A MULTICENTER, RANDOMIZED, CONTROLLED STUDY TO EVALUATE THE EFFICACY AND SAFETY OF SHORT-TERM PLASMA EXCHANGE FOLLOWED BY LONG-TERM PLASMAPHERESIS WITH INFUSION OF HUMAN ALBUMIN COMBINED WITH INTRAVENOUS IMMUNOGLOBULIN IN PATIENTS WITH MILD-MODERATE ALZHEIMER'S DISEASE

Sponsor for the Clinical Trial:	Instituto Grifols, S.A. Polígono Levante, C/ Can Guasc 2 08150 - Parets del Vallès Barcelona Spain
Contract Resource Organization:	[redacted]
Product:	Albutein Flebogamma® DIF
Type of study:	Multicenter, randomized, controlled, parallel-group.
Protocol Number:	IG1002
Study Phase	2b/3
EudraCT Number	2011-1598-25
Version Number:	5.0 (IND version)
Date:	February 2018

A MULTICENTER, RANDOMIZED, CONTROLLED STUDY TO EVALUATE THE EFFICACY AND SAFETY OF SHORT-TERM PLASMA EXCHANGE FOLLOWED BY LONG-TERM PLASMAPHERESIS WITH INFUSION OF HUMAN ALBUMIN COMBINED WITH INTRAVENOUS IMMUNOGLOBULIN IN PATIENTS WITH MILD-MODERATE ALZHEIMER'S DISEASE

SIGNATURE/APPROVAL PAGE

Sponsor

Laura Núñez, BSc
Clinical Project Manager
Instituto Grifols, S.A.
[redacted]
Date 21 feb 2018

Carlota Grifols i Vilella, MSc
Clinical Research Associate
Instituto Grifols, S.A.
[redacted]
Date 21 feb 2018

Antonio Páez, MD
Clinical Assessment Monitor
Medical and Technical Director
Instituto Grifols, S.A.
[redacted]
Date 21 Feb 2018

A MULTICENTER, RANDOMIZED, CONTROLLED STUDY TO EVALUATE THE EFFICACY AND SAFETY OF SHORT-TERM PLASMA EXCHANGE FOLLOWED BY LONG-TERM PLASMAPHERESIS WITH INFUSION OF HUMAN ALBUMIN COMBINED WITH INTRAVENOUS IMMUNOGLOBULIN IN PATIENTS WITH MILD-MODERATE ALZHEIMER'S DISEASE

Protocol Number: IG1002

Version Number: Version 5.0 (IND version)

Version Date: February 2018

AUTHORIZED SIGNATURE PAGE

I have read this protocol and agree to conduct this trial in accordance with Good Clinical Practice (GCP), all stipulations of the protocol, the Declaration of Helsinki and applicable regulatory requirements.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the conduct of the study.

Investigator: _____

(Print Name)

_____

(Signature) (Date)

PRIMARY CONTACT INFORMATION

For the Sponsor

[redacted]
Clinical Project Manager
Instituto Grifols, S.A.
Can Guasc, 2
Parets del Vallès
08150 Barcelona. SPAIN
[redacted]

For the Sponsor

[redacted]
Clinical Research Associate
Instituto Grifols, S.A.
Can Guasc, 2
Parets del Vallès
08150 Barcelona. SPAIN Phone:
[redacted]

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ABS	Agitated Behavior Scale
ACD-A	Acid Citrate-Dextrose A
ACEI	Angiotensin-Converting Enzyme Inhibitor
AChEI	Acetylcholine Esterase Inhibitor
AD	Alzheimer's Disease
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive
ADCS-ADL	Alzheimer's Disease Cooperative Study-Activities of Daily Living
ADCS-CGIC	Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change
ADL	Activities of Daily Living
AE	Adverse Event
ANCOVA	Analysis of covariance
APP	Amyloid Precursor Protein
Apo E	Apoenzyme E
Apo J	Apoenzyme J
aPPT	activated Partial Thromboplastine Time
AR	Adverse Reaction
Aβ	Beta-amyloid
$A\beta_{1-40}$	Beta-amyloid 40 peptide
$A\beta_{1-42}$	Beta-amyloid 42 peptide
BBB	Blood brain barrier
BNT	Boston Naming Test
BST	Banc de Sang i Teixits
CAT	Computed Axial Tomography
CDR-sb	Clinical Dementia Rating-Sum of boxes
CI	Confidence Interval
CK	Creatine Kinase
cm	Centimeter
CNS	Central Nervous System
CHMP	Committee for Human Medicinal Products
CRF	Case Report Form
CSDD	Cornell Scale for Depression in Dementia
C-SSRS	Columbia-Suicide Severity Rating Scale
CSF	Cerebral Spinal Fluid
3D-SPGR	Three-Dimensional SPoiled Gradient-Recalled
DICOM	Digital Imaging and Communications in Medicine
dL	Deciliter
EC	European Commission
ECG	Electrocardiogram
EMEA	European Medicines Agency
FDA	Food and Drug Administration
FFP	Fresh Frozen Plasma

