CLINICAL PERFORMANCE STUDY PLAN

TransGen Biotech Co., Ltd

SARS-CoV-2 Nucleic Acid Detection Kit

Version 2.0

10-Jun-2022

CONTACT INFORMATION

Sponsor and legal manufacturer:

TransGen Biotech Co., Ltd
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Investigational Site (Biobank)

Biobanco del Hospital Universitario Puerta de Hierro Majadahonda (HUPHM) / Instituto de Investigación Sanitaria Puerta de Hierro-Segovia de Arana (IDIPHISA)
C. Joaquín Rodrigo, 1, 28222 Majadahonda,
Madrid

COMPLIANCE STATEMENT:

This clinical investigation will be conducted in accordance with this Clinical Performance Study Plan (CPSP), the Declaration of Helsinki, law 14/2007 of 3 July 2007 on Biomedical Research, ISO 20916:2019 standards and the Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices (IVDR) and repealing Directive 98/79/EC and Commission Decision 2010/227/EU and EU General Data Protection Regulation. The most stringent requirements, guidelines or regulations must always be followed. The conduct of the clinical performance study will be approved by the appropriate Competent Authority (if applicable) and Ethics Committee (EC) of the respective clinical site and as specified by local regulations before study initiation. Any additional requirement imposed by the EC will be followed.

CLINICAL PERFORMANCE STUDY PROTOCOL SUMMARY

Version Number	V 2.0
Date	10-Jun-2022
Sponsor	**TransGen Biotech Co., Ltd** Building No.4, Zhongguancun Dongsheng International Science Park, North Yongtaizhuang Road, Haidian District, 100192 Beijing P.R.C., China **EU authorized representative:** Riomavix S.L. ES-AR-000001202 Add: Calle de Almansa 55,1D, Madrid 28039 Spain Email: leis@riomavix.com
Clinical Performance Study Name	SARS-CoV-2 Nucleic Acid Detection Kit Clinical Performance Study
Clinical Performance Study Code	CW008
Objective	The primary objective is to determine the diagnostic accuracy (sensitivity and specificity) of the SARS-CoV-2 Nucleic Acid Detection Kit
Device Under Investigation	SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR)
Number of Specimens Required	600 leftover samples
Planned Number of Sites	1 site located in Spain
Study Design	Non-interventional, retrospective, non-randomized, open-label and single-center Clinical Performance Study
Primary Endpoint	The primary accuracy endpoint is to evaluate the sensitivity and specificity of SARS-CoV-2 NAD Kit, defined as the positive SARS-CoV-2 detection rate of the confirmed positive samples, and the negative detection rate for confirmed negative samples, respectively.
Secondary Endpoint	No secondary endpoint in this CPS
Estimated duration	6 months
Inclusion Criteria	1. Specimen collected with a nasopharyngeal swab.

	2. Negative samples from specimens confirmed SARS-CoV-2 negative by PCR, or positive samples from specimens confirmed SARS-CoV-2 positive by PCR.
Exclusion Criteria	1. Specimens that have been stored at 4ºC for more than 24h 2. Specimens stored at -70ºC that have been under more than 2 freeze/thaw cycles 3. Specimens that have been stored at -20 for more than 10 days 4. Contamination and/or deterioration of the specimen that, in the investigator's opinion, may impact its handling and/or analysis
Electronic Data Capture Software	Castor EDC
Investigational site (Biobank)	Biobanco del Hospital Universitario Puerta de Hierro Majadahonda (HUPHM) / Instituto de Investigación Sanitaria Puerta de Hierro-Segovia de Arana (IDIPHISA) C. Joaquín Rodrigo, 1, 28222 Majadahonda, Madrid
Central Laboratory	Biobanco del Hospital Universitario Puerta de Hierro Majadahonda (HUPHM) / Instituto de Investigación Sanitaria Puerta de Hierro-Segovia de Arana (IDIPHISA) C. Joaquín Rodrigo, 1, 28222 Majadahonda, Madrid

INVESTIGATOR SIGNATURE PAGE

I have read the Clinical Performance Study Plan (CPSP) and I agree to conduct this clinical investigation as outlined and in accordance with all applicable laws and regulations, including Good Clinical Practice (GCP) guidelines. In addition, I agree to provide all the information requested in the case report forms presented to me by the Sponsor in a manner to assure completeness, legibility and accuracy.

Investigator Signature	Date (DD-MMM-YYYY)
Investigator Printed Name	Study Role
Investigational Site Name	

SPONSOR SIGNATURE PAGE

The sponsor will conduct the clinical study in compliance with GCP, with the appropriate regulatory requirements and with the Clinical Performance Study Plan (CPSP) agreed to by the sponsor and given approval/favorable opinion by the EC. The sponsor will comply with the procedures for data recording/reporting, and to permit monitoring, auditing and inspection. The sponsor is to retain the essential documents that should be in the investigator files. The sponsor should obtain a signed Clinical Trial Agreement of the institution.

Sponsor Signature	Date (DD-MMM-YYYY)
Sponsor Printed Name	Study Role

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1. INTRODUCTION

Nucleic Acid Detection (NAD) is the primary method to identify the specific nucleic acid sequence of SARS-CoV-2 virus in human specimens. NAD techniques include those based on nucleic acid amplification, such as Polymerase Chain Reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), nested PCR and real-time/quantitative PCR, among others.

