctions in the duodenum. There was a decrease in the level of LPS in the portal vein ($P \leq 0.05$) and a tendency towards a decrease in the expression level of TNF-α, IL-8R, and IL-1b mRNA in the liver of animals receiving the high fructose feed with LGG supplement. Moreover, mice fed a high fructose feed with LGG supplement showed less pronounced fat accumulation in the liver and lower concentration of alanine aminotransferase in the portal vein ($P \leq 0.05$).

The obtained results indicate the potential benefit of LGG strain bacteria in the case of NAFLD, as well as, in general, for the balance of intestinal microflora. The mechanisms of this protective effect may include an increase in the population of beneficial bacteria, recovery of the intestinal barrier function, and a subsequent decrease in inflammation and liver steatosis.

The anti-inflammatory properties of LGG strain bacteria were also studied by Sawada et al. (2007) in the model of human atopic dermatitis in NC/Nga mice. Female mice and their offspring were given a feed with or without the supplement of thermally inactivated LGG strain during pregnancy and breastfeeding, as well as after ablactation. A spontaneous appearance of typical skin lesions, very similar to those in patients with atopic dermatitis, was noted in control animals of NC/Nga line, which were kept under conditions without control of air pollution. On the other hand, the introduction of feed with the supplement of thermally inactivated LGG strain inhibited the appearance and development of atopic skin manifestations, which was accompanied by a lower number of mast cells and eosinophils in the skin lesion sites. A significant increase in IL-10 plasma level was determined in mice receiving the feed with LGG strain supplement, compared with the control animals, while the proportion of CD4+CD25+ regulatory T-cells in the spleen did not significantly differ between the group of LGG strain administration and the control group. A higher level of IL-10 mRNA expression was determined in Peyer's glands and mesenteric lymph nodes

of mice fed with LGG supplement. These observations suggest that some components of the thermally inactivated bacteria of LGG strain are able to delay the onset and prevent the development of atopic dermatitis, probably by means of the mechanism of pronounced induction of IL-10 in lymphoid organs and systemic blood flow.

Isolauri et al. (1993) studied the effect of lactobacilli on the intestinal mucosa in suckling rats aged 14 days. In addition to ordinary mother's milk, animals from "milk" group received cow milk daily by means of a probe, animals from "milk-GG" group received cow milk with the supplement of *Lactobacillus* casei GG strain, and the animals from the control group were administered with the same volume water by means of the probe. After 21 days, the absorption of horseradish peroxidase was evaluated in the segments of jejunum without Peyer's glands and in the segments with Peyer's glands using Ussing chambers. A significant intergroup difference in the average level of absorption of intact horseradish peroxidase, expressed in ng • h^{-1}• • cm^{-2}, was found both in the segments without Peyer's glands (control group: 9 [95% confidence interval: 7–12]; «milk» group: 72 [60–87]; «milk-GG» group: 15 [4–52]), and in the segments with Peyer's glands (control group: 3 [1–17]; «milk» group: 80 [43–151]; «milk-GG» group: 15 [4–56]). There was a significant increase in the frequency of cells secreting antibodies to beta-lactoglobulin (according to the data of enzyme-linked immunospot assay) in "milk-GG" group. Prolonged administration of cow milk to the suckling rats led to an increase in permeability of intestinal wall for intact proteins, while *Lactobacillus* GG strain counteracted the permeability increase. The obtained results indicate a relationship between the intensity of the antigen-specific immune response and stabilization of the mucous barrier.

Lee et al. (2000) studied the bacterial translocation of the ampicillin-resistant Escherichia coli K1 strain and the effects of L. rhamnosus GG (LGG) strain in 77 newborn New Zealand albino rabbits. On the day of birth, the newborn rabbits were separated from the females and distributed into the groups of probe feeding with placebo, 10^8 KOE LGG (n = 8), 10^8 KOE K1 (n = 26) or a combination of both bacterial strains at a dose of 10^8 CFU (n = 33) twice a day for two days. These newborn rabbits were sacrificed on the 3rd day and tissue samples were taken, which were incubated under aerobic conditions for 48 hours, and then the number of colonies of LGG and K1 strains was counted. In newborn rabbits fed with the LGG strain supplement, significantly lower intestinal colonization with the K1 strain (25%) was determined compared to animals not receiving LGG strain. Also, a significantly lower degree of bacterial translocation of strain K1 to the mesenteric lymph nodes, spleen, and liver was noted in this group. Damages of the small intestine mucous membrane were detected in none of the rabbits. Bacterial translocation of the LGG strain to these extraintestinal foci was detected in 1 of 8 animals in the LGG group and in 4 of 33 animals

in the LGG + K1 group. According to the authors, LGG strain inhibited the colonization of E. coli K1 strain bacteria and their translocation into extraintestinal foci. However, despite the fact that for newborns rabbits had a tendency to immaturity of the immune function and underdevelopment of the mucous membrane, mucous layer and intestinal epithelium, as well as, despite the absence of visible clinical manifestations of infecting by LGG strain, the authors expressed concern about the even rarer problem of LGG strain translocation.

Naaber et al. (1998) studied the effects of the L. rhamnosus GG (LGG) strain in 10 adult Syrian hamsters who were given with ampicillin at a single dose of 3 mg, and after 24 hours — an infecting dose of *Clostridium difficile*. Starting 20 hours before the introduction of C. difficile and up to 5 days after the sacrifice, 0 or 0.5 ml of LGG strain in the broth culture (the CFU value was not specified) was introduced once a day into the test animals. According to the results of stool culture, LGG strain bacteria were isolated from all animals receiving active therapy, however, they did not prevail in the intestinal microflora and were not observed among translocating lactobacilli. Four of the five hamsters treated with the probiotic LGG strain remained healthy and had milder inflammatory changes in the intestinal mucosa compared with animals from the control group. No adverse effects were reported.

3.2.2.3 Pharmacological safety studies

There were no reports in preclinical studies about safety hazards associated with the use of LGG strain. In toxicity tests of extremely high doses of the Lactobacillus rhamnosus GG strain and other strains, no adverse effects on overall health, general clinical and biochemical blood values, histological parameters of the intestinal mucosa, or bacterial translocation frequency were determined. There were several reports about bacterial translocation (Link et al., 1995, Wagner et al., 1997, Lee et al., 2000). Although we considered it was normal and even beneficial (Bengmark and Jeppsson, 1995), there is a probability of developing septicemia and generalized infections in subjects with impaired immunity. However, this effect was rarely observed, and clear clinical evidence was not found. According to Herzberg et al. (1992), infecting New Zealand albino rabbits with lactic acid bacterium (LAB) strains that can induce platelet aggregation (Agg+) invariably caused infectious endocarditis with a more severe course and higher mortality compared to infection with Agg- strains. As a result, the lack of platelet aggregation potential is indicated as one of the most important criteria for choosing a probiotic strain (Harty et al., 1993, Kirjavainen et al., 1999.). Based on this, Zhou et al. (2005**)** studied the ability of strains of L. rhamnosus HN001, GG and B. animalis ssp. lactis HN019 to the induction of platelet aggregation in humans in vitro. Three probiotic strains and Streptococcus sanguis 133-79 strain, used as a positive control, were cultured, purified and concentrated into a suspension containing approximately 10^9 CFU/ml.

Blood samples were took from six healthy volunteers aged 24 to 45 years old, then the samples were centrifuged, exposed to bacterial cells with 1:1 ratio of platelets to bacterial cells (the optimal ratio for inducing platelet aggregation) and incubated at room temperature for 30 minutes. Platelet samples were analyzed by flow cytometry for the average fluorescence intensity and the percentage of cells twice positive for mC-CD41a and PECD62p (markers of normal and activated platelets, respectively). Additionally, the following clinical blood values were measured: the total red blood cell and platelet counts, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. None of the listed clinical blood values changed under the influence of bacterial cells. S. sanguis strain induced platelet activity to a higher degree; more than 91% of the cells were CD62p positive. On the other hand, probiotic bacterial strains did not affect platelet activity; the degree of platelet activation did not significantly differ between platelets exposed to probiotic strains and intact platelets. By analogy, the evaluation of platelets exposed to the S. sanguis strain showed a significant increase in the size of thrombocytic particles and aggregates. Probiotic bacteria did not affect the size of thrombocytic particles, which indicates that L. rhamnosus HN001 strain and other studied probiotic strains did not induce or enhance platelet aggregation. In addition, Vankerckhoven et al. (2007) studied the potential infectivity of 10 isolates of L. rhamnosus and 7 isolates of L. paracasei in a model of experimental infectious endocarditis in rats. All test organisms were obtained from PROSAFE strain collection. Four strains of L. rhamnosus were commonly used probiotics, other four strains were isolated in patients with endocarditis, one strain was used in fermentation processes, and another strain was under consideration as a potential probiotic. As part of the study, experimental lesion was induced in female Wistar rats with a body mass of 180–200 g by leading a catheter through aortic valve. After 24 hours, rats were injected intravenously with 10^4–10^8 CFU of each test microorganism in groups of 7–11 animals per strain and inoculum. After 72 hours, the rats were sacrificed, after which the heart and infected valves were removed to count microorganisms. Strains of Staphylococcus aureus Newman, Streptococcus gordonii Challis and Lactococcus lactis 1363 were used as a positive and negative control of infectivity. Additionally, adhesion of lactobacilli to immobilized matrix proteins of host organism and their sensitivity to thrombocytic proteins with microbiocidal activity were evaluated. 90% infectious doses of S. aureus and S. gordonii for tissue culture (SCID90) were 10^4 and 10^5 CFU, respectively, indicating a high infectivity of the true pathogens of endocarditis, whereas SCID90 of the control strain of Lactococcus lactis was 10^7 CFU. The SCID90 value of L. rhamnosus isolates varied from 10^6 to 10^7 CFU. SCID90 of LGG strain was 10^6–10^7. According to the authors, investigational probiotic, nutritional and probiotic isolates of lactobacilli are less able to induce experimental endocarditis compared to isolates, which are common causative agents of

endocarditis in humans. The number of lactobacilli necessary for infection of 290% of individuals was from 100 to 1000 times higher than the number of S. aureus or S. gordonii bacteria. They additionally suggested that lactobacilli can act as the causative agent of endocarditis under certain factors on the part of the host organism, since the corresponding cases were recorded mainly in patients with severe background diseases or reduced immunocompetence. Shu et al. (1999) studied the safety of different strains of lactobacilli in BALB/c mice by introducing different doses of the corresponding bacteria (5×10^7, 10^9 or 5×10^{10}CFU per individual a day) for 7 days. During the trial, abnormal clinical manifestations were observed in none of the groups. There were no significant differences in the feed and water intake levels or liveweight gain between mice receiving various probiotic strains and animals from the control group not receiving LAB. No bacteria were found in spleen tissues in any animals. Despite the fact that lactic acid bacteria were detected in the kidney tissues of one of animals in each of the groups receiving the strains of Lb. rhamnosus HN001 or B. lactis HN019, analysis by DNA fingerprinting showed the difference between the recovered isolates and tested probiotic strains. Histological and hematological indicators also showed the absence of adverse effects of lactic acid bacteria strains on mice health condition.

3.2.2.4 Secondary pharmacological properties

Lactobacillus rhamnosus strain was successfully used in the treatment of alcohol-induced liver injury in animals, for example in rats and mice. These studies identified additional anti-inflammatory effects that are also important for understanding the basic pharmacological properties of LGG strain.

Forsyth et al. (2009) suggested that probiotics can maintain normal barrier function in the animal model of ALD by preventing alcohol-induced oxidative stress and, as a result, inhibiting the development of increased permeability and subsequent alcoholic steatohepatitis(ASH). Male Sprague-Dawley rats were gavaged alcohol twice a day (8 g/kg) for 10 weeks. Rats that received alcohol were also gavaged 2.5×10^7 live bacteria of the *L. rhamnosus* (Gorbach–Goldin) strain (LGG) or vehicle (V) once a day. Intestinal permeability (initially and after 10 weeks) was studied by bolus injection of sugar and subsequent analysis of sugar in the urine by GC. Intestinal and liver tissues were analyzed for oxidative stress and inflammation markers. A histological evaluation of the liver for the ASH severity and total fat content (steatosis) was also performed. Rats treated with alcohol and LGG strain (ALC+LGG) showed a significantly lower severity of ASH ($P \leq 0.05$) compared with rats from the ALC+V group. The *L. rhamnosus* (Gorbach–Goldin) strain also reduced alcohol-induced "leakage" of the intestine and significantly reduced alcohol-induced oxidative stress and inflammation in the intestines and liver. Probe administration of the

probiotic *L. rhamnosus* (Gorbach–Goldin) strain significantly reduced the manifestations of ASH in rats. The observed improvement was accompanied with a decrease in the level of oxidative stress and inflammation markers in the intestines and liver and the intestinal barrier function preservation.

Wange et al. (2011) carried out a study in C57BJ/6N mice, which received Lieber DeCarli feed with the 5% alcohol supplement for 8 weeks. The animals also received the supplement of *Lactobacillus rhamnosus* GG (LGG) strain for the last two weeks. The introduction of LGG supplement led to a significant reduction in alcohol-induced endotoxemia and liver steatosis and resulted in liver function improvement. LGG strain restored alcohol-induced decrease in the level of HIF-2α and intestinal trefoil factors. It was demonstrated in *in vitro* studies using a Caco-2 cell culture model that the addition of the supernatant of LGG strain prevented alcohol-induced dysfunction of the intestinal barrier formed by the epithelial monolayer. Moreover, HIF-1α/2α gene silencing eliminated the effects of LGG, which indicated the dependence of the protective effect of LGG strain on HIF.

This study examined the mechanisms that underlie the probiotics effectiveness in the treatment of ALD, and suggests a critical role of intestinal hypoxia and decrease in the trefoil factor level for ALD development.

Wang et al. (2013) studied the effect of probiotics on the tumor necrosis factor α (TNF-α) synthesis in the liver and inflammatory response to chronic alcohol consumption. The mice received Lieber DeCarli liquid feed with 5% alcohol supplement for 8 weeks, and additionally Lactobacillus rhamnosus GG strain (LGG) was introduced to them for the last 2 weeks. The introduction of alcohol for eight weeks led to a significant increase in the inflammatory response in the liver, as was shown in the results of histological evaluation and analysis of myeloperoxidase activity in liver tissues. The introduction of the LGG strain supplement for two weeks reduced the inflammatory response in the liver and the liver damage, and also significantly reduced TNF-α expression. The introduction of alcohol led to an increase in the expression level of mRNA of toll-like receptors (TLR) and CYP2E1 in the liver and a decrease in the expression level of nuclear factor erythroid 2-related factor 2. Administration of LGG supplement reduced the extent of these changes. Using macrophages obtained from human peripheral blood monocytes, we also showed that incubation with ethanol triggers lipopolysaccharide- and flagellin-induced synthesis of TNF-α, and the supernatant of LGG strain culture dose-dependently reduced this inducing effect. In addition, the introduction of the LGG strain also significantly reduced the degree of alcohol-induced phosphorylation of p38 MAP kinase. Thus, the probiotic strain LGG reduced

alcohol-induced liver inflammation by reducing the synthesis of TNF-α through inhibition of TLR4 and TLR5-mediated activation of endotoxins.

3.2.2.5 Pharmacodynamic drug Interactions

Data about pharmacodynamic drug interactions was not reported.

It is known that the effectiveness of LGG strain may decrease on treatment of antibiotics (Radulovic et al., 2012).

3.2.2.6 Pharmacokinetics

The pharmacokinetics of probiotics mainly describes a change in their condition (survival and association with the host organism) in the gastrointestinal tract. Several in vitro models were developed to predict in vitro survival of probiotics or their adhesion to intestinal epithelium. Static models can provide information on the sensitivity of strains to a constant pH or bile concentration. Multicompartment dynamic models were also developed in which using a computer program the dynamics of secretion of gastric juice and bile, as well as the passage of chyme through the intestines can be reproduced. These models can more accurately predict in vivo situations, as well as examine the effects of various indicators, such as bile concentration or variability in gastric acid secretion (Marteau and Vesa 1998).

3.2.2.7 Adhesion to intestinal epithelium

The lactobacilli adhesion to the mucous membrane is considered one of the first components of the digestive tract colonization process in the host organism.

Nishiyama et al. (2016) provided a comprehensive overview of the lactic acid bacteria adhesion properties. The main segment of the intestine colonized by the LAB, especially *Lactobacillus* genus bacteria, is the segment between the duodenum and the terminal part of the ileum, in which the intestinal wall is lined with a mucous layer consisting mainly of mucin-like glycoproteins. The mucous layer contributes to the protection of intestinal epithelial cells against damage but is also a critical factor in the lactobacilli adhesion in the gastrointestinal tract. *Lactobacilli* exhibit different adhesiveness to mucin and mucin carbohydrate chains due to the wide scatter of molecular structures. This means that lactobacilli adapt to the constantly changing intestinal environment of the host organism, and additionally indicates that the lactobacilli adhesion factors have a specific affinity for binding, which allows them to efficiently colonize the intestines of the host organism and at the same time avoid competition with other bacteria.

Two main adhesion factors were determined for the Lactobacillus rhamnosus GG strain: mucus-specific adhesin (von Ossowski et al., 2011, Nishiyama et al., 2015) and fimbria

(Nishiyama et al., 2016, Kanakinen et al., 2009, Reunanen et al., 2012, Rintahaka et al., 2014, von Ossowski et al., 2010).

Conway et al. (1987) studied the adhesion mechanism of different strains of lactobacilli. Based on the fact that all strains showed a certain adhesive ability to human and porcine intestinal cells, nonspecific attachment was suggested as a possible adhesion mechanism. This was further confirmed in a study by Sillanpää (2001). This study demonstrated that the ability to adhere to target human tissues, established for lactobacilli, is typical for LAB from different sources and different types of the *Lactobacillus* genus bacteria.

3.2.2.8 Survival in gastrointestinal tract and excretion

Goldin et al. (1992) carried out a study in which 76 volunteers took the *Lactobacillus* GG strain as a frozen concentrate or fermented preparation in milk or serum. The studied strain was isolated from feces of all volunteers who took fermented milk or serum, and 86% of volunteers who took the frozen concentrate, according to the results of inoculation on one feces sample from the volunteer. The studied microorganism was also present in the feces of subjects taking ampicillin at the time of the study. After stopping the administration of a probiotic supplement with food, the *Lactobacillus* GG strain continued to be excreted in feces in 87% of volunteers after four days and in 33% of participants after seven days. *Lactobacillus* GG strain reduced the activity of fecal bacterial beta-glucuronidase by about 80% in volunteers who received it with food for four weeks. It was shown in these studies that the Lactobacillus GG strain survives in the human gastrointestinal tract and temporarily colonizes it, as well as the fact that it affects the metabolic activity of resident microflora.

Moreover, according to Marteau and Vesa (1998), the strains of L. acidophilus, L. reuteri, and L. rhamnosus GG show a higher survival ability in acidic medium compared to other strains of lactobacilli. After the L. rhamnosus GG supplement administration, the concentration of this strain in feces reached 10^6 CFU/g. After stopping the probiotic strains oral administration, their excretion kinetics approached the inert marker excretion kinetics, which indicates the absence of colonization.

Charteris et al. (1998) developed an in vitro method for modeling a transit in the human upper gastrointestinal tract. The survival of potential probiotic strains of Lactobacillus and Bifidobacterium genera during transit through the gastrointestinal tract was studied by exposure to washed cell suspensions at 37 °C in artificial gastric juice (pH 2.0) containing pepsin (0.3% m/v) and sodium chloride (0,5% m/v), as well as artificial small intestinal juice (pH 8.0) containing USP pancreatine (1 g/L) and sodium chloride (5 g/L), and periodic monitoring of changes in the

total viable bacteria count. This method was also used to evaluate the effect of adding milk proteins (1 g/L), gastric mucus of domestic pigs (1 g/L), and soybean trypsin and chymotrypsin inhibitor [SBTCI] (1 g/L) for survival during transit in gastrointestinal tract. A limited survival was established for the Lactobacillus rhamnosus GG strain during transit through simulated gastric juice, namely, after 1.5 hours, 0.1% of the exposed cells survived. This observation confirms the results of Goldin and Gorbach (1989), according to which the number of GG strain bacteria decreased from 10^9 to 10^3 after 2 hours in the human stomach at pH 1-2. The authors also determined a decrease in the number of bacteria of GG strain from 10^8 to 10^6 after holding them in normal human gastric juice for 1.5 hours at pH 2.5. Nevertheless, it was shown that Lactobacillus rhamnosus GG strain tolerates the transit through the stomach (Gorbach and Goldin, 1989; Goldin et al., 1992; Charteris et al., 1994, 1996), the effects of biliary salts (Gorbach and Goldin, 1989) and has the ability to adhere to the cells of ileum adenocarcinoma (Silva et al., 1987) and human colon in the culture (Elo et al. 1991; Chauviere et al., 1992). The current study also demonstrated the natural resistance of the LGG strain to intestinal proteases, since its viability did not decrease during simulated transit through the small intestine.

Corocan et al. (2005) studied the effect of glucose on the survival of L.*rhamnosus* GG strain in artificial gastric juice at pH 2.0. It was found that in the presence of 19.4 mmol glucose, the survival of the studied strain increased by 6 $\log_{10}$times after 90-minute exposure. An additional study in the diluted HCl confirmed that this effect is mediated exclusively by glucose. In the course of comparative analysis with other strains of *Lactobacillus*, it was determined that survival is increased in all strains, but at different pH values. In the presence of glucose in concentration from 1 to 19.4 mmol/L, the survival of *L. rhamnosus* GG strain in artificial gastric juice increased by 6.4–8 $\log_{10}$ CFU/ml. The mechanisms of this protective effect of glucose were studied. According to observations, the addition of *N*′,*N*-dicyclohexylcarbodiimide to artificial gastric juice deprived the bacterium of its ability to survive, which indicates a significant role of F_0F_1-ATPase inhibition. The results of an additional test with neomycin-resistant mutants, which had F_0F_1-ATPase activity from 38 to 48% of initial level, support this observation. The survival of these mutants decreased 3×10^6- fold in the presence of glucose compared with the survival of the non-mutant strain (8.02 $\log_{10}$ CFU/ml). Bacteria of *L. rhamnosus* GG strain survived in an acidic environment only in the presence of sugars, which they could efficiently metabolize. In order to confirm the role of the glycolysis process in the considered glucose effect, iodoacetic acid was used to inhibit the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The decrease in GAPDH activity was accompanied by a decrease in survival by 8.30 $\log_{10}$ CFU/ml in the presence of glucose. The obtained observations indicate that glucose serves as a source of ATP for F_0F_1-ATPase during

glycolysis, which ensures the process of proton exclusion and, thereby, increases survival during transit through the stomach.

3.2.2.9 Pharmacokinetic drug interactions

Bacteria of LGG strain are not absorbed after oral administration. However, they affect the absorption of other substances in the intestine. The most important consequence of this observation is the potential use of LGG strain and other probiotics as adjunctive therapy in cases of acute or chronic poisoning.

Gratz et al. (2006) demonstrated that oral administration of LGG strain to rats leads to a change in the uptake of B1 oral aflatoxin. An increase in AFB1 excretion with feces due to binding to bacteria was noted. In turn, this therapy prevented a body mass loss and reduced the hepatotoxic effect of high doses of AFB1.

Moreover, Deabes et al. (2012) also studied the effects of the oral LGG supplement on subacute exposure to aflatoxin in male albino mice. According to observations, the oral administration of LGG culture to mice led to a significant reduction in mycotoxin-induced toxicity by preventing oxidative stress, maintaining glutathione levels and stable activity of SOD, and also preventing mycotoxin-induced genotoxicity and toxic effects on sperm

Trinder et al. (2016) studied the effect of bacteria of Lactobacillus rhamnosus species on the absorption of organophosphorus compounds (parathion and chlorpyrifos (CP)) in *Drosophila melanogaster*. According to observations, strains of *Lactobacillus rhamnosus* GG (LGG) and L. rhamnosus GR-1 (LGR-1) are capable of significant sequestration of organophosphorus pesticides from solution after 24-hour co-incubation. The indicated effect was independent on metabolic activity due to absence of hydrolytic activity against CP in the L. rhamnosus GG strain and the absence of differences in sequestration of organophosphorus compounds between live and thermally inactivated strains. Moreover, LGR-1 and LGG strains reduced the absorption of 100 mol of parathion or CP in the Caco-2 Transwell model of small intestine epithelium. In order to determine the effect of sequestration on acute toxicity, recently caught *Drosophila melanogaster* flies were given with a feed containing 10 mol CP with or without the LGG strain live bacteria supplement. With the simultaneous introduction of LGG strain, but not simultaneously with the CP administered three days before (preventive therapy), a decrease in CP-induced mortality was observed.

3.2.2.10 Antibiotic resistance genes and probability of their transfer

Salminen et al. (1998) performed a review of lactic acid bacteria safety, during which it was noted that these bacteria have a long history of safe use in food products. Lactic acid bacteria are naturally resistant to many antibiotics. However, in many cases, resistance is not transferred, and the studied species are also sensitive to many antibiotics used in clinical practice, even in case of opportunistic infections associated with lactic acid bacteria. Thus, the presence of natural resistance does not raise any particular concerns about safety.

Like all bacteria, LAB are prone to gene exchange in order to increase survival in a medium containing antibiotics (Teuber et al. 1999). The main concern associated with ability of phenotypic antibiotic resistance in probiotic bacteria is their potential for in vivo transferring this resistance to pathogenic or potentially pathogenic microorganisms.

Borriello et al. stated that (2003) many strains of lactobacilli are naturally resistant to vancomycin. Nevertheless, it is generally accepted that insensitivity or resistance to antibiotics alone does not cause concern if it does not prevent the treatment in rare cases of infections caused by a probiotic, or if it can not be transferred to potential pathogens, resistance to which may cause therapeutic consequences.

By analogy, Temmerman et al. (2003) established resistance to kanamycin of all studied strains of L. rhamnosus and other strains of lactobacilli and bifidobacteria. Genetic grounds for resistance to kanamycin were not determined, and therefore this property is classified as natural.

In the framework a review of the antibiotic resistance of enterococci, Arthur and Courvalin (1993) noted that back in 1986, plasmid-mediated resistance to the glycopeptide antibiotics vancomycin and teicoplanin was found (Leclercq et al., 1988; Uttley et al., 1989), and also that the induced resistance to high doses of vancomycin and teicoplanin determines the manifestation of the vun(A) phenotype. According to the authors, the nucleotide sequences associated with the vun(A) gene were not found in gram-positive bacteria with natural resistance to glycopeptides, including Lactobacillus spp. Thus, resistance genes are not part of the chromosomes of these species and are not susceptible to gene transfer.

Based on materials from Teuber et al. (1999): despite the theoretically possible transfer of antibiotic resistance genes between LAB and bacteriophages and prophages, this has not been proved to be practically relevant. Moreover, the scatter of such a transfer in theory should be limited to closely related strains of the same species.

3.2.2.11 Susceptibility and resistance to antibiotics

Zhou et al. (2005b) assessed the resistance of lactobacilli to antimicrobial drugs. They studied resistance to 18 commonly used antibiotics by the disk diffusion method. The three studied lactobacilli strains had an antibiotic resistance profile similar to that of Lactobacillus plantarum HN045 strain and two commercial probiotic Lactobacillus strains, namely GG and LA-1. The antibiotic resistance profile of B. lactis HN019 was similar to that of three commercial probiotic B. lactis strains (Bb12, HN049 and HN098). All 10 strains were sensitive to antibiotics with activity against gram-positive bacteria, such as erythromycin and novobiocin, broad-spectrum antibiotics - rifampicin, spectinomycin, tetracycline and chloramphenicol, as well as beta-lactam antibiotics penicillin, ampicillin and cephalotin. At the same time, most strains were resistant to antibiotics active against gram-negative bacteria, such as fusidic acid, nalidixic acid and polymyxin B, and aminoglycosides neomycin, gentamicin, kanamycin and streptomycin. All three strains of L. rhamnosus (HN001, HN067 and GG) were resistant to vancomycin, and several strains also showed resistance to cloxacillin.

Klein et al. (2000) compared the genetic basis of lactobacilli and enterococci resistance to glycopeptides in order to determine their similarity. L. rhamnosus GG (ATCC 53103) strain, as well as five L. reuteri strains and 4 control Enterococcus strains were analyzed for the presence of the van(A) gene cluster, van(B) gene, and van(C) gene by PCR, Southern blot-hybridization, and DNA-DNA hybridization. Their antibiotic resistance and plasmid profiles were also determined. All strains of lactobacilli showed a certain resistance to vancomycin, oxacillin, gentamicin, ciprofloxacin, and trimethoprim/sulfamethoxazole, but were sensitive to a wide range of antibiotics, including clindamycin, imipenem, chloramphenicol, and rifampin. The authors noted that resistance of L. rhamnosus and L. reuteri strains to glycopeptide antibiotics is a natural property, and that there was no evidence of a possible transfer of this property of lactobacilli to other bacterial strains. The genomes of the studied lactobacilli strains did not contain the van(A), van(B) or van(C) genes.

According to these observations, the mechanism of resistance to glycopeptide antibiotics of the examined strains of lactobacilli differs from that of enterococci. The authors concluded that the study supports the safety of the presence of resistance to vancomycin in lactobacilli strains.

Kastner et al. (2006) in Switzerland, conducted a review of starter and probiotic cultures in order to study the current status of antibiotic resistance of nutritional supplements of bacterial nature. They screened 200 isolates from 90 different sources for phenotypic resistance to 20 antibiotics using the disk diffusion method. According to observations, the L. rhamnosus GG strain (the only

studied strain of L. rhamnosus species) showed resistance to 8 antibiotics (fusidic acid, kanamycin, nalidixic acid, tobramycin, nitrofurantoin, oxacillin, streptomycin and vancomycin), but not to the other studied antibiotics (cefotaxime , clindamycin, ofloxacin, penicillin G, tetracycline, lincomycin, meticillin, erythromycin, chloramphenicol, rifampicin, novobiocin and gentamicin). The last observation, namely the sensitivity of LGG strain to gentamicin, differs from the mentioned above Zhou et al. results. (2005b).

Twenty-seven isolates with an identified antibiotic resistance, which is not a natural characteristic of the corresponding genera, were further studied by microarray hybridization in order to identify phenotypic resistance to certain genetic determinants. No antibiotic resistance genes were found in the genome of the L. rhamnosus GG strain. Klare et al. (2007) evaluated 131 isolates of L. rhamnosus strains for resistance to 16 antimicrobials, which covered almost all important classes, in order to determine the distribution of minimum inhibitory concentration (MIC) for each isolate. This study revealed a surprisingly small proportion of cases of acquired antibiotic resistance. According to the authors, this could be due to the fact that all studied isolates were well-known and recognized completely harmless strains. Acquired resistance was observed only to streptomycin, erythromycin, clindamycin (3 isolates for each antibiotic) and oxytetracycline (12 isolates). Five of 131 isolates of L. rhamnosus showed acquired resistance, but no known antibiotic resistance genes were found in the genome of any of them.

3.2.2.12 Toxicity study

3.2.2.12.1 Acute toxicity

In acute toxicity studies, the same procedures as in chemicals toxicity tests were used. In these trials, high doses of probiotic strains are orally administered to animals, usually mice, for a short period of time. One of the important studied indicators is the translocation of the probiotic strain from the intestine to other tissues, especially intestinal lymph nodes, since this is one of the necessary conditions for the development of potential opportunistic infections. Zhou et al. (2000a) evaluated the acute oral toxicity of three strains of lactobacilli (Lactobacillus rhamnosus HN001, Lb. acidophilus HN017 and Bifidobacterium lactis HN019) in BALB/c mice that were administered with HN019, HN001 or HN017 strains in a high dose of 10^{11} CFU per animal per day for 8 consecutive days. According to the obtained results, the listed probiotic strains did not have adverse effects in relation to overall health, food intake, weight gain and morphology of the intestinal mucosa (villus height, crypt depth, epithelial cell height and mucous membrane thickness). No viable bacteria were identified in the blood and tissue samples of mice (mesenteric lymph nodes, liver and spleen). Also, no development of any diseases or deaths associated with

treatment was reported. The results indicate that the oral LD50 of HN019, HN001 and HN017 strains in mice exceeds 50 g/kg/day, which is 700 times higher than the usual dose of probiotic in humans.

Donohue et al. (1993) studied the acute toxicity of several strains of lactobacilli, including LGG, in mice. LGG strain at a dose of 0, 1.5, 3, 6 or 9×10^{11} CFU/kg was administered by means of a probe to twenty-five adult male house mice. Significant changes in behavior, food intake and body mass were not observed. Treatment-related deaths and toxic effects were absent, as well as noticeable macroscopic changes in internal organs. According to the authors, the acute oral LD50 of each of the studied bacterial strains is $>9 \times 10^{11}$ CFU/kg bm for male house mice. A dose of 6 g/kg/day was established as LD50 for the LGG strain, which corresponds to the maximum dose that can technically be administered to mice. (Bioactive Foods in Promoting Health: Probiotics и Prebiotics 2010; Kabeir et al., 2008).

3.2.2.12.2 Repeated dose toxicity studies

Zhou et al. (2000b) studied the overall safety of immunostimulating strains of lactic acid bacteria (LAB) *Lactobacillus rhamnosus* HN001, *Lb. acidophilus* HN017 and *Bifidobacterium lactis* HN019. In groups of BALB/c mice, test strains of LAB or a commercial control *Lb. acidophilus* LA-1 strain were orally administered at a dose of 2.5×10^9, 5×10^{10} or 2.5×10^{12} colony forming units (CFU) per kg of body mass per day for 4 weeks. During the period of the supplement administration, the intake of food, water, and live weight was monitored. At the end of the 4-week-long follow-up, samples of blood, liver, spleen, kidney, mesenteric lymph nodes and intestines (ileum, cecum and colon) were taken to determine the following indicators: hematological values (the total red blood cell and platelet counts, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration), Arneth formula, blood chemistry values ((total protein, plasma albumin, plasma cholesterol and plasmatic glucose), histological characteristics of the mucous membrane (epithelial cell height, mucous membrane thickness, villus height), bacterial translocation into extraintestinal tissues (blood, spleen liver, kidneys and mesenteric lymph nodes). Identification of viable bacterial strains isolated from these tissues was performed by DNA fingerprinting. According to the obtained results, the introduction of these strains of LAB for 4 weeks was not accompanied by adverse effects on overall health, hematological values, blood chemistry values, histological characteristics of the intestinal mucosa and the frequency of bacterial translocation.

A small number of viable LAB were isolated from the tissues of the control and test group animals, but none of them was identified as inoculated strain based on the results of DNA fingerprinting analysis. According to the results obtained in this study, the LAB HN001, HN017 and HN019 potentially probiotic strains are not toxic to mice.

Zhou (2000) studied the potential adverse effects of a dietary supplement based on two LAB strains, HN001 and HN019, in a model of immunocompromised animals. For the purpose of immunosuppression induction, dexamethasone was administered subcutaneously to animals 24 hours before the administration of probiotic (200 micrograms per individual for 48 hours). A freshly prepared strain of LAB was administered at a dose of 1.5-2.5 × 10^7 CFU per individual per day for 7 days. A pathogenic strain of Salmonella typhimurium was used as a positive control. At the end of the test, animal tissue and blood samples were taken to determine hematological parameters and bacterial translocation. None of the considered safety indicators was significantly changed. The introduction of the LAB supplement was not accompanied with adverse effect on animals, and there were no cases of bacterial translocation and systemic infections.

To study the effects of LAB strains in individuals with initial immunological dysfunction in a group of female CBA/CaH mice with experimentally induced autoimmune thyroiditis, a freshly prepared LAB strain (HN001 4.2 × 10^8 CFU per individual per day; HN019 2.16 ×10^8 CFU per individual per day) was administered for 5-8 weeks. Probiotic administration was started a week before immunization with autoantigens (MTg; mouse thyroglobulin). According to the results obtained, the introduction of LAB did not cause increased proliferative responses of splenocytes to MTg or lymphocytic infiltration of thyroid tissue. The LAB strains did not have adverse effects on the induction or course of autoimmune reactions in mice.

3.2.2.12.3 Genotoxicity

No studies demonstrating the lactobacilli genotoxic potential have been performed. On the other hand, there is an evidence of a genoprotective effect of the LGG strain administered simultaneously with known genotoxic substances.

Burns and Rowlan (2004) studied the effect of six strains of lactic acid bacteria (LAB) on genotoxicity against cells of fecal extract from the colon through a comet DNA analysis that measures single-strand DNA breaks. All the studied bacteria, with the exception of Streptococcus thermophilus, significantly reduced the DNA damage. The decrease in genotoxicity was associated with cell density, and it was found that viable probiotic cells did not affect the genotoxicity of fecal waters.

Pool-Zobel et al. (1996) evaluated the potential of different strains of lactobacilli to prevent DNA damage induced by N-methyl-N'- nitro-N-nitrosoguanidine (MNNG, 7.5 mg/kg of body weight) in rat colon cells. The results showed that most of the studied strains of LAB significantly inhibited genotoxicity against the GI tract of rats, and that only viable LAB can show a protective effect in vivo. The studies presented above were also summarized and reviewed in Rowlans' article (2007).

Farag et al. (2010) conducted a study to assess the possible protective effect of the Lactobacillus rhamnosus GG (LGG) strain on OTA-induced toxicity in mice. The study included four groups of 30 mice each: the control group, the LGG administration group (1×10^{10} CFU), the OTA administration group (1.8 mg/kg body weight), and the group of LGG administration two hours before the OTA probe. The study measured the activity of malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) in the liver and kidneys. Genotoxicity was studied by means of a micronuclear test on bone marrow cells, analysis of chromosomal aberrations in spermatocytes, as well as mitotic and meiotic activity; additionally, sperm parameters were evaluated. In mice treated with the LGG strain prior to OTA probe administration, there was a significant decrease in lipid peroxidation (POL) in the liver and kidneys due to increased GSH content and SOD activity. Cytogenetic analyses showed that the introduction of the LGG strain before the OTA probe led to a significant decrease in the incidence of MYAPCHE in the bone marrow and chromosomal aberrations in spermatocytes, as well as to the restoration of mitotic and meiotic activity. Moreover, when the LGG strain was gavaged before OTA, there was a significant recovery of all the studied sperm parameters.