GCP	Good Clinical Practice
GOT	Aspartate aminotransferase (Glutamate Oxaloacetate Transaminase)
GPT	Alanine aminotransferase (Glutamic Pyruvic Transaminase)
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HDL	High Density Lipoprotein
ICH	The International Conference on Harmonisation (of Technical Requirements for Registration of Pharmaceuticals for Human Use)
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1	Interleukin-1
IL-6	Interleukin-6
IP	Investigational Product
IRB	Institutional Review Board
i.v.	Intravenous
IVIG	Intravenous Immunoglobulin
kg	Kilogram
L	Liter
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
MBq	Megabecquerel
MEDRA	Medical Dictionary for Regulatory Activities
µg	Microgram
mg	Milligram
min.	Minute
µL	Microliter
mL	Milliliter
mm	Millimeter
MMSE	Mini-Mental Status Examination
MRI	Magnetic Resonance Imaging
mT/m	Millitesla per meter
NGF	Nerve Growth Factor
NINCDS-ADRDA	National Institute of Neurological and Communication Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NPI	Neuropyschiatric Inventory
NPS Battery	Neuropyschiatric Battery
OAS	Overt Aggression Scale
OSEM	Object Search Engine Mapping
pg	Picogram
p.o.	By mouth (Latin “per os”)
PP	Per protocol

p-Tau	Phosphorylated Tau protein
QoL-AD	Quality of Life Alzheimer Disease
RUD-Lite®	Resource Utilization in Dementia
s.c.	Subcutaneous
SDMT	Symbol Digit Modalities Test
SPECT	Single Photon Emission Computed Tomography
SPM	Statistical Parametric Mapping
^{99m}Tc-ECD	Technetium-99m Ethyl Cysteinate Dimer
TGF	Transforming growth factor
T/m/s	Tesla per meter per second
T-Tau	Total Tau protein
UBC	United Biosource Corporation
ULN	Upper Limit of Normal
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization

PROTOCOL SYNOPSIS (ALBUMIN GRIFOLS + FLEBOGAMMADIF)

STUDY TITLE	A multicenter, randomized, controlled study to evaluate the efficacy and safety of short-term plasma exchange followed by long-term plasmapheresis with infusion of human albumin combined with intravenous immunoglobulin in patients with mild-moderate Alzheimer's disease.
SPONSOR	Instituto Grifols, S.A.
INVESTIGATIONAL PRODUCT AND FORMULATION	Treatment Group: Albutein® Flebogamma® DIF Albutein® and Flebogamma® DIF are approved in USA. Control group: Sham procedure.
INDICATIONS	Probable mild to moderate Alzheimer's disease
OBJECTIVES	**Primary objective** To evaluate the changes in the cognitive, functional, behavioral and global domains based on the different applicable psychometric batteries and scales. **Secondary objectives** • To determine the changes in the concentration of beta-amyloid peptide in plasma and cerebrospinal fluid (CSF) in the treatment group of patients with Alzheimer's disease (AD). • To evaluate the structural changes in volume of the hippocampus, posterior cingulate area, and other associated areas based on neuroimaging studies with Magnetic Resonance Imaging (MRI) (variations versus baseline). • To determine functional brain functional changes through FDG-PET (fluordeoxyglucose-PET). • To determine whether plasma exchange with human albumin combined with intravenous immunoglobulin (IVIG) is safe, taking into account the following factors: - Type, severity and frequency of adverse reactions during and after the procedure and infusions. - Changes in vital signs and clinically relevant changes, according to the laboratory test findings. - Control of episodes of cerebrovascular accidents with MRI.
METHODOLOGY	A clinical trial comprised of 364 subjects with probable mild to moderate AD will be conducted primarily to determine whether short-term followed by long-term, low-volume plasma exchange with human albumin combined with IVIG is able to modify patient's cognitive, functional, behavioral