The PCR is a laboratory technique used to amplify DNA sequences from a DNA template or RNA previously converted to cDNA. The method makes use of short DNA sequences named primers to select the portion of the genetic material to be amplified.

SARS-CoV-2 Nucleic Acid Detection Kit is an *In Vitro* Diagnostic (IVD) medical device intended for RNA detection of SARS-CoV-2 in human specimens using multiplex real-time polymerase chain reaction (RT-PCR) technology. The conserved regions of *ORF1ab* and *N* genes are used as target sites of the primers and probes according to the indications provided in the Instructions for Use (IFU).

1.1 Background and rationale

Corona viruses are a diverse group of viruses infecting many different animals causing mild to severe respiratory infections in humans. In 2002 and 2012, respectively, two highly pathogenic corona viruses with zoonotic origin, named as severe acute respiratory syndrome corona virus (SARS-CoV) and Middle East respiratory syndrome corona virus (MERS-CoV), emerged in humans and caused fatal respiratory illness, making outbreaks of corona viruses a new public health concern in the twenty-first century[1]. This concern was confirmed in 2019 when a novel corona virus, designated as SARS-CoV-2 and associated with unusual viral pneumonia named Corona virus Disease 19 (COVID-19), sprouted in the city of Wuhan, China, and rapidly spread to create a global pandemic.

SARS-CoV-2 is a positive-sense single-stranded-RNA virus (+ssRNA), whose sequence is approximately 30,000 bases in length. As a novel beta-corona virus, it presents 79% genome sequence identity with SARS-CoV and 50% with MERS-CoV[2]. Besides, its genome organization is shared with other beta-corona viruses, and the six functional open reading frames (ORFs) are arranged in order from 5′ to 3′: replicase (*ORF1a* and *ORF1b*), spike (*S*), envelope (*E*), membrane (*M*) and nucleocapsid (*N*). In addition, seven putative ORFs encoding accessory proteins are interspersed between the structural genes[3].

Regarding the infectious capacity of SARS-CoV-2, any age group is susceptible to contagion, with a median age of infection around 50 years[4–8].

The high transmissibility of SARS-CoV-2 may be attributed to its unique virological features. Transmission of SARS-CoV occurs mainly after illness onset and peaks following disease severity[12]. However, the SARS-CoV-2 viral load in upper respiratory tract samples is highest during the first week of symptoms, and thus, the risk of pharyngeal virus shedding is very high at the beginning of the infection[13,14]. This translates into a high transmissibility during mild disease or the asymptomatic period[15]

Early diagnosis is crucial for controlling the spread of COVID-19 to minimize its impact on economic and health systems. The current diagnostic procedures for the identification of SARS-CoV-2-infected individuals are primarily based on three distinct approaches[16]:

1. Immunological tests detect the host's immune response triggered by the virus through the detection of specific antibodies (IgM, IgG and IgA) directed against nucleocapsid (N) and spike (S) viral proteins[16]. Different types of assays can be used to determine different aspects of the adaptive immune response and functionality of antibodies.
2. Antigen tests are immunoassays that detect the presence of a specific viral antigen, which implies current viral infection.
3. Nucleic Acid Amplification Test (NAAT) is the gold standard method for diagnosis of COVID-19. NAATs for SARS-CoV-2 identify the RNA sequences that comprise the genetic material of the virus. This method

amplifies the virus' genetic material, if present. Amplification enables NAATs to detect very small amounts of SARS-CoV-2 RNA in a specimen, making these tests highly sensitive for COVID-19 detection.

NAATs can use different methods to amplify nucleic acids and detect the virus, and the detection time ranges from several minutes to hours depending on the technology[21,22,24–26].Currently, RT-PCR tests for COVID-19 are often based on specimens collected from the upper respiratory system using swabs. The first step to detect SARS-CoV-2 virus is the conversion of viral genomic RNA into DNA by a reverse transcriptase. This reaction relies on small DNA sequence primers designed to specifically recognize complementary sequences on the RNA viral genome, and on the reverse transcriptase to generate a short complementary DNA copy (cDNA) of the viral RNA. In real-time RT-PCR, the amplification of DNA is monitored as the PCR reaction progresses. This quantification is enabled by a fluorescent dye or a sequence-specific DNA probe labeled with a fluorescent molecule and a quencher molecule (e.g., TaqMan assays). An automated system repeats the amplification process for approximately 40 cycles until the viral cDNA can be detected by a fluorescent or electrical signal[27].

SARS-CoV-2 Nucleic Acid Detection Kit is presented as a method to detect covid cases in a short time as the procedure to obtain results only takes a little more than 6 min. In addition, this kit is intended to accurate the diagnosis of strong positives and strong negatives.

2. IN-VITRO DIAGNOSTIC (IVD) MEDICAL DEVICE OVERVIEW

2.1 IVD Medical Device Description

The SARS-CoV-2 Nucleic Acid Detection Kit is an *In Vitro* Diagnostic (IVD) medical device intended for Qualitative RNA detection of SARS-CoV-2 using multiplex real time RT-PCR technology for professional use. The kit is available in two different formats (48 tests/kit or 200tests/kit), and it can be stored at -20±5ºC away from light for 12 months. This kit includes a PCR Reaction Mix, PCR Enzyme Mix, SARS-CoV-2 PCR Primer/Probe Mix and a negative/positive control (Figure 1).