Chiu at al. (2013) studied the genotoxicity on lyophilized powder of cultured probiotic strains that included *Lactobacillus rhamnosus LCR177*, *Bifidobacterium adolescentis* BA286, and *Pediococcus acidilactici* PA318. The Ames test, *in vitro* analysis of chromosomal aberrations in mammalian cells, and micronuclear tests on mouse peripheral blood cells were performed. For five strains of *Salmonella* typhimurium, the Ames tests did not show an increase in the number of reverse mutations after exposure to the test substance. The frequency of chromosomal aberrations in CHO cells did not increase in response to the introduction of probiotics. Similarly, the occurrence of micronuclear reticulocytes in mice treated with probiotic did not differ from that in individuals from the negative control group. Generalized results of toxicity studies indicate that multi-species probiotic mixtures do not exhibit mutagenic activity against different species.

3.2.2.12.4 Carcinogenicity

There are no studies that demonstrate the carcinogenic potential of lactobacilli. On the contrary, there is evidence that the LGG strain has anti-carcinogenic potential. However, some strains of

LAB synthesize enzymes necessary for deconjugation or dehydroxylation of bile acid salts (Eyssen 1973, Gilliland and Speck 1977), which is a concern because dehydroxylated bile acid salts exhibit cytotoxicity and cocancerogenicity (Marteau et al., 1995). However, more recent studies have concluded that the biological effect of probiotics is minimal and therefore not considered dangerous or clinically significant (Marteau and Salminen 1999, Zhou 2000c).

Banna et al. (2017) summarized the available evidence for the anti-carcinogenic effect of the LGG strain. The authors claim a positive effect of the LGG strain on inflammatory responses, epithelial damage, invasiveness, and proliferation of malignancies, based on the results of in vitro and in vivo studies.

Table 1. Effects of the *Lactobacillus rhamnosus* GG (LGG) strain on intestinal disorders associated with cancer (based on Banna et al., 2017)

Medical condition	**Model of the study**		**LGG strain effect**	**Control**
	In vitro	**In vivo**		
Gastric cancer	HGC-27		Displacement of the polyamine ionization profile to the acidic side	Linsalata et al., 2010
	GAC		Immunoregulation IL-8 H. pylori-dependent action	Rokka at al., 2008
Colon cancer	Caco-2, HT29, SW480		Proapoptotic agent in combination with vitamin K	Orlando et al., 2016
	Caco-2		Immunoregulation IL-8 Flagellin-induced action	Lopez et al., 2008
		Male rats of the Fischer-344 line	Antiproliferative action	Goldin et al., 1996
		Sprague Dawley rats	Inhibition of angiogenesis and inflammation	Gamallat et al., 2016
Metastatic CRC	HCT-116		Inhibition of invasiveness	Escamilla et al., 2012

		Female mice of the C57BL/6 line	Tumor regression	Seow et al., 2010
Loss of intestinal crypts		The mouse of line C57BL/6	Reduction of epithelial cell apoptosis	Ciorba et al., 2012
Inflammation		The mouse of line C57BL/6	Activation of the mucosal immune response	Wang et al., 2017
RIIT and CIIT		Human	Reducing the frequency of diarrhea by 15 %	Osterlund et al., 2007

GAC — gastric adenocarcinoma; H. pylori — Helicobacter pylori; DMG — dimethylhydrazine; CRC — colorectal cancer; RIIT — radiation-induced intestinal toxicity; CIIT — chemo-induced intestinal toxicity.

3.2.2.12.5 Reproductive Toxicity

There have been no preclinical studies of the effect of the LGG strain or other probiotic strains on reproductive functions.

3.2.2.13 Local tolerance

Zhou et al. (2000) studied pathological changes in the intestinal mucosa in BALB/c mice inoculated with three probiotic strains (Lactobacillus rhamnosus HNOO 1-DR20™, Lactobacillus acidophilus HNOl 7 and Bifidobacterium lactis HN019-DR10®). According to observations, the introduction of Lb. rhamnosus HN001, Lb. acidophilus HN017 or B. lactis HN019 LAB strains to mice in a high dose (2 times 10^{12} CFU/ml in skimmed milk [50 µl] per day [equivalent to 10^{11} CFU per individual per day]) for 8 days did not result in histologically distinguishable damage of the intestinal mucosa. No adverse effects were observed on the morphology of the intestinal mucosa (villi height, crypt depth, epithelial cell height and mucosal thickness), as well as on the orientation and location of the mucosal epithelial cells. According to the conclusion, the HN019,

HN001 and HN017 strains do not violate the integrity of the gastrointestinal mucosa when taken in an acceptable daily dose (ADD) of 35 g of dried bacteria per day for a person weighing 70 kg.

Kozakova et al. (2016) evaluated the effect of a mixture of three Lactobacillus strains (*Lactobacillus (L.) rhamnosus* LOCK0900, *L. rhamnosus* LOCK0908, and *L. casei* LOCK0919) on the integrity of the intestinal mucosa and allergic sensitization. The results showed that oral administration of a mixture of three strains of lactobacilli improved the intestinal epithelial barrier and reduced allergic sensitization in a model of gnotobiotic mice. These observations clearly indicate the positive effect of certain strains of lactobacilli on the process of allergic sensitization and even support their use for early prevention of allergies.

3.3 Summary of clinical studies

The beneficial effect of probiotic bacteria on human health is presumably based on three main mechanisms of action (Lebeer et al. 2008, Segers and Lebeer 2014). First, certain probiotic bacteria can displace or inhibit pathogens through direct action or influence on synanthropic microorganisms (Lebeer et al. 2008, Corr and Hill 2009). Second, some probiotic strains are able to strengthen the epithelial barrier by modulating signaling pathways, for example, mediated by nuclear factor-kB (NF-kB), Akt, and mitogen-activated protein kinase (MAPK), which result in mucus production induction (Mack et al. 2003) or strengthening close contacts (Rao et al. 2013). Third, most probiotic strains are also able to modulate the host's immune response, which is manifested by strain-specific local and systemic effects (Wells 2011). Most interactions between probiotic bacteria and intestinal epithelial and immune cells are presumably mediated by molecular structures known as microbial molecular patterns (MAMP), which can be recognized by specific image-identifying receptors (PRR), such as Toll-like receptors (TLR) (Abreu 2010).

A strain can be recognized as probiotic if it meets a number of criteria (Dunne et al. 2001; Zuccotti et al. 2008):

1. has a human origin;
2. is non-pathogenic;
3. is not destroyed during processing;
4. is not destroyed with gastric juice and bile;
5. is capable of adhesion to the intestinal epithelium;
6. is capable of colonizing the gastrointestinal tract, even in a short time;
7. produces antimicrobial substances;
8. modulates the immune response;

9. affects metabolic processes in the human body (cholesterol assimilation, lactase activity, vitamin production).

The Lactobacillus genus is represented by non-pathogenic rod-shaped LAB and consists of more than one hundred species. The November 2007 report of the European food safety authority (EFSA 2007) lists 112 species, whereas according to Bernardeau et al. (2008), this genus includes about 135 species and 27 subspecies. Bacteria of the Lactobacillus genus have heterogeneous phenotypic, biochemical and physiological characteristics; the extreme diversity of genomes of bacteria of the Lactobacillus genus is proposed to be used as a justification for the allocation of new subgenus divisions (Bernardeau et al. 2008). Lactobacilli are rod-shaped fixed non-spore-forming microorganisms. Growth of lactobacilli is possible in an environment with a reduced oxygen content, provided that there is sufficient nutrient content. This genus includes both homo- and heterofermentative bacteria. The first ones convert carbohydrates into lactic acid via the glycolytic pathway, and the second, with the participation of phosphoketolase, convert carbohydrates into lactic acid, acetic acid, ethyl alcohol and carbon dioxide. L. rhamnosus bacteria are optional heterofermenters of hexose (GRAS status notification # 288).

The strain of *L. rhamnosus* GG (ATCC 53103) has acid and bile resistance, which is a mandatory characteristic of functionally probiotic strains (Pant et al. 1996). In addition, this strain shows a high affinity for the cells of the human intestinal mucosa and produces lactic acid. The GG strain of lactobacilli has been studied as a probiotic in mice and humans, and its probiotic properties determine its effectiveness in the treatment of various forms of gastrointestinal and respiratory disorders and diseases (Papizadeh et al. 2016). The *Lactobacillus* GG strain showed no invasiveness, despite a high degree of adhesion to human intestinal cells (Elo et al. 1991, English and Dean 2013). According to a safety study, the *Lactobacillus GG* strain slowed the development of dimethylhydrazine-induced colon tumors in rats fed a high-fat diet (Goldin et al. 1996).

Since recently, it has been known that *Lactobacillus*, like L.cmei, is a synanthropic microorganism that colonizes the intestines, vagina, and distal ureters of a healthy person (Anukam et al. 2006; Voravuthikunchai et al. 2006). This type is often used for foodstuff preparation (for example, in the production of cheese, milk fermentation). Since *Lactobacillus* is found in the digestive tract of humans and mammals, it is obvious that the bacteria of this species enter the body during normal meals for a long period. As a result, it can be concluded that a person has swallowed bacteria of the species L.rhamnosus with food for a long time, which does not cause any visible undesirable effects.

The *Lactobacillus rhamnosus* GG strain has been used everywhere since 1990 as a component of food and dietary supplements (Salminen et al. 2002). The US food and drug administration (FDA) assigned the LGG strain the "generally recognized as safe" status (GRAS) (FDA notifications of GRAS status # 281 and 288) in Europe, and the European food safety administration (EFSA) assigned *Lactobacillus rhamnosus* the QPS status ("qualified presumption of safety") in 2007 at the species level (EFSA 2007).

Based on the available data, the following significant aspects can be identified that justify the need and feasibility of studying the effectiveness and safety of Lactobacillus rhamnosus in relation to AAD (antibiotic-associated diarrhea):

1. The increase in the prevalence of AAD in recent decades and the particular acuteness of the problem in the Russian Federation.
2. The predominant use of a large number of beta-lactam antibiotics, which have a proven depressing effect on the intestinal microbiota.
3. The risk of developing CdAD is one of the most severe manifestations of AAD.
4. A well-founded therapeutic tactic for prescribing probiotics for the prevention and treatment of AAD, with proven efficacy and safety of *Lactobacillus* GG (ATCC 53103).

Probiotics are living microorganisms that, when used in adequate quantities, are beneficial to the health of the host organism (report of the United Nations Food and Agriculture Organization (FAO)/World Health Organization (WHO), 2001). Therefore, to achieve a positive effect, they should be taken in fairly high doses. The proposed dose of Lactobacillus rhamnosus GG is within the range of doses that have been proven effective in well-planned and randomized controlled trials, and also corresponds to the doses recommended in the published guidelines of the world gastroenterological organization (WGO, 2017) and the European society of pediatric gastroenterologists, hepatologists and nutritionists (ESPGHAN, 2016).

According to the recommendations of the World Gastroenterological Organization, given in the international guidelines on probiotics and prebiotics (WGO, 2017) considering dosage in the case of antibiotic-induced diarrhea, the recommended dose of Lactobacillus rhamnosus GG for children is 1–2 × 10^{10} CFU, and for adults −10^{10} CFU twice daily. The developed product contains at least 10^{10} CFU of Lactobacillus rhamnosus GG per capsule. The recommended dosage is one or two capsules per day, depending on indications.

The choice of this dosage is also consistent with the data obtained during the acute toxicity study by Donohue et al. (1993), in which it was shown that the acute oral LD_{50} for the LGG strain is 6 g/kg/day, which corresponds to the maximum dose that can technically be administered to mice.

3.3.1 Overview of biopharmaceutics

Klu et al. (2012) studied the effect of storage conditions and the drug matrix on the survival of the L.rhamnosus GG strain. L. rhamnosus GG cells in an amount of 10^7CFU/g were inoculated on a layer of full- or reduced-fat peanut oil. Inoculated peanut oil was stored at 4, 25, and 37 °C for 48 weeks. Samples were collected periodically to count populations of L.rhamnosus GG. The results indicated that there was no significant reduction in the number of viable L. rhamnosus GG cells in preparations stored at 4°C. The survival rate of L. rhamnosus GG decreased with increasing temperature and storage time. The drug matrix had no significant effect on the survival of L.rhamnosus GG, except for storage conditions at 37 °C. Populations of L. rhamnosus GG were maintained at > 6 log in preparations stored at 4 °C for 48 weeks and at 25 °C for 23-27 weeks. At a storage temperature of 37 °C, populations of 6 log size were not preserved even for 6 weeks.

In a 6-month human study, Tannock et al. (2000) performed the repeated analysis of the concentration of viable L.rhamnosus HN001 cells in a lyophilized powder stored in a nitrogen atmosphere at room temperature. The powder contained 5.3×10^7 CFU at the beginning of the study and maintained this concentration after 6 months of storage; other bacteria other than lactobacilli were absent, and the viability of lactobacilli did not change for 6 months. In a study of the stability of the L. rhamnosus HN001 strain in infant formula stored at 30 °C, a decrease in bacterial viability was determined at a rate of 0.4 log per year.

3.3.2 Overview of clinical and pharmacological research data

3.3.2.1 Pharmacokinetic characteristics

The pharmacokinetic characteristics of probiotics can be broadly divided into three main groups:
- survival in the gastric environment;
- ability to adhere to the intestinal wall (colonization);
- metabolic pathway of active ingredients.

The active ingredients of probiotics are not always well known, so most studies are aimed at assessing the survival of probiotics in the human gastrointestinal tract (Marteau and Vesa 1998). One of the main conditions for assigning a bacterial strain to probiotics is its ability to survive in the gastrointestinal tract. The role of probiotic strain survival for its effectiveness is not absolute, since even inactivated and dead cells exhibit immunological and health-promoting effects (Linares et al. 2017, Zhang et al. 2005). Despite this, viability is still considered a prerequisite for optimal functioning of the probiotic strain (Lahtinen 2012).

Goldin et al. (1992) studied the Lactobacillus rhamnosus GG strain for survival in the human gastrointestinal tract. In this trial, 76 volunteers took the Lactobacillus GG strain as a frozen

concentrate or fermented preparation in milk or serum. The strain was isolated from feces of all volunteers who took fermented milk or serum, and 86% of volunteers who took the frozen concentrate, according to the results of inoculation on one sample of feces from the volunteer. The studied microorganism was also present in the feces of subjects taking ampicillin at the time of the study. After stopping the introduction of this microorganism with food, Lactobacillus GG was preserved in the feces of 87% of volunteers after four days and 33% of participants after seven days. Lactobacillus GG strain reduced the activity of fecal bacterial beta-glucuronidase by about 80% in volunteers who received this strain with food for four weeks. This indicates that the Lactobacillus GG strain survives in the human gastrointestinal tract and temporarily colonizes it, as well as the fact that it affects the metabolic activity of resident microflora.

Saxelin et al. (1995) studied discharge of the L.rhamnosus GG strain with feces after oral administration. Twenty healthy volunteers received the Lactobacillus GG strain as gelatin capsules for 7 days in daily doses of 1.6×10^8 CFU and 1.2×10^{10} CFU. All volunteers in the high-dose group had a distinguishable amount of Lactobacillus GG in their feces during the trial period. The studied strain was detected in the feces of all volunteers 3 days after administration. The effect on the total number of lactobacilli in the feces was not determined.

Stool was sampled before L.rhamnosus GG administration and on days 3, 5 and 7. Fecal samples were immediately frozen and stored in the refrigerator at -45 °C. Samples were analyzed for the presence of Lactobacillus and Lactobacillus GG on cups with MRS agar at pH 6.2. The plates were incubated anaerobically for 3 days at 37 °C and the total number of Lactobacillus and typical Lactobacilus GG colonies was evaluated.

Lactobacillus GG was detected in the feces of all 10 volunteers in the higher dose group (1.2×10^{10} CFU) during the test period (table 2). A slight decrease in Lactobacillus GG was observed on the seventh day, but the change was not statistically significant. In the lower-dose group, Lactobacilus GG was registered in feces of only one volunteer at the end of the treatment period (table 2). The introduction of Lactobacillus GG did not appear to affect the total number of lactobacilli in the feces (see the table below). It is worth mentioning that none of the volunteers had typical Lactobacillus GG colonies or any morphologically similar colonies in the fecal samples prior to the test (day 0, table 2).

The number of lactobacilli in stool samples (in CFU/g of feces) in healthy volunteers after oral administration of Lactobacillus GG for 7 days at doses of 1.6×10^8 CFU/day or 1.2×10^{10} CFU/day.

Table 2. The number of Lactobacillus GG and the total number of lactobacilli in fecal samples of 20 healthy volunteers received the Lactobacillus GG strain as gelatin capsules for 7 days in daily doses of 1.6×10^8 CFU and 1.2×10^{10} CFU.

Dose	The number of Lactobacillus GG and the total number of lactobacilli in stool samples							
CFU/ day	Day 0		Day 3		Day 5		Day 7	
	Lb	Lb GG	Lb	Lb GG	Lb	Lb GG	Lb	Lb GG
1.6×10^8	1.5×10^8	ND	1.7×10^7	ND	2.8×10^8	ND	8.0×10^7	10^3
1.2×10^{10}	9.2×10^7	ND	2.1×10^8	2.0×10^6	2.5×10^8	1.5×10^6	9.8×10^7	1.2×10^5

Note: Lb – Lactobacili, ND – not detected.

The study showed that orally administered Lactobacillus GG in gelatin capsules can be detected in the feces of healthy human volunteers when taken at higher doses (1.2×10^{10} CFU).

Hibberd et al. (2014) conducted a study of a drug containing Lactobacillus rhamnosus GG in elderly healthy volunteers.

Fifteen elderly volunteers aged 66-80 received Lactobacillus rhamnosus GG capsules containing 1×10^{10} CFU twice a day for 28 days, and were monitored for 56 days.

The main goal of this open-label clinical study was to evaluate the safety and tolerability of 1×10^{10} CFU of Lactobacillus rhamnosus GG taken orally twice a day in elderly volunteers for 28 days. The study also determined the concentration of Lactobacillus rhamnosus GG excreted in the feces by day 28 and 56 of the study.

Lactobacillus rhamnosus GG was found in the feces of 11 out of 15 (73%) volunteers at the end of the treatment period (study day 28), although the number of colonies in the feces varied significantly from 1.4×10^3 to 1.3×10^8. None of the volunteers was found to have Lactobacillus rhamnosus GG in the feces at the beginning of the study or by day 56 of the study.

No serious adverse events were reported in healthy volunteers during the study. A total of 47 adverse events were reported in 15 volunteers (1 to 7 per volunteer), 39 (83%) of which were assessed as mild, and 40% were assessed as possibly related to the use of Lactobacillus rhamnosus GG. The most common adverse events were recorded from the gastrointestinal tract (bloating, gas, and nausea), 27 of which were assessed as mild adverse events, and 3 as moderate. In the first week of taking LGG, mild and passing gastrointestinal symptoms were recorded, which did not affect the daily activity of the volunteers. After the first week, most of the gastrointestinal symptoms appeared periodically during treatment and during the follow-up period with no identified Association with LGG.

All AEs registered in this study are described in detail in table 3.

Table 3. AE description in healthy volunteers after taking Lactobacillus rhamnosus GG capsules containing 1×10^{10} CFU twice a day for 28 days

Study day	1–3	4–7	8–14	15–28	29–64	All time
Symptoms	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Expected AE	5 (11)	4 (9)	6 (13)	7 (15)	9 (19)	31 (66)
Gastrointestinal disorders						
Abdomen distention	0 (0)	1 (2)	1 (2)	0 (0)	0 (0)	2 (4)
Flatus	3 (6)	1 (2)	2 (4)	2 (4)	2 (4)	10 (21)
Borborygmus	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Diarrhea	0 (0)	0 (0)	1 (2)	1 (2)	1 (2)	3 (6)
Abdominal spasms or pain	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)
Nausea	1 (2)	0 (0)	2 (4)	0 (0)	2 (4)	5 (11)
Vomiting	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)	2 (4)
Loss of appetite	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)
Epigastric burning	0 (0)	1 (2)	0 (0)	1 (2)	1 (2)	3 (6)
Constipation	0 (0)	1 (2)	0 (0)	1 (2)	0 (0)	2 (4)
Others						
Rash	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)
Unexpected AE	0 (0)	1 (2)	1 (2)	2 (4)	2 (4)	6 (13)
Actinic keratosis	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)
Fever	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)
Headache	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Knee pain	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)	2 (4)
Kidney stones	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)
				28	56	All time
Unexpected AE (based on the results of physical examination or laboratory tests)				7 (15)	3 (6)	10 (21)
Vital signs						
High blood pressure				0 (0)	1 (2)	1 (2)
Low blood pressure				1 (2)	0 (0)	1 (2)

Laboratory parameters			
Decrease in hemoglobin concentration	2 (4)	0 (0)	2 (4)
Increased white blood cell concentration	0 (0)	1 (2)	1 (2)
Increased blood urea nitrogen	2 (4)	1 (2)	3 (6)
Decrease in glucose concentration	1 (2)	0 (0)	1 (2)
Increase of glucose concentration	1 (2)	0 (0)	1 (2)

Ninety-four percent (116) of the symptoms were disappearing, lasted for a short time, and did not require additional therapy. The study showed that Lactobacillus rhamnosus GG is safe and well tolerated by healthy adult volunteers aged 65 and older.

Pitino et al. (2010) studied six strains of Lactobacillus rhamnosus for their ability to survive in the upper parts of the human gastrointestinal tract. MRS broth was used as a carrier, and survival was evaluated in vitro by gastric digestion separately and in combination with duodenal digestion. The results indicated a high survival rate of all studied strains during both gastric and duodenal digestion. In particular, a very high survival rate was determined for three strains, which was evidenced by the degree of extraction in the range from 117 to 276 %. In accordance with the data on survival, a high level of lactic acid production was determined for all strains, which confirms the preservation of their metabolic activity during digestion.

Jacobsen et al. (1999) conducted an in vitro study on 47 strains of lactobacilli and additionally studied 5 selected strains in vivo. Selected strains were studied for resistance to pH 2.5 and 0.3% bovine bile, adhesion to Caco-2 cells, and antimicrobial activity against pathogenic intestinal bacteria in model systems. For LGG, the high adhesion capacity was determined, as well as resistance to gastric acid and bile salts (simulated by a medium with a pH of 2.5 and 0.3 % bovine bile). Under these conditions, the bacteria survived, but lost the ability to replicate. LGG was one of the strains selected for in vivo testing. Twelve healthy volunteers took two doses daily of 10^{10} lyophilized bacteria of the selected strains for 18 days, followed by a washout period of 17 days. Fecal samples were collected on days 0 and 18, as well as on days 5 and 11 of the washout period. Bacteria were re-isolated mainly during the administration period (day 18; 7 cases out of 12). On day 23, only 2 of 12 cases were re-isolated with the LGG strain. No bacteria were re-isolated on day 29. The LGG strain also inhibited a number of pathogenic bacteria and had minimal effect on the normal intestinal microflora.

According to the results obtained in this study, the bacteria of the LGG strain survive during passage through the gastrointestinal tract and can be re-isolated during the introduction period. However, there was no long-term colonization of any of the studied bacterial strains.

Dommels at al. (2009) conducted a double-blind randomized placebo-controlled interventional clinical trial to assess the survival of Lactobacillus reuteri DSM 17938 and Lactobacillus rhamnosus GG strains in the human gastrointestinal tract after daily use of low-fat probiotic spread using traditional cultivation methods as well as molecular methods. Forty-two healthy volunteers were randomly assigned to one of three treatment groups in which they received 20 g of placebo spread (n = 13), 20 g of spread with L. reuteri DSM 17938 at a target dose of 10^9 CFU (n = 13), and 20 g of spread with L. rhamnosus GG at a target dose of 5×10^9 CFU (n = 16) daily for 3 weeks. Fecal samples were collected before and after the intervention period. After three weeks of daily use of the spread containing the strain L. reuteri DSM 17938 or L. rhamnosus GG, a significant increase in the degree of recovery of viable probiotic lactobacilli in stool samples relative to the baseline level was determined according to selective counting. No such increase was observed in the placebo group. The results obtained indicate the ability of the LGG strain to survive in the human gastrointestinal tract. The number of viable bacteria of the L. rhamnosus GG strain in fecal samples was at the level of 6.6 $\log_{10}$ CFU/g after 3 weeks of using the L. rhamnosus GG spread, which contained, on average, from 3.3×10^{10} to 5.6×10^{10} CFU of L. rhamnosus GG per 20 g of spread. These numbers are comparable to the results of the evaluation of the extraction degree of L. rhamnosus GG bacteria from feces after taking them with different foods, which were published earlier.

Saxelin et al. (1993) studied the concentration of Lactobacillus GG bacteria in human feces after the oral administration. In this study, 44 healthy volunteers received Lactobacillus GG bacteria for 7 days in the form of enteric-coated tablets at a daily dose of 1×10^9, 4×10^9, and 8×10^9 CFU, as well as fermented milk at a daily dose of 2×10^9 and 1.2×10^{10} CFU. All volunteers were observed for the excretion of the studied strain with feces on the 3rd day of the test period. The average content of LGG bacteria in feces did not significantly differ between the groups receiving tablets. When using fermented milk, there was a clear statistically significant increase in the average content of Lactobacillus GG bacteria in the feces after taking a dose of 1.2×10^{10} CFU/day compared to a dose of 2×10^9 CFU/day. According to the results obtained, fermented milk and tablets with an enteric coating are effective carriers for the Lactobacillus GG bacteria strain used as a probiotic.

Alander et al. (1999) studied colon biopsies to demonstrate the adhesion of lactobacilli of the GG strain to the human intestinal mucosa and the persistence of this connection after discontinuation of this probiotic. In this study, volunteers took 100 ml of a commercial drink based on whey with hydrolyzed lactose fermented with the GG strain twice a day for 12 days. The daily dose of GG strain bacteria was about 6×10^{10} CFU. After taking the GG strain bacteria, the volunteers were

divided into three groups: those who underwent a colonoscopy immediately after the 12-day intake period, 1 week after stopping taking it, and 2 weeks after stopping taking the probiotic, respectively. Patients collected stool samples, which were first stored in home freezers at -20 °C (for up to 3 weeks), and then in the laboratory at -20 °C before analysis. Biopsies of the descending colon were immediately placed in a thioglycolate medium and stored at 4 °C until analysis was performed (within one day). Confirmatory PCR analysis was also performed on L. rhamnosus isolates.

There was a high level of matching (88%) between the results of the PCR analysis and the screening analysis based on the assessment of colony morphology and the lactose fermentation test, which confirms the overall reliability of strain identification. The number of colonies of the GG strain decreased as a function of time after stopping the probiotic to be taken.

GG strain bacteria were found in biopsies and last stool samples of all volunteers in group A. In group B, colonies of the strain L. rhamnosus GG were found in seven of the eight biopsies. However, only two of the eight subjects had colonies of the GG strain at a discernible level in the last fecal samples. None of the seven subjects from group C had colonies of the GG strain in the last fecal samples. However, colonies of the GG strain were found in the biopsies of two of the seven volunteers. According to the results obtained, the bacteria of the strain L.rhamnosus GG are capable of adhesion to the colon mucosa in vivo, where they remain for a long period of time after stopping taking the GG strain bacteria.

Saxelin et al. (1991) conducted a dose-response study to determine fecal colonization by bacteria of the LGG strain. Forty healthy adult volunteers took LGG strain bacteria in the form of lyophilized powder once a day for 7 days in doses $1,5 \times 10^6$, $1,5 \times 10^7$, $1,5 \times 10^8$, $1,5 \times 10^9$, $1,5 \times 10^{10}$ and 1.1×10^{11} CFU/day. Fecal samples collected on day 0 and on each day of the treatment period were analyzed for the total number of lactobacilli and LGG strain bacteria. Bacteria of the LGG strain were not detected in fecal samples before the probiotic period and were absent in the fecal samples of subjects from groups with dosages of 10^6–10^8 during the probiotic period. Two of seven subjects from the group with a dosage of 10^9 during the tests was periodically observed the colonization of feces with bacteria of LGG strain on distinguishable level, and all subjects of the groups with doses of 10^{10} and 10^{11} were observed colonization of stool with these bacteria at the level of 10^5–10^7 CFU/g. There was no influence of level of intake of the bacterial strain LGG on the total number of lactobacilli, as well as the dominance of strain LGG in the feces. No adverse effects were observed.

3.3.2.2 Pharmacokinetics in special groups

Millar et al. (1999) studied the ability of a probiotic strain of Lactobacillus GG to colonize the immature intestines of premature infants and, if found, the possibility of reducing the size of the intestinal reservoir of nosocomial pathogens, such as enterobacteria, enterococci, yeast fungi or staphylococci, as well as the effect of colonization by bacteria of the Lactobacillus GG strain on clinical progress and outcome. Twenty premature babies at gestational age of no more than 33 weeks, who had been in the neonatal unit for a long time, were monitored from the beginning of feeding with milk until discharge. Infants were randomized to groups of feeding with simple infant formula or with the addition of bacteria of strain Lactobacillus GG at a dose of 10^8 colony-forming units twice a day for two weeks. The clinical characteristics of infants from the two groups were similar. The orally administered strain of Lactobacillus GG was well tolerated and did not colonize the intestines of premature babies. However, colonization by bacteria of the Lactobacillus GG strain did not reduce the fecal reservoir of potential pathogens, and there was no evidence of a positive clinical effect of colonization in this group of infants.

Ling et al. (1992) evaluated the use of a whey beverage fermented with bacteria of LGG strain by elderly people from nursing homes,

which noted difficulties with defecation for 2 weeks, which included a 2-week introduction period and a 2-week washout period. All participants had distinguishable levels of bacteria of LGG strain in fecal samples and significantly reduced levels of glycochol, hydrolase, and tryptic activity. A slight normalization

of stool consistency was determined, but there was no effect on the frequency, mass, and pH of the stool. According to the authors, bacteria of the LGG strain can change the metabolism of bacteria, but do not have a significant effect on intestinal function.

In a study evaluating the possible induction of colonization of the intestines of their infants against the background of intake of LGG strain bacteria by pregnant women (Schultz et al. 2004) 6 women were identified who took 2×10^9 CFU LGG/day in weeks 30-36 of pregnancy. Four pregnancies ended in vaginal delivery, and two in cesarean section. In all four children born by vaginal delivery, and one of the two children born by caesarean section, the LGG strain bacteria (according to DNA sequencing analysis) were isolated from the feces at the age of 1 and 6 months. Bacteria of the LGG strain were isolated in the feces of 3 children aged 12 months and 2 children aged 24 months. At the age of 36 months, the LGG strain of bacteria was not detected in the feces of children. According to the authors, temporary colonization of the intestines of infants with LGG bacteria is possible by colonizing the intestines of a pregnant woman before delivery.

3.3.2.3 Pharmacodynamics

Siitonen et al. (1990) studied the effectiveness of introducing LGG strain bacteria into yogurt to reduce erythromycin-associated diarrhea in 16 healthy adult men. Participants took 400 mg of erythromycin acistrate 3 times a day for 7 days; half of them consumed 125 ml of regular yogurt twice a day (control group), and the test group received yogurt with LGG. Subjects who received yogurt with addition of LGG, had significantly less pronounced diarrhea, abdominal discomfort, stomach pain, and flatulence compared to those who consumed regular yogurt. Despite the fact that the total number of lactobacilli in the feces remained unchanged in the test and control groups, colonies with morphological characteristics of LGG were found in the feces of subjects from the LGG group and were absent in the feces of subjects from the control group. The authors did not report any adverse effects (GRAS status notification # 288).

Fang et al. (2000) randomly assigned 30 healthy adults (15 of each sex, aged 20-50 years) to three groups: a group of oral administration of lyophilized LGG at a dose of 4×10^{10} CFU/day, B.lactis at a dose of 3.4×10^{11} CFU/day, or a placebo for 7 days. They took an oral vaccine based on a weakened strain of *Salmonella typhimurium* TY21A in capsule form on days 1, 3, and 5. On days -1 and +14, blood samples were taken and analyzed for immunoglobulin-secreting cells and cells that secrete antibodies to S.typhimurium.

Significant differences in the amount of IgA, IgG, or IgM - secreting cells between groups

were not detected. The authors did not report any adverse effects (GRAS status notification # 288).

Reid et al. (2001) conducted a study of bacterial vaginosis. Forty-two healthy women were randomized to groups of oral intake of a combination of L.rhamnosus GR-1 and L.reuteri RC-14 bacteria at a dose of up to 6×10^9 CFU/day, or only LGG bacteria at a dose of 10^{10} CFU/day for 28 days. The data obtained was difficult to interpret, but it was clear that the combination of probiotic strains had a generally favorable effect on the vaginal microflora population. None of the patients had clinically expressed vaginitis or urinary tract infections, or any adverse side effects during or after the study. Discharge of all tested strains of bacteria with feces was stopped on the 14th day after stopping taking the Supplement.

Reid et al. (2003a) conducted a double-blind placebo-controlled trial to study the effect of oral probiotic administration on the vaginal microflora during and after antibiotic therapy. Twenty-four women diagnosed with respiratory or oral infections who received antibiotic therapy were randomly assigned to a placebo or a combination of L.rhamnosus GR-1 and L.reuteri RC-14 bacteria at a dose of 2×10^9 CFU/day for 21 days starting from the first day of taking antibiotics.

Cases of vaginitis or diarrhea were absent in the probiotic group and were observed in 3 women from the placebo group. This difference was not statistically significant. No adverse effects of probiotic therapy were registered.

The effect of taking probiotics on women with asymptomatic bacterial vaginitis was further studied by Reid et al. (2003b) in a randomized placebo-controlled trial in which 64 women aged 35 on average received a placebo or a combination of probiotic strains of L.reuteri RC-14 and L.rhamnosus at a dose of 2×10^9 CFU/day for 60 days. Evaluation of vaginal smears on days 0, 7, 28, 60 and 90 showed an improvement in the populations of lactobacilli in the vaginal microflora and a decrease in the content of pathogenic bacteria and yeast fungi. No adverse effects of probiotic therapy were registered.

Elmadfa et al. in 2001, conducted a cross-sectional study with 12 healthy adults (6 men and 6 women aged 25-36 years) who applied 500 g of yogurt per day, which corresponds to a dose of LGG 2.5×10^{10} CFU/day, for 4 weeks. The studied yogurt also contained strains of *Streptococcus thermophilus* and *Lactobacillus acidophilus*. In the first two weeks, yogurt cultures underwent thermal inactivation. Urine samples were collected daily, and blood samples were collected on days 1, 15, and 29; urine and blood samples were analyzed for group B vitamins content.

There was a significant and continuous decrease in the level of vitamin B1 in blood plasma during the study, as well as a slight but relevant decrease in this vitamin in the urine. These changes were associated with a 25% decrease in the bioavailable vitamin B1 in the subjects' diet. The content of vitamins B2 and B6 in blood plasma and urine changed slightly throughout the study, namely, most of these values decreased, but did not go beyond the normal ranges. According to the authors' conclusion, the bacterial flora of the studied yogurt did not affect the content of vitamins B1, B2 and B6 in the human body. No adverse effects were reported.

Schultz et al. (2003) conducted a study of the immunomodulatory effects of LGG. Ten healthy adults (6 men and 4 women) aged 21 to 43 (average age 29.9) took a single dose of lyophilized LGG strain 2×10^9 CFU in capsules daily for 35 days. Stool samples were collected 3 weeks before the start of the study, the day before the first dose, and the day after the last dose. Blood samples were also collected during the last two designated periods. As part of this study, the reaction of peripheral blood cells to "own" and "allogenic" fecal samples was evaluated, the content of microorganisms of the Bacteroides fragilis, Escherichia coli group, pro- and anti-inflammatory cytokines (IL-10, IL-4, IL-6, IFN-γ, TNF-α) in the culture's supravascular fluid was measured, as well as the activation of CD4 T-lymphocytes.

Lactobacilli not distinguishable from LGG were isolated from the feces of all subjects at the end of the administration period. Three subjects had mild bloating, but no other adverse effects. There was a slight modulation of the cellular immune response, which was manifested mainly by an increase in CD4 T-lymphocyte activity and a decrease in the secretion of cytokines such as TNF-α and IL-6.

Di Caro et al. (2005) studied the effects of the L.rhamnosus GG (LGG) strain on the gene expression profile on the small intestine mucosa. Six men (average age is 38) with endoscopically confirmed esophagitis received esomeprazole with placebo or LGG at a dose of 1.2×10^{10} CFU/day in the form of lyophilized powder in sachets twice a day for 1 month. During endoscopic procedures, biopsies of the distal duodenal mucosa were taken before and after the treatment. The extraction of all DNA and RNA was performed and the expression of more than 22,000 genes was studied. In the LGG strain group, but not in the placebo group, there was an upregulation of 316 genes and a downregulation of 78 genes. Genes that mediate the immune response and inflammation (for example, TGF-b, members of the TNF family, cytokines, nitric oxide synthase), apoptosis, cell growth and differentiation (for example, cyclins and caspases, oncogenes), intercellular signaling, cell adhesion, signal transcription and transduction were most affected. The authors did not report any adverse effects associated with taking LGG.

To study the effects of three probiotics on immune response indicators, Kekkonen et al. (2008) conducted a prospective double-blind randomized placebo-controlled intervention study in parallel groups involving 62 healthy adults (17 men and 45 women aged 23-58; average age was 44). After a three-week introduction period, subjects were randomly assigned to 1 of 4 groups for the indicated intervention (in the form of a fruit milk drink) for 3 weeks: placebo (n = 16), LGG at a dose of 1.6×10^{10} CFU/day (n = 13), Bifidobacterium animalis ssp. lactis Bb12 at a dose of 3.5×10^{10} CFU/day (n = 16) or Propionibacterium freudenreichii ssp. shermanii JS at a dose of 3.3×10^{10} CFU/day (n = 17). Initially, on days 1, 7, and 21, as well as after a 3-week washout period, venous blood samples were taken for analysis for leukocytes, monocytes, neutrophils, basophils, eosinophils, lymphocytes, IgA, IgG, IgM, C-reactive protein, and cytokines (TNF - α, IL-6, IFN-γ, and IL-10). At each designated evaluation period, an unstimulated saliva sample was collected for analysis for secretory IgA. Initially and on day 21, the fecal samples were collected to identify and count probiotic strains. Participants kept a diary in which they recorded symptoms from the respiratory system, gastrointestinal tract, other symptoms, and medication. The content of probiotic strains studied in feces significantly increased relative to the initial values in all groups of probiotic therapy. The use of the LGG strain, but not other probiotic strains, resulted in a significant decrease in the level of C-reactive protein. The content of white blood cells,

immunoglobulins, and cytokines did not differ significantly between the groups. No adverse effects were reported.