	and global domains. There will be 3 treatment groups and one control group. The subjects will be randomized in a 1:1:1:1 proportion. After screening and randomization, treatment groups will proceed as follows: • One month and a half (6 weeks) of intensive treatment with one plasma exchange per week. All 3 treatment groups are the same during the intensive treatment phase. • Two of the three groups will follow 12 months of maintenance treatment with one low-volume plasma exchange every month combined with IVIG every 4 months administered at the end of the corresponding plasmapheresis instead of albumin (that is, 9 plasmaphereses with Albumin replacement and 3 plasmaphereses with IVIG replacement). During this year patients will follow one of the two different pre-allocated treatment arms: 1) with the doses of albumin and IVIG needed to replace those removed during the plasmapheresis, 2) with half of the doses of albumin and IVIG, (see **Investigational Product Dosage** below for specific dosage and schedule). • One of the three groups will follow the same schedule as the above two groups but with half of the doses of albumin alone (without IVIG), that is, 12 plasmaphereses with half-dose albumin alone. Patients in the control group will undergo sham procedures mimicking plasmaphereses but with neither fluid exchange nor albumin or IVIG administrations.	
CLINICAL STUDY DESIGN AND DESCRIPTION	A multicenter, randomized, controlled, parallel-group study	
POPULATION SIZE AND GROUPS	364 subjects with probable mild to moderate AD (NINCDS-ADRDA criterion; McKhann, *et al.* 1984.) and Mini-mental Status Examination (MMSE) score between ≥18 and ≤26. There will be sites in Spain and USA. The site of the Coordinating Investigator (Fundació ACE/Hospital Vall d'Hebron, Barcelona, SPAIN) will be the first site to be included. The rest of the sites will be incorporated progressively after the first site. Several months can elapse between the inclusion of the Spanish sites and the rest of sites.	
CLINICAL STUDY DURATION	Enrollment period:	Aproximately 12 months
	Subject participation (since enrollment):	Up to 14 months
MAIN SUBJECT INCLUSION CRITERIA	In order to be eligible for participation in the trial, the patients must meet the following requirements:	

	1. Males or females between 55-85 years of age at the time of signing of the informed consent document. 2. A diagnosis of AD (NINCDS-ADRDA criterion), and Mini-mental Status Examination (MMSE) score between ≥18 and ≤26. 3. Current stable treatment with acetylcholine esterase inhibitors (AChEIs) and/or memantine for the previous three months. 4. The patient and a close relative or legal representative must read the patient information sheet, agree to participation in the trial, and then sign the informed consent document (the patient personally and the close relative/legal representative). 5. The patient must be able to follow the study protocol, receive the treatment in the established time period, and continue during the follow-up interval. 6. A brain Computed Axial Tomography (CAT) or Magnetic Resonance Imaging (MRI) study, obtained in the 12 months prior to recruitment, showing the absence of cerebrovascular disease, should be available. Nevertheless, it is mandatory to use the MRI obtained during the screening period to rule out any cerebrovascular disease such as microhemorrages, infarction, hematoma, stroke, meningioma or other finding that could affect patient safety. 7. A stable care taker must be available, and must attend the patient study visits. Patients meeting any of the following criteria will not be able to participate in the trial: 1. Any contraindication for plasma exchange due to behavioral disorders or abnormal coagulation parameters, such as for example: • Hypocalcemia (Ca^{++} < 8.7 mg/dL). • Thrombocytopenia (<100,000/μL). • Fibrinogen <1.5 g/L. • Prothrombin time (Quick) p<60% versus control (INR>1.5). • Beta-blocker treatment and bradycardia <55/min. • Treatment with angiotensin-converting enzyme inhibitors (ACEIs) (increased risk of allergic reactions). 2. Hemoglobin < 10 g/dL 3. Difficult venous access precluding plasma exchange. 4. A history of frequent adverse reactions (serious or