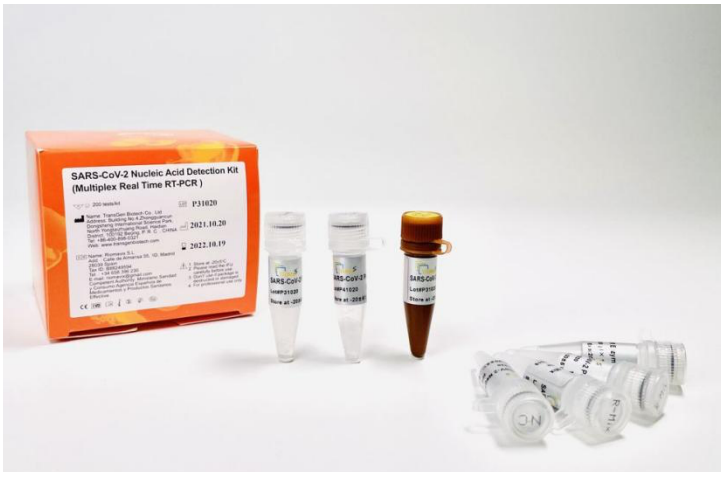

Figure 1: SARS-CoV-2 Nucleic Acid Detection Kit components

Further information about the device and its components can be found in Table 1.

Table 1: Detail of SARS-CoV-2 Nucleic Acid Detection Kit components

Component Name	Main Constituents	Quantity (48 tests)	Quantity (200 tests)
2019-nCoV PCR Reaction Mix	Reaction buffer, dNTPs, etc.	720 μL×1 tube	1000 μL×3 tube
2019-nCoV PCR Enzyme Mix	Reverse transcriptase, RNaseinhibitor, Taqpolymerase, uracil-DNA glycosylase (UDG)	48 μL×1 tube	200 μL×1 tube
2019-nCoV PCR Primer/Probe Mix	Primers and probes for *ORF1ab* and *N* genes; primers and probes for the control-RNase P gene (RP)	192 μL×1 tube	800 μL×1 tube
2019-nCoV Positive Control	*in vitro* transcribed RNA with *ORF1ab* and *N* gene sequences; *in vitro* transcribed RNA with the control-RP gene sequence	50 μL×1 tube	200 μL×1 tube
2019-nCoV Negative Control	RNase-free Water	50 μL×1 tube	200 μL×1 tube

2.2 Intended Use

TransGen SARS-CoV-2 Nucleic Acid Detection Kit is intended for in vitro qualitative detection of *ORF1ab* and *N* genes from the SARS-CoV-2 virus in nasopharyngeal swab specimens collected from COVID-19 suspected cases, suspected clusters of cases or other individuals who need SARS-CoV-2 infection diagnosis or differentiation diagnosis.

The definitions of COVID-19-related groups, such as “suspected cases” or “suspected clusters of cases”, are detailed in the Diagnosis and Treatment Protocol for Novel Corona virus Pneumonia, Surveillance Protocol for Novel Corona virus Pneumonia or other COVID-19 documents from the Centers for Disease Control and Prevention (CDC).

The TransGen SARS-CoV-2 Nucleic Acid Detection Kit should comply with the requirements of Diagnosis and Treatment Protocol for Novel Corona virus Pneumonia, Protocol for Prevention and Control of COVID-19 and other COVID-19 documents from the CDC. The biosafety requirements should be strictly followed as well.

2.3 Intended User

The laboratory personnel for SARS-CoV-2 detection shall be professionally trained on DNA amplification or molecular biology detection, and qualified for related experimental operations. Biosafety protective equipment and programs are required for the laboratories.

2.4 IVD medical device storage

All information on the storage of the kit is detailed in the instructions for use. Briefly, the TransGen SARS-CoV-2 Nucleic Acid Detection Kit should be stored as sealed at -15˚C | -25˚C, which is kept away from light for 12 months. Stability has been previously tested under the following conditions and the results indicate that the shelf life of the reagent is 1 day only at 37˚C

3. CLINICAL PERFORMANCE STUDY OVERVIEW

3.1 Clinical performance study objective

The main and only objective of this clinical performance study (CPS) is to evaluate the SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR) diagnostic accuracy, defined in terms of sensitivity and specificity.

3.2 Clinical performance study duration

The estimated duration of this CPS are 6 months.

4. CLINICAL PERFORMANCE STUDY DESIGN AND PROCEDURES

4.1 Clinical performance study design

This clinical performance study is designed as, non-interventional, retrospective, non-randomized, open-label and single-center study. A total amount of 600 nasopharyngeal swab leftovers from the biobank of Hospital Puerta del Hierro (Spain) will be needed to evaluate the study endpoint.

The clinical performance study has been designed to be conducted at 1 site in Spain. The rationale for the number of sites and sample size is provided in Section 9 Statistical Considerations of this plan.