3.3.2.4 General conclusions on clinical pharmacology

The results of clinical and pharmacological studies support preclinical observations. Thus, the Lactobacillus GG strain was shown to survive in the human gastrointestinal tract and temporarily colonize it, as well as affect the metabolic activity of resident microflora. This strain was detected in the feces of volunteers after three days of administration and did not affect the total number of lactobacilli in the feces. Various studies have demonstrated high survival and, which is more important, maintenance of metabolic activity. There was no long-term colonization of any of the studied bacterial strains.

Increasingly, the results obtained also confirmed that the bacteria of the strain L.rhamnosus GG are capable of adhesion to the colon mucosa in vivo, where they remain for a long period of time after stopping taking the GG strain bacteria. The results of pharmacodynamic studies in healthy subjects showed that the L.rhamnosus GG strain is able to regulate the host's immune response through a variety of microorganisms, which confirms preclinical observations. Through this immunomodulatory effect, probiotics can influence the course of various diseases, which was further demonstrated in clinical studies of efficacy and safety (see section 3.3). No adverse effects were observed in any of the presented studies. Thus, the ability of the L.rhamnosus strain to survive in the gastrointestinal tract and to adhere to the intestinal mucosa has been demonstrated, which is one of the main aspects of the pharmacokinetics of probiotic strains. It has also been shown that the pharmacodynamic properties of probiotics identified in in vitro and in vivo studies can be reflected in favorable clinical effects, especially in gastrointestinal and immune diseases.

3.3.3. Review of efficiency data in children and adults

3.3.3.1 Treatment of acute diarrhea in adults

Allen et al. (2010) conducted a systematic review of clinical trials to study the effects of probiotic strains in confirmed or suspected acute infectious diarrhea. The review included 63 studies conducted from 1961 to 2010, involving a total of 8,014 participants. 56 studies of these were conducted with infants and younger children. The selected studies differed in the definition of acute diarrhoea used and the completion of diarrhoeal disorder, as well as the risk of systematic error. These studies were conducted under different conditions and varied significantly as to the microorganisms studied, the doses used, and the characteristics of the participants.

No adverse events of probiotic therapy were registered. Probiotics reduced the duration of diarrhea, although the magnitude of this effect varied significantly between studies. Significant average effects were determined for the average duration of diarrhea (average difference of 24.76 h; 95% confidence interval from 15.9 to 33.6 h; n = 4555, 35 studies), diarrhoeal episodes lasting more than 4 days (risk ratio of 0.41; CI: 0.32 to 0.53; n = 2853; 29 studies), and stool frequency on day 2 (average difference of 0.80; CI: 0.45 to 1.44; n = 2751; 20 studies). The difference in the effects size between studies was not related to the quality of studies, the probiotic strains studied, the number of different strains, microbial vitality, microbial doses, the causes of diarrhea, the severity of diarrhea or the country of study (developed or developing country). According to the authors' conclusion, probiotics used on the background of rehydration therapy are safe and have clear beneficial effects, which is manifested in the reduction of diarrhea duration and stool frequency in case of acute infectious diarrhea.

Sazawal et. al. (2006) analyzed available data from medical publications and concluded that this data was sufficient to confirm the role of probiotics in the prevention of acute diarrhea. Positive effects of probiotics have also been observed in adults, however to a lesser extent than in children. The most pronounced effect was observed for antibiotic-associated diarrhea, but also for diarrhea not associated with antibiotics and travel.

Gross et. al. (2010) conducted an open randomized controlled trial in parallel groups involving 174 patients with acute diarrhea who were treated in primary health care facilities. The two groups studied initially had homogeneous distribution of prognostic variables. The average duration of diarrhea since the start of treatment was 4.24 days (CO 2.73) in the Flortec drug group (L. paracasei B-21060) and 5.09 days (CO 3.72) in the FlorVis drug group (L. rhamnosus GG) (P = 0.09). Clinical efficacy rates expressed by absence of abdominal pain and diarrhea (less than two defecations by watery or loose stool), which were recorded at different times, were statistically significantly higher in the Flortec group (P = 0.05 for Kaplan-Meier assessment for both symptoms). According to the medical report, the overall effectiveness was high or very high in 91.8% of Flortec patients. In FlorVis group, the corresponding figure was 83.7% (P = 0.003). In the two groups studied, a very good tolerance profile was demonstrated, based on a low and similar frequency of adverse events and a similar frequency of consumption of concomitant drugs.

Bennet et. al. (1996) evaluated the effectiveness of the L. rhamnosus GG strain in treating C. difficile-induced acute diarrhoea in adults. Thirty-two patients with recurrent C. difficile-induced diarrhea received the Lactobacillus GG bacteria in the form of lyophilized powder; of these, 23 were outpatients from Boston, Massachusetts, and 9 were residents of a nursing home in Baltimore, Maryland state. All subjects who took the Lactobacillus GG strain had a symptomatic

improvement after administration. Twenty-seven (84%) patients have registered a recovery after one course of therapy according to the data of the observation period of at least 2 months. Five patients had a relapse within 10 days of the first course of Lactobacillus GG therapy; of these, three were re-treated and cured and two were dropped out of observation and placed in the group of ineffective therapy. The authors conclude that the use of probiotic strain Lactobacillus GG is a safe and sufficiently effective alternative to antibiotic therapy for recurrent diarrhea caused by C. difficile.

3.3.3.2 Antibiotic-associated diarrhea: data on prevention and treatment in adults and children

Doses of Lactobacillus rhamnosus GG used in therapy of antibiotic-associated diarrhea

During the study of the efficacy and safety of Lactobacillus rhamnosus GG in the treatment of antibiotic-associated diarrhea, several meta-analyses were carried out, all of which indicated a significant effect in the use of *Lactobacillus rhamnosus drugs.* Children and infants were more likely to have significant effects, but positive effects were also observed in adults. Information on the doses of Lactobacillus rhamnosus GG used in clinical trials in patients with antibiotic-associated diarrhea is given in Table 4 below.

Table 4. Information on the doses of Lactobacillus rhamnosus GG used in clinical trials in patients with antibiotic-associated diarrhea

Reference	Study Population	Duration of study	Applied dose
Szajewska, 2015	Adults and children, meta-analysis of 12 randomized controlled trials (RCTs)	From 10 days to 3- months	Doses in the range from 4 x 10^8 to 12 x 10^{10} CFU
Hempel, 2012	Adults (including the elderly people) and children, meta-analysis 82 RCTs	Different	Different doses. Dose data used in the studies are not included in the meta-analysis.
McFarland, 2006	Adults and children, meta-analysis of 25 RCTs	Different	Different doses range from 1 × 10^7 to 1 × 10^{11} CFU, with the mean value of 3 × 10^9 CFU. The use of high-dose probiotic (≥10^{10}/day) has been associated with significant

Reference	Study Population	Duration of study	Applied dose
			efficacy in curing of antibiotics-induced diarrhea.
Vanderhoof, 1999	Children aged from 6 months to 10 years receiving antibiotics	Equivalent duration of antibiotic therapy	LGG 1 x 10^{10} - 2 x 10^{10} CFU per day
Arvola, 1999	Children aged from 2- weeks to 12.8 years receiving antibiotics	Equivalent duration of antibiotic therapy	LGG 2 x 10^{10} CFU
Johnston, 2006	Children aged from 2- weeks to 15 years, meta-analysis of 6 clinical trials	7-14 days	Johnston et. al. analyzed the subgroups based on the applied dose of probiotic. Primary results were compared for daily doses of 5 × 10^9 CFU and higher with results for lower doses. In 4- studies in which children received 5.5-40 × 10^9 bacteria or yeast cells per day, evidence of the prophylactic effects of probiotics (RR = 0.36, 95% CI: 0.25-0.53) was obtained, while in one study in which significantly lower daily dose of probiotic bacteria (2 × 10^9) is used, the effect was negligible.
Goldenberg, 2015	Children, systematic review of 23 studies	Different	Goldenberg et. al in their systematic review analyzed subgroups based on the applied dose of probiotic. It is reported that when administered in doses greater than 5 x 10^9 CFU RR (random effects model) is 0.37 (95% CI): 0.27-0.51), while at lower doses (less than 5 x 10^9 CFU) RR is 0.62 (95% CI: 0.41-0.92). In both cases the level of statistical significance was achieved, but the probiotic had

Reference	Study Population	Duration of study	Applied dose
			a significantly better effect when used in higher doses.

A large number of studies have been conducted on the role of probiotics in the prevention and treatment of antibiotic-associated diarrhoea. Due to the large volume of publications, this section presents basic meta-analyses, systematic reviews and guidelines.

Szajewska and Kolodziej (2015) conducted a meta-analysis of 12 RCTs to assess the impact of the L. rhamnosus GG strain on antibiotic-associated diarrhea performed between 1998 and 2008. According to the results of the review, treatment with LGG strain reduced the risk of AAD in patients treated with antibiotics from 22.4% to 12.3% compared to placebo or no additional therapy (11 RCTs, n = 1308, relative risk (RR) 0.49, 95% confidence interval (CI) 0.29-0.83, low number of sick days). However, when children and adults were evaluated separately, the difference was significant only in children (5 RCTs; n = 445; RR: 0.48; 95% CI: 0.26-0.89, moderate number of sick days). In adults the difference was insignificant (6 RCTs; n = 863; OR: 0.48; 95% CI: 0.20-1.15; low CHD), except for a subgroup of patients receiving antibiotics as part of the Helicobacter pylori eradication therapy (4 RCTs; n = 280; RR: 0.26; 95% CI: 0.11-0.59; low number of sick days).

Hempel et. al. (2012) performed a meta-analysis of 82 RCTs. Most of these studies used only lactobacillus or lactobacterial interventions in combination with bacteria from other genera; the strains studied were not documented in detail. The value of the combined relative risk in the meta-analysis of random effects by the DerSimonian and Laird model for 63 RCTs, including 11,811 participants, indicated a statistically significant connection between taking probiotic and reducing the severity of AAD (relative risk 0.58; 95% of MDIs): 0.50-0.68; P = 0.001; I2, 54% [risk difference -0.07; 95% CI: -0.10 to -0.05] [number of patients requiring treatment - 13; 95% CI: 10.3-19.1]) in studies that recorded the number of patients with AAD.

The main observation from this review is that the use of probiotics as an adjunctive therapy reduces the risk of AAD (RR: 0,58). This result was comparable for several subgroups and sensitivity analyses. The therapeutic effect corresponds to the value of NNT = 13. The main limitations of the result obtained include unexplained residual heterogeneity, inadequate documentation of probiotic strains and failure to assess probiotic specific adverse events.

McFarland (2006) compared the efficacy of probiotics in the prevention of antibiotic-associated diarrhoea (AAD) and the treatment of diarrhea induced by Clostridium difficile (CSD), according to published randomized controlled clinical trials by meta-analysis. In 25 randomized controlled

trials (RCTs) against the background of taking probiotics a significant decrease in relative risk of AAD was determined (RR = 0.43; 95% CI: 0.31-0.58; p < 0.001). In 6 randomized studies, the significant efficacy of probiotics for CsD was found (RR = 0.59; 95% CI: 0.41-0.85; p = 0.005). It has been observed that three types of probiotics (Saccharomyces boulardii, Lactobacillus rhamnosus GG and probiotic mixtures) significantly reduced the risk of antibiotic-associated diarrhea. Based on stratified data for L. rhamnosus GG strain, the value of the combined RR = 0.31 (p = 0.006) for 6 RCTs was determined. Only the S. boulardii strain was effective in CsD.

Vanderhoof et. al. (1999) studied the effectiveness of Lactobacillus rhamnosus (Lactobacillus GG; LGGG) strain in reducing the incidence of antibiotic-associated diarrhea when used in combination with oral antibiotics in children with acute infectious diseases. The study enrolled 202 children aged 6 months to 10 years, of whom 188 completed all phases of the protocol. In a double blind randomized trial, children receiving oral antibacterial therapy in outpatient settings were injected with LGG at a dose of 1×10^{10} to 2×10^{10} colony-forming units per day or a comparable placebo. Principal caregivers were asked questions every three days about the frequency of gastrointestinal symptoms, mainly the frequency and consistency of stool, during a telephone conversation with researchers who did not have access to patient data. Diarrhoea, which was defined as loose stool 2 or more times per day, was maintained in 25 placebo group subjects and only 7 subjects in the LGG group. Compared to the placebo group, the Lactobacillus GG bacteria as a whole reliably reduced the stool frequency and improved its consistency against the background of antibacterial therapy on day 10.

Arvola et. al. (1999) studied the role of the L. rhamnosus GG strain in preventing diarrhea in children who were prescribed antibiotics for acute respiratory infections. Patients were randomly identified as groups of placebo or Lactobacillus GG in a dose of 2×10^{10} CFU in capsules 2 times/day during antibiotic treatment. Diarrhoea was established when there was a watery or loose stool with a frequency of at least three times a day for at least two consecutive days. In case of diarrhea, feces were tested for viral (adenovirus, rotavirus, calcivirus and astrovirus) and bacterial (Salmonella, Shigella, Yersinia, Campylobacter, Clostridium difficile, Staphylococcus aureus and yeast) etiology. Metabolic activity of intestinal microflora by analyzing faecal urease, β-glucosidase and β-glucuronidase was studied. The main indicator of outcome was diarrhea in the first 2 weeks after the beginning of antibiotic therapy, as this period is most likely to characterize the effects of antibiotics. The activity of faecal urease, β-glucosidase and β-glucuronidase were considered as secondary endpoints of the study.

Within the observation period 80% of all gastrointestinal symptoms were observed in the first 2 weeks after the start of antibiotic therapy. The incidence of diarrhea during the two-week period

of antibiotic therapy was 5% in the Lactobacillus GG strain group and 16% (x^2 5 3.82). The authors have concluded that Lactobacillus GG strain is effective in preventing diarrhea in children receiving antibacterial therapy for respiratory infections.

Szajevska et. al. (2016) published clinical guidelines developed by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition's (EU) Working Group (WG) on Probiotics concerning the use of probiotics to prevent antibiotic-associated diarrhoea (AAD) in children, based on a systematic review of previously performed systematic reviews and randomised controlled trials published after these reviews. The following recommendations are given for the L. rhamnosus GG strain: *If the use of probiotics to prevent AAD in children is considered, the working group recommends the use of L. rhamnosus GG.* This recommendation has an average quality of evidence, although the credibility of the recommendation is considered to be high.

Johnston et al. (2006) carried out a review to assess the effectiveness of probiotics (any identified strain or dose) in preventing antibiotic-associated diarrhoea in children and adverse events associated with the use of probiotics against the background of antibiotic therapy in children. Six studies were included in this review (total n = 707 patients). The summary results, analyzed in the population of patients who fulfilled the requirements of the protocol with the registration of the incidence of diarrhea during antibiotic therapy, indicated a significant benefit of using probiotics in comparison with placebo (relative risk [RR] = 0.43, 95% confidence interval [CI] 0.25-0.75, I2 = 70.1%). In contrast, the results of the analysis in the population according to the prescribed treatment were generally insignificant (RR: 1.01; 95% CI: 0.64-1.61). In subgroup analysis for 4 studies in which probiotic doses of at least 5 billion single-stage colony-forming units (CFU) were applied daily (range 5.5-40 × 10^9 Lactobacillus GG, L. or Saccharomyces boulardii), convincing evidence with narrow CI for the preventive effects of probiotics on antibiotic-associated diarrhea has been obtained (RR: 0.36; 95% CI: 0.25-0.53; I2 = 3.5%). There were no serious adverse events reported. According to the authors' conclusion, regardless of the unsuccessful analysis in the ITT population, promising results have been obtained for Lactobacillus GG, L. sporogenes and S. boulardii strains, which should be further studied.

Doron et al. (2008) conducted five meta-analyses of studies on the role of probiotics in AAD prevention. The results indicated an overall reduction in the risk of AAD when administered simultaneously with antibiotics. Moreover, McFarland (2006) performed the largest meta-analysis to date on 25 randomized controlled trials of the role of probiotics in AAD prevention, involving 2,810 participants. The effectiveness of the probiotic strain under study has been demonstrated in more than half of the studies. In particular, the efficiency of *Lactobacillus* GG, *Saccharomyces boulardii* strains and probiotic mixtures was determined.

Goldenberger et al. (2015) published a systematic review and meta-analysis of RCTs to assess the use of probiotics to prevent antibiotic-associated diarrhoea in children. 23 studies (3938 participants) met the inclusion criteria. In these studies the use of Bacillus spp., Bifidobacterium spp., Clostridium butyricum, Lactobacillus spp., Lactococcus spp., Leuconostoc cremoris, Saccharomyces spp. or Streptococcus spp. has been investigated individually or in combination. In eleven studies used a single strain probiotic has been used, in four studies a combination of two probiotic strains has been used, in three studies a combination of three probiotic strains has been used, in one study a combination of four probiotic strains has been used, in two studies a combination of seven probiotic strains has been used, in one study a combination of ten probiotic strains has been used, and the last study included two groups of probiotic therapy based on three and two strains respectively. The risk of systematic error was high or uncertain in 13 studies and low in 10 studies. Available case data (patients who did not complete their studies were not included in the analysis) for 22 of the 23 studies that recorded the incidence of diarrhea indicates a clear benefit of probiotics versus active control, placebo and untreated control. AAD occurrence rate was 8% in the group of probiotic therapy (163 from 1992) and 19% (364 from 1906) in the control group (RR: 0,46; 95% CI: 0,35-0,61; I2 55%; 3898 participants). The GRADE analysis indicated an average level of overall quality of evidence for this outcome. The revealed benefit remained statistically significant in the sensitivity analysis of extreme plausible variants (diarrhea in 60% and 20% of children who dropped out of observation in the probiotic therapy group and the control group, respectively), namely, the frequency of AAD was 14% in the probiotic therapy group (330 out of 2294) and 19% in the control group (426 out of 2235) (RR): 0.69; 95% CI: 0.54-0.89; I2 63%; 4529 participants). None of the 16 studies that registered adverse events (n = 2455) reported the development of serious adverse events in the background of taking probiotics. All differences in adverse events between active treatment and control groups were included in the meta-analysis, except for extremely small differences (RR 0.00; 95% CI: -0.01-0.01). Most of the undesired effects have been observed in placebo, standard therapy or non-therapy groups. Adverse events in these studies included rash, nausea, flatus, meteorism, tympanites, abdominal pain, vomiting, increased mucus secretion, chest pain, constipation, distortion of the sense of taste and decreased appetite.

Based on the evidence of medium quality, it was suggested that probiotics were effective in AAD preventing. The summary data indicate an exact probiotic effect (RR: 0.46; 95% CI: 0.35-0.61) corresponding to the value of NNT = 10. Among the various probiotics studied, the most appropriate is the strain Lactobacillus rhamnosus or Saccharomyces boulardii at a dose of 5 to 40 billion colony-forming units per day due to the modest size of the NNT and very low probable frequency of undesirable events.

Hojsak (2017) summarized the available data on probiotic use for clearly defined clinical indications of importance in paediatrics. Individual probiotic strains (Lactobacillus rhamnosus GG and Saccharomyces boulardii) are reported to have proven efficacy in treating acute gastroenteritis and preventing antibiotic-associated diarrhea. Moreover, the effectiveness of the LGG strain in preventing nosocomial diarrhea and respiratory tract infections in nursing homes has been confirmed. The author has concluded that probiotic strains have different efficacy depending on the clinical indication and therefore recommends the use of strains with proven efficacy and safety.

Thomas et al. (2001) studied the effectiveness of the LGG strain in preventing antibiotic-associated diarrhea in adults. In a prospective, double-blind, randomized, placebo-controlled trial, 302 hospitalized patients (143 males and 159 females, average age 55.8 years, range 18-90 years) undergoing intravenous or oral antibiotic therapy were randomized to placebo groups in a daily dose of 2×10^9 CFU 2 times/day in capsule form or strain LGG for 14 days. The main outcome was the proportion of patients who had diarrhea within 21 days of recruitment, defined as the presence of watery or loose stool for two consecutive days or at three times the usual frequency for a particular patient. Patients were also asked to record unwanted effects such as nausea, abdominal cramping, flatus or tympanites.

Against the background of the LGG strain, the incidence of diarrhea did not change; diarrhea occurred in 29-30% of patients in the trial and control groups. The study group and placebo group did not differ in the number, type and severity of reported undesirable effects.

Ruszczynski et al. (2008) studied the safety, tolerability and efficacy of a combination of 3 strains of L. rhamnosus in the prevention of antibiotic-associated diarrhea in children in a prospective, double-blind, randomized, placebo-controlled study. A total of 240 children, 130 boys and 110 girls, aged 3 months to 14 years, who received standard antibiotic treatment for common infectious diseases also received a combination of L. rhamnosus E/N, Oxy and Pen' strains in a dose of 2×10^{10} CFU 2 times/day (n = 120) or placebo, sucrose in skimmed milk (n = 120), during antibiotic treatment. Children or their parents recorded the frequency of the stool per day as well as any symptoms they considered to be important. The frequency and consistency of the stool was

recorded daily. All samples of loose stool were analyzed for viral pathogens. Children received antibiotic therapy (and L. rhamnosus in the test group) for 3 to 30 days, an average of 8 days. The addition of L. rhamnosus to antibiotic therapy has resulted in a significant reduction in the incidence of diarrhea. Two children in the control group and one child in the probiotic group required intravenous rehydration. No adverse or undesirable effects were observed. The authors noted high tolerability of Lactobacillus rhamnosus and absence of undesirable phenomena associated with antibiotic therapy (or placebo).

3.3.3.3 Coadjuvant therapy for the eradication of Helicobacter pylori in adults

Cremonini et al. (2002) studied the effect of probiotics on the side effects of eradication therapy with Helicobacter pylori, namely the probiotic strains LGG, Saccharomyces boulardii and the combination of Lactobacillus spp. and bifidobacteria. A total of 97 H. pylori-infected patients with no clinical manifestations (43 men and 54 women aged from 18 to 61 years) were randomized to one of the probiotic therapy groups or placebo for 2 weeks. The trial period was preceded by a three-week induction period during which 12 individuals were excluded from the trial due to symptoms or medications related to gastrointestinal side effects. All patients received eradication therapy based on rabeprazole, clarithromycin and tinidazole for 7 days and probiotic or placebo 2 times/day for the same 7 days and the following week. The daily dose of the LGG strain was 1.2×10^{10} CFU. Patients filled out side effects assessment questionnaires at the end of the week of antibiotic treatment and weekly for the next three weeks.

No side effects requiring early exclusion from the study were observed. All three probiotic treatment options resulted in a reduction in observed side effects compared to placebo group; the intergroup difference was significant only in the first week and gradually decreased until week 4. The most noticeable effect was a significant reduction in diarrhea.

Tong et al. (2007) performed a meta-analysis of 14 RCTs and compared different outcomes, such as the frequency of eradication and the frequency of side effects. According to the obtained results, the frequency of eradication was slightly higher during probiotic therapy against the background of standard triple eradicative therapy in the analysis samples assuming that all patients received the prescribed intervention (83.6 vs. 74.8%; OR 1.84; CI: 1.34-2.54) and that all patients met the protocol requirements (85.4 vs. 77.6%; OR 1.82; 95% CI: 1.30-2.56). The meta-analysis also demonstrated the potential effectiveness of probiotics in treating patients with unsuccessful eradication therapy. When estimating the eradication frequency against the background of L. rhamnosus GG strain reception, the value of OR 2.09 was determined in favor of probiotic (χ^2 = 4.01, d.f.). = 3 (P = 0.26) and I^2 = 25.1%).

Also in meta-analysis the effect of probiotic therapy on antibiotic-associated side effects of the gastrointestinal tract on the background of therapy aimed at eradication of H. pylori was studied. The results showed a positive effect on the overall frequency of side effects associated with eradication therapy, especially diarrhea and taste disturbances. Subanalysis demonstrated that the frequency of H. pylori eradication did not depend on age, but was higher in patients with clinical manifestations.

Dang et al. (2014) conducted a similar meta-analysis of the RCTs that took place between 2000 and 2013. Analysis by subgroups was performed to compare different probiotic strains and antibiotic treatment schemes of different efficacy in control subjects. This analysis evaluated the systematic error of publication using funnel charts and the Harbor criterion. The quality of research was assessed using the Cochrane Collaboration tool to assess the risk of systematic error. 33 RCTs with 4459 patients met the inclusion criteria based on the eradication frequency, including 20 studies evaluating the total frequency of side effects. The total frequency of eradication was generally significantly higher in probiotic therapy groups than in control groups (analysis in ITT sample: RR: 1,122; 95% CI: 1,086-1,159; analysis in PP- sample: 1,114; 95% CI: 1,070-1,159). However, subgroup analysis confirmed this observation for only four strains (Lactobacillus acidophilus, Lactobacillus casei DN-114001, Lactobacillus gasseri, and Bifidobacterium infantis 2036) and for relatively ineffective antibiotic treatment regimens. The total frequency of side effects differed significantly between the groups (RR: 0.735; 95% of CI: 0.598-0.902). However, this result was only confirmed for studies without masking. The combined data indicate that the use of additives of individual probiotic strains, in comparison with traditional regimens of eradication therapy, can be considered an option to increase the frequency of eradication, especially when antibiotic therapy regimens are relatively ineffective. The impact on side effects remains unclear.

Armuzzi et al. (2001) conducted a prospective, double-blind, randomized, placebo-controlled study to evaluate the possibility of using the LGG strain in the eradication therapy of Helicobacter pylori. Sixty healthy non-clinical patients (25 men and 35 women, average age 40 years) with positive screening results on H. pylori received rabeprazole, clarithromycin and tinidazole for 7 days in combination with placebo or LGG in a dose of 1.2×10^{10} CFU/day in the form of lyophilized powder in sachets. Patients continued to take LGG or an appropriate placebo for 7 days after completion of eradication therapy. During the first week of therapy and the following three weeks, patients filled out acceptability and side effects assessment questionnaires. Patients in the LGG group reported significantly lower rates of nausea and diarrhea than placebo patients and gave significantly higher scores on therapy tolerance. The LGG strain and placebo did not differ

in the influence on the success of H. pylori eradication, and no side effects were observed against the background of LGG intake.

3.3.3.4 Treatment of acute gastroenteritis in children

Kaila et. al. (1992) studied the role of the LGG strain in the recovery of children with acute rotavirus diarrhea in a prospective double-blind randomized placebo-controlled study. After rehydration, 39 children aged from 7 to 37 months (average age 16 months) with satisfactory nutritional status who had diarrhea for less than 7 days, were given 125 g of pasteurized usual yogurt (n = 17) or fermented dairy product with LGG supplement (n = 22) 2 times/day (10^{10}-10^{11} CFU/day), for 5 days. Children were weighed daily, their parents filled out stool quality questionnaires during the study and observation period, and on days 1 and 21 blood samples were taken to assess their immune status.

The duration of diarrhea was significantly lower in the study group than in the control group, and there was no disease recurrence in the three-week follow-up period. According to the number of IgG-, IgA- and IgM-secreting cells, patients who received the LGG strain had a statistically significantly higher nonspecific humoral response during the acute phase of infection; at the end of the observation period, a response from cells secreting specific IgA antibodies to the rotavirus was observed in a significantly higher number of patients in the test group than in the control group (90% vs. 46%). The authors have not reported any undesirable phenomena.

As part of a prospective double blind randomized placebo-controlled study (Isolauri et al. 1994) 42 children aged from 5 to 28 months (average age 14 months) with satisfactory nutritional status who had severe diarrhea were given the LGG strain in a dose of 10^{10} CFU in the form of lyophilized powder or placebo 2 times/day, for 5 days. The Lactobacillus GG strain was isolated from feces of 83% of patients in the test group. In this group, a reduction in the phase of diarrhea was noted. Administration of lactobacillus supplement had a significant effect on the enzymatic profile of bacteria: in the control group, urease activity increased temporarily against the background of diarrhea, but it did not change in the test group ($F = 8.6$, $P = 0.01$). There were no intergroup differences in the activity of beta-glucuronidase, beta-glucosidase and glycocholic acid hydrolase. According to our assumption, rotavirus infection is accompanied by two-phase diarrhea: in the first phase, osmotic diarrhea is developing, while in the second phase an excess growth of specific urease-producing bacteria is observed. Oral bacteriotherapy is a promising method for impaired microflora balance restoration.

Kaila et. al. (1995) conducted a double blind randomized study to assess the effect of a viable or heat-inactivated LGG strain on the immune response to rotavirus acute diarrhea in children. Forty-one children aged from 1 to 38 months (average age 13 months) with satisfactory nutritional status, who had acute gastroenteritis for less than 7 days, were given a viable (n = 20) or heat-inactivated strain of LGG (n = 21) in a dose of 10^{10}-10^{11} CFU with water 2 times/day, for 5 days. The patients were weighed daily, stool quality was recorded throughout the study and during the follow-up period, and blood samples were collected on days 1 and 30 after recruitment, and tested for immune status parameters. The groups studied did not significantly differ in terms of recovery from diarrhea. However, a viable LGG strain stimulated a significantly higher response from the IgA antibodies to the rotavirus than the heat-activated strain. No security indicators have been reported by the authors.

Majamaa et. al. (1995) compared different strains of lactobacteria in terms of their effect on the immune response to rotavirus in children with acute rotavirus gastroenteritis. After initial oral rehydration, 49 children aged from 6 to 35 months, with rotavirus gastroenteritis were randomly distributed to obtain Lactobacillus casei subsp., a strain of casei GG (LGG), L. casei subsp. rhamnosus (Lactophilus) or a combination of Streptococcus thermophilus and L. delbrückii subsp. bulgaricus (Yalacta) twice a day, for 5 days. At the acute stage of the disease and at the stage of recovery serum antibodies to rotavirus were determined, the total number of cells secreting immunoglobulin (CSI) and cells secreting specific antibodies to rotavirus (CSSA). The average duration of diarrhea (AD) was 1.8 (0.8) days in children in the LGG group, 2.8 (1.2) days in children in the Lactophilus group and 2.6 (1.4) days in children in the Yalacta group ($F = 3.3$, $p = 0.04$). The response from the ISC was comparable in the three study groups, but the rotavirus-specific immune response varied. LGG intake was accompanied by an increase of the level of IgA to the rotavirus secreted by the CSSA and the level of serum IgA antibodies in the recovery phase. The authors concluded that certain strains of lactobacteria, especially LGG, stimulate serum and intestinal immune response to rotavirus and, as a result, play an important role in developing immunity to repeated rotavirus infections.

Raza et al. (1995) conducted a prospective triple blind placebo-controlled clinical study in Pakistan to determine the effect of the Lactobacillus GG strain on progression of acute diarrhea in hospitalized children. Forty children (average age 13 months) were enrolled in the study and were given Lactobacillus GG (n = 21) or placebo (n = 19) 2 times/day after rehydration therapy for 2 days in addition to the usual diet. The clinical course of diarrhea was followed-up during treatment. Patients had similar characteristics in the distribution of treatment groups that included severe diarrhea, nutritional deficiencies and inappropriate treatment before admission. The

response to the treatment was detected on the 2nd day, when a lower incidence of vomiting and diarrhea was determined in the lactobacillus group. Among children hospitalized with acute, bloodless diarrhea (n = 32), the percentage of children with persistent watery diarrhea 48 hours later was significantly lower in the lactobacillus group: 31 vs. 75% ($P < 0.01$). Among children with diarrhea with an admixture of blood, this figure was not significantly different after 48 hours.

Guarino et al. (1997) conducted a prospective randomized controlled study to assess the effect of the LGG strain on the duration of diarrhea in hospitalized children aged from 3 to 36 months (average age 19 months).

100 children (51 boys and 49 girls) were enrolled in the study and randomized into the study group (n = 52) and the control group (n = 48). After primary oral rehydration, children were injected with an additional solution

separately or in combination with LGG additives in a dose of 3×10^9 CFU, in 200 ml of milk or mixture of 2 times/day, for up to 5 days. After primary rehydration children were provided an age-appropriate complete diet as soon as possible. At the time of enrollment and 6 days after the onset of diarrhea, fecal samples were taken for rotavirus analysis by ELISA. At the time of enrollment, a narrow majority of children (61%) had positivity for rotavirus. Diarrhea was determined as frequency of watery stool three or more times a day.

Recovery from diarrhea was determined as the period of time from the last thin or loose stool. The outcome of diarrhea was assessed by the mothers of the enrolled children, who received instructions and were interviewed daily.

The duration of diarrhea was significantly reduced (by 50%) in all children, regardless of the rotavirus test results, who received the LGG strain compared to the control group. Among children with initially positive rotavirus test results, the number of children who excreted rotavirus at the end of the treatment period was significantly lower in the LGG intake group than in the control group (4 children from the active treatment group and 26 children from the control group). No adverse effects were reported.

In a controlled study conducted by Shornikova et al. (1997) in Petrozavodsk (Karelia), the effects of oral rehydration and the introduction of Lactobacillus GG (LGG) strain on recovery from acute diarrhea were studied in 123 children aged 1 to 36 months (27% cases of rotavirus, 21% cases of bacterial etiology). On admission to hospital, children were first randomized to the group of isotonic oral rehydration solution (ORS) with osmolarity of 311 mOsmol/l and sodium content of 90 mmol/l (WHO-ORS) or the group of hypotonic ORS with osmolarity of 224 mOsmol/l and

sodium content of 60 mmol/l (Light-ORS), then they were assigned either to a LGG strain (in a dose of 5 × 10^9 CFU) administration group or a corresponding placebo group. The two ORS studied had similar efficacy in emergency rehydration, in addition, oral rehydration by either ORS resulted in a shorter duration of diarrhea than in case of intravenous rehydration (p = 0.036). Patients who received the LGG strain had significantly shorter duration of watery diarrhea (2.7 [2.2] days on average) than patients from placebo group (3.7 [2.8] days, p = 0.03). The introduction of LGG significantly reduced the duration of rotavirus diarrhea, but not diarrhea with confirmed bacterial etiology.

Within the scope of a multicentre prospective double blind randomized placebo-controlled study, Guandalini et al. (2000) studied the effect of the LGG strain introduced into oral rehydration solution on the course of acute diarrhea in children aged from 1 to 36 months (average age 12.3 months). A total of 287 children hospitalized with acute diarrhea of any etiology received an oral rehydration solution in the first 4-6 hours containing the strain LGG in a dose of 10^{10} CFU per 250 ml (n = 147), or placebo (n = 140).

At the time of admission, anthropometric measures, including weight, were measured and feces were sampled; additional feces were sampled 48 hours later and, if possible, 7 and 30 days later. During the observation period liquid intake, frequency and characteristics of the stool and the frequency of vomiting were monitored. The incidence of diarrhea over 7 days long was significantly lower in the test group than in the control group (2.7 vs. 10.7%), as was the average hospitalization term.

The weight gain in the first 24 h of rehydration therapy did not differ between the groups.

Despite the fact that adverse reactions were not discussed separately, the authors conclude that the use of oral rehydration solution with Lactobacillus GG supplement in children with acute diarrhea is safe and results in shorter duration of diarrhea, lower probability of protracted diarrhea and reduction of hospital discharge term.

Szajevska et. al. (2013) published a meta-analysis on the efficacy of LGG strain in the treatment of acute gastroenteritis (AGE) in children. Fifteen RCTs (2963 participants) met the criteria for inclusion in this updated meta-analysis. Combined data from 11 RCTs (n = 2444) showed that taking the LGG strain significantly reduced the duration of diarrhea compared to placebo or non-treatment (mean difference [MD] -1.05 days, 95% of CI: -1.7 to -0.4). The use of LGG was more effective with a daily dose of ≥ 10^{10} CFU (eight RCTs, n = 1488, MD - 1.11 days, 95% CI: -1.91 to -0.31) than with a dose of < 10^{10} CFU (three RCTs, n = 956, MD - 0.9 days, 95% CI: -2.5 to 0.69). LGG use was effective in children treated in Europe (five RCTs, n = 744, SR -1.27 days,

95% CI: 2.04 to -0.49); in non-European studies, the difference between the LGG group and the control group was of borderline statistical significance (six RCTs, n = 1700, MD -0.87, 95% CI: from -1.81 to 0.08). The authors concluded that taking Lactobacillus GG strain reduces the duration of diarrhea. The number of patients who are more likely to benefit from such treatment includes patients with a high daily dose of LGG (≥ 10^{10} CFU/day) undergoing inpatient or outpatient treatment in Europe.

The ESPGHAN position statement contains data from Szajevska et al. (2014) on the effectiveness of probiotics in treating acute gastroenteritis in children. The following recommendation has been published for the L. rhamnosus GG strain: "The use of Lactobacillus GG strain is possible in the treatment of children with AGE as an adjunct to rehydration therapy"; this is a recommendation of "high" strength despite the poor quality of the evidence. The quality of the evidence base for this recommendation has been lowered due to methodological limitations in many of the studies included.

Szajevska et al. (2001) studied the effectiveness of the LGG strain in preventing nosocomial diarrhea in younger children admitted to children's hospitals. 81 children aged from 1 to 36 months who were hospitalized for reasons other than diarrhea were enrolled in the double-blind study. At the time of admission, children were randomly assigned to LGG injection groups (n = 45) at a dose of 6×10^9 CFU or a corresponding placebo (n = 36) 2 times/day ingestion during the entire period of hospitalization. Taking the LGG strain reduced the risk of nosocomial diarrhea (≥ 3 loose or watery stools/24 h) compared to placebo (6.7 and 33.3%; relative risk: 0.2; [95% CI: 0.06-0.6]; number of patients requiring treatment: 4 [95% CI: 2-10]). The prevalence rate of rotavirus infection was similar in the LGG and placebo injection groups (20 vs. 27.8% respectively; relative risk 0.72; 95% CI 0.33-1.56). However, the use of LGG in comparison with placebo has significantly reduced the risk of rotavirus gastroenteritis (1/45 [2.2%] and 6/36 [16.7%] respectively; relative risk: 0.13; 95% CI: 0.02-0.79; number of patients requiring treatment: 7; 95% CI: 3-40).