	otherwise) to blood products. 5. Hypersensitivity to albumin or allergies to any of the components of Albutein®. 6. History of immunoglobulin A (IgA) deficiency. 7. Known allergies to Flebogamma® DIF components such as sorbitol. 8. History of thromboembolic complications of intravenous immunoglobulins. 9. Plasma creatinine > 2 mg/dL. 10. Uncontrolled high blood pressure (systolic blood pressure of 160 mmHg or higher and/or diastolic blood pressure of 100 mmHg or higher despite regular treatment during the last 3 months). 11. Liver cirrhosis or any liver problem with alanine aminotransferase (GPT) > 2.5 x upper limit of normal (ULN), or bilirubin > 2 mg/dL. 12. Heart diseases as evidenced by myocardial infarction, severe or unstable angina, or heart failure (New York Heart Association Class II, III or IV) in the past 12 months. 13. Participation in other clinical trials, or the reception of any other investigational drug in the three months prior to the start of the study. 14. Any condition complicating adherence to the study protocol (illness with less than one year of expected survival, known drug or alcohol abuse, etc.). 15. Pregnant or nursing women or women not using effective contraceptive methods for at least one month after plasma exchange. 16. Fewer than six years of education (exclusion criteria under medical criterion). 17. Less than three months with stable treatment for behavioral disorders insomnia. 18. Patients being treated with anticoagulants or antiplatelet therapy (antiaggregants) should not be recruited in the study.
INVESTIGATIONAL PRODUCT DOSAGE	During the treatment phase, and after the screening visits, the subject will undergo the following plasma exchanges: • a maximum of six full replacements with albumin 5% (Albutein®, an approved medicinal product) within six weeks during the intensive treatment period (all groups) • a maximum of 9 plasmaphereses with albumin 20% (Albutein®, an approved medicinal product) replacement plus 3 plasmaphereses with IVIG replacement during the maintenance period (two groups). • one group will undergo 12 plasmaphereses with albumin

	20%, without IVIG The six full replacement sessions of the intensive treatment period will be completed in six weeks (one session per week). Conventional plasma-exchange devices will be used in this intensive period. The 12 low-volume replacement sessions (plasmapheresis + albumin or IVIG replacement) of the maintenance period will be completed in 12 months (one session per month). A prototype based on the Auto-C™ device (Fenwal Inc, Lake Zurich, IL, USA. FDA PMA approval # BP850001) or the Aurora™ device (Fenwal Inc, Lake Zurich, IL, USA. FDA 510(k) clearance # BK110072) will be used in all sites where the low-volume replacement sessions will be performed. The volume of plasma taken on each full plasma exchange during the intensive period will be approximately that of the plasma volume of the subject as calculated from body weight, height and hematocrit (approximately 35-45 mL/kg, corresponding to a volume of 2500-3000 mL). Approximately the same volume will be replaced with albumin 5%. During the maintenance period the volume of plasma taken on each low-volume replacement will be that of a regular plasmapheresis (maximun of 880 mL, depending on the patient weight) which will be replaced with albumin 20% or IVIG, according to the treatment arm, as follows: - 1st arm: a maximum of 200 mL of albumin 20% since this volume of albumin 20% contains approximately the same amount of albumin as that of 880 mL of plasma. When IVIG is used, 20 g of IVIG will be administered after plasmapheresis for the same reason as above. - 2nd arm: a maximum of 100 mL of albumin 20% and 10 g of IVIG. - 3rd arm: a maximum of 100 mL of albumin 20% alone, without IVIG. Albumin doses will be adjusted according to the actual volume of plasma removed (depending on the patient weight). IVIG doses will be fixed for each group.
COMPARATOR PRODUCTS	N/A
MODALITIES OF TREATMENT ADMINISTRATION	Albutein® administered intravenously. Flebogamma® DIF administered intravenously.
MAIN STUDY PARAMETERS	**Efficacy variables:** • Change from baseline in the cognitive scores as measured by ADAS-Cog (6 measurements: weeks -3, -2