4.2 Clinical performance study procedures

4.2.1 Specimen overview

4.2.1.1 Specimen identification

Specimens used for this clinical performance study will be remnants of specimens taken for purposes of standard of care (leftover or archived) through a nasopharyngeal swab from male and female subjects. Specimens may come from persons suspected of SARS-CoV-2 infection but also from those who need a diagnostic test due to other reasons such as medical intervention, blood donors, or travel for which a negative test is requested. No demographic data will be required for this CPS.

4.2.1.2 Specimen collection process

The specimen collection will take place in the biobank Hospital Puerta del Hierro. This procedure will be carried out by professionally trained and qualified for related operations laboratory staff in compliance with the GDPR. The person responsible for collecting specimens shall also be equipped with the appropriate Biosafety protective equipment.

4.2.1.3 Specimen storage

Laboratory personnel shall ensure proper storage of specimens in accordance with the GCP. According with IFU, specimens to be used immediately or within 24 hours should be stored at 4ºC. Specimens which cannot be used within 24 hours should be stored at or below -70ºC. If -70ºC is not possible, the specimens to be subsequently tested can be stored at -20ºC for 10 days. Isolated nucleic acid can be stored at -20ºC ± 5ºC for 15 days. Repeated freeze-thaw cycles should be avoided.

- **Specimen handling and transport**

Specimens should be shipped on ice in sealed foam boxes for transportation or adding ice constantly on the way. The results of transport stability evaluation indicate that the negative control and positive control both conform to the requirements of the kit, demonstrating that the strike and vibration during the transportation have no influence on the packaging and detection results.

- **Specimens' disposal**

After the end of the study, specimens will be placed in collection of biobank: The registration number of the collection or name of the biobank, if applicable, will be submitted.

4.2.2 Sample analysis overview

After collecting the specimens in the above manner, the corresponding samples shall be extracted. This process shall be carried out in two steps; in the first step, RNA shall be extracted as per the biobank´s common practice, and then, the RNA will be converted to cDNA and amplified.

4.2.2.1 RNA isolation process

The RNA isolation process will be performed as per the biobank´s Standard of Care, using the RNA extraction kit used in their common practice.The study laboratory personnel will determine if the RNA specimen is appropriate for the qPCR analysis. Isolated RNA can be used directly for detection or can be stored at -70ºC.

4.2.2.2 Nucleic Acid Amplification

- ***Laboratory standard method (gold standard)***

Once the RNA has been extracted, each sample will be analysed by the gold standard (the laboratory standard PCR kit) to determine whether it is classified as positive or negative. In case the sample has already been analysed by PCR that result (Ct or copies/ml) must be used as reference.

- ***Device under investigation***

Samples should be prepared as detailed in the IFU for PCR amplification. In the case of samples stored at -70ºC, avoid repeated freeze-thaw cycles. The PCR amplification process is performed as detailed in Table 2. Prior to running the PCR, select the FAM and VIC channels to detect the SARS-CoV-2 genes *ORF1ab* and *N* respectively, and the Cy5 channel to detect the internal control gene RP. Amplification will be performed on a real-time multiplex PCR instrument with a readout in the FAM and VIC channels to detect both genes simultaneously and in the Cy5 channel to detect the internal control. In case the real-time PCR instrument requests a "Quencher dye" and/or "passive reference", they must be set to "none".

Table 2: PCR steps

STEPS	TEMPERATURE	REACTION TIME	CYCLES
Reverse Transcription	50ºC	5 min	1
Pre-denaturation	95ºC	30 s	1
Denaturation	95ºC	5 s	45
PCR cycling	60ºC	34 s	

For specific information about the interpretation of the results, please refer to the IFU. To consider the experiment as valid, each control in the kit should meet the requirements listed in Table 3.

Table 3: Results interpretation

	Positive Control	Negative Control
FAM channel (ORF1ab gene)	Ct ≤ 32	No Ct value or Ct>40
VIC channel (N gene)	Ct ≤ 32	No Ct value or Ct>40
Cy5 channel (internal standard gene)	Typical S-shaped curve, and Ct ≤ 32	No Ct value or Ct>40

If a typical S-shaped curve is observed after amplification in Cy5 channel of the specimen tested and Ct ≤ 38, the results can be determined as described in Table 4. For specimens tested as positive, when the Ct values of a target

gene are between 38-40, it is necessary to observe whether the amplification curve of the target gene is in sigmoidal shape. The suspected positive specimens should be verified (both amplification curves of FAM and VIC channels).

Table 4: Interpretation for test results

VIC channel \ FAM channel	Ct<=38	38<Ct<=40 (in a sigmoidal shape)	Ct>40
Ct<=38	Positive	Positive	Suspected Positive
38<Ct<=40 (in a sigmoidal shape)	Positive	Suspected Positive	Suspected Positive
Ct>40	Suspected Positive	Suspected Positive	Negative

If discrepant results between the gold standard and the device under investigation, are identified as part of a performance evaluation, these results should be resolved as far as possible, by one or more of the following: evaluation of the discrepant sample in further devices; use of an alternative method or marker; a review of the clinical status and diagnosis of the patient; testing of follow-up samples.

5. STUDY ENDPOINTS

5.1 Primary Endpoint

The primary endpoint of this study is to evaluate the sensitivity and specificity of the SARS-CoV-2 Nucleic Acid Detection Kit in the diagnosis of COVID-19. Their analysis will be performed independently.