According to the authors' conclusion, prophylactic administration of LGG strain significantly reduced the risk of nosocomial diarrhea in infants, especially nosocomial rotavirus gastroenteritis.

Salazar-Lindo et al. (2004) studied the effect of the introduction of the LGG strain suspended in the milk formula on the duration and severity of non-rotavirus acute diarrhea in Peruvian boys. A total of 179 boys aged from 3 to 36 months received oral rehydration therapy with an oral rehydration solution and were randomly assigned to milk formula feeding groups with LGG supplement in the dose of 10^9 CFU/ml (n = 90) or without LGG supplement (n = 89). The first

portion of the mixture was given immediately after rehydration therapy, and subsequent portions were given every four hours until the diarrhea ceased, but not longer than for five days. Each child was given a mixture of 150 to 1000 ml per body weight per day. In this study, LGG administration was studied at a dose of 6-8 × 10^{11} CFU/day. Before treatment, clinical analysis was collected, physical examination was performed and blood samples were taken, and additional blood samples were taken 24 hours after the start of treatment. The feces volume was measured periodically, and the duration of diarrhea was calculated based on the physical characteristics of the feces. Eight boys from the LGG administration group and 11 boys from the control group were prematurely excluded from the study for various reasons not related to treatment (blood admixtures in the feces in the first 24 hours after admission, failure to comply with the scheme of treatment by parents, etc.). The volume of feces was significantly higher in the LGG injection group than in the control group, although there was no significant difference in the duration of diarrhea. Approximately 12% of patients did not have diarrhea resolved and a further 20% were found to have had unsuccessful treatment, mainly due to high severity of diarrhea. The percentage did not differ between the two groups. The authors concluded that this study did not find a beneficial effect of the LGG strain on the clinical course of acute watery diarrhea, but there were no adverse effects associated with the intake of the test milk formula in both groups during the study.

Canani et. al. (2007) conducted a major (571 participants) simple blind clinical study to evaluate the use of probiotics in the management of acute diarrhea in children

from 3 to 36 months old. Five options for probiotic drugs were studied: 1) LGG; 2) Saccharomyces boulardii; 3) Bacillus clausii; 4) a mixture of L. delbrueckii ssp. bulgaricus, Streptococcus thermophilus, L. acidophilus, Bifidobacterium bifidum; 5) the strain Enterococcus faecium SF68. Oral rehydration solution was administered for 3–6 hours in children (282 boys and 289 girls; aged from 8 to 28 months; average age 17.5 months) with acute diarrhea having developed less than 48 hours before, and then lactose- or cow's milk (depending on age)-based formula was administered. The enrolled children were randomly assigned to groups of oral rehydration (n = 92), LGG administration at a dose of 6 × 10^{9} CFU/day (n = 100) or one of four probiotic therapy options (n = 91, 100, 97 and 91, respectively). At the time of enrollment, the duration and severity of diarrhea and associated clinical characteristics were assessed for each child, and parents received a special form for daily recording of the frequency and consistency of the stool, the presence of vomiting and fever, as well as adverse events. The parents purchased probiotics at a pharmacy; family doctors were responsible for prescribing treatment, providing written instructions to the parents and monitoring compliance; researchers who collected reports did not have information about the treatment prescribed.

Two studied options for probiotic therapy, namely LGG and a mixture of L. delbrueckii and other bacteria, significantly reduced the duration and severity of diarrhea, while the other three options did not have significant effects. The parents of the children who received experimental probiotic therapy settings did not report any adverse effects.

Basu et al. (2007) conducted a randomized controlled study to assess the role of Lactobacillus rhamnosus GG (LGG) as a probiotic in resistant diarrhea (RD) in children in West Bengal, India. The study included all RD patients hospitalized within a two-year period, based on predetermined inclusion criteria. In this double-blind controlled study, patients were randomized into oral rehydration solution (ORS) or powder-supplemented ORS (60 million cells), twice a day, for at least 7 days or until diarrhea ceases with rehydration using ORS and/or intravenous solutions in accordance with the WHO protocol, and antibiotic therapy in patients with positive culture results. The study included 235 patients randomized into 2 groups: active treatment group (117 people) and control group (118). Both groups were comparable as to the age, the number of breastfed children, dehydration rate on admission to hospital, degree of protein-energy deficiency and distribution of infections by etiology. Stool seeding was positive in 90 (38.3%) patients, with Escherichia coli and, more rarely, Shigella spp. and Clostridium difficile. The average duration of diarrhea was significantly lower in the active treatment group than in the control group (5.3 versus 9.2 days). The average duration of hospitalization was also significantly lower in the active treatment group. No complications associated with the application of the LGG strain studied dose have been observed. The introduction of the LGG strain (at a dose of 120 million CFU) resulted in a decrease in the frequency and duration of diarrhea and vomiting, and the hospital stay reduction in patients with persistent diarrhea.

Basu et al. (2009) conducted a randomized controlled study to evaluate the effective dose of the Lactobacillus rhamnosus GG (LGG) strain as a probiotic for acute watery diarrhea (AWD) in children from India. All hospitalized AWD patients, older than 1 year, were randomized into 3 groups: oral rehydration solution (ORS) administration group without additives (group A / control, n = 185), ORS administration group with LGG powder supplement in a dose of 10^{10} CFU (CFU) (group B, n = 188) and ORS administration group with LGG powder supplement in a dose of 10^{12} CFU (group C, n = 186) twice a day for at least 7 days or until diarrhea ceases against the background of rehydration therapy. The duration and frequency of diarrhea and vomiting were assessed in the study. The frequency and duration of diarrhea, the need for intravenous therapy and the duration of hospitalization were significantly lower in both active therapy groups, compared with the control group. There was no significant difference between the two active

therapy groups. No complications associated with the application of the studied dose of LGG strain have been observed.

Nixon et al. (2012) conducted a double blind randomized controlled study in children (aged from 6 months to 6 years) who were admitted to the admission office of the children's hospital with a complaint of diarrhea. Patients were randomized into placebo or LGG, in powder form, groups, 2 times/day for 5 days. After each dose was administered, the parents recorded the characteristics of the stool in their home diary and the secret researcher looked through the diaries daily. The study compared time to stool normalization and frequency of diarrheal stools between groups. The study was completed by 129 of 155 recruited patients: 63 patients in the LGG group and 66 patients in the placebo group. No significant differences in median (midspread) time before stool normalization (60 h [37-111] in the LGG administration group and 74 h [43-120] in the placebo group; P = 0.37) and diarrheal stool frequency (5.0 [1-10] in the LGG administration group and 6.5 [2-14] in the placebo group; P = 0.19) were observed. Among children admitted after at least 2 days of diarrhea, the LGG administration group showed earlier normalization of stool (51 h [32-78] in the LGG administration group and 74 h [45-120] in the placebo group; P = 0.02), fewer episodes of diarrhea (3.5 [1.0-7.5] in the LGG administration group and 7 [3.0-16.3] in the placebo group; P = 0.02) and 2.2 times higher probability of normalization of stool (95% confidence interval: 1,3-3,9; P = 0.01) in comparison with placebo group. According to the authors' conclusions, the introduction of Lactobacillus GG strain may reduce the duration of acute diarrheal disease in children with symptoms that last longer than 2 days.

Pieścik-Lech et al. (2013) published a review article on the case management in children with acute gastroenteritis. Attempts to improve the taste and/or efficacy of oral rehydration solution (ORS) are ongoing, and some interventions are promising. So far, the standard (more than 24 hours) nasogastric rehydration has been applied, but new evidence of the rapid (more than 4 hours) rehydration effectiveness has been obtained. As for intravenous rehydration, new data have been obtained concerning the comparison of fast or ultra-fast rehydration and rehydration of large and standard volumes. Due to the inconsistency of new observations, rehydration will continue at 20 ml/kg until new data are available. Strong evidence that ondansetron reduces the risk of vomiting has been obtained, but proof of the drug's safety in children is needed. New data have confirmed the fact that in Europe, where zinc deficiency is rare, zinc intake can help in diarrheal syndrome. Also new data, obtained mainly in non-European countries, confirmed the efficacy of smectite or racecadotril as a supplement to oral rehydration. The effectiveness of certain probiotics such as Lactobacillus GG or S. boulardii has been proven.

3.3.3.5 Functional disorders of the gastrointestinal tract associated with abdominal pain in children

Apart from diarrhea, gastrointestinal disorders can manifest themselves in a variety of symptoms. Probiotics, in particular Lactobacillus rhamnosus GG, have been shown to relieve abdominal pain in children, regardless of diagnosis. Abdominal discomfort may be associated with inflammation, and the strain Lactobacillus rhamnosus GG reduces the inflammatory response.

Francavilla et al. (2010) conducted a randomized controlled study of Lactobacillus GG in children with functional abdominal pain. The study involved 141 children with irritable bowel syndrome or functional pain. Children received LGG (in a dose of 6×10^9 CFU/day) or placebo for 8 weeks after a 4-week induction period, after which they were followed-up for another 8 weeks. The LGG strain administration caused the significant decrease in occurrence ($P < 0.01$) and severity ($P < 0.01$) of abdominal pain from baseline. During the follow-up period the difference found was still significant ($P < 0.02$ and $P < 0.001$ respectively). By the 12th week, improvement was observed in 48 children in the LGG treatment group, compared with 37 children from the placebo group ($P < 0.03$). The difference remained also significant until the end of the follow-up period (the 16th week, $P < 0.03$). In the LGG treatment group, the results of the intestinal permeability assessment, mainly in children with IBS, improved significantly ($P < 0.03$). The administration of the LGG strain resulted in significant decrease in the abdominal pain occurrence and severity in children with IBS; this effect maintained and could be related to the improvement of intestinal barrier.

Gawronska et. al. (2007) performed the double-blind randomized placebo-controlled study of the role of the Lactobacillus GG strain in abdominal pain-associated diseases in children. A total of 104 children were enrolled to this double-blind randomized placebo-controlled study, they met the Roman II criteria for functional dyspepsia (FD), irritated bowel syndrome (IBS) or functional abdominal pain (FAP) wherein they received LGG (n = 52) or placebo (n = 52) for 4 weeks.

In the general study group, the probability of successful treatment (pain disappearance) was higher in patients from the LGG treatment group than in patients from the placebo group (25 vs 9.6 %, relative benefit (RB) 2.6, 95 % confidence interval (CI): 1.05–6.6, the number of patients needed to treat (NNT) 7, 95 % CI: 4–123). In the group of children with IBS (n = 37) the probability of successful treatment in the LGG treatment group was higher than in the placebo group (33 vs 5 %, RB 6.3, 95 % CI: 1.2–38, NNT 4, 95 % CI: 2–36); furthermore, the LGG treatment resulted in the abdominal pain occurrence ($P = 0.02$) but not severity ($P = 0.10$). No difference between patient groups with FD (n = 20) and FAP (n = 47) was found.

As a part of the double-blind randomized placebo-controlled study, Bausserman и Michail (2005) investigated the administration of LGG strain at a dosage of 2×10^{10} CFU/day or placebo (inulin) during 6 weeks in 50 children with irritated bowel syndrome (IBS). 64 children (12 boys and 52 girls, the average age was 12 years old, the age range was 6–20 years old) diagnosed with IBS were enrolled in the study. Patients were clinically examined at enrollment and at the end of the study. Six participants (4 in the LGG treatment group, 2 in the control group) were withdrawn before the study start, 3 patients from each group were lost from the follow-up, and 2 patients in the placebo group did not comply with the treatment regimen, so the analysis included 50 patients (25 in each group). The group of analysis included patients aged between 6 and 17 years old (the average age is 12 years old), 10 boys and 40 girls.

No significant difference in the rate of abdominal pain decrease between the LGG and placebo groups was observed, but it has been established that the use of the LGG supplement allows to reduce such symptoms as abdominal distension. Authors pointed out the absence of the Lactobacillus GG strain treatment-associated adverse events.

Horvath et al. (2011) performed the meta-analysis aimed at systematic evaluation of the Lactobacillus rhamnosus GG (LGG) strain role in the treatment of functional gastrointestinal disorders associated with abdominal pain in children. In the LGG intake the higher treatment response rate (absence of pain or decrease of its intensity) compared with placebo intake was observed in the total population of patients with functional gastrointestinal disorders associated with abdominal pain (three RCT n = 290; risk ratio [RR]: 1.31; 95 % CI: 1.08–1.59; number of patients in need of treatment [NNT]: 7; 95 % CI: 4–22) and irritated bowel syndrome (IBS) (three RCT; n = 167; RR: 1.70; 95 % CI: 1.27–2.27; NNT: 4; 95 % CI: 3–8). But there was no difference in treatment respondents among those children who had functional abdominal pain or functional dyspepsia and received placebo or LGG. In the general study group and in the IBS patients subgroup the pain intensity decreased significantly. Only in the IBS patients subgroup, the pain occurrence decreased significantly. The use of the Lactobacillus rhamnosus GG strain results in a moderate increase in treatment success in children with functional gastrointestinal disorders associated with abdominal pain, particularly in children with IBS.

3.3.3.6 Traveler's diarrhea

Oksanen et al. (1990) investigated the efficacy of the Lactobacillus GG strain in the prophylaxis of traveler's diarrhea. They performed the double-blind randomized placebo-controlled study with 820 participants (aged between 10 and 80 years old), who traveled in two directions in South Turkey. The participants were randomized into two groups: receiving the Lactobacillus GG

treatment (at a dose of 2×10^9 CFU/day) or placebo in identical sachets. During the overflight home each participant completed the questionnaire, where the diarrhea and associated symptoms occurrence during the travel was stated. In the first group 756 (92 %) participants completed the study properly. The total occurrence of diarrhea was 43.8 % (331 event). In the placebo group, the total occurrence of diarrhea was 46.5 %, and in the treatment group with the Lactobacillus GG strain — 41.0 %, which indicates the mean protective effect value of 11.8 %. Protective effect values varied between two target places, and the maximum value was 39.5 %. There were fewer diarrhea events in older participants compared with younger ones. The treatment with the Lactobacillus GG strain appears to be efficient for the decrease of diarrhea occurrence in travelers in one of two target points, and is not associated with any adverse events.

Riddle et al. (2017) published the guidance for the prophylaxis and treatment of traveler's diarrhea. According to the authors' conclusion, the use of prebiotics and probiotics designed for the prophylaxis and treatment of acute infectious diarrhea is an attractive strategy for its simplicity and safety. The concept of host organism resistance to intestinal infections, commonly referred as colonization resistance as well, describes the capability of microflora for prophylaxis and limitation of pathogens' colonization and growth. In recent decades our understanding of different colonization resistance mechanisms has extended to elucidation of main direct and indirect mechanisms. In spite of the studies on the efficacy of prebiotics and probiotics in the prophylaxis of acute diarrhea infections in traveling adults having been performed, they vary in test conditions, reasons of the acute diarrhea development, probiotic strains used, have a short follow-up period and don't include any analysis of person-days. These studies also have great differences of dosages, administration frequency and probiotic forms. Times and routes of administration of these preparations relative to various factors, including traveler's groups and target places, as well as concomitant antibiotics use are also different. In spite of the establishment of the probiotics margin effect for the DT prophylaxis in two meta-analyses, the data collected in both studies were insufficient to extrapolate them to International recommendations.

Vae (2018) published the meta-analysis of RCT to evaluate the role of probiotics in the prophylaxis of travel's diarrhea. Eleven papers were selected for this meta-analysis. The standardized relative risk value (SRR) was 0.85 (95 % CI 0.79–0.91), indicating the statistical significance of the results. No heterogeneity of results (I2 = 28.4 %) or systematic error due to the publication of results were noted. The authors observed the statistically significant efficacy of probiotics in the DT prophylaxis based on which the guidance for the prophylaxis of traveler's diarrhea should be updated (International Society of Travel Medicine, 2017).

3.3.3.7 Study of occurrence and severity of respiratory and gastrointestinal infections in children

Hatakka et al. (2001) studied the effect of the LGG prolonged use with cow milk occurrence and severity of gastrointestinal and respiratory infections in children at day-care services. As a part of the multicentre, prospective, double-blind, randomized, placebo-controlled study 571 children, aged between 1 and 6, received milk supplemented with LGG (n = 282) or without LGG (n = 289). Supplemented milk contained 5–10 × 10^5 CFU of LGG/ml, and the mean daily milk exposure (5 days a week) for the active treatment group and the control group was 260 mL (1.3–2.6 × 10^7 CFU). During the study, children's parents kept daily records of respiratory symptoms (fever, rhinorrhea, sore throat, cough, wheezing, ear pain) and gastrointestinal symptoms (diarrhea, vomiting, abdominal pain) as well as absence from school due to the disease. Stool samples were taken at the start, in the middle and at the end of the study for measuring of the LGG recovery and assessment of compliance with the therapy. The study included the 8-month follow-up period, though 58 children did not complete this phase of the study.

At the end of the study period the LGG strain was recovered from the stool of 97 % patients receiving the therapy (versus 9 % of patients in the control group). In children from the group of the LGG treatment, the statistically significant lower number of absence from day-care facilities was established (in average 4.9 days vs 5.8 days in the control group). In the active therapy group, the statistically significant decrease in the complication-associated respiratory infections and lower respiratory tract infections as well as the significant relative decrease of the antibiotic use for respiratory infections was established as well. There was no difference observed between the group of the LGG strain treatment and the control group, including stool frequency and consistence, abdominal pain, allergic symptoms or adverse effects.

Hojsak et al. (2010a) performed the double-blind, randomized, placebo-controlled study with participation of 281 children attending day-care facilities. The children were randomly distributed into the LGG treatment group at a dose of 10^9 CFU per 100 mL of the fermented milk product (LGG treatment group, n = 139) or the placebo group, wherein the same pasteurized fermented milk product without the LGG strain supplement (placebo group, n = 142) for 3-month interventional period. The children from the LGG treatment group had significantly lower risk of the upper respiratory tract infections occurrence compared to the children in the placebo group (RR: 0.66; 95 % CI: 0.52–0.82; NNT: 5; 95 % CI: 4–10), lowered risk of respiratory tract infections with more than 3-day duration (RR: 0.57; 95 % CI: 0.41–0.78; NNT: 5; 95 % CI: 4–11) and the significantly lower number of days with respiratory symptoms ($p < 0.001$). No lower risk

for the lower respiratory tract infections occurrence was established (RR: 0.82; 95 % CI: 0.24–2.76). When compared with the placebo group, the children from the group of LGG treatment had significantly lower risk of gastrointestinal infections (RR: 0.63; 95 % CI: 0.38–1.06), vomiting episodes (RR: 0.60; 95 % CI: 0.29–1.24) and diarrhea episodes (RR: 0.63; 95 % CI: 0.35–1.11) as well as fewer days of gastrointestinal symptoms (p = 0.063). According to the authors' conclusion, the LGG administration may be recommended as an efficient action to lower the risk of upper respiratory tract infections in children attending kindergartens.

Hojsak et al. (2010b) performed the double-blind, randomized, placebo-controlled study with participation of 742 hospitalized children. For the in-patient period, the children were randomly distributed into the LGG treatment group at a dose of 10^9 CFU per 100 mL of the fermented milk product (LGG treatment group, n = 376) or the placebo group, wherein the same pasteurized fermented milk product was used without the LGG strain supplement (placebo group, n = 366). When compared with the placebo group we found the significant decrease of the risk of gastrointestinal infections (relative risk [RR]: 0.40; [95 % confidence interval (CI): 0.25–0.70]; NNT: 15; [95 % CI: 9–34]), respiratory infections (RR: 0.38; [95 % CI: 0.18–0.85]; NNT: 30 [95 % CI: 16–159]), vomiting episodes (RR: 0.5; [95 % CI: 0,3–0,9]), diarrhea episodes (RR: 0,24; [95 % CI: 0.10–0.50]), gastrointestinal infection episodes of the duration of more than two days (RR: 0,40; [95 % CI: 0.25–0.70]) and respiratory infection episodes of the duration of more than three days (RR: 0,4; [95 % CI: 0.32–0.9]). Groups did not differ by the in-patient period (P = 0.1). According to the authors' conclusion, the LGG administration may be recommended as an efficient action to lower the risk of nosocomial gastrointestinal and respiratory infections in children at day-care facilities.

Liu et al. (2013) performed the systematic review on efficacy of the Lactobacillus rhamnosus GG (LGG) strain in the prophylaxis of respiratory infections in children. Four RCT with 1805 study subjects met the inclusion criteria. When compared with placebo, the LGG strain administration was associated with the decrease in the occurrence of acute otitis medium (four RCT; n = 1805; RR: 0.76; 95 % CI: 0.64–0.91; the constant-effect model; NNT: 17; 95 % CI: 11–46), the risk of upper respiratory tract infections (one RCT; n = 281; RR: 0.62; 95 % CI: 0.50–0.78; NNT: 4; 95 % CI: 3–8) and the frequency of antibiotic use (four RCT; n = 1805; RR: 0,80; 95 % CI: 0.71–0.91; the constant-effect model). The LGG group and the control group did not significantly differ in the risk of respiratory infections in total and the occurrence of lower respiratory tract infections. But the subgroup analysis of the two studies with participation of children of more than 1 year old showed the major risk lowering of total respiratory infections (two RCT; n = 794; RR: 0.73; 95 % CI: 0.57–0.92; the random-effect model; NNT: 8; 95 % CI: 5–14). Adverse events were similar in

both groups. There were no serious adverse events reported. According to the authors' conclusion, the administration of the Lactobacillus rhamnosus GG strain allows to lower the occurrence of acute otitis medium, upper respiratory tract infections and antibiotic use in children, when compared with placebo.

3.3.4 Other effects studied in human

3.3.4.1 Immunological effects

According to assessments, more than two-thirds of all lymphocytes of the organism are located in epithelial and sub-epithelial layers of the intestine wall. The immune system of the mucous membrane is the first-line defense and decreases the requirement of the systemic immunity involvement in the host organism maintenance. The immune system of the mucous membrane carries out a complicated task of balance maintenance between the mediation of various protective immune responses to infectious agents and the limitation of antigenic load present in the intestinal lumen. The Lactobacillus rhamnosus GG strain was shown to support innate and acquired immunity reaction by providing the integrity of the intestinal epithelial barrier and mediating the cellular immune response (Bäckhed et al. 2005; Gogineni et al. 2013).

Hatakka et al. (2003) performed the pilot double-blind, randomized, placebo-controlled study to evaluate the efficacy of the LGG strain in mild rheumatoid arthritis. Totally 21 patients aged from 18 to 64 years old were randomly distributed into the LGG treatment group or the placebo group, 2 capsules BID for 12 months; the daily dose of LGG was 10^{10} CFU. Feces samples were examined to confirm the compliance with the treatment. The arthritis activity was assessed based on the results of clinical examination and laboratory investigations. The treatment had no effect on any arthritis characteristics, but in the LGG group the patients' significantly improved general well-being was observed. There was no reports of any cases of the treatment discontinuation because of adverse events.

Szachta et al. (2010) performed the simple blind, randomized, placebo-controlled study to evaluate the efficacy of the *Lactobacillus rhamnosus* GG (LGG) strain in elimination of vancomycin-resistant enterococci (VRE) from the gastrointestinal tract of the children with confirmed colonization, as well to evaluate the effect of the probiotic strain on the colony count of *Lactobacillus spp.* in the gastrointestinal tract. Children (aged between 0 and 18) who were hospitalized to the children's hospital, and carried VRE in their gastrointestinal tract, were randomized into the group of LGG treatment at a dose of 3 bln. CFU daily or placebo for 21 consecutive days. In total 61 children completed the study (32 in the active treatment group and 29 in the control group). Samples by means of rectal tampons for the VRE and *Lactobacillus*

spp. analyses were taken at the baseline, once a week during the supplement administration and one month after the supplement administration completed. The antibiotic control was performed during the whole period of analysis. After 3 weeks, the significant intergroup difference in the numbers of children with gastrointestinal colonization by VRE was found ($P = 0.002$). The VRE carrier status was lost in 20 of 32 study subjects in the active treatment group and in 7 of 29 study subjects in the control group. The increase of *Lactobacillus spp.* colony count in the gastrointestinal tract of the children receiving LGG. Starting from the first week, the statistically significant difference in the frequency of the detection of bacteria was found, while their growth intensity began to differ starting from the 2nd week. When compared with placebo, LGG temporarily eliminates VRE carrying and increases the count of the *Lactobacillus spp.* strain in the children's gastrointestinal tract.

Bruzzese et al. (2007) performed the prospective, randomized, placebo-controlled cross-over study aimed to examine effects of the Lactobacillus GG (LGG) strain on pulmonary exacerbations in cystic fibrosis (CF). Nineteen children received LGG for 6 months, after which they were switched to the oral rehydration solution (ORS) for 6 months. In parallel, nineteen children initially received ORS, and then were switched to LGG. The main studied outcomes included the following: the occurrence of pulmonary exacerbations and hospitalizations, forced expiratory volume (FEV1) and body weight changes. In children receiving the LGG supplement the lower number of pulmonary exacerbations (median 1 vs 2; range 4 vs 4; median difference 1; 95 % CI: 0.5–1.5; p = 0.0035) and hospitalizations (median 0 vs 1; range 3 vs 2; median difference 1; 95 % CI: 1.0–1.5; p = 0.001) compared with the children from the ORS group. During the LGG administration the higher extent of FEV1 (3.6 ± 5.2 % vs 0.9 ± 5 %; p = 0.02) and body weight increase (1.5 ± 1.8 kg vs 0.7 ± 1.8 kg; p = 0.02) were noted. The LGG administration resulted in decrease of occurrences of pulmonary exacerbations and hospitalizations in patients with CF. Based on that, the delay of pulmonary function disturbances during the probiotic intake and the association between inflammatory processes in the intestine and lungs were proposed.

Kekkonen et al. (2008) performed double-blind, randomized, placebo-controlled interventional study for evaluation of effects of three potentially anti-inflammatory bacteria of three genera on immune function parameters in healthy adults in clinical practice settings. In this double-blind, randomized, placebo-controlled interventional study, the total of 62 volunteers participated. Study subjects were randomized into the groups using a diary beverage with the supplement of Lactobacillus rhamnosus GG (LGG), Bifidobacterium animalis ssp. lactis Bb12 (Bb12) or Propionibacterium freudenreichii ssp. shermanii JS (PJS) strains, or the placebo beverage for 3 weeks. Samples of venous blood and saliva were taken initially and on Days 1, 7 and 21. Stool

samples were taken initially and at the end of the intervention period. The serum hs-CRP level expressed as the median $AUC_{0–21}$ (relative to the baseline), was 0.018 mg/L in the placebo group, -0.240 mg/L — in the LGG group, 0.090 mg/L — in the Bb12 and -0.085 mg/L — in the PJS group (P = 0.014). In vitro synthesis of TNF-α in peripheral blood mononuclear cells (PBMC) cultured in vitro was significantly lower than in study subjects from the LGG group compared with the placebo group. Synthesis of IL-2 by PBMC in the Bb12 group. Thus, probiotic bacterial strains demonstrate strain-specific anti-inflammatory effects in healthy adults.

3.3.4.2 Efficacy in inflammatory intestine diseases

As a part of the prospective, double-blind, randomized, placebo-controlled study Prantera et al. (2002) studied the efficacy of the LGG strain in the prophylaxis of symptom recurrence after the radical intestine resection for Crown disease. Forty-five patients with Crown disease who underwent the total resection of all affected parts of the intestine within previous 10 days (29 men and 16 women aged from 22 to 71, average age 37 years old) were randomized into groups receiving the LGG strain at a dose $1,2 \times 10^{10}$ CFU or placebo in two sachets per day for 1 year. After 13, 26, 39 and 52 weeks of the treatment start control visits were performed; for the purpose of control, urine and blood samples were collected and analyzed for blood cell number, serum levels of iron and ferritin, creatinine, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Ileocolonoscopy was performed at the end of the study. As it was observed, the LGG strain intake for 1 year had no effect on the Crown disease recurrence rate or the severity of intestine affection in recurrences. In total thirteen patients did not complete the study until the end; nine discontinued patients were withdrawn due to adverse events. The reasons for discontinuation did not differ between the active treatment group and the control group. The treatment groups did not differ in such symptoms as diarrhea, abdominal distension and flatulence. No effects on liver enzyme activities were noted. Adverse events occurred in 2 patients from the LGG and 6 patients in the placebo group; none of them was considered as study-associated and did not result in discontinuation.

The study for evaluation of the efficacy of the combination of 4 probiotic strains, L. rhamnosus GG, L. rhamnosus LC-705, Propionibacterium freudenreichii ssp. shermanii JS and Bifidobacterium breve Bb99 for the decrease of irritated bowel syndrome (IBS) was performed (Kajander and Korpela (2006)). As a part of the double-blind, randomized, placebo-controlled study 103 patients with RCT were randomized into the placebo group or the group receiving the probiotic combination in one capsule (containing $8–9 \times 10^9$ CFU of each strain) QD for 6 months. Patients kept a diary of abdominal symptoms and stool characteristics. The study was completed by 86 patients. According to observations, the probiotic intake significantly decreased IBS

symptoms: by 42 % in the probiotic therapy group and by 6 % in the placebo group. No changes in stool characteristics or quality of life were observed. The reasons for discontinuation were not reported, as well as the data on distribution of withdrawn patients by groups were absent. Detected undesirable effects of the probiotic therapy were not discussed.

Baroja et. al. (2007) performed the open-label study (with the control group) in which 20 adults with clinically manifested inflammatory bowel disease (IBD) (15 patients with Crown disease and 5 patients with ulcerous colitis) and 20 healthy control persons consumed the yogurt supplemented with 2×10^7 CFU/mL of L. rhamnosus GR-1 and 10^3 CFU/mL of L. reuteri RC-14 corresponding to the daily dose of L. rhamnosus and L. reuteri 2.5×10^9 CFU and 1.25×10^5 CFU, respectively. The intake of probiotic yogurt was associated with beneficial anti-inflammatory effects in patients with IBD in combination with an extending pool of regulatory T-cells in peripheral blood. In other eight patients with IBD, who consumed yogurt without supplements, no anti-inflammatory effects were observed. According to the authors' conclusion, "the short-time consumption of yogurt supplemented with Lactobacillus GR-1 and RC-14 strains induced the formation of beneficial anti-inflammatory environment in peripheral blood of IBD patients and was not associated with any adverse effects". It's particularly noticeable, that in this study there were no detected effects which could indicate the penetration of affected gastrointestinal barrier in patients with IBD, supporting the previous evidence for the infectivity absence in these strains.

3.3.5 Overview of safety data

In studies performed in different groups (adults, children) reviewed above, most authors did not observe and report any adverse reactions to the LGG administration even in such high dose as 10^{12} CFU/day. There are anecdotal reports of mild gastrointestinal symptoms such as nausea, abdominal cramping, flatulence or abdominal distension, but no cases of discontinuation due to adverse events were reported in any of the studies. Even in these cases the occurrence of AE was comparable with that in the control group. No serious adverse reactions were encountered in any of clinical studies.

In total these observations indicate the safety of the LGG supplement used at recommended doses. However due to the fact that there are live bacteria, the probability for the development of adverse effects, associated with their pharmacokinetic properties, remains. As stated above, the bacteria of the LGG strain are metabolically active and are able to regulate the immune response in the host organism. This capability is commonly characterized as positive, but in some cases it is the reason of adverse effects. In this section we'll discuss relevant cases and perform the overview of adverse reactions reported.

According to the report of 2002, collectively published by the World Health Organization (WHO) and Food and Agricultural Organization (FAO) which are structural divisions of the United Nations Organization, probiotics can theoretically induce the following four types of side effects:

1. Systemic infections

2. Adverse metabolic activity

3. Immune hyperstimulation in sensitive persons

4. Gene transfer

Slight gastrointestinal symptoms were reported as well. The Working group of WHO/FAO recommends to perform the safety assessment of novel probiotic strains based on antibiotic resistance, toxin production and hemolytic potential, metabolic activity, including the synthesis of D-lactate and deconjugation of bile salts; to perform studies in human for adverse effects evaluation and post-marketing observation of commercial users; and, ideally, to assess their use in immunocompromized animals in order to determine the probiotic organism's infectivity in the particular host (Doron et al. 2015).

Hibberd et al. (2014) published the data from the safety study of a drug containing Lactobacillus rhamnosus GG in elderly healthy volunteers. The main goal of this open-label clinical study was to evaluate the safety and tolerability of 1×10^{10} CFU of Lactobacillus rhamnosus GG taken orally twice a day in elderly volunteers for 28 days.

Fifteen elderly volunteers aged 66-80 received Lactobacillus rhamnosus GG capsules containing 1×10^{10} CFU twice a day for 28 days, and were monitored for 56 days.

No serious adverse events were reported in healthy volunteers during the study. A total of 47 adverse events were reported in 15 volunteers (1 to 7 per volunteer), 39 (83%) of which were assessed as mild, and 40% were assessed as possibly related to the use of Lactobacillus rhamnosus GG. The most common adverse events were recorded from the gastrointestinal tract (bloating, gas, and nausea), 27 of which were assessed as mild adverse events, and 3 as moderate. The study showed that Lactobacillus rhamnosus GG is safe and well tolerated by healthy adult volunteers aged 65 and older. In the first week of taking LGG, mild and passing gastrointestinal symptoms were recorded, which did not affect the daily activity of the volunteers. After the first week, most of the gastrointestinal symptoms appeared periodically during treatment and during the follow-up period with no identified Association with LGG.

In studies with healthy participants, the AE occurrence in the use of Lactobacillus rhamnosus GG at doses 3.6×10^{11} CFU are usually not reported. Only in the study by Schultz et al. (2004) in 3 study subjects, abdominal distension was registered; there were no reports about other AE in this

study. Summarized information on AE occurrence during the Lactobacillus rhamnosus GG intake by healthy subjects is represented in the Table 5.

Table 5. Summarized safety data of Lactobacillus rhamnosus GG in healthy subjects

Author	Study Population (N)	Study goal	Duration	Strain/Dose	AE
Goldin 1992	Healthy volunteers (N=76)	Survival in GIT	7, 28 and 35 days	LGG 4 x 10^{10}, 1.6 x 10^{11}, and 3.6 x 10^{11} CFU	No reports
Saxelin 1995	Healthy volunteers (N=20)	Survival in GIT	7 days	LGG 1.6 x 10^{8} CFU and 1.2 x 10^{10} CFU	No reports
Jacobsen 1999	Healthy volunteers (N=12)	Survival in GIT	18 days	LGG 10^{10} CFU/day	No reports
Saxelin 1993	Healthy volunteers (N=44)	Determination of LGG concentration in feces	7 days	1 x 10^{9} CFU, 4 x 10^{9} and 8 x 10^{9} in tablets/ 2 x 10^{9} CFU and 1,2 x 10^{10} CFU with fermented milk	No reports
Alander 1999	Healthy volunteers (N=24)	GIT colonization	12 days	LGG 6 x 10^{10} CFU	No reports
Saxelin 1999	Healthy volunteers (N=40)	GIT colonization	7 days	LGG 1.5 x 10^{6}, 1.5 x 10^{7}, 1.5 x 10^{8}, 1.5 x 10^{9}, 1.5 x 10^{10}, and 1.1 x 10^{11} CFU/day	No reports
Millar 1993	Prematurely-born children (N=20)	GIT colonization	14 days	LGG 2 x 10^{8} CFU	No reports
Schultz 2004	Pregnant women (N=6)	LGG colonization in children through the intake by pregnant women	6 weeks	LGG 2 x 10^{9} CFU/day	Mild abdominal distension in 3 study subjects
Elmadfa 2001	Healthy volunteers (N=12)	Vitamin Serum Levels	4 weeks	LGG 2.5 x 10^{10} CFU/day	No reports
Hatakka 2001	Children at kindergartens (1-6 years, N=571)	Prevention of infections	30 weeks	1.3-2.6 x 10^{7} CFU	No differences in AE, including stool rate and consistency, abdominal

Author	Study Population (N)	Study goal	Duration	Strain/Dose	AE
					pain, allergy symptoms between groups were observed.
Hojsak 2010a	Children at kindergartens (1-6 years, N=281)	Prevention of infections	3 months	LGG 10^9 CFU/day	No reports
Oksanen 1990	Adults and children (10-80 years old, N=820)	Prophylaxis of traveler's diarrhea	During the travel	LGG $2x10^9$ CFU/day	No reports
Kalliomäki 2003	Children aged 4 years (N=107)	Prophylaxis of atopic dermatitis	4 weeks	LGG 2 x 10^{10} CFU	No reports
Galpin 2005	Children aged 36-60 months (N=164)	Effect of LGG on intestinal wall integrity	30 days	10^{11} CFU/day	No reports
Pelucchi 2012	Pregnant women in the 3rd trimester, children aged less than 2 years (NA)	Prophylaxis of atopic dermatitis	varied	LGG 2 x 10^{10} CFU/day	No reports
Panduru 2015	Pregnant women in the 1st and 3rd trimester, children aged less than 9 years (NA)	Prophylaxis of atopic dermatitis	varied	LGG 1-2 x 10^{10} CFU/day	No reports

3.3.5.1 Systemic infections

In a number of reports on adverse reactions, episodes of infections caused by microorganisms similar to probiotic strains, were described in patients who used probiotics before the occurrence of symptoms. However, most of those cases can be explained by characteristics of the host organism, namely by the immunodeficiency or the immunity weakening due to serious underlying diseases. DeGroote et al. (2005) described a bacteriemia case, caused by L. rhamnosus GG, in use of this probiotic in a child with "short gut" syndrome. Barton et al. (2001) reported three cases in infants with gastrointestinal congenital abnormalities requiring a surgical correction, who developed sepsis, caused by Pediococcus sp.; the pathogenesis of these infections could be associated with probiotic strains. Tommasi et al. (2008) described a bacteriemia case, caused by Lactobacillus casei, in 66 years old, non-immunocompromized man with the history of unclear genesis fever. The blood culture showed bacteriemia caused by leuconostoc, however the

subsequent analysis by means of polymerase chain reaction with 16S RNA sequencing the Lactobacillus casei was identified, and the successful antibiotic therapy was performed. According to the authors' conclusion, bacteriemia, caused by probiotic strains, is rarely-occurring, however it is commonly underestimated because of being related to contaminating species and being not considered to be the main causative agent. Vahabnezhad et al. (2013) described a bacteriemia case, caused by Lactobacillus, in 17 years old boy with ulcerous colitis, being treated with systemic corticosteroids and infliximab. The patient was admitted with complaints for body temperature rise to 38.9 °C, flushing and shivers after 1 week after starting with the Lactobacillus rhamnosus GG probiotic. The results of the first blood culture on the 2nd- day of the temperature rise Lactobacillus had been identified, however the subsequent blood cultures on 3rd and 5th days were negative. The patient was administered with the empiric antibiotic therapy for 5 days, wherein the body temperature normalized up to the 8th day of the disease. This report indicates a potential risk of the bacteriemia development caused by Lactobacillus bacteria, in immunocompromised patients with underlying highly severe ulcerous colitis.