	or -1, and 7-8, months 6, 9, 12 and 14) within the 3 treatment arms. • Change from baseline in ADCS-ADL inventory (6 measurements: weeks -3, -2 or -1 and 7-8; months 6, 9, 12 and 14) within the 3 treatment arms. **Secondary variables:** • Change from baseline in the cognitive, functional and neuropsychiatric scores and overall development as measured by MMSE, NPS battery, NPI, CDR-Sb, ADCS-CGIC, CSDD, C-SSRS. (6 measurements: weeks -3, -2 or -1 and 7-8; months 6, 9, 12 and 14) and QoL-AD, RUD-Lite® (5 measurements: weeks -3, -2 or -1; months 6, 9, 12 and 14). Moreover the optional questionnaires like OAS and ABS could be used at any visit in case any sign of aggression or restlessness. • Variation in levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in CSF in the period between baseline lumbar puncture (before the start of the Intensive treatment period) and lumbar puncture immediately after the end of the last low-volume plasmapheresis (whenever this may be) • Variation in the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in CSF between the finalization and beginning of each of the two treatment periods. • Levels of β_{1-40}, $A\beta_{1-42}$, T-tau and P-tau in CSF throughout the study. • Plasma levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ before and after each plasma exchange for both treatment periods. • Structural changes in volume of the hippocampus, posterior cingulate area, and other associated areas by MRI. Five measurements will be made (weeks -3, -2 or -1 and 7-8; months 6, 9 and 14). • Variation in FDG-PET patterns (4 measurements: weeks -3, -2 or -1 and 7-8; months 9 and 14). **Safety variables:** • The primary criterion of safety will be the percentage of plasma exchanges associated with at least one adverse event that may be related to the study procedure (adverse reactions) within 72h after infusion completion (or after the infusion stops). In addition, the percentage of plasma exchanges involving some adverse event, whether or not related to the procedure, will be considered in general. • Vital signs will be recorded before, during and after each plasma exchange session, where required. Various laboratory test parameters (blood cell counts, platelet count, prothrombin time (Quick), aPTT, fibrinogen, total proteins, and calcium) will also be assessed when

	necessary. • During the treatment periods (before each plasma exchange) and on the days when there is no replacement, anxiety and restlessness tests will be made based on the Overt Aggression Scale (OAS) and the Agitated Behavior Scale (ABS), whenever considered opportune. • According to the criterion of the investigator, all clinically relevant changes in vital function, laboratory test parameters and neuroimaging findings will be evaluated.
ANALYTICAL PLAN / STATISTICAL METHOD	All subjects included in the study and subjected to at least one plasma exchange session (i.e., randomized and treated) during the intensive treatment phase (the six first weeks of treatment) will form part of the efficacy population. Control group subjects will also be included (without the plasma exchange) if they attended at least 1 of the 6 intensive treatment phase visits. A second efficacy analysis will also be carried out (per protocol analysis, PP) with the subjects who complete the treatment without major breaches in the study protocol. The statistical tests will be performed with a 5% significance level and will be two-sided. In addition to the tests, two-sided 95% confidence intervals (95% CI) will be reported. The analysis of tolerability will be based on description of the safety variables according to their nature. The adverse reactions will be coded according to the adverse events classification of the World Health Organization (WHO) (MedDRA current version), and will be described by a synonym (Lowest Level Term) and the affected organ / system, the intensity, causality and seriousness. The statistical analysis will be carried out by UBC (United Biosource Corporation).

INTRODUCTION

1.1 Rationale

1.1.1 Characteristics of Alzheimer's disease and Its Relation to β-Amyloid Peptide

Dementia, a mental condition that is related to aging, has become a major public health problem. The proportion of elderly people is increasing in the population, and in future the number of individuals with dementia inevitably will increase as well. This in turn will imply an increased burden for health care systems. Over half of all patients with dementia suffer Alzheimer's disease (AD). At present, the prevalence of AD in Europe is 2% for the 65-69 years age interval, 4% for the 70-74 years age interval, and 8% in the 75-80 years age group. The incidence increases exponentially with advancing age and doubles every five years.

AD is characterized by progressive memory and cognitive impairment, with an important impact upon patient social and occupational activities. It is currently accepted that there is no cure for AD, and its molecular causes have not been fully clarified to date. It is assumed that the clinical phase is preceded by a 15- to 30-year preclinical period. However, no existing AD diagnostic or follow-up methods based on biological markers or tests are able to detect all early cases of the disease. Indeed, the detection and analysis of the histopathological changes must be based on the brain findings at autopsy, thus, diagnosis can only be confirmed post-mortem. Current treatment is not curative but symptomatic, and its objective is to improve patient memory and cognitive functions, or at least to stabilize them to ensure maximum patient autonomy[1]. It is assumed that in the future treatment will not be based on a single management modality; rather, simultaneous interventions will focus on several therapeutic targets.

At present it is only possible to evaluate progression of the disease on the basis of the clinical data, cognitive function scales and imaging and biochemical marker (i.e., subrogate marker) diagnostic tests.