5.1.1 Sensitivity

The sensitivity of the SARS-CoV-2 NAD Kit is defined as the capacity to correctly detect confirmed positive specimens of SARS-CoV-2 infection (True Positive, TP).

To comply with the MDCG 2021-21, sensitivity will be assessed in 100 patient samples determined as SARS-CoV-2 RNA positive by the laboratory standard method (golden standard). So different SARS-CoV-2 genotypes/subtypes and mutants are considered, specimens from different stages (from 2020 to 2022) and outbreak clusters of the COVID19 pandemic will be selected from the biobank leftover material.

5.1.2 Specificity

The specificity of the SARS-CoV-2 NAD Kit is the ability to correctly classify specimens with no SARS-CoV-2 infection as negative (True Negative, TN).

To comply with the MDCG 2021-21 specificity will be assessed in 500 SARS-CoV-2 RNA negative human specimens defined so as to reflect the target population for which the device is intended, such as blood donors, hospitalised patients or pregnant women. These specimens must be confirmed in advance as a strong negative by the laboratory standard method.

6. SELECTION OF SPECIMENS

6.1 Specimens and subjects providing specimens

The specimens used in this clinical performance study will be leftovers previously collected from male and female subjects suspected of SARS-CoV-2 infection through a nasopharyngeal swab. In this study, the requirement for informed consent may not apply or may be waived. If necessary, during sample collection, informed consent will be a general form to cover the use of the specimens in this clinical performance study. For further information about the informed consent process, see section 7.

The Principal Investigator or designee, previously trained in this CPSP, will revise specimens' eligibility according to the inclusion/exclusion criteria. Specimens meeting general inclusion criteria and no exclusion criteria shall be suitable to participate in the study.

6.2 Eligibility criteria

The specimens must meet all of the inclusion criteria to be considered for the study. If any of the exclusion criteria are met, the specimen is excluded from the clinical performance study.

6.2.1 Inclusion Criteria

- Specimen collected with a nasopharyngeal swab.
- Negative samples from specimens confirmed SARS-CoV-2 negative by the gold standard, or positive samples from specimens confirmed SARS-CoV-2 positive by the gold standard.

6.2.2 Exclusion Criteria

- Specimens that have been stored at 4ºC for more than 24h
- Specimens stored at -70ºC that have been under more than 2 freeze/thaw cycles
- Specimens that have been stored at -20 for more than 10 days
- Contamination and/or deterioration of the specimen that, in the investigator's opinion, may impact its handling and/or analysis

7. INFORMED CONSENT PROCESS

The requirement for patient informed consent should be based on the risk posed to subjects participating in the clinical performance study. This clinical performance study of an in vitro diagnostic (IVD) medical device does not involve the enrolment of patients. For IVD medical devices, informed consent is only required for specimens specifically collected for the purpose of a clinical performance study.

However, if the clinical performance study will solely use leftover or archived specimens, as in the case of this study, that are not individually identifiable (i.e., devoid of information that would otherwise permit traceability to the patient of origin), the requirement for informed consent may not apply or may be waived.

For this reason, an informed consent process is not necessary.

8. ADVERSE EVENTS AND DEVICE DEFICIENCIES

8.1 Adverse events

An adverse event is any untoward medical occurrence, inappropriate patient management decision, unintended disease or injury, or untoward clinical signs in subjects, users, or other persons, with any connection to study related activities, whether or not related to the IVD medical device under investigation.

Note 1: Adverse events can be caused by, for instance, insufficient or inadequate instructions for use, deployment, installation, operation, or any malfunction of the IVD medical device under investigation.

Note 2: This definition is not intended to be used in determining whether an event is reportable to a regulatory authority.

Note 3: For users or other persons, this definition is restricted to events related to investigational (IVD) medical devices.

Note 4: False negative or false positive results are not considered an adverse event unless in an interventional study, inappropriate patient management decisions are made based on those false results.

The study has been designed to involve as little pain, discomfort, fear, and any other foreseeable risk as possible for subjects. The samples used in the clinical performance study will be obtained from biobank leftover specimens. In this case, the use of leftover samples will pose none additional risks to the subjects, therefore, no adverse events are contemplated for this study.

8.2 Device deficiencies

Device deficiency is defined as inadequacy of a medical device with respect to its identity, quality, durability, reliability, usability, safety or performance

Note 1: Device deficiencies include malfunctions, use errors, and inadequacy in the information supplied by the manufacturer including labelling.

Note 2: This definition includes device deficiencies related to the investigational medical device or the comparator.

8.2.1 Device Deficiency Reporting

To comply with ISO20916:2016 all device deficiencies must to be reported to the sponsor as soon as possible but no later than outlined below.

Clinical Sites	Reporting timelines
All Sites	Device deficiencies/malfunctions must be reported to the Sponsor no later than 3 calendar days from the day the site personnel became aware of the event or as per the investigative site's local requirements, if the requirement is more stringent than those outlined.

Device deficiencies should be reported to the EC per the investigative site's local requirements.

An offline form will be made available to allow the investigator to report device deficiencies/malfunctions in the event that the entry cannot be made in the EDC system. This does not replace the EDC reporting system. All information must still be entered in the EDC system as soon as feasible.