Kunz et al. (2004) published two cases of bacteriemia, caused by Lactobacillus bacteria, with the background probiotic therapy associated with the "short gut" syndrome. This publication was commented by Young and Vanderhoof (2004), who had demonstrated in several studies, that the *L. rhamnosus* probiotic strain strengthens intercellular contacts of the mucous membrane and subsequently decreases the intestinal penetrance, which may be the main factor responsible for the efficacy of this strain in the prophylaxis of food allergy in infants. In view of above mentioned observations bacterial translocation is less likely factor in the development of sepsis. It appears to be possible that one of etiological factors is the contamination of the central catheter, especially in hospitalized patients characterized by a high incidence of nosocomial infections in principle, since opening capsules is necessary to administer the preparation to infants. Caregivers must take special precautions changing diapers for an infant with the installed central catheter; obligatory measures include the thorough handwashing to prevent any bacterial dissemination. These precautions are usually ignored in opening of capsules containing powder and its preparing for administration, nevertheless this powder contains live bacteria in much higher concentration than feces do. Authors did not indicate by what means blood samples had been drawn for culturing (from the central catheter or peripheral vein), while it could help to establish the source of the bacterial invasion. This publication underlines the importance of the observation obtained, namely that all probiotics are necessary to be used with caution in patients with weak health, which especially relates to patients with the central catheter.

Land et. al. (2005) described two patients receiving Lactobacillus probiotic strains, who had subsequently developed bacteriemia and sepsis due to Lactobacillus bacteria. The molecular analysis by the DNA fingerprinting method had shown that the Lactobacillus strain isolated from blood samples was undistinguishable from one consumed by patients. This report for the first time demonstrated, that invasive disease may be caused by Lactobacillus. Authors highlighted that this report is not a base to refuse from the proper use of Lactobacillus or other probiotic strains, rather it should serve as a reminder that in selected populations these medications may cause invasive disease. Mackay et al. (1999) published a case of 67-year-old man with the diagnosis of mild mitral valve regurgitation due to the mitral valve prolapse, who developed bacterial endocarditis, caused by L. rhamnosus on the blood culture data. The complete recovery was the result of intravenous administration of beta-lactams. Furthermore, Presterl et al. (2001) published the report on bacterial endocarditis and septic arthritis in man who consumed a large amount of probiotic yogurt.

In 2018, Naqvi et al. published the report on fatal endocarditis, caused by Lactobacillus, in a young patient with complicated cirrhosis and past colitis, caused by Clostridium difficile. In the same publication the literature review is provided. There were entering reports on the development of invasive infections, such as meningitis, endometritis, peritonitis, pneumonia, bacteriemia and endocarditis. Lactobacillus are the causative factor in 0.05–0.4 % of all endocarditis cases. As the tendency for the development of said infections in immunocompromised patients was noted, the related mortality varies from 23 to 29 %.

Griffiths et al. (1992) reviewed 2 Lactobacillus-induced endocarditis cases and 39 cases from literature, and according to the results the lower (39 %) response rate to isolated drug therapy and mortality in the studied cohort on the level of 27 % had been established. As possible reasons low-quality studies of antibiotic sensitivity and the absence of standardized therapy were proposed, including the use of semi-optimal antibiotics inactive for Lactobacillus (Griffiths et al. 1992; Husni et al. 1997). Similarly, Cannon et al. (2005) reviewed 241 cases of clinical Lactobacillus-induced infections. 73 patients in this sample had endocarditis. Most of these patients initially had the structural cardiac abnormalities (63 %), dental problems or underwent a dental procedure recently (47 %). The development of clinical infections was also related to invasive interventions, such as endoscopy and colonoscopy. Lactobacillus have a capability for collagen and fibrinogen binding, platelet aggregation and glucosidase and protease enzymes production which may facilitate colonization of the vascular endothelium (Harty et al. 1994; Oakey et al. 1995). In indicated cases the most commonly identified species were L. casei and, more rarely, L. rhamnosus and L. plantarum. There are only 11 reports in literature of the development of endocarditis, caused by L.

rhamnosus, in adults, two of them were associated with the probiotic use (Mackay 1990, Presterl et al. 2001).

As several cases of the development of Lactobacillus-induced bacteriemia are known in patients with the central catheter in intense therapy settings, experts recommend to maintain the thorough hand hygiene during manipulations with the central catheter after manipulations with probiotic medications.

Furthermore, Forestier et al. (2008) performed the pilot prospective, double blind, randomized, placebo-controlled study in patients of the resuscitation and intensive care unit (ICU). Starting from the 3rd day after the admission to ICU and until the discharge patients received Lactobacillus casei rhamnosus 35 at a dose of 10^9 CFU BID; the number of treatment days is not provided. Totally 208 patients were involved in the study (146 men and 62 women, average age was 58.4 years old): 102 in the probiotic therapy group and 106 in the placebo group. The goal of this study was to determine the potential of probiotic therapy to decrease the occurrence of respiratory infections, caused by Pseudomonas aeruginosa. The most patients had undergone a surgery or had an injury or respiratory disorders. During the probiotic therapy the significant decrease in the occurrence of infections caused by P. aeruginosa was noted, and the time to the development of infections, whereas no effect on the occurrence of infections caused by other microorganisms was determined. No cases of sepsis caused by Lactobacillus, were observed. In spite of the fact that in the study the measurement of the occurrence of L. rhamnosus-induced bacteriemia was not contemplated, it was shown that the probiotic use is safe and may provide the protective effect on pseudomonas infections. However, the study group was relatively small, and the study was performed on the base of the single center.

Strong evidence for the safety of the L. rhamnosus GG (LGG) strain (one of the most popular probiotic strains in Finland) was obtained in the Finnish review indicating the absence of increase in Lactobacillus-induced bacteriemia cases over ten years from 1990 to 2000, in spite of increasing popularity of this probiotic (Salminen et al. 2002). Lactobacillus were identified as causative factors in 0.02 % of all positive blood cultures. There were no time variations of said occurrence parameter over the considered ten years. Eleven of 89 strains isolated from blood were similar to the probiotic LGG strain according to the results of pulse-field gel electrophoresis (Salminen et al. 2004), however within the subsequent study the difference between LGG-like isolates from the blood culture and the probiotic LGG strain based on phenotypical characteristics was established (Ouwehand et al. 2004) according to the results of studies demonstrating pathogenicity, including adhesion parameters in vitro and induction of respiratory burst.

There are known studies of the safe probiotic use in recipients of solid organ grafts and other immunocompromised patients, wherein no systemic infections were reported (Rayes et al. 2002; Rayes et al. 2005; Doron et al. 2015).

Anukam et al. (2008) studied the efficacy of probiotics in 24 adult women (between 18 and 44 years old) with HIV infection / AIDS with clinical manifestations of moderate diarrhea, CD4-cell count more than 200, receiving no antiretroviral medications or dietary supplements. Patients consumed 100 mL of yogurt with or without supplements daily for 15 days. The hematologic profile, CD4-cell count and quality of life were evaluated on the baseline and after 15 and 30 days of the probiotic yogurt consumption. No significant differences in hematologic parameters before and after the probiotic yogurt consumption were noticed in either of groups. In the probiotic yogurt group, the mean WBC quantity was measured as $5.8 \pm 0.76 \times 10^9$/L, $6.0 \pm 1/02 \times 10^9$/L and $5.4 \pm 0.14 \times 10^9$ L on the baseline, after 15 and 30 days, respectively. However, the mean CD4-cell count remained unchanged or increased to 15th and 30th day in 11 of 12 probiotic-consuming patients compared with 3 of 12 patients in the control group. Diarrhea, flatulence and nausea resolved in 12 of 12 patients from the probiotic yogurt group within 2 days compared with 2 of 12 patients from the conventional yogurt group after 15 days. In this study the beneficial effect of probiotic yogurt on the quality of life in patients with HIV infection / AIDS was demonstrated, and a little benefit of simple fermented products in the AIDS treatment was established.

Within the prospective, double-blind, randomized, placebo-controlled study of the effect of the LGG on the intestine wall integrity the evaluation of clinically healthy children from Malavi with the increased risk of enteropathy was performed (Galpin et al. 2005). Tropic enteropathy is a condition of asymptomatic atrophy of intestinal villi, being common in developing countries. 164 children were enrolled in the study, 161 of them completed it. Enrollment criteria included the age between 36 and 60 months; the average age of children (76 boys and 88 girls) was 46.4 months at the entrance. At the time of enrollment, children were clinically examined, and their parents completed the questionnaire on demographic data and sanitary conditions. Children were given 2 capsules of the LGG strain each at a dose of 5×10^{10} CFU daily (daily dose = 10^{11} CFU) or placebo for 30 days. Children were examined twice a week during the entire study period.

The main outcome of the study was the absorption parameters for mannitol, lactulose and sucrose according to urinalysis results. As observed, the administration of the LGG strain had no effects on mannitol, lactulose and sucrose absorption.

Caregivers did not report any vomiting development or other undesirable effects during the study. There were no differences in growth parameters or the occurrence of fever, cough and diarrhea.

Honeycutt et al. (2007) undertook the prospective, double-blind, randomized, placebo-controlled study in the pediatric resuscitation and intensive care unit for 16 in-patient beds to in order to evaluate the LGG strain potential for the decrease in the occurrence of nosocomial infections. During the period from April 2004 to December 2004 61 children were enrolled in the study. The everyday screening of all newly admitted patients was performed. Patients were withdrawn from the study if the following criteria were met: confirmed or suspected intestine perforation, confirmed or suspected mechanical intestinal obstruction, absolute neutrophils count < 0.5 × 10^9 cells/L, intolerance of required enteral nutrition volume on the attending physician's opinion, use of the probiotic medication in any day of the week before the enrollment in the study, participation in another clinical study, absence of parents or their consent. Patients were randomized into the groups of Lactobacillus rhamnosus GG strain treatment (Culturelle, ConAgra Foods, Omaha, Nebraska) or placebo (inulin) one capsule daily, until the discharge from the hospital. 61 patients were randomized: 31 into the active treatment group and 30 into the placebo group. In the control group, four infectious diseases were registered in three patients. In the active treatment group 11 infectious diseases were registered in six patients. The relative risk of infection development in the active treatment group was 1.94 (confidence interval [CI]: 0.53–7.04; p = 0.31). The mean number of infectious diseases was 1.83 and 1.33 in the active treatment group and in the control group respectively (difference = 0.5; p = 0.52). There were no adverse events in the study group. But due to recent safety concerns for the L. rhamnosus GG strain use and the absence of benefits according to the interim analysis data, the study was discontinued on the decision of Investigators.

Salminen et al. (2004) published the review article on the clinical relevance of Lactobacillus-induced bacteriemia with a special attention for the L. rhamnosus GG strain. Authors performed the review of 89 cases of L. rhamnosus-induced bacteriemia in order to evaluate risk factors and outcomes. In 53 % of cases the analysis of causative species was performed. In 11 cases the isolated species from the isolate was identical to the species being used in the probiotic composition. Most patients in all groups had underlying diseases with inevitable or soon lethal outcome (Class 3 or 4 on McCabe criteria). These are 91 % of patients from the LGG group. Predisposing factors for bacteriemia development included immunosupression, previous prolonged hospitalization and underwent surgery. No significant difference in said predisposing factors and clinical characteristics between patients with bacteriemia caused by different Lactobacillus species except the higher level of C-reactive protein in patients with L. rhamnosus-induced bacteriemia, was determined. The mortality rate was 26 and 48 % after 1 month and 1 year respectively. The contribution of other concomitant bacteria to the results obtained could be

completely excluded, because in 39 % of cases bacteriemia had the polymicrobial etiology, but respective cases were not associated with higher mortality rate. Within the study period serious septicemic complications were detected in several cases only.

3.3.5.2 Unfavorable metabolic effects

In the double-blind, randomized, placebo-controlled study (Besselink et al. 2008) the evaluation of the multistrain probiotic efficacy in the prophylaxis of infection complications in 296 patients with severe pancreatitis was performed. In the probiotic therapy group the higher mortality rate associated with intestine ischemia was registered. As a proposed reason of intestine ischemia in critically ill patients receiving the contemplated combination of 6 probiotics, authors stated a possible growth in oxygen requirement of the intestine mucous under the conditions of already reduced blood supply. It's also possible that probiotics trigger an inflammatory reaction in small intestine which results in the decrease of capillary blood flow.

The results of this study caused serious concerns and stimulated the detailed study of this issue by health authorities of Netherlands. According to the results of evaluation it was established that the represented study had a number of significant drawbacks in the design, approval and performance, so these results should be interpreted with caution (the comment by Lancet editors; 2010).

In two previously performed less-scaled studies (Olah et al. 2002; Olah et al. 2007) the decrease in septic complications occurrence, surgical interventions and infected necrosis was observed in patients with pancreatitis receiving the symbiotic medication, containing lactic acid bacteria and cellulose. Intestine ischemia was not mentioned by authors.

At the same time, in two other studies in critically ill adults (Jain et al. 2004) and children (Honeycutt et al. 2007) the insignificant but potential increase in the occurrence of infectious complications was stated during the probiotic therapy.

Other metabolic risks include effects of D-lactate, being synthetized by probiotic strains, and bile acid deconjugation. There are five reports in literature on acidosis induced by D-lactate (Kee et al. 2006; Munakata et al. 2010; Oh et al. 1979), one of which had been reported for the patient with the "short gut" syndrome.

In 2017, Vitetta et al. published the review article related to D-lactate-acidosis and strain specificity. Authors criticize the discipline-specific approach to the safety of probiotic medications and unwillingness to consider scientifically justified evidence. However, there is an experience of the satisfactory use of the probiotic L. rhamnosus strain in studies, and authors are aware of its efficacy and safety.

3.3.5.3 Immune hyperstimulation

Because of probiotics' influence on congenital and acquired immunity reactions, including effects on cytokine secretion and dendrite cell function (Vaarala et al. 2003; Veckman et al. 2004; Braat et. 2004; Drakes et al. 2004), the concerns about the risk of immune response hyperstimulation in some persons possibly leading to autoimmune or inflammatory reactions arises. Theoretical concerns are not supported by any clinical cases.

3.3.5.4 Gene transfer

Lactic acid bacteria carry plasmids with genes, conferring the resistance to tetracyclines, erythromycin, chloramphenicol or lincosamide, macrolids, streptomycin and streptogramin (Lin et al. 1996; Gevers et al. 2003). The capability for conjugation transfer of genes from enterococci to Lactobacillus and Lactococci in animal intestine and in vitro was observed; but the gene transfer to Lactobacillus is observed rather rarely (Morelli et al. 1988). The attempts were made for molecular identification of genes for vancomycin resistance in the Lactobacillus genome. Related genes were not found.

Based on the results of hybridization analysis or polymerase chain reaction no genes Van A, B, H, X, Z, Y or S were identified (Klein et al. 2000; Tynkkynen et al. 1998). Despite the theoretical possibility of lateral gene transfer between probiotic bacteria and other bacteria living in or outside the intestine, there is no clinical evidence of the possibility of antimicrobial resistance transfer. This observation is especially important in view of the widespread use of probiotics simultaneously with antibiotics.

3.3.5.5 Gastrointestinal adverse effects

In studies, minor symptoms of the gastrointestinal tract, such as abdominal cramps, nausea, soft stools, flatulence and distortion of taste sensations, were recorded against the background of probiotic therapy. Nevertheless, according to a meta-analysis and a systematic review of the use of probiotics aimed at prevention of diarrhea caused by Clostridium difficile, the probability of developing these adverse effects is 18–20% lower in patients receiving probiotics than in control individuals (Johnston et al. 2012; Goldenberg et al. 2013)

3.4 A brief description of the known and potential risks and benefits to research subjects, if there are any

Preclinical and clinical studies of the components of the products planned for use in this study indicate a good safety profile and good tolerability of LRG-002, capsules. A list of known adverse effects of these products is presented in section 3.2.

During the study, venous blood is supposed to be taken for analysis - about 12 ml (about 1 tablespoon) on day 1, day 7 ± 2 and day 15 ± 2, i.e. the total volume of blood that will be taken for the study shall not exceed 36 ml for the entire period of the study. This is a clinically insignificant amount and rate of blood loss for an adult without severe concomitant diseases. Potentially, a bruise or hematoma is possible due to venipuncture, as well as the development of phlebitis or thrombophlebitis at the injection site.

The research center staff will make every possible effort to minimize the risks for patients associated with the research procedures and treatment within the study. In the case of adverse events or other health problems, the patient will be provided with the necessary medical care.

The benefits of participating in the study are related to the possibility of conducting a series of laboratory and instrumental examinations, treatment under the supervision of highly qualified doctors, and treatment with the investigational drugs free of charge for the study participant.

3.5 Description and justification of the method of administration, dosage, dosing regimen and course of treatment

3.5.1 Description and justification of the method of administration, dosage, dosage regimen and course of treatment with the investigational drug

To achieve a positive effect, probiotics should be taken in sufficiently high doses. The proposed dose of Lactobacillus rhamnosus GG is within the range of doses that have proven effective in well-planned and conducted randomized controlled trials described in the metanalysis Szajewska, 2015, Hempel, 2012, McFarland, 2006, as well as in the studies, carried out by Vanderhoof, 1999, Arvola, 1999, Johnston, 2006, Goldenberg, 2015. Besides, a dose of Lactobacillus rhamnosus GG in LRG-002 product corresponds to the doses recommended in the published guidelines of the World Gastroenterological Organization (WGO, 2017) and the European Society of Pediatric Gastroenterologists, Hepatologists and Nutritionists (ESPGHAN, 2016).

According to the recommendations of the World Gastroenterological Organization, given in the international guidelines on probiotics and prebiotics (WGO, 2017) considering dosage in the case of antibiotic-induced diarrhea, the recommended dose of Lactobacillus rhamnosus GG for children is 1–2 × 10^{10} CFU, and for adults −10^{10} CFU twice daily.

The developed product contains at least 10^{10} CFU of Lactobacillus rhamnosus GG per capsule.

The investigational medicinal product and placebo in the frameworks of this study in accordance with the draft instructions for medical use of LRG-002 medicinal product will be prescribed according to the following scheme:

Single dose - 1 capsule; the investigational medicinal product/placebo will be taken orally 2 times a day (morning and evening) for 14 days. The tested product / placebo capsule is swallowed with some water. In case Visit 1 took place in the afternoon, only one capsule is allowed on the first day.

The medicinal product is recommended to be taken with meals, as this ensures its maximum efficacy, not less than 3 hours after taking antibiotics.

It must not be taken concomitantly with alcohol, fruit juices or hot beverages.

3.5.2 Justification of placebo use

Due to the need for blinding of this study, a placebo will be used in the form of capsules for oral administration. The use of placebo is necessary for formation of objective conclusions about the efficacy and safety of the investigational medicinal product and is a standard practice. Efficacy of probiotic strains in comparison with placebo is shown in a number of clinical studies.

In this study, patients will not be exposed to the excess risk associated with placebo, as they will receive antibacterial therapy of the underlying disease.

Thus, the use of placebo in the study does not threaten the safety of patients and is fully consistent with international practice in clinical trials.

3.5.3 Regulatory frameworks

This document is a clinical trial protocol, which is planned in accordance with the principles of the World Medical Association Declaration of Helsinki (Adopted by the 18th World Medical Assembly in Helsinki in June 1964, the latest revision was approved at the 64th Assembly in Fortaleza in October 2013), the Tripartite Guideline for Good Clinical Practice (GCP, ICH E6 (R2) dated 11/09/2016) and is regulated by the current legislation of the Eurasian Economic Union (EAEU) and the Russian Federation (RF):

- The rules of marketing authorization and assessment of medicinal products for medical use (approved by Decision No.78 of the Council of the Eurasian Economic Commission dated November 3, 2016);
- The rules of Good clinical practice of the Eurasian Economic Union (approved by Decision of the Council of the Eurasian Economic Commission dated November 3, 2016 No. 79);
- Federal law No. 61-FZ dated 12.04.2010 “On circulation of medicines” (in the current edition);
- Federal law No. 152-FZ dated 27.07.2006 "On personal data” (in the current edition);

- Order of the Ministry of Health of the Russian Federation dated April 1, 2016, N 200n "On Good Clinical Practice Approval";
- Russian Federation national standard GOST R 52379-2005 "Good clinical practice";
- Resolution of the government of the Russian Federation No.714 dated 13.09.2010 (as amended and supplemented) "On approval of standard rules of compulsory life and health insurance of a patient participating in clinical trials of a medicinal product";
- The order of the Ministry of health of the Russian Federation No. 986n dated November 29, 2012 "Approval of the Regulations of the Board of Ethics" (in the current edition).
- The order of the Federal Service for Surveillance in the Health Care Sector ("Roszdravnadzor") of the Russian Federation No. 1071 dated February 15, 2017, "On approval of the pharmacovigilance procedure"

3.6 Description of study population

Men and women aged 18 to 65 years old with acute respiratory disease who signed an informed consent to participate in the study, meeting the inclusion criteria (see section 6.1.) and not having the criteria for non-inclusion (see section 6.2.)

4 Study aims and objectives

4.1 Study goal

The goal of this study is to investigate the efficacy and safety of LRG-002 investigational medicinal product, capsules (Lek d.d., Slovenia) in comparison with placebo, as an adjunct treatment for prophylaxis of antibiotic-associated diarrhea (AAD) in patients with acute respiratory diseases (ARDs) receiving a standard antimicrobial therapy.

4.2 Study objectives

Main objective:

To evaluate the incidence of AAD while taking LRG-002, hard capsules (Lek d.d., Slovenia), as compared to placebo in antibiotic-treated patients with ARDs, receiving standard antimicrobial therapy;

Secondary objective:

To evaluate the safety of LRG-002 therapy, hard capsules (Lek d.d, Slovenia), as compared with placebo in antibiotic-treated patients with ARDs, receiving standard antimicrobial therapy.

5. Study Design

5.1 Main and additional studied parameters

5.1.1 Efficacy parameters

5.1.1.1 Primary efficacy parameter

An assessment of frequency of occurrence of AAD from 1 to the last day of LRG-002 administration compared with placebo in antibiotic-treated patients with ARDs, receiving standard antimicrobial therapy is selected as the primary efficacy endpoint. The primary efficacy criterion will be considered achieved if the odds ratio for the AAD incidence between two groups is significantly higher than 1 (i.e. AAD incidence in the LRG-002 group is lower than in placebo group).

AAD is defined as diarrhea associated with the AB use caused by C. difficile or of otherwise not identified etiology, upon analysis of stool samples and differential diagnostics according to investigator's judgment.

Diarrhea is defined as loose or watery stool (Type 5-7 according to Bristol Stool Form Scale) three times a day (frequent bowel movements with formed stool is not considered as diarrhea) in accordance with WHO criteria; based on the diary data (BSFS) and confirmation of AAD by the investigator.

5.1.1.2 Secondary efficacy parameters

The following criteria were selected as secondary efficacy criteria of the therapy in all study groups for a comparative assessment of the therapeutic effect:

• The occurrence of bowel movements per day (according to the diary data) in the treatment group compared with the placebo group;
• The incidence of any diarrhea in the treatment group compared with the placebo group;
• The incidence of C. difficile-associated AAD in the treatment group compared with the placebo group;
• The incidence of AAD not associated with C. difficile in the treatment group compared with the placebo group;
• The duration of AAD (the time from the onset of AAD to the normalization of stool form according to BSFS (types 1, 2, 3 and 4) and the presence of normal stool within 48 hours)) in the treatment group as compared to the placebo group;

• Duration of any diarrhea (the period from the diarrhea onset to the normalization of stool form according to BSFS (types 1, 2, 3 and 4) and the presence of normal stool within 48 hours)) in the treatment group vs the placebo group;
• The changes in stool consistency (according to Bristol Stool Form Scale) (according to the diary data) in the treatment group as compared to the placebo group;
• The changes and severity of gastrointestinal symptoms, including nausea, vomiting, flatulence, abdominal pain and decreased appetite (according to the diary data) in the treatment group as compared to the placebo group;
• Change in body weight at Visit 3 compared with Visit 1 in the placebo group as compared to the treatment group;
• Hospitalization rate in the treatment group compared with the placebo group;
• The number of days of using standard symptomatic therapy (as "rescue medication") to relieve symptoms of acute diarrhea in the treatment group as compared to the placebo group;
• The number of patients using standard symptomatic therapy (as "rescue medication") to relieve symptoms of acute diarrhea in the treatment group as compared to the placebo group.

5.1.2 Safety parameters

Safety evaluation will be based on the following parameters (between Visits 1 and 3), compared between the investigational medicinal product and placebo:

Frequency of adverse events (AEs) and/or serious adverse events (SAEs) in the treatment groups, including:

- Overall AE rate as compared to placebo;
- Frequency of AEs according to the results of physical examinations during each visit compared with placebo;
- AE incidence according to the results of the vital signs assessment (HR, BP, BT, RR) at each visit compared with placebo;
- AE incidence according to the ECG results on Day 15 (± 2) compared with placebo;
- AEs incidence according to the results of laboratory examinations on Days 7(± 2) and 15(± 2) compared with placebo;
- Frequency of AEs according to the everyday patients' records of well-being self-evaluation in the diary.

Diary records will be reviewed by the Investigator. In case the researcher considers that the records in the diary meet criteria of AE, the patient will be questioned about this event, and all information (including an evaluation of causality) will be documented.

Adverse event will be described according to the following scheme:

- Description of adverse event;
- Seriousness of adverse event (compliance with seriousness criteria);
- severity;
- duration;
- causal relationship with the investigational product;
- actions taken in relation to the study drug;
- actions taken in relation to the patient;
- outcome.

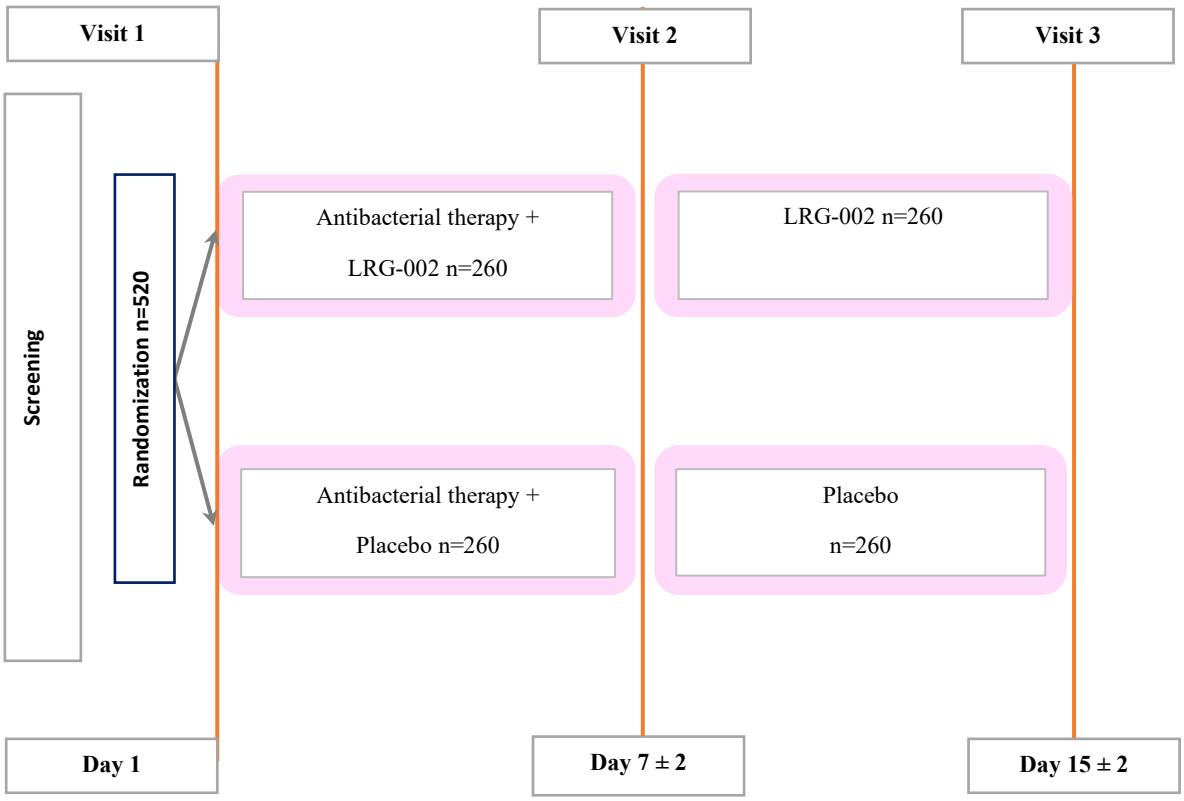
Adverse events will be coded according to MedDRA terms.

5.2 Description of study design

5.2.1 Study type

A multi-center, double-blind, randomized, placebo-controlled, parallel-group clinical study in two parallel groups.

5.2.2 Flowchart of study design



5.2.3 Study procedures

5.2.3.1 Schedule of research procedures in tabular form

Table 6. Visit schedule

Study period	Screening/ randomization/ start of treatment	Treatment		Unscheduled visits
Visits	Visit 1	Visit 2	Visit 3	
Day after starting therapy	Day 1	Day 7 ± 2	Day 15 ± 2	
Study procedures				
Signing the Informed Consent Form	●			
Demographic data (date of birth, sex, age)	●			
Body weight measurements	●	●	●	
Height measurement	●			
Medical history	●			
Complaints collection, medical history update	●	●	●	●
Reporting concomitant treatment	●	●	●	●
Physical examination	●	●	●	●
Vital signs assessment[1]	●	●	●	●
Complete blood count[2]	●	●	●	
Blood chemistry profile[3]	●	●	●	
Urine pregnancy test[4]	●			
Rapid test for the qualitative detection of immunoglobulin G (IgG)	●			

[1] SAD (mmHg), DAD (mmHg), respiratory rate (per minute), heart rate (beats per minute), axillary body temperature (°C)
[2] hemoglobin content, red blood cells, white blood cells, leukogram, ESR) will be carried out in the laboratory of the study site
[3] total protein, ALT, AST, total bilirubin
[4] only for women with preserved reproductive potential

Study period	Screening/ randomization/ start of treatment	Treatment		Unscheduled visits
Visits	Visit 1	Visit 2	Visit 3	
Day after starting therapy	Day 1	Day 7 ± 2	Day 15 ± 2	
Study procedures				
and immunoglobulin M (IgM) antibodies to COVID-19				
A 12-lead electrocardiogram	●		●	●
Evaluation of the inclusion/non-inclusion criteria	●			
Randomization	●			
Dispensing of the investigational medicinal product	●			
Dispensing of patient diary and thermometer	●			
Dispensing a laboratory stool collection kit	●	●		
Collection of unused medication			●	
Collection of patient diary			●	
Collection of unused lab kits			●	
Assessment of adverse events	●	●	●	●
Evaluation of exclusion criteria		●	●	●
Assessment of the adherence to study		●	●	●

Study period	Screening/ randomization/ start of treatment	Treatment		Unscheduled visits
Visits	Visit 1	Visit 2	Visit 3	
Day after starting therapy	Day 1	Day 7 ± 2	Day 15 ± 2	
Study procedures				
procedures (compliance with diary completion and study treatment)				
Assessment of diarrhea, if documented in the diary and face-to-face interview regarding its classification as AAD (according to investigator's judgment)		●	●	●

5.2.3.2 Description of study procedures

5.2.3.2.1. Collection of medical history, demographic data, anthropometry

When compiling medical history, it is necessary to pay attention to past diseases, concomitant chronic diseases, hereditary background, habitual intoxications (smoking status, alcohol consumption, drug addiction), allergic history, previous operations and injuries, occupational history, and constant or periodic medicinal therapy. In relation to this disease: the date and time of the onset of the first symptoms, the diagnostic and therapeutic measures carried out at the time of the examination, their effectiveness. The data about contraception method used by the male/female patient should be clarified; upon signing the informed consent, the male/female patient agrees to use one of the methods of contraception recommended in accordance with the study protocol throughout the study and a month after the completion of the study.

The primary documentation indicates gender, age of the patient, race and ethnicity of the patient. Patient's height is measured in centimeters using a height meter, and body weight in kilograms – using balance.

Height measurement should be performed for all patients (the reasons that make it impossible to measure height include hairstyle features (e.g., mohawk), the patient's refusal to take off his

headdress for various reasons, - for example, a turban, the patient's inability to stand, and exceedence of height meter scale). Height is determined with the help of a height meter - a vertically placed board with divisions by centimeters and a horizontal ruler sliding on it. Scale error according to the standard should not exceed two millimeters. The patient is asked to remove shoes, heavy clothes, hairpins, and hair ornaments. The patient should touch the vertical board with the back of the head, back, buttocks, calves and heels; feet together. The upper border of the external auditory meatus should be on the same horizontal level with the lower border of the orbit (zygomatic bone). The patient is asked to look straight ahead. If the height of patient above the one of the researchers, the latter stands on the platform. The ruler of height meter must touch the head, then the divisions are counted. Reading of height meter should be taken while the patient is still under the ruler. The error should not exceed half a centimeter.

Patients should be weighed in the morning on an empty stomach, after urination and stool, in underwear (followed by a subtraction of the average clothes weight from a readout). The balance must stand on a rigid base, horizontally. The measurement error according to standards should not exceed 0.5 kg. Patient stands strictly in the center of balance with both foots. It is not allowed to record the body weight based on patient's words even in cases where weight measurement is not possible.

5.2.3.2.2. Physical examination and vital signs assessment

Physical examination will be carried out according to the general rules of propaedeutics of internal diseases in the following sequence: general examination, examination of mucous membranes, including pharyngoscopy, palpation of the lymph nodes, assessment of the musculoskeletal system; palpation, percussion and auscultation of the main organ systems (cardiovascular, respiratory, digestive, urinary systems).

Determination of the vital signs (measuring HR, RR, BP, body temperature) should be performed before a physical examination at rest (after 15 minutes of rest, not earlier than an hour after smoking cigarettes and 2 hours after eating). The pulse rate should be determined on the radial artery (or on the carotid artery if pulsation on the radial artery is weak) for a minute in a sitting position. The respiratory rate (RR) should be measured for a minute at rest in a sitting position, noting the respiratory movements of the chest or abdominal wall without attracting the attention of the patient.

Blood pressure should be measured on the brachial artery in the patient's sitting position according to the Korotkoff's method using a certified sphygmomanometer or tonometer and cuff with length and width to fit the length and circumference of the patient's shoulder.

Body thermometry should be measured using the same type of thermometer (mercury-filled or electronic one) and the method (axillary temperature), which will be constant throughout the study.

5.2.3.2.3. Laboratory Tests

Blood sampling for analysis will be performed not earlier than 4 hours after the last meal from the cubital vein with a disposable sterile syringe subject to aseptic/antiseptic conditions. A sampling of approximately 12 ml of blood (1 tablespoon) on visits 1, 2 and 3 is necessary for analysis.

Complete blood count (hemoglobin content, red blood cells, white blood cells, differential, ESR) and blood biochemistry profile (includes the following parameters: total protein, ALT, AST, total bilirubin) will be carried out in the laboratory of the study site.

Urine pregnancy test using a test strip (immunochromatographic determination of beta-chorionic gonadotropin in the urine) is performed in all women with preserved reproductive potential at the screening visit.

5.2.3.2.4. Electrocardiography

Electrocardiography (ECG) will be performed at visit 1 and at visit 3. The ECG includes the registration of an electrocardiogram in 12 leads (in 3 standard and 3 augmented limb leads, as well as, in 6 precordial leads) at rest in the supine position. ECG interpretation will be performed by a cardiologist, therapist or functional diagnostics practitioner.

5.2.3.2.5. Collection of stool samples

Stool samples are collected in case of diarrhea by patients of both groups. Samples will be collected in containers dispensed in advance with the appropriate labeling:

- 1 container - a stool sample for presence of common diarrheal pathogens - Rotavirus, Adenovirus, Norovirus, Campylobacter spp, Salmonella spp, Shigella spp.;
- 1 container – stool sample for presence of C. difficile toxins A and B;
- 1 container - collection of stool samples for the culture of opportunistic flora: Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis, Candida spp, E. Coli, and culture of Yersinia spp. (special container). A detailed description of the procedure of stool sample collection and instructions for the patients are provided in Appendix 2.

The drugs for the treatment of acute diarrhea can be used only in case of acute diarrhea, namely: the presence of liquid, watery stools (Type 6, 7 on the Bristol scale) with a frequency of 3 or more times within 24 hours).

Patients will be instructed that in the case of acute diarrhea (the presence of liquid, watery stools (Type 6, 7 on the Bristol scale) with a frequency of 3 or more times within 24 hours), the physician

investigator will prescribe an oral rehydration therapy to the patient, as well as give diet recommendations.

After the third stool of type 6 and/or 7, the patient should consult the physician investigator and get medical advice before using any remedy for acute diarrhea.

If the patient has stools of type 6 and/or 7 according to the Bristol scale at least three times a day, the physician investigator will prescribe an oral rehydration therapy to the patient, give recommendations on the diet and can instruct the patient about the possibility of using additional drugs to relieve symptoms of acute diarrhea.