AD, as a neurodegenerative process, is expressed by cognitive impairment, which manifests in the form of memory loss, speech alterations, visual-spatial deficits, impaired recognition capacity and attentional loss among the most relevant neuropsychological aspects. The cognitive alterations are correlated to the evolutional stage of AD and the capacity of the affected individual to perform activities of daily living (ADL). Non-cognitive symptoms or neuropsychiatric alterations often accompany AD, depression and behavioral disorders being the most prevalent manifestations in the early stages of the disease. The neuropsychological defects and the functional capacities and behavioral disorders or mood states are assessed by means of neuropsychological batteries and scales (designed ad hoc). The differences in pre- and post-interventional measures are the parameters used to evaluate the efficacy of the currently employed pharmacological treatments and the drugs being evaluated by the different Medicinal Agencies.

On the other hand, the diagnostic utility of different neuroimaging techniques is being explored. Computed Axial Tomography (CAT) is useful for detecting some causes of dementia, though it is unable to precisely distinguish between early AD and normal aging. In a way similar to CAT, Single-Photon Emission Computed Tomography (SPECT) may prove useful for establishing a differential diagnosis in dementia. Lastly, Magnetic Resonance Imaging (MRI) offers superior anatomical discrimination, and generates a more precise image of the affected brain areas. Other technologies and other ways of evaluating the images obtained are also being investigated.

Regarding the biochemical markers, a number of studies suggest that the concentration of β-amyloid 42 peptide ($A\beta_{1-42}$) and of tau protein (total, T-tau and phosphorylated, P-tau) in cerebrospinal fluid (CSF) may be of diagnostic value. Thus, the CSF concentration of T-tau and P-tau is known to be increased in AD patients, while $A\beta_{1-42}$ is lower than in healthy subjects[2,3].

The accumulation of β-amyloid (Aβ) in the extracellular spaces of the brain and in the walls of the cerebral blood vessels is one of the most significant characteristics of AD, and presently constitutes one of the most important targets for the development of new therapies[4]. Two predominant types of Aβ plaques are found in the brain of AD patients: neuritic plaques and diffuse plaques. Neuritic plaques contain dense bundles of Aβ fibers surrounding the dystrophic neurites, astrocytes and microglia. The diffuse plaques in turn contain non-structured Aβ and are not surrounded by dystrophic neurites. It is believed that the neuritic plaques may develop from diffuse plaques[3]. The number of neuritic plaques has not shown a clear correlation to the severity of dementia in AD; a high density of these structures has also been found in elderly persons without dementia[3].

A number of researchers believe that some options for treatment may be to prevent the accumulation of such deposits, inhibiting binding of the Aβ monomers, favoring their clearance or reducing monomer production. Unfortunately, there are no adequate animal models for reproducing the pathology of Alzheimer's disease[5,6]. Nevertheless, treatments are still being investigated to reduce the brain deposits of Aβ. Recently, studies have been made involving active and passive immunization in both transgenic mice and in patients - reductions in Aβ being achieved in both cases[7,8,9,10].

In humans, approximately 100-150 mL of cerebrospinal fluid (CSF) circulates within the brain ventricles and subarachnoid space. Each day, 400-500 mL of CSF are generated, resulting in replacement of the total CSF volume four to five times a day. As to the composition of CSF, 99% is water, and approximately 20% of its protein content is synthesized in the brain. From the proteic perspective, CSF may be regarded as a window to the brain. As a result, studies have centered on the changes in its composition. If a protein quantified in CSF is also present in significant amounts in plasma, and the protein is able to cross the blood-brain barrier (BBB), then the concentration in CSF may be influenced by changes both within the central nervous system (CNS) and in plasma. The concentration of a particular agent in CSF is a net effect of diffusion from blood, synthesis in the brain, clearance and degradation of the agent in the extracellular space, and its final dilution in the total volume of CSF.

1.1.2 Existence of a Dynamic Equilibrium between Brain and Plasma Aβ

Both tau protein and $A\beta_{1-42}$ peptide are able to cross the BBB. Aβ is normally detectable in plasma, though the levels are 100-fold lower than in CSF, suggesting that $A\beta_{1-42}$ is predominantly produced in the CNS[3]. Earlier studies in both animal models and humans have detected the presence of a bidirectional flow of Aβ peptide between the CSF compartment and plasma[11-19], with involvement of the subependymal and choroid plexus capillaries[3]. In some cases, it has even been shown that the elimination half-life of Aβ between CSF and plasma is about 30 minutes[11,18]. On the other hand, some researchers have demonstrated that the plasma levels of Aβ decrease as the deposits of the latter within the brain increase[20,21], while other investigators have reported high plasma levels of Aβ both after active immunization with Aβ[7] and after passive immunization with anti-Aβ antibodies[8, 22].