9. STATISTICAL CONSIDERATIONS

The following section describes the statistical methods for the clinical performance study. Additional details on statistical analyses, including justification of study design, sensitivity analyses, poolability analyses, subgroup analyses and analysis of descriptive endpoints, may be maintained in a separate Statistical Analysis Plan (SAP).

This clinical performance study will be conducted at only one site in order to maintain consistency in collecting, storing and processing the large number of specimens required. This is to ensure proper execution of the activities detailed in this CPSP.

9.1 Statistical design, method and analytical procedures

9.1.1 Primary Diagnostic Accuracy Endpoint Analysis

The evaluation of the accuracy of the SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR) in the diagnosis of COVID-19 will be analysed through the definition of the sensitivity and specificity capacity of this test.

- The sensitivity is the ability to detect a target marker (genetic material) associated with SARS-CoV-2. The sensitivity means the capacity to detect positive samples of COVID-19. Therefore, sensitivity will be analysed as the result of the ratio of the True Positive measurements (TP) to total known positive measurements (True Positive + False Negative).

$$Sensitivity = \frac{True\ Positive}{(True\ Positive + False\ Negative)}$$

- The specificity is the ability to recognise the absence of a target marker (for this kit, genetic material), associated with SARS-CoV-2, which means the capacity to detect true negative samples of COVID-19. Consequently, specificity will be determined as the ratio of the True Negative measurements (TN) to total known negative measurements (True Negative + False Positive).

$$Specificity = \frac{True\ Negative}{(True\ Negative + False\ Positive)}$$

9.2 Sample size Calculation and assumptions

Sample size calculation is based on the purpose to determine the diagnostic accuracy of the SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR). According to the requirements defined in the MDCG2021-21, a total number of 600 samples is sufficient to prove these parameters, which will be divided considering different purposes:

- 100 positive samples of SARS-CoV-2 to test sensitivity.
- 500 negative samples of SARS-CoV-2 to test specificity.

No drop-out ratio is expected in this clinical performance study.

9.3 Success criteria

This clinical performance study will be considered successful when the primary objective of diagnosis accuracy, in terms of sensitivity and specificity, is met.

9.4 Subgroup Analysis

Subgroup and stratified analyses may be performed as detailed in the Statistical Analysis Plan (SAP).

9.5 Multiplicity

There is no simultaneous multiple hypothesis analysis testing in this clinical performance study. Therefore, no multiplicity control is required.

9.6 Pooling Strategy

No pooling strategy is planned or necessary for the analysis of the data of this performance study

9.7 Planned Interim Analysis

Interim analysis for this clinical performance study will be completed as per Sponsor discretion.

9.8 Statistical considerations for early termination

There are no statistical criteria for termination of this clinical performance study.

9.9 Procedures for accounting of missing, unusued or spurious data

All variables and endpoints will be assessed as recorded in the database. Therefore, there will not be any imputation of missing data, unless otherwise specified.

10. QUALITY CONTROL AND QUALITY ASSURANCE

10.1 Finances and Agreements

The clinical performance study will be financed by TransGen Biotech Co., LTD. Investigational sites will be compensated by TransGen Biotech Co., LTD for participation in the study per the conditions of agreement between the Sponsor and the Investigational site.

10.2 CPSP Amendments

Any substantial CPSP amendment will be reviewed and approved by the EC prior to its implementation.

Acknowledgement/approval by the EC of the CPSP amendment must be documented in writing prior to implementation of the CPSP amendment. Copies of this documentation must also be provided to the Sponsor.

10.3 Training

10.3.1 Site Training

All Investigators and clinical investigation personnel are required to attend Sponsor training sessions. Over-the-phone or self-training may take place as required. Training of Investigators and clinical investigation personnel will include, but is not limited to, the CPSP requirements, investigational device usage, electronic case report form completion and clinical investigation personnel responsibilities. All Investigators and clinical investigation personnel that are trained must sign a training log (or an equivalent) upon completion of the training. Prior to signing the training log, Investigators and clinical investigation personnel must not perform any CPSP-related activities that are not considered standard of care at the site.

10.3.2 Training Required for the Use of the Device

The laboratory personnel for SARS-CoV-2 detection should be professionally trained with gene amplification or molecular biology detection and qualified for related experimental operations.

Biosafety protective equipment and programs are required for the laboratories.

10.4 Monitoring

Sponsor and/or designee will monitor the clinical investigation over its duration according to the CPSP-specific monitoring plan which will include the planned extent of source data verification. This monitoring plan will be a separate document that can be updated during the course of the study.

Prior to initiating any procedure, the Sponsor monitor (or delegate) will ensure that the following criteria are met:

- The investigator understands and accepts the obligation to conduct the clinical investigation according to the CPSP and applicable regulations, and has signed the Clinical Trial Agreement.
- The Principal Investigator and his/her staff should have sufficient time and facilities to conduct the clinical investigation and should have access to an adequate number of appropriate subjects to conduct the clinical investigation.
- Source documentation (including original medical records) must be available to substantiate proper informed consent procedures, adherence to CPSP procedures, accuracy of data collected on case report forms, and device information.
- The Principal Investigator/site will permit access to such records. A monitoring visit sign-in log will be maintained at the site. The Principal Investigator will agree to dedicate an adequate amount of time to the monitoring process. The Principal Investigator and/or research coordinator will be available for monitoring visits. It is expected that the Principal Investigator will provide the monitor with a suitable working environment for review of clinical investigation-related documents.