5.2.3.2.6. Evaluation of patient diary

Evaluation of patient diary is done by investigator. The assessed parameters include:

1. Dosing regimen of investigational medicinal products;
2. Dosing regimen of concomitant treatment;
3. Occurrence of bowel movements a day;
4. Stool consistency;
5. Occurrence of gastrointestinal symptoms;
6. Body temperature.

At each visit, diarrhea is assessed and classified as AAD or non-AAD by the investigator (in the absence of fecal analysis results at the time of the visit, the assessment is carried out after the investigator receives the results from the laboratory).

5.2.4 Study Periods

It is allowed to conduct visits in the form of outpatient visit of a doctor and a nurse at the patient's home.

5.2.4.1 Screening/randomization/treatment initiation (Visit 1)

Visit 1 is carried out on Day 1. At this visit, the physician evaluates the patient's compliance with the criteria for participation in the study on the same day. If non-inclusion criteria are not identified, randomization is performed, the patient is given the investigational medicinal product/reference product, containers for collecting stool samples with the appropriate instructions, the patient's diary, and training is carried out to fill out the patient's diary (Appendix 3).

Procedures at Visit 1:

Visit 1 (Day 1)

- Collection of demographic data (year of birth, sex, age);
- Body weight and height measurements;

- Medical history;
- Reporting concomitant treatment (including medications, food supplements and medical devices);
- Complaints reporting, medical history update;
- Physical examination;
- Measurement of vital signs (BP, HR, RR, BT);
- Complete blood count;
- Blood chemistry profile;
- Rapid test for the qualitative detection of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to COVID-19;
- Urine pregnancy test;
- A 12-lead electrocardiogram;
- The assessment of inclusion/non-selection criteria;
- Randomization;
- Dispensing of the investigational medicinal product (IMP)/placebo;
- Dispensing of patient diary and relevant training;
- Dispensing of a laboratory kit with the necessary consumables for collecting stool samples;
- Adverse event assessment.

5.2.4.2 Intermediate Follow-up Visit

Visit 2 (Day 7 ± 2)

Procedures at Visit 2 in each study group:

- Complaints reporting, medical history update;
- Reporting concomitant medications;
- Body weight measurements;
- Physical examination;
- Vital signs assessment;
- Complete blood count;
- Blood chemistry profile;
- Adverse event recording;
- Evaluation of exclusion criteria;
- Dispensing of a laboratory kit with the necessary consumables for collecting stool samples (if necessary);
- Assessment of the adherence to study procedures (compliance with diary completion and study treatment);

- Diarrhea assessment based on patient diary entries and face-to-face interviews in relation to the use of antibiotics (AAD or non-AAD) as assessed by the investigator (in the absence of results of stool analysis at the time of the visit, the assessment is carried out after the investigator receives the results from the laboratory).

5.2.4.3 Treatment completion visit (Visit 3, Day 15 ± 2)

Visit 3 is carried out in the morning on the day 15 ± 2, the patient must come to the study site on an empty stomach, bring a completed diary and an unused product.

- Complaints reporting, medical history update;
- Body weight measurements;
- Reporting concomitant medications;
- Physical examination;
- Vital signs assessment;
- Adverse event recording;
- Evaluation of exclusion criteria;
- Assessment of the adherence to study procedures (compliance with diary completion and study treatment);
- Complete blood count;
- Blood chemistry profile;
- A 12-lead electrocardiogram;
- Diarrhea assessment based on patient diary entries and face-to-face interviews for classification as AAD as assessed by medical doctor (in the absence of results of stool analysis at the time of the visit, the assessment is carried out after the investigator receives the results from the laboratory);
- Collection of unused medication;
- Collection of unused containers for stool samples;
- Collection of patient diary.

5.2.4.4 Unscheduled Visits

The unscheduled visits will be conducted when necessary, e.g., in case of the index disease deterioration, AEs development, or intolerability to therapy of LRG-002 medicinal product or placebo.

Every unscheduled visit, irrespective of its cause, must include the procedures listed below with completion of the relevant eCRF pages (Unscheduled Visit):

- Physical examination;
- Measurement of vital signs (BP, HR, RR, body temperature);
- A 12-lead ECG;
- AE assessment;
- Evaluation of the concomitant treatment;
- If indicated, any of the study procedures may be additionally performed upon the decision of the Principal Investigator.

Taking into account frequency of visits, unscheduled visits may be conducted on the same days as the scheduled ones but at a different time (e.g., in case of worsening the patient may visit the trial site in the afternoon).

Procedures for planned clinical study visits are the same in each treatment group during each study period.

5.2.5 Early study termination

Sponsor has the right to terminate the trial, and Investigator has the right to stop enrolling patients at any time. In case of early discontinuation of the site/study, all completed and unused CRFs (including unused pages of partially filled CRFs) and other documentation (excluding the documentation that should be kept on site) must be returned to the Sponsor. Materials of study may be destroyed by the permission of the Sponsor only.

5.3 Handling of investigational medicinal products

5.3.1 Supply of the medicinal products to the study site

LRG-002 and placebo are provided to study sites by the Sponsor. The sponsor is responsible for the production, packaging, labeling and supply of medicinal products, determination of storage conditions and shelf life of the medicinal products. Primary and secondary packaging of the medicinal products will contain the labeling “For clinical trial use only”.

At each study site, the investigator or authorized person will accept supplies of the investigational medicinal products. The drugs should be stored in a room with limited access, the temperature regime of which corresponds to the storage conditions of the medicinal products. Each supply of medicinal products to the study site will be confirmed by Delivery-Acceptance Act.

Investigator is responsible for the proper storage of the medicinal product, its disposal to the study participants and accounting. In case of breach of the storage conditions of the medicinal product,

as well as if defects or damage to the investigational medicinal product or packaging are detected upon supply to the site, the investigator should immediately inform the study monitor.

5.3.2 Quality complaints

5.3.2.1 Terms and definitions

Nonconformities of the studied medicinal product (investigational medicinal product and reference product) or medical device may include:

1) Complaints related to the quality of the medicinal product or medical device, such as:

- any deficiency of quality and (or) effectiveness, for example, a change in appearance, a change in the quantity, damaged tablets or capsules, the presence of foreign matter.
- any defect of containers and outer packaging, for example, a surface damage, leakage of a container, a broken syringe or piston, lack of contents, malfunction of the device.
- any defect of labeling, such as, a missing label or an unreadable label.
- any falsification of a pharmaceutical product or medical device, such as suspicion on drug mixing, opening-up of packages or counterfeiting.

2) Deviations associated with the transportation of the product, such as a damaged transport package and/or damaged secondary packaging after receipt of the goods, missing quantity of pharmaceutical product in transport package, improper transportation conditions.

5.3.2.2 Terms and definitions

If any of the nonconformities listed above are identified or information on these nonconformities is received, a filled-up *Non-conformity Report* must be sent to the Sponsor *(Study Manager or Clinical Study Manager)* within 24 hours. *Simultaneously On-site Monitor or Clinical Research Associate (CRA) of corresponding study should be informed of nonconformities.* If possible, a photograph of the damaged material should be attached to the report. Damaged material should be saved and stored in accordance with the storage conditions indicated on the label and/or returned to the sponsor upon sponsor's request.

5.3.3 Dispensing and collection of medical products

The investigational medical products are intended only for patients participating in this study and should be used in accordance with the protocol.

The study drugs will be dispensed to patients at the first visit in an amount sufficient for the entire course of therapy in the study. Patients will be asked to bring with them all used and unused drug packages to the study site at each visit.

5.3.4 Product Accountability

The investigator or authorized person must keep records of medicinal products at the site during the study, by filling out special accounting forms for the medicinal products provided by the Sponsor. The aspects of account of the study products will be regularly checked by the monitor.

The investigator or any authorized person should calculate the number of packages of the used and unused product returned by patient and write down these data in the accounting forms for the medicinal products and in CRFs. Besides, the number of days of skipping the administration of the investigational medicinal products, changes in their dosage (if there are any, according to patient's diary) should be recorded in CRFs.

5.3.5 Collection and destruction of the medicinal products

After completion of the study or in case of premature termination of the site participation in the study, all unused investigational medicinal products and packaging of the used medicinal products shall be returned to the Sponsor. The sponsor is responsible for the destruction of the study medicinal product. After the packages of the used and unused medicinal products are counted and the labels on the packages are checked, the monitor arranges the dispatch of the medicinal products to the Sponsor for subsequent destruction.

5.4 Measures aimed at minimizing/eliminating subjectivity

5.4.1 Randomization

The study will be double-blinded, randomized.

Patients will be included by applying stratified randomization in two strata:

- age <50 years
- age ≥ 50 years

Each treatment group will be balanced at a ratio of 1: 1 within each stratum.

Stratified randomization is a two-stage procedure in which patients who enter a clinical trial are first grouped into strata. Within each stratum, patients are then assigned to a treatment according to separate randomization schedules. Randomization module of EDC system assigns patient unique randomization code based on group of treatment and stratum. Randomization schedules will be hidden from medical personnel and all other persons involved in the study (monitors, data management, biometry, sponsor etc.), except the specialists responsible for labeling of the investigational medicinal products, and the emergency unblinding.

5.4.2 Blinding methods

Within the framework of this study, neither investigators nor patients should be aware whether the investigational medicinal product or placebo is used in a patient.

Investigator will receive LRG-002 product/placebo for dispensing to the patient in identical packaging, which will differ by individual numbers. The lot of the medicinal product is individual for the patient and includes the medicinal product for the entire blinded period of treatment of the patient.

When the patient is included in the study (randomization), Investigator receives from the Sponsor the patient identification number and the corresponding lot number of the medicinal product that the patient will take. In case of using an electronic randomization system, all the necessary numbers will be automatically generated after data entry by Investigator. The investigator records the numbers obtained in the primary documentation and eCRF.

The sets with the medicinal product in the required quantity will be formed for patients of each group.

The set provides additional packs in case of damage or loss of the medicinal product/placebo.

5.5 Dosage regimen of the study medicinal products

Investigational medicinal product LRG-002 is used according to the following dosage regimen: a single dose is 1 capsule for oral administration during meals with a small amount of water; the medicinal product will be taken orally 2 times a day for 14 days.

Placebo is used according to the following dosage regimen: a single dose is 1 capsule for oral administration during meals with a small amount of water; the medicinal product will be taken orally 2 times a day for 14 days.

If treatment order is received in the afternoon, the protocol allows only one capsule to be taken on the first day. The interval between taking the oral antibiotic and investigational medicinal product should be at least 3 hours in both groups.

Dosing regimen of investigational medicinal products:

Day No.	Date	Dosage frequency of LRG-002 product or placebo	Intake of antibiotic
1	__/__/_____	1 or 2	Yes
2	__/__/_____	2	Yes
3	__/__/_____	2	Yes
4	__/__/_____	2	Yes
5	__/__/_____	2	Yes
6	__/__/_____	2	Yes
7	__/__/_____	2	Yes
8	__/__/_____	2	No
9	__/__/_____	2	No
10	__/__/_____	2	No
11	__/__/_____	2	No
12	__/__/_____	2	No
13	__/__/_____	2	No
14	__/__/_____	2	No

5.6 Dosage form, packaging and labeling of investigational medicinal products

5.6.1 Dosage form

Investigational medicinal product: LRG-002, capsules

Reference product: Placebo, capsules.

5.6.2 Packaging

Investigational medicinal product: The product will be packaged in blisters

Reference products: The product will be packaged in blisters

5.6.3 Labeling of investigational medicinal product

The study is double blinded. The research cite will be supplied with blinded packages of study drugs.

Each package will contain information about the name, composition, expiration date and manufacturer of the medicinal product, the inscription "for clinical trials only", the clinical trial protocol number, the name and contact information of the trial sponsor.

5.7 Duration of patient participation in the study

Duration of study will be for patients not more than 17 days:
Screening/randomisation/study treatment initiation– Day 1.
Intermediate Follow-up Visit – Day 7 ± 2.
Study Termination Visit - Day 15 ± 2
The overall enrollment period is at least 8 months.

5.8 Rules for stopping the parts of clinical study and/or the clinical study in general

The study can be stopped for the following reasons:

1. At the initiative of Sponsor:
   a. obtaining new toxicological or pharmacological data, or data about SAE, which require to reconsider the earlier assessment of the benefit/risk of participation in the study;
   b. the frequency of AEs and/or their severity do not allow to continue the study;
   c. other reasons, including administrative ones.
2. At the initiative of Investigator: the frequency of AEs and/or their severity unacceptably increases the risk of the participation of patients in the study.
3. By decision of regulatory authorities.

If the study is prematurely stopped, the sponsor is obliged to notify the staff of the study sites and regulatory authorities, indicating the reason for the premature termination of the study.

5.9 Accounting procedures of the investigational medicinal products

The study sites will receive the number of the study medicinal products (the investigational medicinal products, the reference product), sufficient for the study, taking into account the planned number of patients.

The responsible employee of the study site maintains the drug accounting journal, where deliveries of the medicinal products to the site, dispensing of the medicinal products to the study participants, and the collection of unused medicinal products are recorded. Storage conditions are also noted. The study medicinal products can only be used for the purposes of this clinical study.

Specialists of the Sponsor or regulatory authorities during the monitoring/audit of the study site can check the drug accounting journal, as well as the availability of the medicinal products. The medicinal products should be stored in compliance with the storage conditions in the room, access to which is available only to the specialists of the study site responsible for dispensing the medicinal product.

5.10 Storage of randomized codes and procedure for their disclosure

The table containing the therapy randomization codes will be maintained by the CRO (Data Management Department) and by the provider providing the eCRF platform. Access to the randomization table will be restricted to authorized persons.

In the course of the study, it may be necessary to unblind the therapy group to which the patient belongs, for the safety of the patient's life and health (for example, when serious adverse events are identified). The subject will be unblinded directly in the eCRF system by authorized users who have been assigned an access level for unblinding subjects. The treatment group unblinding right will be given to:

- CRO Data Management Manager;
- Designated Research Center Team member.

In case of urgent need for a patient to be unblinded by the research center, but in the absence of technical feasibility to implement this process, the researchers will be provided with an emergency support phone number, full name and contact details of the CRO Data Management Manager.

5.11 List of data recorded in Individual Case Record Form (without prior written or electronic recording) and considered as primary data

All study data will be entered in the primary documentation, then in the eCRF.

6. Patient inclusion and exclusion

6.1 Inclusion criteria

1. Voluntarily signed Informed Consent Form for participation in this clinical study;
2. Male and female subjects, aged 18 to 65 years inclusive;

3. Antibacterial treatment for active ARDs started/to be started on the first day of the study (7-day course of oral beta-lactam antibiotic) (see Background antibacterial therapy). Only one AB should be used per subject, in an outpatient setting. Diagnostic procedures of acute respiratory infections and the prescription of antibiotic therapy should be completed before signing of the Informed Consent Form;
4. Female patients are eligible for inclusion in the study if they:
   - Unable to become pregnant (i.e. postmenopausal women or those after surgical sterilization). Surgically sterile women are defined as patients with documented hysterectomy, and/or bilateral oophorectomy, and/or tubal ligation. Postmenopausal women are defined as women with amenorrhea for more than 1 year with the relevant clinical profile (e.g., age > 45 years, and absence of hormone replacement therapy). However, in controversial cases, it is recommended to confirm menopause by drawing a blood sample which would show an FSH level of >40 IU/mL and estradiol level of <40 pg/mL (<140 pmol/L).

     OR
   - **Capable of childbirth,** but with negative pregnancy test at the screening visit, and the patient agrees to continuously and properly use one of the following suitable methods of contraception (i.e., in accordance with the approved Package Insert for the medicinal product and physician instructions) during the participation period:
     a. Complete abstinence
     b. Oral contraceptives (combination drugs containing progestogen, or progestogen alone)
     c. Injectable progestogen
     d. Levonorgestrel implants
     e. Estrogen-containing vaginal ring
     f. Transdermal contraceptive patches
     g. Intrauterine device or intrauterine system
     h. A male partner has been sterilized (vasectomy with documented azoospermia) prior to inclusion of a woman, provided that he is the only partner of the female patient. The definition "documented" is related to the result of a subject's medical examination performed by Investigator/responsible person or subject's past medical history review for assessment of eligibility for enrollment, obtained during the interview with the subject or from his medical records.
     i. Double barrier method: a condom or an occlusive cap (diaphragm or cervical/vault caps) with a spermicide (foam/gel/film/cream/suppository).

5. Male participants are obliged to use appropriate contraception during the entire study period starting from signing of the Informed Consent Form and until the study end, and for 30 days after study completion;
6. The ability to understand the information about the clinical study, readiness to comply with the study protocol requirements, ability to take the investigational products and evaluate symptoms on his (or her) own using diary/questionnaires as per protocol;
7. Ability to maintain the habitual lifestyle throughout the study, including diet.
8. Willingness not to consume any products containing probiotics during participation in the clinical trial.
9. Willingness not to take part in any other study during the present trial.

6.2 Non-inclusion criteria

Patients will not be eligible for study enrollment if they have one or more of the following criteria:

1. Any treatment (including medications, medical devices and food supplements) that can influence the stool consistency, according to the Investigator's opinion should not be used within 14 days prior to Visit 1;
2. Use of anti-rejection medication after stem cell or solid organ transplant;
3. Use of glucocorticosteroids within the last 8 weeks prior to the study start;
4. Use of proton pump inhibitors within 3 months prior to Visit 1;
5. Chemotherapy or radiation;
6. History of recurrent diarrhea;
7. Patient has diarrhea or loose stool in the last 2 days prior to the study start;
8. Patient has severe ARD expected to require an administration of additional antibiotics or antibiotics therapy for more than 7 days;
9. Use of antimicrobials in the last 3 months prior to the study start;
10. Use of yeast/probiotic/fermented products within the last 2 weeks prior to the first visit;
11. Known allergy/sensitivity to the investigational medicinal product;
12. Immunocompromised patients;
13. Patients with digestive / absorption disorders of the gastrointestinal tract (for example, inflammatory bowel disease, celiac disease, pancreatitis, motility disorders, etc.) and / or patients who underwent surgery on the organs of the gastrointestinal tract (with the exception of appendectomy more than 3 months prior to Visit 1);
14. Known irritable bowel syndrome;
15. Known small intestinal bacterial overgrowth;
16. Patients with pyrexia over 38°C;

17. Pregnant and/or breastfeeding women;
18. Participation in other clinical trials of medicinal products or medical devices at the screening Visit or for 30 days before the screening Visit;
19. Surgical intervention within 30 days before the screening visit or planned surgical treatment during the trial (before a follow-up visit is completed), including diagnostic procedures or inpatient stay;
20. Known or suspected alcohol and/or drug addiction;
21. A suspected low compliance or incapability of the patient to perform the procedures and comply with restrictions according to the trial protocol (e.g., due to mental disorders);
22. Potential for translocation of probiotic across bowel wall (active bowel leak, acute abdomen, active intestinal disease including colitis, or significant bowel dysfunction; presence of neutropenia or anticipation of neutropenia after chemotherapy; radiation therapy);
23. Any diseases of cardiovascular, renal, hepatic, gastrointestinal, endocrine and nervous systems, or other conditions/diseases which, in the Investigator's opinion, may render study participation unsafe for a patient or may affect a test result;
24. A positive result of a rapid test for the detection of immunoglobulin M (IgM) antibodies to COVID-19.

6.3 Exclusion Criteria

6.3.1 Timing and terms of subject exclusion from the study

Subjects can be excluded from the study of their own free will at any time without explanation, as well as by decision of the physician investigator or Sponsor, in cases where continued participation in the study poses a threat to the health and/or life of the patient. In addition, the patient will not be able to continue participating in the study for the following reasons:

Subject participation will be stopped in case any of the following reasons occur:

1. Disease progression (including pyrexia above 38°C for 3 days or longer);
2. Positive results of culture or PCR for the dysentery group, typhoid fever group, pathogenic E. coli and other pathogenic groups (incl. Cl. dificille), the treatment of which requires the prescription of additional antibiotic therapy;
3. Need to change tactics of antibacterial therapy (extension course of antibacterial therapy or change of antibiotic group);

4. The Ethics Committee, regulatory authorities, or Sponsor can terminate the clinical study or participation of the specific trial site in the study for any reason;
5. The investigator decided that the patient must be withdrawn from the study for the own interests of the patient;
6. Informed consent withdrawal (patient reluctance to continue to participate in the study);
7. Individual intolerability of Investigational medicinal products according to Investigator's judgment;
8. Occurrence of clinically significant adverse event or serious adverse event in the patient;
9. Patient's non-compliance;
10. Error enrollment (e.g., the patient was included with violation of the inclusion/non-inclusion criteria);
11. The patient complies with the non-inclusion criteria during the study;
12. The patient receives/requires an additional treatment which might influence the study results or patient's safety (see "Prohibited concomitant therapy" section);
13. Other conditions or events which, in the Investigator's judgement, require the patient's exclusion from the study.

6.3.1.1 Timing and volume of data collection for excluded subjects.

If the subject is excluded from the study at his/her request, the investigator should try to find out the reason for the termination of the study. If the study is terminated due to the occurrence of AE/SAE, monitoring of the subject continues until AE/SAE resolution. In case of premature termination of the patient's participation in the study, the patient is invited to the study site to carry out the procedures of premature study termination visit (carried out within the frameworks of unscheduled visit); if the patient refuses to visit the study site, the premature study termination visit is carried out by phone, while as much as possible the available information is recorded.

6.3.2 Terms and conditions of stopping/termination by the subject of treatment with the medicinal product under study

Patients stop taking the study medicinal products upon completion of their participation in the study in accordance with the protocol or upon their exclusion from the study.

6.3.3 Replacing the discontinued patients

Replacement of the patients who discontinued their study participation is not provided.

7. Information about the medicinal products used in the clinical study

7.1 Investigational medicinal product and reference product

Investigational medicinal product: LRG-002

Manufacturer: Lek d.d., Verovskova 57, 1526 Ljubljana, Slovenia.

Dosage form, dosage: capsules

Description: Each capsule contains live lyophilized probiotic bacteria of Lactobacillus genus.

Composition per 1 capsule: each capsule contains at least 1 x 10^{10} CFU of Lactobacillus rhamnosus GG as a lyophilizate.

Excipients: sucrose, maltodextrin, silicon dioxide, magnesium stearate, sodium ascorbate

Capsule shell: hydroxypropyl methylcellulose, titanium dioxide (E171), gellan gum, water.

Reference product: Placebo

Duration of treatment

Patients will take the investigational medicinal products in all groups daily for 14 days.

7.2 Compliance control

Compliance will be controlled by review of patient's diary and counting the amount of the medicinal product remaining.

7.3 Allowed concomitant treatments

Oral beta-lactam antibiotic (eg, amoxicillin, amoxicillin + clavulanic acid, cefixime, etc.) (See Background antibacterial therapy) according to inclusion criterion 3, at the same dosage as at the study initiation.

Continuation of the drug administration, started prior to the inclusion into the study for treatment of concomitant diseases and not covered by any exclusion criterion. Female participants taking oral contraceptives may continue to take them during the study.

Standard symptomatic treatment agents used to treat acute respiratory infections (in case of medical need), including anilides, triazole derivatives, with the exception of drugs that can induce a laxative effect (for example, throat lozenges with sugar substitutes).

Agents for relieving symptoms of acute diarrhea (in case of medical need), including electrolyte solutions, with the exception of sorbents.

Prescribing therapy for acute diarrhea and symptomatic therapy for ARDs is possible only by the decision of the Investigator.

7.4 Prohibited concomitant therapy

1. Systemic glucocorticosteroids within last 8 weeks prior to and during the study;
2. Any antibacterial agents in the last 3 months prior to the study and during the study, excluding the antibacterials according to inclusion criterion 3 during the study;
3. Use of any proton pump inhibitor within 3 months prior to the study;
4. Use of other probiotics (bacteria or yeast, medications, food supplements, fermented products) and the product Broncho-munal® within 2 weeks prior to and during the study;
5. Any therapy (including medications, medical devices and food supplements) that can influence the stool consistency must not be used, according to the Investigator's opinion, within 14 days prior to Visit 1 (excluding the drugs specified in items 3 and 4 of the "Allowed Concomitant Treatments").

The list of prohibited medicinal products is presented in Appendix 1

8. Efficacy Evaluation

8.1 List of efficacy parameters

Primary efficacy criterion

Evaluation of AAD incidence from the first to the last day of LRG-002 administration vs placebo in patients with ARDs, receiving standard antimicrobial therapy. The primary efficacy criterion will be considered achieved if the odds ratio for the AAD incidence between two groups is significantly higher than 1 (i.e. AAD incidence in the LRG-002 group is lower than in placebo group).

AAD is defined as diarrhea associated with the AB use caused by C. difficile or of otherwise not identified etiology, upon analysis of stool samples and differential diagnostics according to investigator's judgment.
Diarrhea is defined as loose or watery stool (Type 5-7 according to Bristol Stool Form Scale) three times a day (frequent bowel movements with formed stool is not considered as diarrhea) in accordance with WHO criteria; based on the diary data (BSFS) and confirmation of AAD by the investigator.

Secondary efficacy criteria

The following criteria were selected as secondary efficacy criteria of the therapy in all study groups for a comparative assessment of the therapeutic effect:

- The occurrence of bowel movements per day (according to the diary data) in the treatment group compared with the placebo group;
- The incidence of any diarrhea in the treatment group compared with the placebo group;
- The incidence of C. difficile-associated AAD in the treatment group compared with the placebo group;
- The incidence of AAD not associated with C. difficile in the treatment group compared with the placebo group;
- The duration of AAD (the time from the onset of AAD to the normalization of stool form according to BSFS (types 1, 2, 3 and 4) and the presence of normal stool within 48 hours)) in the treatment group as compared to the placebo group;
- Duration of any diarrhea (the period from the diarrhea onset to the normalization of stool form according to BSFS (types 1, 2, 3 and 4) and the presence of normal stool within 48 hours)) in the treatment group vs the placebo group;
- The changes in stool consistency (according to Bristol Stool Form Scale) (according to the diary data) in the treatment group as compared to the placebo group;
- The changes and severity of gastrointestinal symptoms, including nausea, vomiting, flatulence, abdominal pain and decreased appetite (according to the diary data) in the treatment group as compared to the placebo group;
- Change in body weight at Visit 3 compared with Visit 1 in the placebo group as compared to the treatment group;
- Hospitalization rate in the treatment group compared with the placebo group;
- The number of days of using standard symptomatic therapy (as “rescue medication”) to relieve symptoms of acute diarrhea in the treatment group as compared to the placebo group;
- The number of patients using standard symptomatic therapy (as “rescue medication”) to relieve symptoms of acute diarrhea in the treatment group as compared to the placebo group.

8.2 Methods and timing for evaluating, recording and analyzing efficacy parameters

The efficacy of the investigational therapy is based on diary data, laboratory and clinical data.

Primary efficacy endpoint – assessment of frequency of occurrence of AAD from 1 to the last day of LRG-002 administration compared with placebo in antibiotic-treated patients with ARDs, receiving standard antimicrobial therapy. The primary efficacy criterion will be considered

achieved if the odds ratio for the AAD incidence between two groups is significantly higher than 1 (i.e. AAD incidence in the LRG-002 group is lower than in placebo group).

AAD is defined as diarrhea associated with the AB use caused by C. difficile or of otherwise not identified etiology, upon analysis of stool samples and differential diagnostics according to investigator's judgment.

Diarrhea is defined as loose or watery stool (Type 5-7 according to Bristol Stool Form Scale) three times a day (frequent bowel movements with formed stool is not considered as diarrhea) in accordance with WHO criteria; based on the diary data (BSFS) and confirmation of AAD by the investigator.

Secondary efficacy endpoints, assessed based on the data of patient's diary include:

- The occurrence of bowel movements per day (according to the diary data) in the treatment group compared with the placebo group;
- The incidence of any diarrhea in the treatment group compared with the placebo group;
- The duration of AAD (the time from the onset of AAD to the normalization of stool form according to BSFS (types 1, 2, 3 and 4) and the presence of normal stool within 48 hours)) in the treatment group as compared to the placebo group;
- The changes in stool consistency (according to Bristol Stool Form Scale) (according to the diary data) in the treatment group as compared to the placebo group;
- The occurrence and severity of gastrointestinal symptoms, including nausea, vomiting, flatulence, abdominal pain and decreased appetite (according to the diary data) in the treatment group as compared to the placebo group;
- Duration of any diarrhea (the period from the diarrhea onset to the normalization of stool form according to BSFS (types 1, 2, 3 and 4) and the presence of normal stool within 48 hours)) in the treatment group vs the placebo group;

Secondary efficacy endpoints, assessed based on the data of stool analysis include:

- • The incidence of C. difficile-associated AAD in the treatment group compared with the placebo group;
- • The incidence of AAD not associated with C. difficile in the treatment group compared with the placebo group;

Secondary efficacy endpoints, assessed based on the clinical data include:

- Change in body weight at Visit 3 compared with Visit 1 in the placebo group as compared to the treatment group;
- Hospitalization rate in the treatment group compared with the placebo group;
- The number of days of using standard symptomatic therapy (as "rescue medication") to relieve symptoms of acute diarrhea in the treatment group as compared to the placebo group;
- The number of patients using standard symptomatic therapy (as "rescue medication") to relieve symptoms of acute diarrhea in the treatment group as compared to the placebo group.

9. Safety Evaluation

9.1 List of safety parameters

Safety evaluation will be based on the following parameters (between Visits 1 and 3), compared between the investigational medicinal product and placebo:

Frequency of adverse events (AEs) and/or serious adverse events (SAEs) in the treatment groups, including:

- Overall AE rate as compared to placebo;
- Frequency of AEs according to the results of physical examinations during each visit compared with placebo;
- AE incidence according to the results of the vital signs assessment (HR, BP, BT, RR) at each visit compared with placebo;
- AE incidence according to the ECG results on Day 15 (± 2) compared with placebo;
- AEs incidence according to the results of laboratory examinations on Days 7(± 2) and 15(± 2) compared with placebo;
- Frequency of AEs according to the everyday patients' records of well-being self-evaluation in the diary.

9.2 Methods and timing for evaluating, recording and analyzing safety parameters

Safety parameters are evaluated at each visit planned in this study, as well as at unscheduled visits.

Safety evaluation will be based on the following parameters (between Visits 1 and 3), compared between the investigational medicinal product and placebo:

Frequency of adverse events (AEs) and/or serious adverse events (SAEs) in the treatment groups, including:

- Overall AE rate as compared to placebo;
- Frequency of AEs according to the results of physical examinations during each visit compared

with placebo;

- AE incidence according to the results of the vital signs assessment (HR, BP, BT, RR) at each visit compared with placebo;
- AE incidence according to the ECG results on Day 15 (± 2) compared with placebo;
- AEs incidence according to the results of laboratory examinations on Days 7(± 2) and 15(± 2) compared with placebo;
- Frequency of AEs according to the everyday patients' records of well-being self-evaluation in the diary.

Diary records will be reviewed by the Investigator. In case the researcher considers that the records in the diary meet criteria of AE, the patient will be questioned about this event, and all information (including an evaluation of causality) will be documented.

Adverse event will be described according to the following scheme:

- Description of adverse event;
- Seriousness of adverse event (compliance with seriousness criteria);
- severity;
- duration;
- causal relationship with the investigational product;
- actions taken in relation to the study drug;
- actions taken in relation to the patient;
- outcome.

9.3 Requirements for reports, procedures on recording and reporting of adverse events and intercurrent diseases

9.3.1 Terminology of adverse events and serious adverse events

Adverse event (AE) — any medically unfavorable event identified in a patient or subject in a clinical trial after the use of medicinal product, which may not have a causal relationship with its use.

Thus, an adverse event (AE) may be any adverse and undesirable clinical sign (including laboratory deviation from the norm), symptom or disease, the time of occurrence of which does not exclude a causal relationship with the use of the drug, regardless of the presence or absence of such a relationship.

Serious adverse event (SAE) — any adverse medical event that regardless of the dose of the medicinal product:

- causes fatal outcome;
- is life threatening;
- requires hospitalization or its extension;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly or malformation;
- is clinically relevant: the decision about whether the express reporting is appropriate in other situations (e.g., in cases of medically important events that do not pose an immediate threat to the patient's life, do not result in death or hospitalization, but put the patient at risk or require intervention to prevent one of the above outcomes given in the definition) is made on the basis of a medical and scientific assessment. Such cases should also be considered as serious adverse events.

These characteristics / consequences must be considered at the time of event occurrence. For example, the concept of life-threatening AE refers to a medical event in which a subject is at risk of death at the time of the AE development; it does not refer to a medical event that could hypothetically cause death in case of a higher severity condition.

(Serious) adverse reaction ((S)AR) — any (S)AE for which there is a reasonable probability of a causal relationship with the use of the medicinal product in the opinion of the Investigator or Sponsor (see paragraph 8.8.3 below).

(Serious) unexpected adverse reaction — a (serious) adverse reaction whose nature or severity is inconsistent with Reference Safety Information (e.g., Summary of Product Characteristics (SmPC), Pharmacopoeial Product Monograph, Investigator's Brochure). The term "severity" in this context describes the intensity of a particular adverse event. It is necessary to distinguish it from the term "seriousness". Reports that contain additional important information regarding the characteristics of the manifestation, increase in the incidence or severity of a known, documented serious adverse reaction should also be considered as reports of unexpected events.

Information on commonly known adverse effects of the investigational medicinal product (IMP) can be found in the Reference Safety Information (e.g., in the SmPC, Product Monograph, Investigator's Brochure). Otherwise, it shall be provided as a notification to the Investigator. This information will be included in the patient information and should be discussed with the patient.

For more information, see the Brief Guideline for Completing the Report Form on the Serious Adverse Events.

9.3.2 Severity of adverse events

The term "severity" in this context describes the intensity of a particular event. It is necessary to distinguish it from the term "seriousness". In the course of the study, the Investigator should identify any AEs manifested and assess their severity as follows:

- Mild: commonly transient in nature AE, as a rule, not disrupting the usual daily activity.
- Medium: accompanied by significant discomfort that disrupts normal daily activity.
- Severe: interferes with normal daily activities.

9.3.3 Causality to the investigational medicinal product:

The Investigator should evaluate all AEs using all available data every time when new information is received. The term "IMP" includes the evaluated medicinal product and the reference medicinal product that patients received during any phase of the study.

The Investigator should assess whether, in his expert opinion, the association of AE with the medical product is possible, based on the following considerations:

Suspected:	Temporality between the clinical event and the administration of the investigational medicinal product **makes a causal relationship possible**, whereas other medicinal products, therapeutic interventions or underlying disease do not provide a sufficient explanation for the observed event.
Not suspected:	Temporality between the clinical event and the administration of the investigational medicinal product **makes a causal relationship unlikely**, or other medicinal products, therapeutic interventions or underlying disease provide a sufficient explanation for the observed event.

Estimates of causal relationship are critically important and should be provided for each individual AE in relation to each IMP, non-investigational medicinal product (NIMP), or other concomitant medicinal product, if applicable. In the absence of causal relationship assessment, AEs will be considered by the Sponsor to be allegedly associated with the IMP.

Investigators should report to the appropriate Country Organization Patient Safety Team about AEs suspected to be associated with NIMP or other concomitant medicinal products (manufactured by Sandoz or other companies), even if they are not serious (contact details are given in section 9.3.5 for contact details).

9.3.4 Documentation of adverse events

Any AEs (non-serious and serious) observed after the subject has given informed consent to participate in a particular study and prior to the subject's last visit in the course of the study should be documented on the AE page in the case report form (CRF).

Information on AE should be collected by unobtrusive interviewing the subject on each visit during the study. AEs can also be identified if the subject reports them voluntarily during a visit, between visits, or during a physical examination, laboratory tests, or other examinations. An appropriate medical care should be provided in all AEs. The treatment may include one or more of the following: no action taken (i.e., follow-up only); dose adjustment / temporary discontinuation of IMP; complete discontinuation of IMP due to this AE; prescription of concomitant medicinal product; administration of non-drug therapy; hospitalization of the patient / extension of hospitalization. The treatment of AE shall be documented in CRF. Besides, actions taken with respect to IMP should be recorded and classified into one of the following categories: no change, discontinuation, dose reduction, dose increase, temporary discontinuation, unknown and not applicable. Concomitant medications, other treatment methods or changes in the IMP regimen should be clearly indicated and documented.

Medical conditions / diseases that occurred prior to the IMP administration are considered as AE only in the case of exacerbation after inclusion of the patient in the study. Deviations of laboratory parameters and results of other studies from the norm are considered AE (only if they entail the development of clinical signs or symptoms), clinically significant or require treatment.

After the detection of AE, the Investigator should continue the follow-up as indicated below. In all cases, AE outcomes should be documented and classified into one of the following categories: no resolution / unchanged, deterioration, recovery / resolution, improvement / in the process of recovery, recovery / resolution with consequences, fatal outcome or unknown. The AE assessment should be carried out at each scheduled visit (or more often, if necessary). The Investigator should

document in the CRF any changes in seriousness, severity, suspected association with IMP, AE related interventions, and outcome.

Adverse events, observed in the period between giving the consent and the last visit

The Investigator should continue to monitor all the AE that have developed after signing the informed consent to participate in a particular study and until the last visit of the subject, during which the assessment of the outcome will be documented in CRF.

Serious adverse events not resolved at the moment of the last visit

The Investigator should continue to monitor all SAEs not resolved during the last visit, up to the resolution or stabilization / recognition of permanent SAEs that were deemed to be associated with IMP (SAE), or up to 30 days after the last visit of the subject in respect of SAE not associated with IMP. The Investigator shall send the SAE follow-up reports to the recipients in accordance with section 9.3.5 "SAE Reporting" introduced below.

Serious adverse events that have developed since the last visit

SAEs recorded since the last visit should be reported to the Sponsor only if they are assessed by the Physician-Investigator as being related to the medicinal product. The Investigator should send SAE reports to recipients in accordance with section 9.3.5 "SAE Reporting" introduced below.

9.3.5 SAE Reporting

It is **vitally important that the Investigator should immediately** (i.e. not later than 24 hours after receiving the information) report any SAE or update previous SAE reports, even if the Investigator does not believe that the AE is related to the investigational medicinal product.