Taken globally, the above data, i.e., bidirectional flow and deliberate imbalance of the flow in one or the other direction, supports the existence of a dynamic equilibrium between plasma

and brain Aβ. In this context, Sigurdsson et al.[9], among other authors[4], have suggested intervention upon Aβ pertaining to the plasma compartment, to induce a reduction in the peptide and thus alter the aforementioned equilibrium. Specifically, Sigurdsson et al. recommend the adoption of an Aβ peripheral clearance strategy based on the use of IgM type antibodies. This strategy would have the advantage of sequestering peripheral Aβ, with preservation of the brain tissue, since IgM is practically undetectable in CSF[9]. In this way, the above mentioned authors consider that it would be possible to avoid adverse events directly produced by interaction of the antibodies with the brain tissue, as in the documented cases of encephalitis that recently have made it necessary to prematurely interrupt a phase II clinical trial testing active immunization with $A\beta_{1-42}$. Although the precise mechanism underlying this complication is not known, some investigators have demonstrated the existence of brain vascular microhemorrhages after passive immunization in animal studies[23,24].

Other investigators have taken a step further. Likewise based on the dynamic equilibrium principle, they have demonstrated that peripheral sequestering of plasma Aβ using substances other than antibodies - though with high affinity for the peptide - is able to reduce both Aβ in CSF and in the brain tissues. Specifically, Matsuoka et al.[25] demonstrated the above using two different substances, gelsolin and GM1 ganglioside. Neither of these two substances appeared to any appreciable degree in CSF, and both produced the desired imbalance in both CSF and brain Aβ. A relevant observation is the fact that probably encouraged by the expectations raised by these results, the mentioned investigators requested a patent of the described procedure, in the name of the investigators themselves for the United States and in the name of the New York State Office of Mental Health for the rest of the world (WO 03/051374 A3).

Recently, Cleary et al.[10] have demonstrated that the soluble oligomers of Aβ that go on to form part of the amyloid plaques are already neurotoxic before the fibrillar forms are produced; as a result, they would play a relevant role in the early forms of AD.

1.1.3 The Role of Plasma exchange with Albutein® 5% in Alzheimer's disease

The amyloid precursor protein (APP) and Aβ itself are secreted during normal cell metabolism, with small amounts of both being found in CSF and plasma. In both compartments, Aβ is associated to proteins[3] that may contribute to maintain the solubility of Aβ. One such protein is albumin, which is the most abundant protein in both blood and CSF. In this sense, albumin is able to bind to and transport a range of small molecules, including peptides and drugs. Only 40% of body albumin is contained in plasma; the remaining 60% is found in the extracellular fluids. The great majority of Aβ circulating in blood (89%) is bound to albumin in 1:1 proportion[4], and only 5% is bound to high density lipoproteins (apoenzyme E, ApoE and apoenzyme J, ApoJ)[26,27]. It has been shown that albumin is one of the most important binding proteins, and one of the most potent inhibitors of Aβ polymerization[4].

Plasma exchange is a process used to eliminate patient plasma and replace it with another solution maintaining normal volemia and osmotic balance. To this effect, albumin or other colloids have been used, as well as fresh frozen plasma (FFP) and crystalloids. The purpose of this procedure is to eliminate toxic substances from patient plasma, such as: autoantibodies, alloantibodies, immune complexes, proteins, or toxins[28]. The clinical efficacy of plasma exchange depends on many factors, including the volume exchanged, the number and frequency of sessions, the nature of the replacing solution, and the separation technique employed. Data obtained in animal studies have shown human albumin to be neuroprotective

in models of acute ischemic infarction, improving neuron function and reducing both infarct volume and brain edema[29].

Plasma exchange is widely used in treatment of different pathologies. Specifically, the procedure has been applied to the following disorders: Guillain-Barré syndrome (acute treatment)[30], multiple sclerosis (chronic treatment)[28], inflammatory demyelinating polyradiculoneuropathy[31], acute inflammatory demyelinating disease of the CNS[32] and other peripheral neurological alterations[33]. Since 2004, the Banc de Sang i Teixits (BST) (Barcelona, Spain) has carried out 401 plasma exchange procedures and over 5000 aphereses. In comparison with this study, all perform a greater number of replacements, or alternatively the volume replaced is greater or the sessions are performed more frequently.