10.5 Deviations from CPSP

The Investigator should not deviate from the CPSP for any reason except in cases of medical emergencies when the deviation is necessary to protect the rights, safety and well-being of the subject or eliminate an apparent immediate hazard to the subject. In that event, the Investigator will notify Sponsor immediately by phone or in writing.

No waivers for CPSP deviations will be granted by the Sponsor. All deviations must be reported to the Sponsor using the Deviation CRF. The occurrence of CPSP deviations will be monitored by the Sponsor for evaluation of investigator compliance to the CPSP and regulatory requirements and dealt with according to written procedures. Investigators will inform their EC or equivalent committee of all CPSP deviations in accordance with their specific EC or equivalent committee reporting policies and procedures.

In the event of repeated non-compliance, as determined by the Sponsor, a Sponsor's monitor or company representative will attempt to secure compliance by one or more of the following (and not limited to):

- Visiting the investigator and/or delegate
- Telephoning the investigator and/or delegate
- Corresponding with the investigator and/or delegate

Repeated non-compliance with the signed agreement, the CPSP or any other conditions of the clinical investigation may result in further escalation in accordance with the Sponsor's written procedures, including securing compliance or, at its sole discretion, Sponsor may terminate the investigator's participation in the clinical performance study.

10.6 Quality Assurance Audit

A Sponsor representative or designee may request access to all clinical investigation records, including source documentation, for inspection during a Quality Assurance audit.

In the event that an investigator is contacted by a Regulatory Agency in relation to this clinical investigation, the Investigator will notify Sponsor immediately. The Investigator and Research Coordinator should be available to respond to reasonable requests and audit queries made during the audit process. The Investigator should provide Sponsor with copies of all correspondence that may affect the review of the current clinical performance study (e.g., Inspectional Observations, Warning Letters, Inspection Reports, etc.). Sponsor may provide any needed assistance in responding to regulatory audits.

11. DATA HANDLING AND RECORD KEEPING

Sponsor and/or its affiliates will maintain documentation of the systems and procedures used in data collection for the duration of the clinical investigation.

CRF data collection will be performed through a secure web portal and only authorized personnel will access the Electronic Data Capture (EDC) system using a unique username and password to enter, review or correct data. Passwords and electronic signatures will be strictly confidential.

The data will be subjected to consistency and validation checks within the EDC system and supplemental review by the Sponsor.

At the conclusion of the clinical performance study, completed CRF images with the date-and-time stamped electronic audit trail indicating the user, the data entered, and any reason for change (if applicable) will be provided to the investigational sites.

For the duration of the study, the Investigator will maintain complete and accurate documentation including, but not limited to, medical records, study progress records, laboratory reports, CRFs, signed ICFs, device accountability records (if applicable), correspondence with the EC and clinical investigation monitor/Sponsor, adverse event and device deficiency reports, and information regarding discontinuation or completion of the clinical performance study.

11.1 Protection of Personally Identifiable Information

The processing, communication and transfer of personal data of all participants will comply with the EU Regulation 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and the free movement of data, and with the Organic Law 3/2018 of 5 December on the Protection of Personal Data and guarantee of digital rights. The legal basis justifying the processing of your data is the consent you give in this act, in accordance with the provisions of Article 9 of EU Regulation 2016/679.

The data collected for this study will be collected from subjects that will be uniquely identified by a code (to be agreed with the biobank), so no information will be included that would allow participants to be identified. The identity of the participants will not be available to any other person except in the case of a medical emergency or legal requirement.

Access to the identified personal information may be granted to the health authorities, the Research Ethics Committee and personnel authorised by the study sponsor, when necessary to verify study data and procedures, but always maintaining confidentiality in accordance with current legislation.

Only coded data will be transferred to third parties and to other countries, and under no circumstances will they contain information that could directly identify the participant (such as name and surname, initials, address, social security number, etc.). If such a transfer were to take place, it would be for the same purpose as the study described above, and confidentiality would be guaranteed.

As project promoters, we are committed to processing data in accordance with EU Regulation 2016/679 and, therefore, to keep a record of the processing activities we carry out and to carry out a risk assessment of the processing we carry out, in order to know what measures, we will have to apply and how to do it.

In addition to the rights already provided for in the previous legislation (access, modification, opposition and cancellation of data, deletion in the new Regulation), participants can now also limit the processing of data collected for the project that are incorrect, request a copy or that they be transferred to a third party (portability). To exercise these rights, they should contact the principal investigator of the study or the corresponding Data Protection Officer. They also have the right to contact the Data Protection Agency if they are not satisfied.

11.2 Data Management Plan

A Data Management Plan (DMP) will describe procedures used for data entry and collection. Data review and data cleaning, and issuing, resolving data discrepancies and methods for data base lock. The DMP will include procedures for the verification, validation and securing of electronic clinical system, if applicable. As well as procedures for maintaining and protecting subjects' privacy.

If appropriate, the DMP may be updated throughout the duration of the clinical investigation. All revisions will be tracked and document controlled.

11.3 Source Documentation

The investigator/site will permit direct access to source data/documents for the purpose of performing clinical investigation-related monitoring, audits, EC review and regulatory inspections.