The Investigator should send the SAE reports using the Serious Adverse Event Reporting Form (document of Novartis) as an initial or subsequent report by fax or e-mail to the relevant Country Organization Patient Safety Team, with a copy to the Executive Officer of the study (Sponsor / CRO) at the addresses shown in the table below.

The Investigator should also send all updates / new information in the form of a new SAE report as subsequent data to the previously noted SAE. Subsequent data should describe whether the adverse event is resolved or still persists, if the diagnosis is known, if the treatment is provided (specify method of treatment), whether the subject continues to participate in the study or dropped out of it.

Recurring episodes, complications, or progression of the original SAE should be noted in the form of subsequent information to supplement the initial case description, regardless of when the AE occurred.

Any new SAEs (which are considered to be completely independent of previously noted SAEs) should be reported in the form of a new and separate initial SAE report.

The Investigator should answer any questions of the Country Organization Patient Safety Team, contract research organization (CRO), or Sponsor regarding the SAE report within 24 hours.

For more information, see the Brief Guideline for Completing the Report Form on the SAE.

The Investigator should store notifications about SAE report delivery to all recipients in the Investigator's file.

Addresses for the SAE reports

[redacted]
[redacted] **Novartis Pharma**
Name: [redacted]
Address: 72 Leningradsky prospect, bld.3, Moscow, Russia 125315
Ph.: [redacted]
Mob.: [redacted] (24h.)
Fax: [redacted]
E-mail: [redacted]
[redacted], **Sandoz (Novartis division):**
Name: [redacted]
Address: 72 Leningradsky prospect, bld.3, Moscow, Russia 125315
Tel: [redacted]
Fax: [redacted]
E-mail: [redacted]

For further information contact the authorized persons:

[redacted], **ZAO Sandoz:** Name: [redacted] Address: 72 Leningradsky prospect, bld.3, Moscow, Russia 125315 Tel: [redacted] Cell number: [redacted] Fax: [redacted] E-mail: [redacted]	[redacted] Name: [redacted] Address: [redacted] [redacted] Cell number: [redacted] Fax: [redacted] E-mail: [redacted]

If the SNR is not listed in the Reference Safety Information (e.g., in the SmPC, Investigator's Brochure), the Sponsor may urgently request additional information from the Investigator to report to the Health Authorities.

Sponsor may, if applicable, issue a notice to the Investigator and semi-annual tabular lists of suspected unexpected serious adverse reactions (SUSAR) to all Investigators participating in any clinical trial with the same IMP.

According to the contract concerning the study conduction, Investigator or CRO is responsible for submission of this notification for Investigator and semi-annual lists, if applicable, to the local Institutional Review Board / Ethics Committee. CRO is responsible for submission of notification for Investigator and semi-annual lists to the National Ethics Committee, if applicable.

Providing information to the Health Authorities

The Sponsor submits information on all cases to be reported within the required period to all involved Health Authorities.

9.3.6 Emergency SAE Unblinding

The Investigator shall unblind information about the prescribed treatment (e.g., using an envelope for emergency situations, interactive voice response systems) for a specific case only in emergency situations and only if it is required for subsequent treatment of the subject

9.3.7 Pregnancies

The Investigator shall immediately (within 24 hours) inform the Country Organization Patient Safety Team of all pregnancies of the subjects during the study, with a copy to the Officer of the

study (Sponsor / CRO) as described in section 9.3.5. Cases of pregnancy are reported only from the moment of the first dose administration of IMP.

The Investigator should immediately exclude the subject from the study and organize follow-up for each case of pregnancy, report its outcome, including spontaneous or voluntary termination of pregnancy, as well as provide detailed information about the birth, the presence of any malformations, congenital abnormalities and complications in mother and (or) newborn.

Cases of pregnancy should be reported using the Report Form on the Use of the Medicinal Product During Pregnancy (Novartis document). Follow-up information during pregnancy should be reported using the same form. It should include an assessment of the association between any adverse pregnancy outcomes and IMP. Any SAE during pregnancy should be reported to the subject using the Report Form on the SAE, as described in Section 9.3.5. The Investigator should store the delivery notifications in the investigator's file to all recipients of reports in the Report Form on the Drug Exposure During Pregnancy. For more information, see the Brief Guideline for Completing the Report Form on the Drug Exposure During Pregnancy (Novartis document).

9.3.8 Claims related to IMP / medical device

In case of a claims regarding the quality of IMP / medical device, the Investigator shall inform the Sponsor within 24 hours of claim receipt in accordance with the instructions in Annex 5.3.2. Any AEs related to quality claims shall be documented and reported by the Investigator additionally as described in sections 9.3.4 and 9.3.5.

9.3.9 Special cases

Special cases may be serious or not serious (see table below). They should be reported as AEs as specified in sections 9.3.4 and 9.3.5, even if no (other) AEs are associated with them.

Special case	**The Investigator informs Country Organization Patient Safety Team in 24 hours**
Drug exposure during the breast-feeding period	x
Intentional overdose on the initiative of the patient (including suicide attempts, suicide attempt is always regarded as a serious event)	Only if related to SAE

Special case	The Investigator informs Country Organization Patient Safety Team in 24 hours
Interaction with other medicinal products	Only if related to SAE
Syndrome/ discontinuation response	Only if related to SAE
Dependence on the medicinal product, misuse, abuse or drug addiction (always regarded as a serious event)	x
Suspected transmission of infectious agents (always regarded as a serious event)	x
Death (including without other AEs; always regarded as a serious event)	x

9.3.10 Reconciliation

The coordination of information between the Sponsor's safety database and the CRO clinical database is carried out periodically / at the end of the study, as described in the coordination plan (by comparing the lists of the safety database with the data in the clinical database).

Data managers will review the CRF/ clinical database for possible cases that have not been reported. For any case to be reported, the clinical database and the safety database must match exactly the following parameters: study number, research center number, participant / patient number, randomization number, investigational drug, severity, date of death (if applicable), and the existence of a causal relationship according to the investigator's opinion. All other parameters should be consistent with the medical point of view and the most likely to fit each other. For any event to be reported and assessed as suspected to be treatment-related, or for other AE of particular interest (if applicable), more detailed coordination is required, including also dates of treatment, outcomes, history and concomitant therapy.

9.3.11 Training of investigators

By signing the study Protocol, the Principal Investigator confirms that he / she has passed the training provided by the CRO regarding the responsibilities of reporting of pregnancy and AE / SAE cases established by the Sponsor, as specified in the study Protocol.

10. Statistic

10.1 Description of statistical methods to be used, including the timing of each planned interim analysis

Statistical analysis will be carried out using specialized R Project software.
All continuous (quantitative) indicators will be presented in the form of the following parameters of descriptive statistics:

- Number of observations;
- Arithmetic mean;
- 95% confidence interval of the mean;
- Standard deviation;
- Median;
- Interquartile range;
- Minimal:
- Maximum.

All sequential, categorical and qualitative indicators will be presented in the form of the following parameters of descriptive statistics:

- Absolute frequency (number of observations);
- Relative frequency (%);
- •95% CI for proportion.

All performed types of statistical analysis will be identical with respect to the investigational medicinal product LRG-002 and in the placebo group.
Concomitant diseases and AEs will be encoded using the MedDRA terms.
This section briefly describes the planned analysis. A complete analysis will be described in terms of statistical analysis.

10.1.1 Demographic and other source data (comparability of groups for analysis)

All data obtained in groups prior to the administration of the investigational medicinal product or reference medicinal product (demographic data, laboratory data, instrumental and physical examination methods, vital signs, etc.) will be compared between groups in order to determine the comparability of groups for analysis. Fisher exact test $\chi 2$ (chi-square) will be used to compare qualitative data, and the Mann-Whitney-Wilcoxon test will be used for quantitative data.

If any of the source data reveals the incompatibility of the investigational groups (statistically significant differences in demographic and other source data between the groups), an additional analysis of the efficacy and safety parameters will be carried out together with the initial planned analysis using multivariate statistics (ANOVA, ANCOVA or logistic regression analysis depending on the type of the studied parameter) adjusted for the initial indicator(s), which vary between groups.

10.1.2 Primary efficacy parameter analysis

Primary efficacy endpoint:

Evaluation of AAD incidence from the first to the last day of LRG-002 administration vs placebo in patients with ARDs, receiving standard antimicrobial therapy. The primary efficacy criterion will be considered achieved if the odds ratio for the AAD incidence between two groups is significantly higher than 1 (i.e. AAD incidence in the LRG-002 group is lower than in placebo group).

AAD is defined as diarrhea associated with the AB use caused by C. difficile or of otherwise not identified etiology, upon analysis of stool samples and differential diagnostics according to investigator's judgment.
Diarrhea is defined as loose or watery stool (Type 5-7 according to Bristol Stool Form Scale) three times a day (frequent bowel movements with formed stool is not considered as diarrhea) in accordance with WHO criteria; based on the diary data (BSFS) and confirmation of AAD by the investigator.
Each patient will be assessed only once, even if more than one AAD episode occurs in one patient.

The primary efficacy endpoint will be analyzed using a logistic regression model with fixed factors for the treatment group and age group. Treatment exposure will be displayed using the adjusted odds ratio and the corresponding bilateral 95% confidence interval. If necessary, an absolute risk difference will be presented with the bilateral 95% confidence interval and the amount needed for treatment. No central effects are expected in this study. Therefore, the center will not be added to the primary analysis model. However, the central effects will be studied in further sensitivity analysis and subgroups analysis. Additional sensitivity analyzes can be identified in terms of statistical analysis.

The p value for testing the adjusted equality of the equivalence of the odds ratio to 1 will be compared with a significance level of 0.05.

10.1.3 Analysis of secondary efficacy parameters

The entire secondary efficacy analysis will be considered investigational, so the multiplicity adjustment will not be conducted, and a formal capacity calculation will not be provided for the test results.

- The occurrence of bowel movements per day (according to the diary data) in the treatment group compared with the placebo group;
- The incidence of any diarrhea in the treatment group compared with the placebo group;
- • The incidence of C. difficile-associated AAD in the treatment group compared with the placebo group;
- • The incidence of AAD not associated with C. Difficile in the treatment group compared with the placebo group.

Comparison of these parameters between the two groups will be performed using the chi-square test.

- The duration of AAD (the time from the onset of AAD to the normalization of stool form according to BSFS (types 1, 2, 3 and 4) and the presence of normal stool within 48 hours)) in the treatment group as compared to the placebo group;
- Duration of any diarrhea (the period from the diarrhea onset to the normalization of stool form according to BSFS (types 1, 2, 3 and 4) and the presence of normal stool within 48 hours)) in the treatment group vs the placebo group;
- The changes in stool consistency (according to Bristol Stool Form Scale) (according to the diary data) in the treatment group vs the placebo group; for any individual changes in the stool consistency, it will be classified as improved / unchanged / worsened, and the difference between the groups will be analyzed using the chi-square test;
- The incidence and severity of symptoms of the gastrointestinal tract, including nausea, vomiting, flatulence, abdominal pain and poor appetite (according to the diary) in the treatment group vs the placebo group; the incidence of symptoms will be compared using the chi-square test, and the comparison of severity by using the Mann-Whitney-Wilcoxon test;
- Change in body weight at Visit 3 compared with Visit 1 in the placebo group as compared to the treatment group;
- Hospitalization rate in the treatment group vs the placebo group; comparison between the two groups will be performed using the Fisher's exact test or chi-square test depending on the number of expected observations per cell (<5 or ≥5);

- The number of days of using standard symptomatic therapy to relieve signs of acute diarrhea in the treatment group vs the placebo group;
- The number of patients using standard symptomatic therapy to relieve signs of acute diarrhea in the treatment group vs the placebo group.

10.1.4 Analysis of safety parameters

Safety parameters should be evaluated at each visit planned in this study, as well as at unscheduled visits.

Safety evaluation will be based on the following parameters (between Visits 1 and 3), compared between the investigational medicinal product and placebo:

Frequency of adverse events (AEs) and/or serious adverse events (SAEs) in the treatment groups, including:

1. Determination of the overall incidence in:
   I. adverse events (AEs), regardless of their association to the treatment;
   II. AEs related or possibly related to the medicinal product administration;
   III. AEs requiring therapy discontinuation.

AEs recording will be based on spontaneous report by the patient, as well as on the results of a patient survey by a physician.

Adverse events will be encoded according to the last update of MedDRA terminology. They will be represented by a preferred term (PT) and a system organ class (SOC).

2. AEs incidence according to the results of the vital signs evaluation (HR, BP, BT, RR) at each visit vs placebo

Changes in the main vital signs over time and the frequency of pathological abnormalities revealed during physical examination will be summarized in groups, and a comparison will be made between groups using appropriate tests for quantitative and qualitative data (see below). Quantitative data will be compared using the t-test or the Mann-Whitney test, depending on the normality of the distribution (which will be evaluated using the Shapiro-Wilk test). Qualitative data will be compared using the Fisher's exact test or the chi-square test, depending on the number of prospective observations in one cell (<5 or ≥5).

3. AEs incidence according to the results of laboratory examinations on Days 7(± 2) and 15(± 2) and AEs incidence according to the ECG results 15(± 2) vs placebo;

Variations in the results of laboratory tests (carried out in the central laboratory) over time and the rate of pathological deviations from the norm of the study results (based on the central laboratory reference values), as well as the deviations rate of the ECG results at the Visit 3 will be summarized by groups, and comparison between groups shall be made using appropriate tests for quantitative and qualitative data (see paragraph 2).

4. AEs incidence according to the everyday patients' diaries records of well-being self-evaluation.

Patient complaints based on the results of the daily records of well-being self-assessment in the diary will be summarized into groups, and comparison between groups shall be made using appropriate tests for quantitative and qualitative data. Gastrointestinal AEs (determined using standardized MedDRA requests for the corresponding symptoms), regarded by the Investigator as associated with the study therapy, will be analyzed separately. Descriptive statistics methods will be used for the results. Comparison of the incidence of new AEs in the study groups will be carried out using the Fisher's exact test or the chi-square criterion, depending on the number of prospective observations in one cell (<5 or ≥5); the comparison of severity degree (and possible causal relationship between received medicinal product and AE) will be carried out using the Cochran – Armitage test for linear trends for ordered categorical data.

10.2 Interim analysis

Interim data analysis is not planned in this study

10.3 Sample size calculation

Sample size calculation is based on the following actions:

1. Efficacy of probiotics in prevention of acute diarrhea: a meta-analysis of masked, randomized, placebo-controlled trials. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Lancet Infect Dis. 2006 Jun; 6(6):374-82. Review. PMID:16728323. DOI:10.1016/S1473-3099(06)70495-9
2. Chow S, Shao J, Wang H. Sample Size Calculations in Clinical Research. 2nd Ed. Chapman & Hall/CRC Biostatistics Series, 2008
3. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. Am J Gastroenterol. 2006 Apr;101(4):812-22.
4. Videlock EJ, Cremonini F. Meta-analysis: probiotics in antibiotic-associated diarrhoea. Aliment Pharmacol Ther. 2012 Jun;35(12):1355-69. doi: 10.1111/j.1365-2036.2012.05104.x. Epub 2012 Apr 24.

5. H. Szajewska, M. Kołodziej. Systematic review with meta-analysis: Lactobacillus rhamnosus GG in the prevention of antibiotic-associated diarrhoea in children and adults. AP&T, Volume 42, Issue 10, November 2015, Pages 1149-1157.

Primary endpoint is the AAD incidence from 1 to the last day of LRG-002 administration vs placebo in ARD patients treated with standard antibiotic therapy. The primary efficacy criterion will be considered achieved if the odds ratio for the AAD incidence between two groups is significantly higher than 1 (i.e. AAD incidence in the LRG-002 group is lower than in placebo group).

A test based on odd ratio was used for sample size calculation.

The following data were obtained from Figure 2 in the meta-analysis [1] (only data on AAD were used):

- Given only data for AAD studies in adults from the cited publication, the rate for the control group is 0.24, and for the treatment group - 0.14.
- Given only data for LGG studies in adults and children, the rate for the control group is 0.26, and for the treatment group - 0.15.

In meta-analysis [14], figures from Table 1 demonstrate a rate of 0.196 for the treatment group and 0.29 for the placebo group (only trials for adults with LGG were taken into consideration).

In meta-analysis [15], figures from Table 2 demonstrate a rate of 0.188 for the treatment group and 0.269 for the placebo group (only trials for adults with LGG were taken into consideration).

Consideration of AAD studies for LGG from the paper[16], concerning infections in adults (including H.pylori), resulted in 0.14 for the probiotic group and 0.22 for the control group.

Combining all the strains, we obtained a rate of 0.26 for placebo and 0.15 for probiotic group.

The following indicators were used for calculations:

1. Difference of AAD absolute risks in placebo and treatment groups is expected to be positive.
2. Incidence of AAD is assumed 0.26 for a placebo group and 0.15 for the treatment group.
3. Likelihood of type I errors: 0.05;
4. Power of the study is 80%, which corresponds to the type II error: 0.20
5. Statistical hypothesis is the evidence of superior efficacy:

$$H0: OR = 1$$
$$H1: OR \neq 1,$$

wherein OR is the odds ratio between both groups.

The ratio between the treatment and placebo group sizes is 1:1

The number of patients in each group could be calculated using the following formula [2]:

$$n_T = n_T = \left(\frac{Z_{1-\alpha/2} + Z_\beta}{p_T - p_R} \right)^2 \left[p_T (1 - p_T) + p_R (1 - p_R) \right] = 208$$

Thus, we obtain the minimum number of patients required for statistical analysis - 416 (208 patients in each group). Given the 20% attrition rate, there is a necessity to randomize 520 subjects (260 patients in each group).

10.4 Applicable significance level of the clinical study

All statistical tests in this study will be conducted at a 95% significance level (the threshold p value for confirming statistical significance is less than 0.05). Bilateral statistical criteria will be applied for all parameters studied.

10.5 Clinical study termination criteria

The study may be terminated subject to occurrence of terms and conditions set forth in section 5.8 of this protocol.

10.6 Missing, Suspect and Non-Amendable-To-Analysis Data Procedures

During monitoring visits to the clinical center, Clinical Research Associates (Monitors) authorized by the Sponsor will analyze the CRF of the study subjects for the lack of necessary data. If there is any missing data in CRF and the relevant information is available in the source documentation, relevant queries to investigators shall be spelled out, as well as instructions for elimination of discrepancies. During check of the database, the Statistician, authorized by the Sponsor, and Managers for Data Control and Processing will determine whether there were any uncertain, missed, or non-amendable-to-analysis data, based on which queries to investigators can be defined. The investigators, if possible, eliminate the detected errors in CRF, and inform the Principal Investigator and authorized representatives of the Sponsor thereof. If the detected errors in the data cannot be eliminated after the completion of the patient's participation in the study, sensitivity of the resulting parameters to the presence of suspected data will be analyzed during the statistical analysis of the data. Information on missing, suspected and non-amendable-to-analysis data will be presented in the final clinical study report. For the primary endpoint, the method of filling in the missing values will be applied on the basis of the maximum likelihood estimation (MLE), if necessary [64]. For all other types of endpoints and other parameters, the analysis will be carried

out only using actually available information, without data fulfillment, which is associated with a short duration of the clinical study.

10.7 Procedures for reporting any deviations from the original statistical plan

All deviations from the original statistical plan should be described and justified in an amendment to the protocol and / or the final study report (in the latter case, the statistical analysis plan developed prior to the initiation of the final statistical analysis should contain a list of these deviations with justification of reasons for deviating from statistical plan according to the Protocol).

10.8 Selection of subjects for analysis

For the analysis, the following data sets will be used:

- Population of all included patients (intention-to-treat, ITT): all randomized subjects who administered at least one dose of the investigational medicinal product / placebo and completed at least one visit to evaluate the efficacy parameters (i.e. at least all procedures of visit 1).
- Population of patients who completed the study according to the Protocol (per protocol, PP): all randomized subjects who completed participation in the study according to the Protocol (completed the prescribed period of treatment and follow-up without significant deviations from the Protocol).
- Safety population: all randomized subjects who administered at least one dose of the investigational medicinal product / reference medicinal product and completed at least one visit to evaluate safety parameters (i.e. at least Visit 1). Unlike the ITT population, the safety population will be analyzed according to the actual treatment received rather than the prescribed treatment (if the differences are identified between the treatment prescribed and actually received).

11. Description of activities concerning Quality Control and Quality Assurance

11.1 Study Monitoring and Quality Control

Regular visits of the Clinical Research Associate (Monitor), on behalf of the Sponsor and in accordance with standard operating procedures (SOPs), prior to, during and in the end of the study, contribute to the study success, guarantee the collection of accurate data and the timely detection of possible errors, documenting the clinical trial process and ensuring the protection of the study

subject rights and the study relevance to the principles of Good Clinical Practice (ICH GCP) and requirements of international law and applicable legislation of the Russian Federation.

Routine study monitoring includes:

- Confirmation of the proper conduction and documentation of the informed consent process, as well as screening and inclusion of subjects in the study;
- Verification of CRF data and primary medical documentation of the study subjects;
- Confirmation of documentation and timely reporting of information on AEs during the study;
- Confirmation of compliance with the requirements for the implementation of diagnostic and therapeutic procedures of the study protocol by the personnel of the clinical center;
- Confirmation of documentation on delivery, storage, distribution and destruction of the investigational medicinal product / reference medicinal product and study materials;
- Confirmation of the personnel competencies of the clinical center, external laboratory, necessary for the study;
- Confirmation of the conformity of diagnostic and laboratory equipment to the requirements of safe and adequate use during the study;
- Confirmation of the Investigator's interactions with the local Ethics Committee on the study safety issues and the introduction of amendments to the study Protocol, approved by the Sponsor.

Quality control of the study results is carried out by the Sponsor / Sponsor's Qualified Person, maintaining an electronic study database, which determine inconsistencies, wrong data, missing data during the cross-validation of all CRFs. Should any questions or a need of clarification arise, a special form (request for data clarification) is sent to the Investigator in the eCRF system or by e-mail / fax, the request must be answered in written form within 7 days after its delivery.

In accordance with legislative requirements, the Sponsor or authorized government authorities have the right to verify (audit) the material and technical support of the study and its documentation. The Investigator shall provide access to documentation and all necessary information to persons authorized to conduct an audit or inspection.

Monitor visits can be carried out both face-to-face and remotely by agreement with the Sponsor. A detailed description and format of the visits will be provided in the Study Monitoring Plan.

11.2 Protocol amendments

The investigators' signatures on the Protocol signature page mean written confirmation of consent to conduct study in accordance with this Protocol. During the clinical study, amendments and

supplements may be made to the study materials. Such changes and supplements are considered as amendments.

Amendment to the Protocol - a written description of the changes or a formal explanation of the text in the Clinical Trial Protocol. The amendments may be substantial and unsubstantial. Any amendment to the Protocol must be duly approved in accordance with the internal SOPs of the sponsoring company before it comes into effect, then approved by the regulatory authorities, the independent ethics committee and signed by the Investigator.

In the Order of the Ministry of Health of the Russian Federation No. 775 "On Approval of the Procedure for Considering Reports on the Need to Amend the Clinical Trial Protocol of medicinal product for medical use" dated August 31, 2010, a list of substantial / unsubstantial amendments and the procedure for submitting materials for examination are defined.

Amendments to the clinical trial materials are considered substantial if they can affect the goals, forms of organization, methodology, statistical methods for processing the clinical trial results and measures to ensure safety of patients participating in it.

Amendments to the clinical trial materials are considered unsubstantial if they cannot affect the goals, forms of organization, methodology, statistical methods for processing the clinical trial results and measures to ensure safety of patients participating in it.

If it is necessary to amend this Protocol, the study Sponsor reports to the Ministry of Health of the Russian Federation on the need to amend the Clinical Trial Protocol. The decision to introduce amendments or to refuse to introduce them is made by the Ministry of Health of the Russian Federation after examination of the provided updated materials. Amendments to the Protocol should be stored with the original version of the Protocol. The number and date of amendment shall be indicated on the title page of the Protocol.

11.3 Protocol Deviations

A deviation from the Protocol is an unintentional deviation from the approved study Protocol.

A serious deviation from the Protocol is a deviation that may, according to the assessment of the Investigator or the Investigator's Qualified Person, result in the subject dropping out from the study or his data being excluded from the bioanalytical and / or statistical part of the study. Deviations not classified as serious are considered minor deviations from the study Protocol.

Serious deviations from the Protocol should be reported to the Sponsor as soon as possible by the clinical center and/or CRO personnel and Monitor (if present at the center). The Sponsor may

propose to re-classify the deviation from the Protocol (from minor to serious or vice versa) on the basis of the assessment. In such case, the classification made by the Sponsor shall prevail and shall be brought to the notice of CRO together with a written justification.

The Sponsor must be informed of minor deviations from the Protocol within 10 working days, but before the start of the next study period / phase or before the start of the bioanalytic phase / statistical phase.

The exceptions are the following minor deviations from the Protocol, which can be reported in the draft study report:

- Logistical deviations (deviations in time from the scheduled plan of blood sampling; follow-up visits that moved out of the time frame established by the Protocol due to the subject's plans/schedule, etc.).
- Administrative deviations (e.g. name change).

In the case of enrollment of a smaller than planned number of volunteers, the CRO should contact the Sponsor, who may approve the dosing of a smaller than planned number of volunteers. This is not considered as deviation from the Protocol.

Notices and reports about deviations from the Protocol shall be submitted to the Regulatory authorities and relevant Ethics Committees in accordance with applicable requirements / guidelines / laws.

Procedure for documenting Protocol deviations

The Investigator or the Investigator's Responsible Person shall document and explain any Protocol deviation. Sponsor's notification of a deviation from the Protocol may be communicated verbally in exceptional cases (if immediate action / notification is required), followed by written notification (e.g., by e-mail; in the report on the study period). All deviations from the Protocol should be described in the final study report.

12. Description of the clinical trial ethical aspects

12.1 General provisions

The study will be conducted in compliance with the principles and requirements set forth in the following documents:

- WMA Declaration of Helsinki (adopted at the 18th WMA Assembly in Helsinki, June 1964, the latest edition was approved at the 64th Assembly in Fortaleza, October 2013),

- ICH E6 Harmonized Tripartite Guidelines for Good Clinical Practice (R2) dated November 9, 2016
- Rules of Good Clinical Practice of the Eurasian Economic Union (approved by Decision of the Council of the Eurasian Economic Commission No. 79 dated November 3, 2016);
- Federal Law No 61-FZ "On Circulation of Medicines" (current edition) dated April 12, 2010;
- Order of the Ministry of Health of the Russian Federation dated April 1, 2016 No. N 200n "On Good Clinical Practice Approval";
- National Standard of the Russian Federation GOST R 52379-2005 "Good Clinical Practice";

The study will be started in all research centers only after obtaining written permission to conduct the study and approval of the Ethics Council of the Ministry of Health of the Russian Federation and obtaining signatures on the Clinical Trial Protocol of each of the parties involved in the study.

Investigators will be familiarized with study materials in a timely manner before its initiation. Qualification of investigators will meet the requirements necessary for conducting quality clinical study.

The selection of prospective study subjects is voluntary.

12.2 Procedure of obtaining informed consent

Before being included in the study, the subject is provided with written information and an oral explanation of the goals, objectives and methods of the study, as well as the expected benefits and possible risks associated with participation in the study. Besides, subjects should be informed of the voluntary nature of participation in the study and that the subject has the right to refuse to participate in the study at any time and this refusal will not affect the quality of medical care provided for him. Although the subject does not have to report on the reasons that prompted him to interrupt participation in the study, the Investigator should try to find out these reasons without violating the subject's rights. Subject consent must be obtained prior to any study procedures.

The data processing collected during the study is carried out in compliance with the confidentiality of subjects. Subjects should be informed about the goals of the planned computer data processing and terms of publishing this data (e.g., for presentation at medical conferences, in journal articles and other open sources), presented only in an aggregated form that does not allow subject's identification.

Subjects should be informed that authorized representatives of the health authorities and the Sponsor will have access to their confidential medical information for monitoring, inspection and

audit purposes. At the same time, subjects must be guaranteed strict confidentiality of all information that allows to identify the study subject, and non-disclosure of such information.

The Patient Information Leaflet with the informed consent form is to be completed in duplicate, signed and dated by the subject and Investigator in their own hands. The first copy of the signed Patient Information Leaflet with the informed consent form must be stored by the Investigator in the investigator's file, the second copy must be given to the study subject.

12.3 Confidentiality and identification of study subjects

Confidentiality of records allowing for identification of the study subjects is ensured in compliance with the right of privacy and privacy protection in accordance with the regulatory requirements. Records concerning patient's identity are kept secret and can be disclosed only to the extent permitted by the legislation. The confidentiality of the subject personal data will be preserved when the study results are published.

12.4 Selection of subjects from vulnerable and special groups

Inclusion / non-inclusion criteria do not include participation of subjects from vulnerable groups in the study.

Groups with special conditions in this study include women with preserved reproductive potential, who, according to inclusion and non-inclusion criteria, can participate in the study only if they consent to the use of effective contraceptive methods throughout the study, which is reflected in the Patient Information Leaflet and is confirmed through signing an informed consent form.

13. Description of working with data and recording

All records and documents of the research center related to the clinical study, including those in the investigator's file (including informed consent forms, logs, subject lists, etc.), as well as the source medical records of the subject must be stored for 15 years after the end of the study. The study Sponsor should control the preservation and availability of all materials in the clinical trial throughout the life cycle of the IMP. Archival data can be stored in the form of photocopies, as well as in optical and electronic media. The Principal Investigator must immediately inform the Sponsor of the facts of unintentional damage / destruction, as well as the change in the storage location of the archived materials concerning clinical trial. Wilful destruction of archived study materials is possible only with the written permission of the study Sponsor.

As the subjects undergoes the planned visits and the Investigator completes eCRF, source documentation and eCRF will be verified by Sponsor's authorized monitors. Should Manager for Data Control and / or Biostatistics at the stage of evaluating data in the eCRF have questions about the data, all updates and amendments to the eCRF data will be documented by creating request forms to clarify the data.
The study should be conducted in accordance with the Protocol and applicable standard operating procedures of the Sponsor. If there is a need to amend the Protocol, one should follow the procedure described in section 11.2.

For investigators, it is mandatory to fill out the source medical record and eCRFs of all subjects involved in the study.

The Investigator is responsible for the full and accurate completion of the eCRF. All data recorded in the eCRF should also be reflected in the source medical record of the subject in printed form or in the form of records made by the Investigator or other Qualified Person of the clinical center.

In eCRF, according to the source documentation, all significant information about the subject's participation in the study is recorded. The eCRF should also contain information on the subject's completion of participation in the study. The eCRF should be completed no later than 5 days after the subject's visit to the research center.

The Investigator has the right to convey the study information to persons not directly involved in the study, only with the permission of the Sponsor.

The final report, consisting of a statistical and clinical report, is formed after the database is closed and the statistical processing of the study results is completed.

The final report is signed by the Principal Investigator of the clinical center, who confirms the results and conclusions of the study.

Direct Access to the Source Data / Documents

Source data is all information contained in the source medical records and certified copies, regarding the clinical data, follow-ups and other procedures within the framework of the study, and necessary for the reconstruction and evaluation of the study.

The Investigator provides the opportunity to monitor study, audit(s), examination by the Ethics Council and regulatory authorities, provides direct access to source data / records.

Source data should be stored in proper quality for the entire period provided by local and international legislation, as well as in written agreements with the company sponsoring the study. For each involved subject, the Investigator confirms in the source records the fact that the subject is participating in this study, and also records the following information: individual ID, personal data of patients (name, address), dates of medicinal product administration, vital signs, any AEs, study completion dates, and main reasons for discontinuing treatment (if applicable).

It is the responsibility of the Investigator to provide direct access to source data and documentation for the Clinical Research Associate of the sponsoring company and / or its authorized representatives (CRO), the auditor of the competent authorities, representatives of the insurance company, and ethics committees.

14. Funding and Insurance

14.1 Funding

This study is funded by the sponsoring company through CRO. Prior to the study, appropriate agreements will be concluded between the CRO and each research center.

14.2 Insurance

Patient safety during this study is the responsibility of the Principal Investigator of the research center. If the patient experience an AE, the Principal Investigator and his staff will provide medical care and will do everything possible to treat the patient.

If the patient agrees to participate in the study, his participation in this study will be insured by [redacted] in accordance with the Federal Law No. 61-FZ "On Circulation of Medicines " dated April 12, 2010, Decree of the Government of the Russian Federation No. 714 "On Approval of Standard Rules for Compulsory Life and Health Insurance of a Patient Participating in Clinical Trials of a Medicinal Product" dated September 13, 2010 and Decree of the Government of the Russian Federation No. 393 "On Amendments to Standard Rules for Compulsory Life and Health Insurance of a Patient Participating in Clinical Trials of a Medicinal Product" dated May 18, 2011.

According to the Decree of the Government of the Russian Federation No. 714 "On Approval of Standard Rules of Compulsory Life and Health Insurance of a Patient Participating in Clinical Trials of a Medicinal Product", the amount of insurance payment under the contract is:

a) in case of death of the insured person - 2 million rubles. The insurance payment in the indicated amount is distributed among the beneficiaries in proportion to their number in equal shares;

b) if the health of the insured person worsens, which entails:

- group I disability confirmation - 1.5 million rubles;
- group II disability confirmation - 1 million rubles;
- group III disability confirmation - 500 thousand rubles;

c) upon deterioration of the insured person health, which did not entail the disability confirmation - not more than 300 thousand rubles.

Each patient will receive the original insurance policy and a memo with a description of the insurance terms and conditions provided for by the policy and the procedure for dealing with harm to health. In order to maintain anonymity, the personal data of each patient in the policy will be

replaced by the Individual Patient Identification Code, which is assigned during the study in the form established in the Russian Federation.

In case of damage to the patient's health associated with a clinical trial, [redacted] Insurance Company shall compensate all costs for the necessary medical examination and treatment, the need for which will arise as a result of the direct effect of the investigational medicinal product and/or medical manipulations applied according to study Protocol.

For more information, patients can contact the address of the Insurance Company: [redacted]

The study does not provide any additional types of voluntary insurance or other options for the provision of treatment and / or compensation in the event of the patient's death or harm to the patient's health as part of the study.

The Sponsor is not responsible for any loss, harm and / or damage that may be caused to the patient in the event that such loss, harm and / or damage are caused by:

- Administration of prohibited medicinal product during the study;
- Deviations from the study Protocol, study requirements and / or any instructions or directions that the physician-investigator can provide, by the patient;
- The action or inaction of a third party concerning adequate respond to an adverse event or reaction to the investigational medicinal product.

15. Publications

The information contained in this document is the property of the Sponsor, and its transfer to third parties is permitted only with the written permission of the sponsoring company. Only the Investigator(s) and the research center(s) participating in the study, members of the independent ethics committee(s) and health officers authorized to control the clinical study have access to this information. Information on the study to the extent of making a decision on granting consent to participate is provided to subjects who can participate in the study.

Exclusive rights to the study results belong to the Sponsor of the study. No data from this study may be presented or published without the prior written permission of the Sponsor.

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17. Appendices

17.1 Appendix 1. Prohibited Therapy List

Pharmaceutical groups	Active substance
Systemic glucocorticosteroids	hydrocortisone, cortisone, prednisone, methylprednisone, prednisone, triamcynolone, dexamethasone, betamethasone and their analogues and/or combinations and derivatives thereof
Antibacterial agents	aminoglycosides, amphenycols, ansamicines, glycopeptides, carbapenemes, lincosamides, macrolides and azalides, penicillins, tetracyclines, cephalosporins - in monotherapy and/or combinations
Probiotics, including dietary supplements	acidophilic lactobacteria, kefir fungi, bifidobacteria bifidum, Escherichia coli, lactobacteria, lysozyme, enterococcus fecium, longum bifidobacteria, animalis bifidobacteria, lactulose, plantharum lactobacteria, product Broncho-munal® - as monotherapy and as part of the drug / dietary supplement in any combination
Adsorbents	activated carbon, aluminium oxide, attapulgite, colloidal silicon dioxide, lactulose, lignin hydrolised, povidone, polymethylsiloxane polyhydrate, dioctahedral smectite - as monotherapy and as a part of the drug / dietary supplements in any combination
Calcium preparations	calcium glubionate, calcium lactobionate, calcium carbonate, calcium lactogluconate - as monotherapy and as part of the drug / dietary supplement in any combination
Anti-diarrheal medications	attapulgite, bergenia rhizome, bacillus subtilis, bifidobacteria bifidum, snakeweed rhizome, burnet rhizome with roots, loperamide+symeticone, loperamide, oval plantain seed coating, racecadotryl, dioctahedral smectite, hackberry fruit, blueberry fruit - as monotherapy and as part of the drug / dietary supplements in any combination
Myotropic spasmolytics	bendazole, metamizole, papaverine, drotaverine, theobromine, bencyclane, darifenacin, dicyclovenerine, codeine, camylophin, mecloxamine, propyphenazole, ergotamine, mebeverine, oxybutynin, otilonium bromide, platyphylline, pynaverium bromide, trimebutine, as well as plant spasmolytics - as monotherapy and as part of the drug / dietary supplements in any combination

Antiemetics and drugs that affect gastrointestinal motility	pyridostigmine, neostigmine, itoprid, cisapride, aprepitant, bromopride, granisetron, dimenhydrinate, domperidone, levomenthol solution in menthyl isovalerate, meclozine, moxastine, ondansetron, palonosetron, perphenazine, thiethylperazine, trifluoperazine, tropisetron, fosaprepitant, as well as medications of plant origin - as monotherapy and as part of the drug / dietary supplements in any combination

17.2.Appendix 2. Prescribing on stool sample collection for patient

In case of diarrhea, collect samples of the stool according to the following instructions:

Container 1 (sterile container with spoon and lid).

Collect a stool sample of no more than 1/3 of the container volume (from several places). During collection, try to avoid impurities of urine and urethral and vaginal discharge. Close the container, place it to the fridge (NOT to the freezer).

Container 2 (sterile container with spoon and lid).

Collect a stool sample of no more than 1/3 of the container volume (from several places). During collection, try to avoid impurities of urine and urethral and vaginal discharge. Close the container, place it to the fridge (NOT to the freezer).

Container 3 (transport kit consisting of a Cary-Blair liquid tube with a green lid and a swab on the applicator)

Collect the sample by immersion of the tampon in the feces alternately in several places, then place the swab to the tube and break off the surplus of applicator on the red mark.

Screw the lid tightly to prevent material from leaking. Close the container, place it in the fridge (NOT in the freezer).

Contact the research center within 1 hour of collecting the samples and notify it on the need to transport the containers to the laboratory. Keep samples in the fridge (NOT in the freezer!) before the employee arrives.

17.3. Appendix 3. Patient diary

Filled by the patient: Day No. 1 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

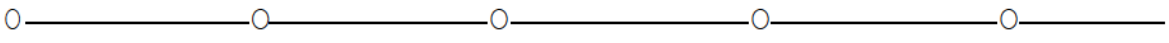
Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

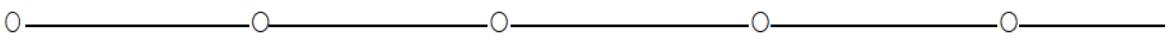
Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: □ Yes □ No



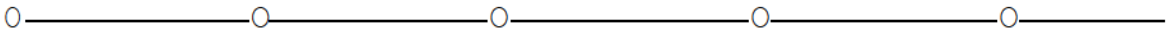
0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Vomiting: □ Yes □ No



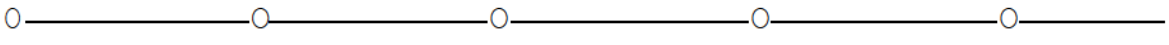
0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Flatulence: ☐ Yes ☐ No



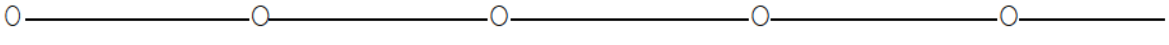
0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Decreased appetite: ☐ Yes ☐ No



0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Comments and complaints:_____

_____

6. **Symptomatic therapy**

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

Filled by the patient: Day No. 2 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

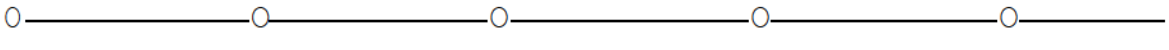
Vomiting: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Flatulence: ☐ Yes ☐ No

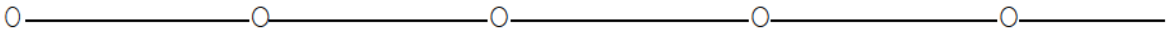
0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Decreased appetite: ☐ Yes ☐ No



0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

6. **Symptomatic therapy**

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 3 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

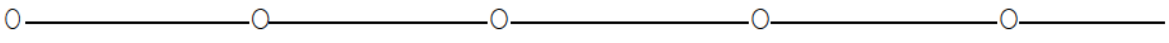
Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

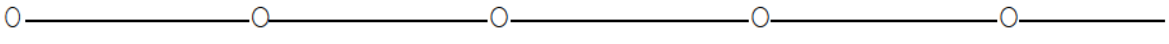
Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No



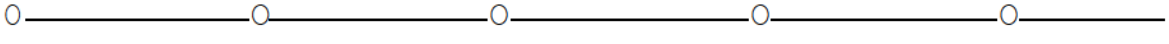
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Vomiting: ☐ Yes ☐ No



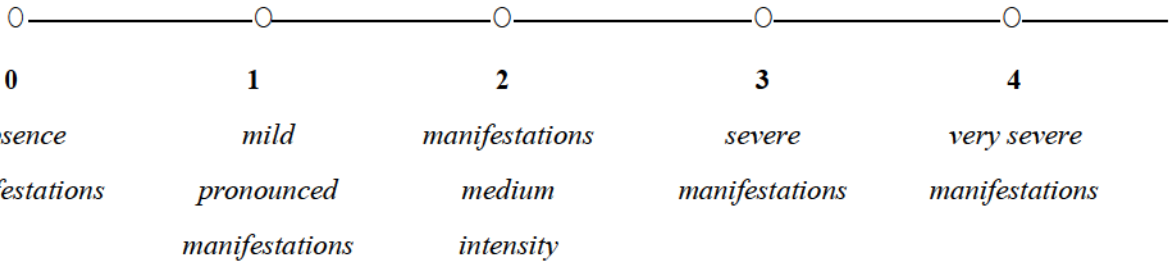
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Flatulence: ☐ Yes ☐ No

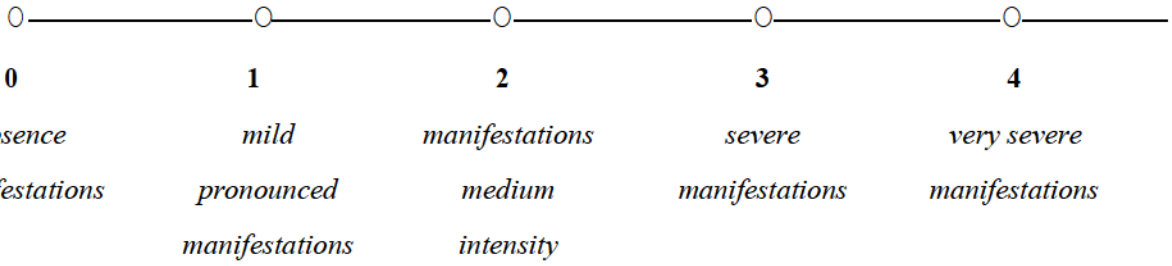


0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. **Symptomatic therapy**

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 4 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

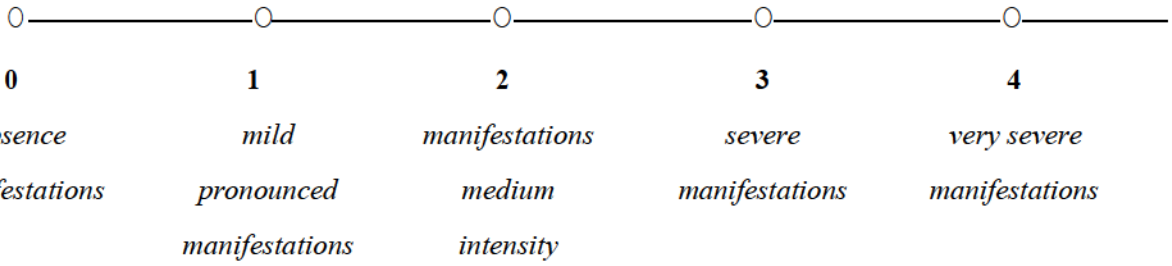
Vomiting: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

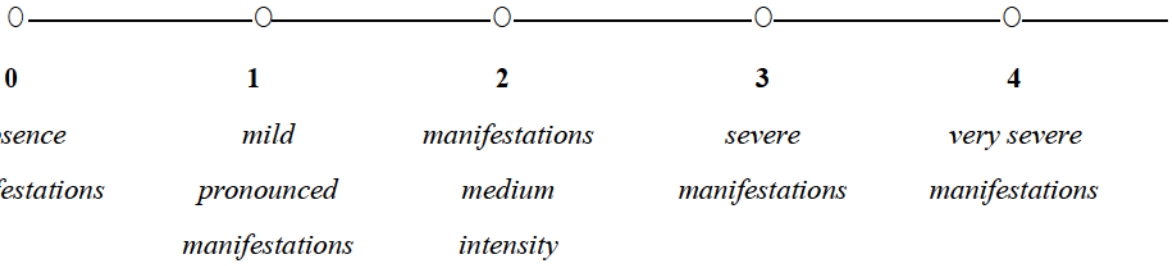
Flatulence: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 5 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

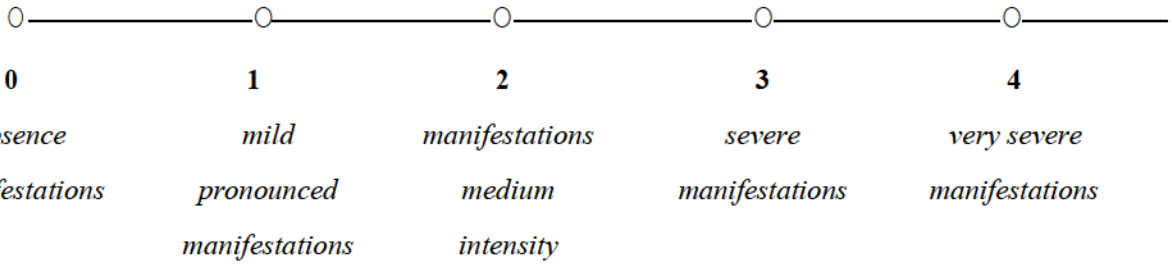
Vomiting: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

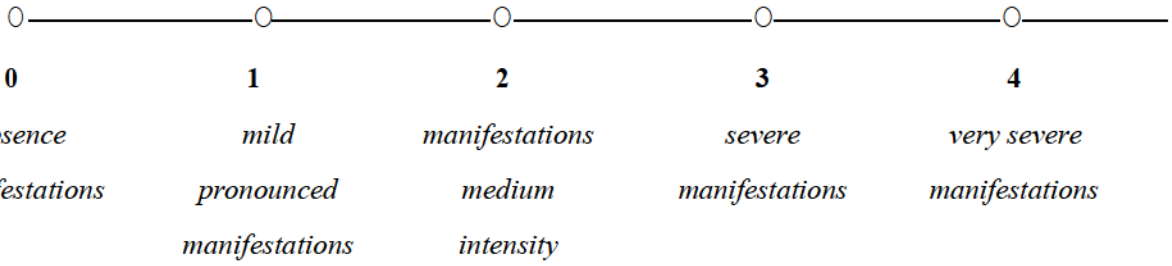
Flatulence: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 6 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

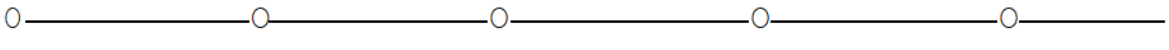
Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

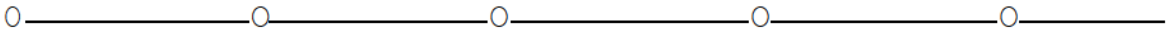
Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No



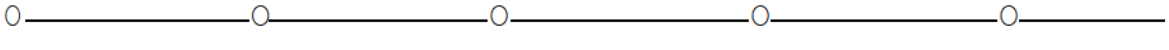
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Vomiting: ☐ Yes ☐ No



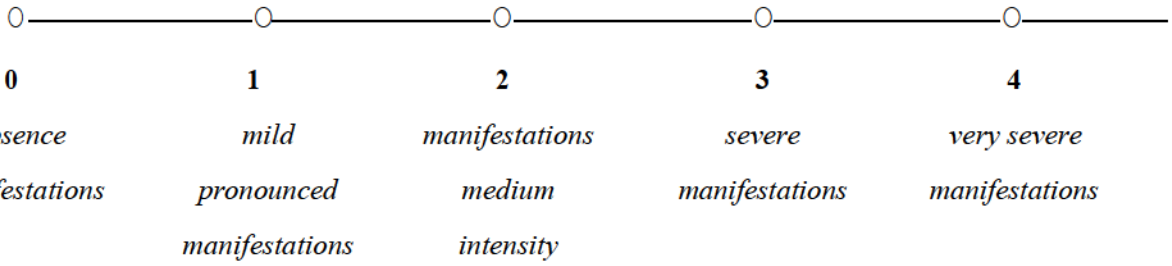
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Flatulence: ☐ Yes ☐ No

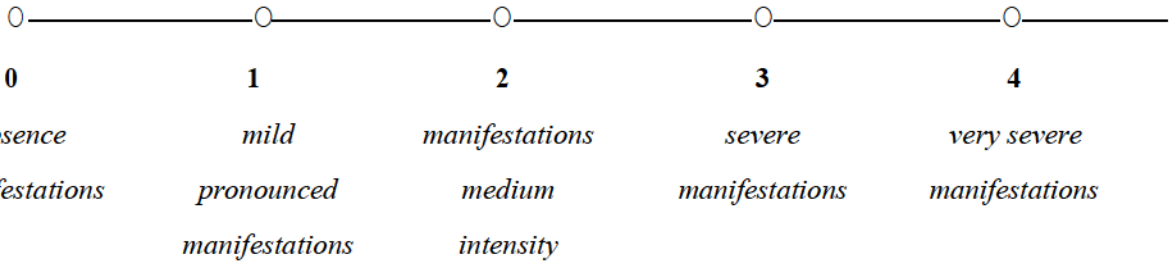


0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 7 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

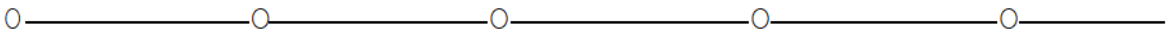
Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

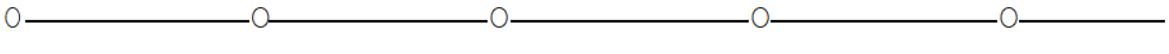
Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No



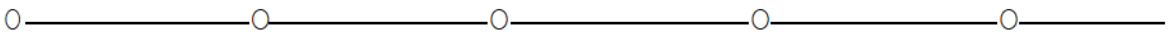
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Vomiting: ☐ Yes ☐ No



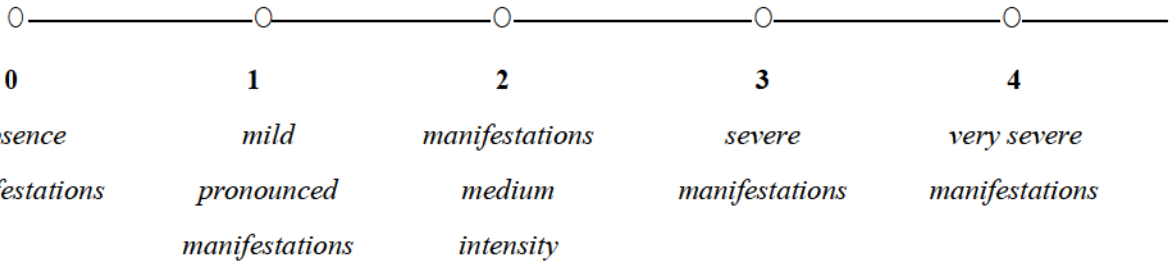
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Flatulence: ☐ Yes ☐ No

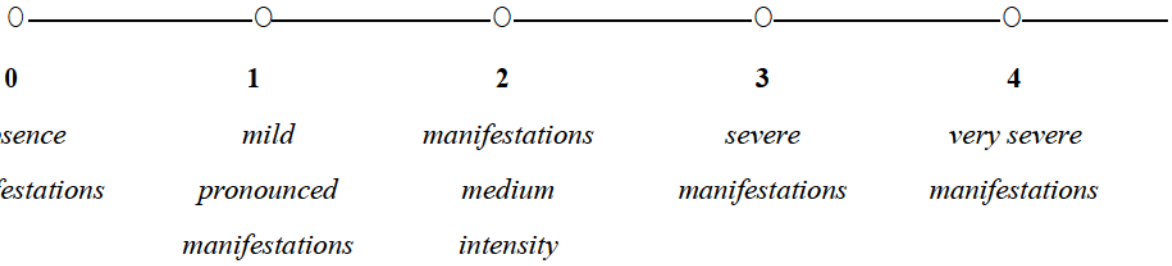


0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 8 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

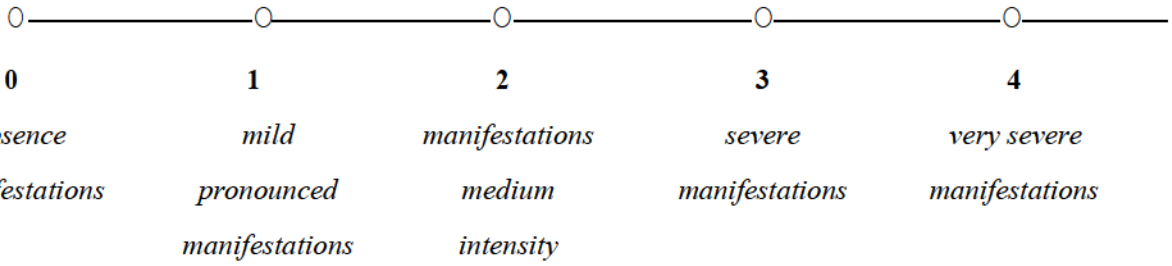
Vomiting: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Flatulence: ☐ Yes ☐ No

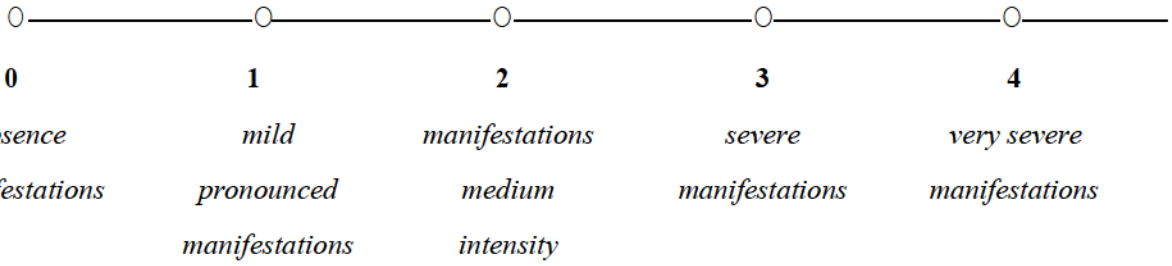
0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Decreased appetite: ☐ Yes ☐ No



0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 9 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

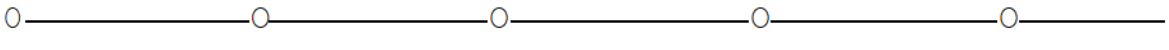
Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

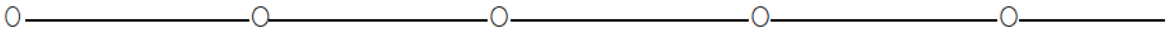
Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No



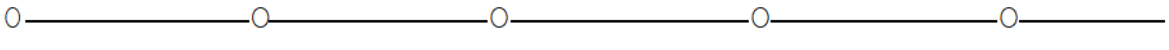
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Vomiting: ☐ Yes ☐ No



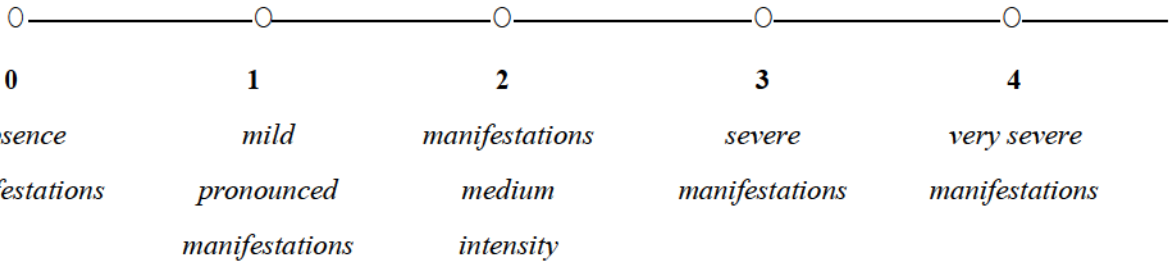
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Flatulence: ☐ Yes ☐ No

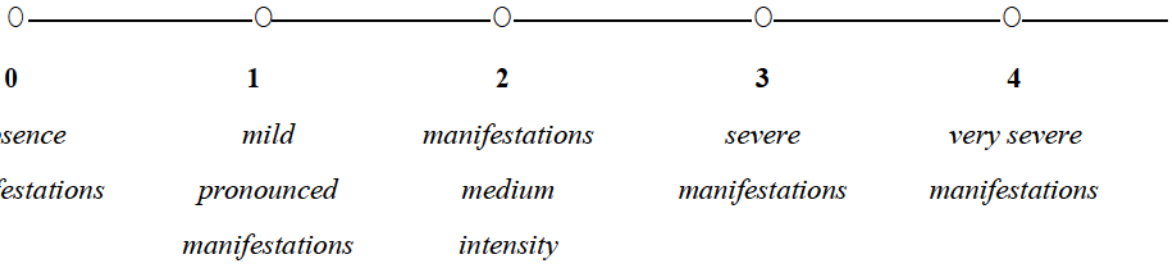


0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 10 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

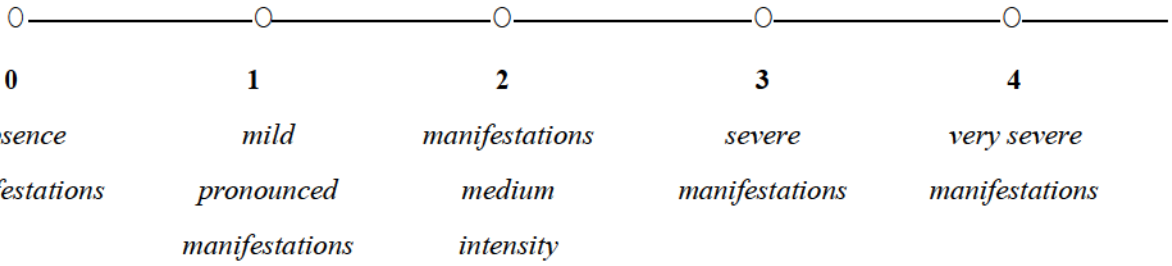
Vomiting: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

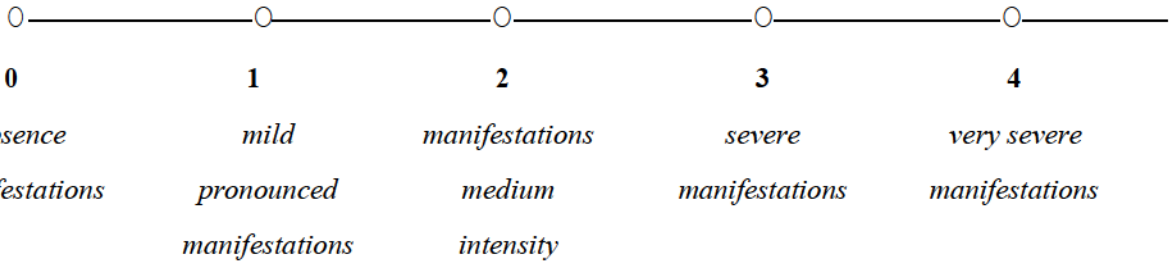
Flatulence: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 11 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

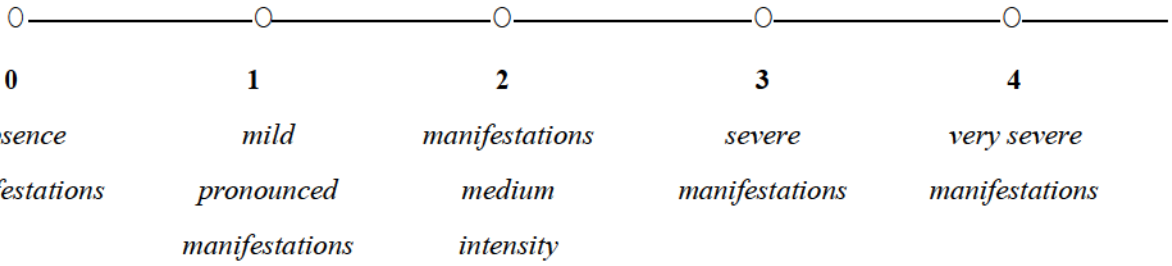
Vomiting: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

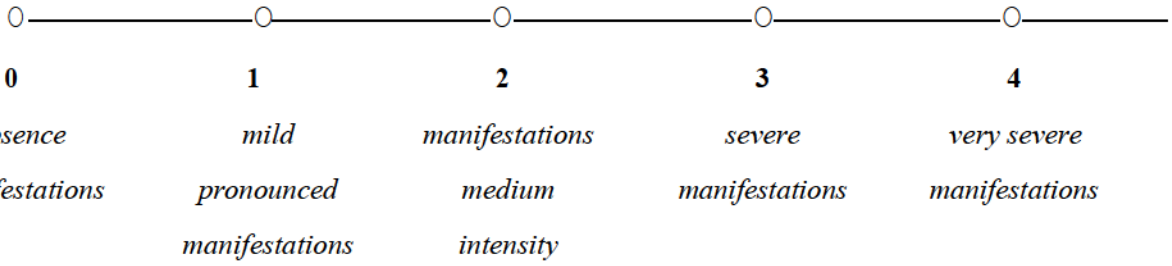
Flatulence: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 12 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

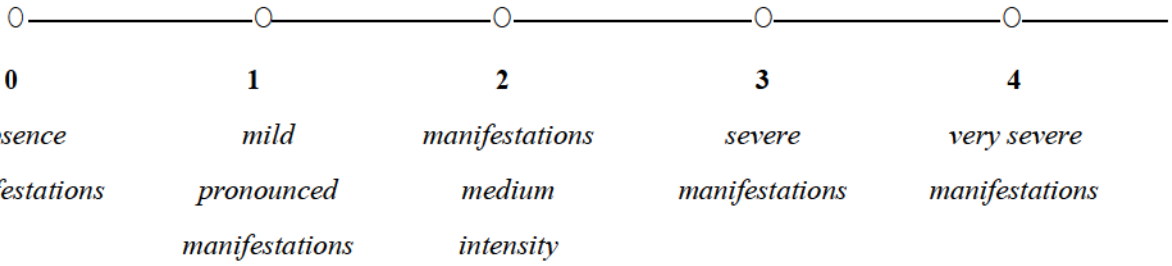
Vomiting: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

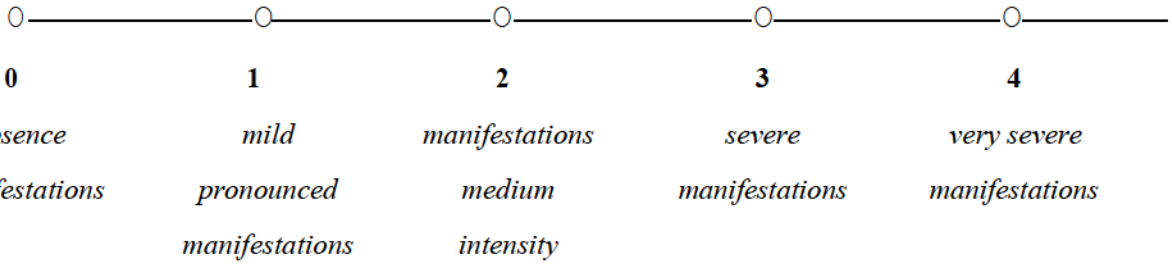
Flatulence: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 13 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

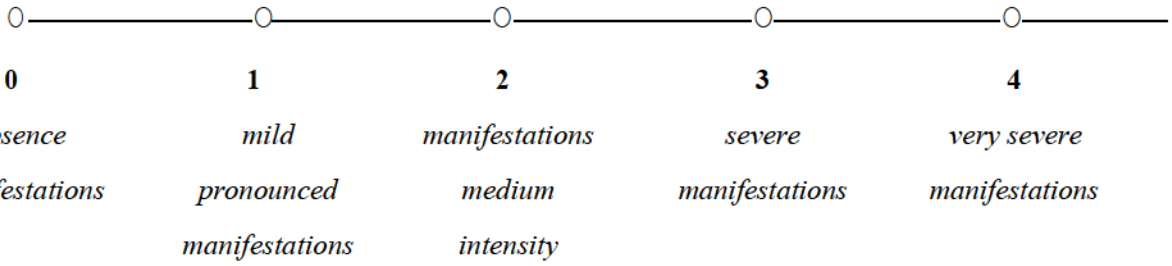
Vomiting: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

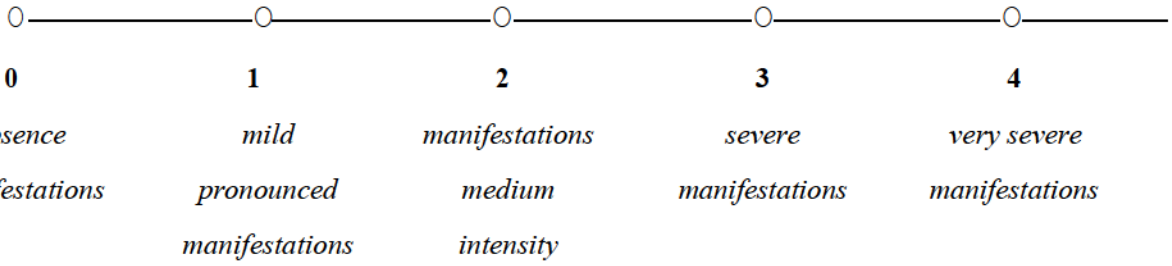
Flatulence: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 14 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced*	*manifestations medium*	*severe manifestations*	*very severe manifestations*

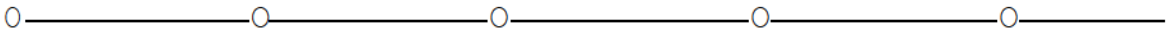
Vomiting: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced*	*manifestations medium*	*severe manifestations*	*very severe manifestations*

Flatulence: ☐ Yes ☐ No

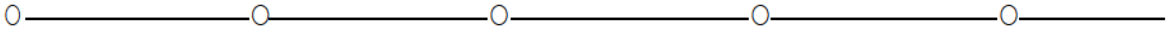
0	1	2	3	4
absence manifestations	*mild pronounced*	*manifestations medium*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



0	1	2	3	4
absence manifestations	*mild pronounced*	*manifestations medium*	*severe manifestations*	*very severe manifestations*

Decreased appetite: ☐ Yes ☐ No



0	1	2	3	4
absence manifestations	*mild pronounced*	*manifestations medium*	*severe manifestations*	*very severe manifestations*

Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 15 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

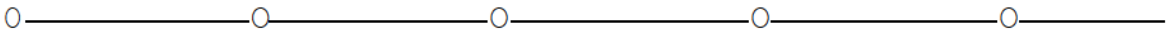
Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

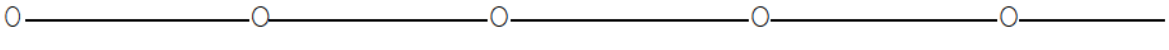
Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No



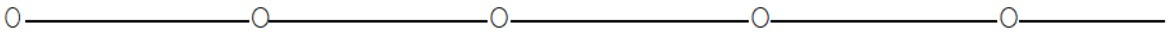
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Vomiting: ☐ Yes ☐ No



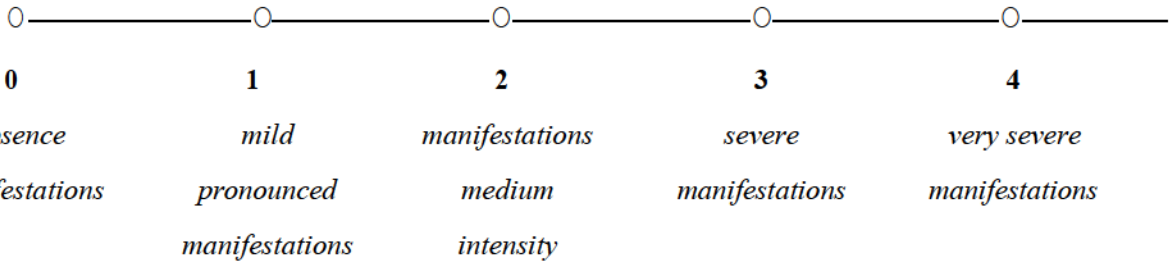
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Flatulence: ☐ Yes ☐ No

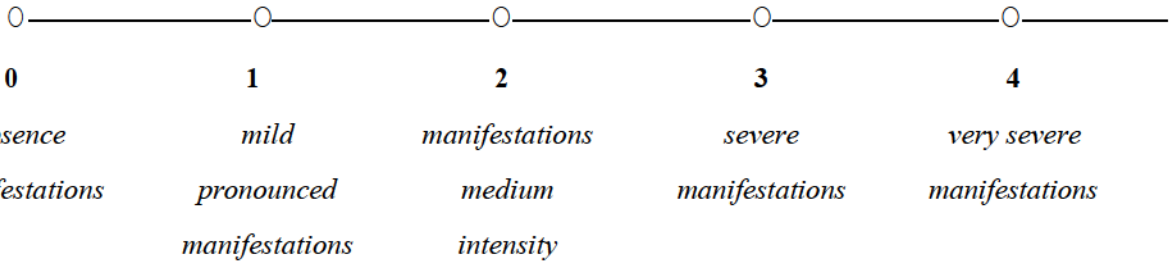


0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 16 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

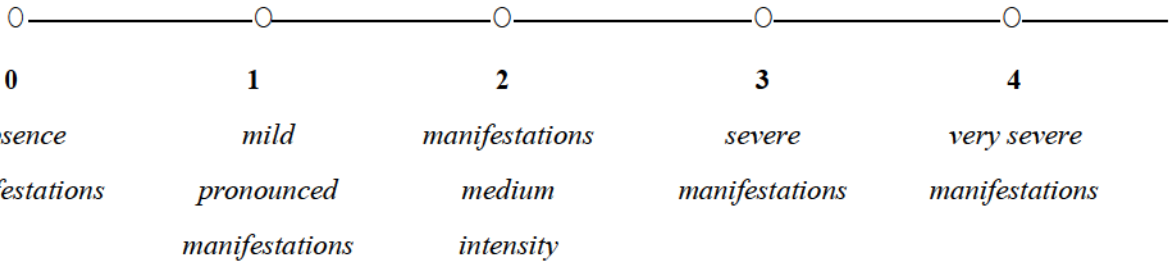
Vomiting: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

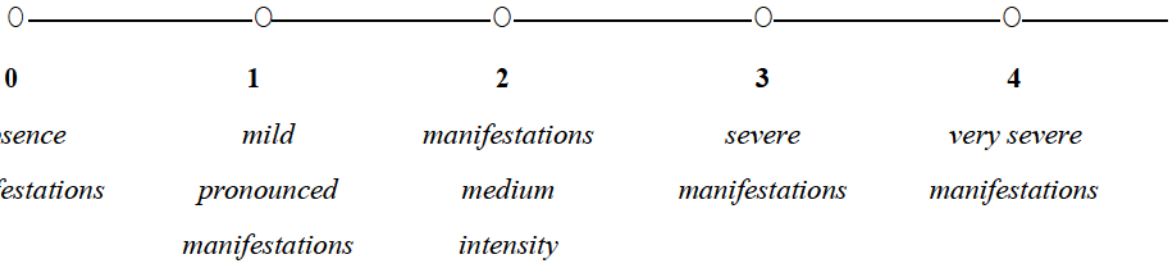
Flatulence: ☐ Yes ☐ No

0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



Decreased appetite: ☐ Yes ☐ No



Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

Filled by the patient: Day No. 17 Date __/__/_____

1. Dosing regimen of investigational medicinal products

Did you take your research drug the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

2. Antibacterial therapy regimen

Did you take the antibiotic the way your doctor prescribed it? ☐ Yes ☐ No:

Comments and complaints:_____

_____

3. Occurrence of stool a day; consistency of stool

Mark the time each chair episode occurred today and indicate the type of consistency (see Figure 1).

1. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
2. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
3. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
4. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
5. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
6. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
7. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
8. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
9. Time: _____
   Type ☐1☐2☐3☐4☐5☐6☐7
10. Time: _____
    Type ☐1☐2☐3☐4☐5☐6☐7

Comments and complaints:_____

_____

If you notice a loose stool or watery shapeless stool with a frequency of 3 or more times a day, you should call and inform your doctor. Your doctor will recommend the drugs approved for use in this study to eliminate symptoms of your condition.

Please remember that the decision to take additional drugs can only be made by a doctor.

4. **Body temperature**

Measure the body temperature in the axillary cavity with a mercury thermometer and record the result:_____

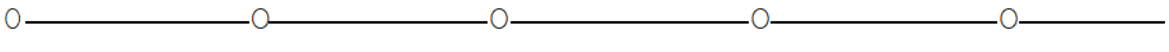
Comments and complaints:_____

_____

5. **Gastrointestinal symptoms**

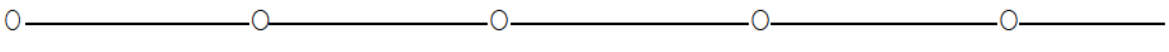
Mark the occurrence of each of these symptoms during the day and rate the severity of symptoms on a 5-point verbal scale (0 to 4):

Nausea: ☐ Yes ☐ No



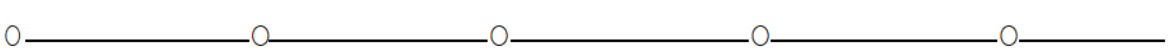
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Vomiting: ☐ Yes ☐ No



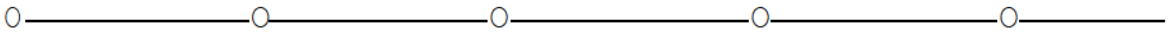
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Flatulence: ☐ Yes ☐ No



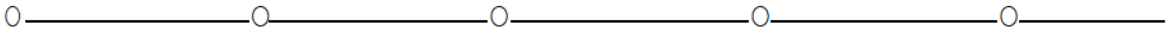
0	**1**	**2**	**3**	**4**
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Abdominal pain: ☐ Yes ☐ No



0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Decreased appetite: ☐ Yes ☐ No



0	1	2	3	4
absence manifestations	*mild pronounced manifestations*	*manifestations medium intensity*	*severe manifestations*	*very severe manifestations*

Comments and complaints:_____

_____

6. Symptomatic therapy

Have you taken any medication to eliminate symptoms of the underlying disease and/or symptoms of diarrhea? ☐ Yes ☐ No:

If yes, provide the name, dose, time and frequency of the medication(s):

_____

_____

_____

_____

_____

17.4. Appendix 4. Bristol Stool Form Scale (according to Lewis S. J. Et al, 1997, adapt. by Kharchenko et al. 2007)

Type of consistency	Parameter	Figure
Type 1	individual hard lumps like nuts (passageway is difficult)	
Type 2	sausage like stool, but lumpy	
Type 3	sausage shaped feces with cracks in the surface	
Type 4	smooth and soft sausage or snake shaped feces	
Type 5	Soft blobs with clear-cut edges	
Type 6	loose pieces with ragged edges	
Type 7	loose stool