Subjects providing informed consent are agreeing to allow clinical investigation monitors or regulatory authorities including foreign countries to review, in confidence, any records identifying the subjects in this clinical investigation. This information may be shared with regulatory agencies; however, Sponsor undertakes not to otherwise release the subject's personal and private information.

11.4 Case Report Form Completion

Primary data collection based on source-documented hospital and/or clinic chart reviews will be performed clearly and accurately by site personnel trained on the CPSP and CRF completion. The investigator will ensure accuracy, completeness, legibility and timeliness of the data reported to the Sponsor on the CRFs and in all required reports.

Data on CRFs will be collected for all specimens to be used into the clinical investigation. Only authorized site personnel will be permitted to enter the CRF data through the EDC system deployed by the Sponsor. An electronic audit trail will be used to track any subsequent changes of the entered data.

11.5 Record Retention

The Sponsor and Investigator/Site will archive and retain all documents pertaining to the clinical performance study for at least 10 (ten) years as per the applicable regulatory record retention requirements. The Investigator must obtain permission from Sponsor in writing before destroying or transferring control of any study records.

11.6 Accountability of IVD medical devices under investigation

The Investigator or an authorized designee may maintain adequate records of the receipt and disposition of each investigational device, including part number, batch number, and serial number (if applicable), date used, subject identification, and treating physician. This documentation shall reflect that investigational device can only be used in the context of this clinical performance study and according to this CPSP.

Storage conditions for the devices at investigational sites shall maintain the requirements and conditions specified in the IFU. Storage locations for the devices at investigational sites must be locked with access restricted only to investigators and authorized personnel.

All investigational devices that are associated with a device failure or device deficiency must be notified immediately to the Sponsor, no later than 3 calendar days.

12. RISK ANALYSIS

According with ISO20916:2019 the studies for IVD medical devices which are performed using samples resulting from the remnants of specimens taken for purposes of standard of care (leftover or archived). There is no risk for

the subjects arising from either the information provided by the IVD medical device or from the collection procedure of the specimen.

13. PUBLICATION POLICY

The data and results from the clinical performance study are the sole property of the Sponsor. The Sponsor shall have the right to access and use all data and results generated during the clinical performance study. The Investigators will not use this clinical investigation-related data without the written consent of the Sponsor for any purpose other than for clinical investigation completion or for generation of publication materials, as referenced in the Clinical Trial Agreement. Any proposals for publications or presentations by the investigators must be reviewed and approved by the Sponsor in a timely manner to enable Sponsor review in compliance with the Sponsor's publication policy set forth in the Clinical Trial Agreement.

The Sponsor will be responsible for registering the clinical investigation in a publicly accessible database, such as www.clinicaltrials.gov, in accordance with the International Committee of Medical Journal Editors guidelines, ISO 20916:2019, and any other applicable international guideline. The Sponsor shall be responsible for any such registration and results posting as required by the ClinicalTrials.gov website. The Site(s) and/or Principal Investigator(s) shall not take any action to register the clinical investigation in public databases.

14. CLINICAL PERFORMANCE STUDY CONCLUSION

The clinical performance study will be concluded when the final report has been provided to investigators or the Sponsor has provided formal documentation of study closure.

To comply with EU IVDR 2017/746 (article 73 - Section 5), irrespective of the outcome of the clinical performance study, within one year of the end of the performance study or within three months of the early termination or temporary halt, the Sponsor shall submit to the Member States in which a performance study was conducted a performance study report.

APPENDIX I: ABBREVIATIONS AND ACRONYMS

Abbreviation/Acronym	Words
AE	Adverse Event
ADE	Adverse device effect
CPSP	Clinical Performance Study Plan
CRF	Case Report Form
CSR	Clinical study report
CTA	Clinical Trial Agreement
DMP	Data Management Plan
EC	Ethics committee
EDC	Electronic Data Capture
ICF	Informed Consent Form
IFU	Instructions for Use
IVD	In vitro diagnostic
NAD	Nucleic Acid Detection
NAT	Nucleic acid tests
PCR	Polymerase chain reaction
PI	Principal Investigator
SAP	Statistical Analysis Plan
SAE	Serious Adverse event
SC	Steering Committee

APPENDIX II: DEFINITIONS

Specimen:

Discrete portion of a body fluid or tissue taken for examination, study, or analysis of one or more quantities or characteristics to determine the character of the whole.

Sample:

One or more representative parts taken from a specimen which are intended to provide information.

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APPENDIX IV: REVISION HISTORY

This CPSP may be amended as appropriate by the Sponsor. Rationale will be included with each amended version in the revision history table below. The version number and date of amendments will be documented.

EC and relevant Regulatory Authorities, if applicable, will be notified of amendments to the CPSP.

Author	Amendment Number	Version	Date	Details	Rationale
AKRN Scientific Consulting	Not Applicable	1.0	03-Mar-2022	First release of CPSP	NA
AKRN Scientific Consulting	I	2.0	10-Jun-2022	Removal of Sponsor's extraction kit and endpoint thresholds	The extraction kit to be used will be as per SOC at the investigational site, instead of one facilitated by the Sponsor. The specificity and sensitivity will be determined as per the SAP, but no thresholds are pre-defined.