On the other hand, in the clinical investigation of AD, methods more aggressive than plasma exchange have been used, such as ventriculoperitoneal shunting, which is a method involving implantation of a catheter connecting the brain ventricles with the peritoneal space for the drainage of CSF[34,35].

Albutein® 5% has been marketed in USA since 1978, though albumin had already been in use in different countries since the 1940s. Since then the product has been continuously used in application to different clinical conditions, and is even employed as placebo in many clinical trials.

1.1.4 Previous Clinical Studies of Plasma exchange with Albutein® 5% in Alzheimer's disease

In order to evaluate the effects of plasma exchange with Albutein® 5% in patients with mild to moderate AD, Grifols S.A. in 2005 started a pilot study directed by Dr. M. Boada of the Service of Neurology of Vall d'Hebron Hospital and of the Fundació ACE in Barcelona, Spain. Ten patients were recruited, of which seven were subjected to plasma exchange, two received no treatment, and one was removed from the study upon personal request. Of the seven subjects treated, three underwent five plasma exchanges, two patients received four exchanges, and two underwent three exchanges during a period of three weeks. The participants were subsequently followed-up on for a period of 12 months.

The most important variables recorded were the following:

- changes in the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in plasma,
- changes in the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in CSF,
- changes in the neuropsychological test results (MMSE and ADAS-Cog), and
- changes in the neuroimaging studies (MRI and SPECT).

Results showed a consistent pattern of Aβ mobilization as well as a trend to stabilization in the MMSE and ADAS-Cog[65].

Based on these results a phase II clinical trial was conducted in 4 sites, 2 in Spain and 2 in the USA, with 42 patients recruited. The trial was a randomized, controlled, single-blind study in which 3 different plasma-exchange schedules were assessed: 1) 6 plasma exchanges in 3 weeks, 2) 6 plasma exchanges in 6 weeks and 3) 6 plasma exchanges in 12 weeks. Therefore, patients that completed all cycles underwent a total of 18 plasma exchanges. The control group underwent sham (simulations) procedures that mimicked the actual procedures. Interim results have recently been published and they show a consistent pattern of Aβ mobilization as well as an improvement of the MMSE and ADAS-Cog of 2.5 and 5.5 points, respectively, at 9 months of follow-up. In terms of Aβ mobilization, the schedule of 6 plasma exchanges in 6 weeks seemed to be more effective than 6 plasma exchanges in 12 weeks and of similar

effectiveness as 6 plasma exchanges in 3 weeks[65]. Thus, 6 plasma exchanges in six weeks were selected as a potential load treatment for future trials.

1.1.5 The role of intravenous immunoglobulin in Alzheimer's disease

In 2004 IVIG was used for the first time in a pilot study of Alzheimer's disease[66]. The study included 5 patients and the results showed an improvement in the ADAS-Cog scale but not in the MMSE. The first randomized clinical trial of IVIG in Alzheimer's disease was performed on 8 patients[67] during a follow up of 18 months and the results showed plasma Aβ mobilization after each IVIG administration as well as a trend to cognitive stabilization. A second randomized clinical trial involving 24 patients has been completed[68] and the preliminary results presented in the *American Academy of Neurology 2010* meeting showed a statistical significant improvement of 6 points in the ADAS-Cog scores. In addition, a phase III study involving 360 patients is currently ongoing[69].

On the other hand, Grifols S.A. has conducted a pilot study of IVIG in 4 patients that had previously participated in the pilot study mentioned in section 1.1.4 and the preliminary results showed a similar pattern of plasma Aβ mobilization as compared to that observed in the plasma-exchange study.

1.1.6 Potential role of a combination of plasma exchange with Albutein® with Flebogamma®DIF

Taking into consideration the results of the clinical studies mentioned above, it can be concluded that both approaches, plasma exchange with albumin and IVIG infusion, act on the peripheral Aβ pool, as seen through the plasma Aβ mobilization observed, and that both approaches show a trend to cognitive improvement. Therefore, it seems reasonable to search for a synergy between the two treatments using lower doses of each of them which will also imply less concern about safety issues. In fact, for selected neurologic diseases, this combination approach has already been used[70, 71].

We propose here a combination of monthly low-volume plasma exchanges (similar to regular plasma donation plasmaphereses) with IVIG every four months. Previously, there will be a load treatment with six plasma exchanges in six weeks to assure the baseline results obtained in previous studies. Albutein® and Flebogamma®DIF, two approved products, will be used as albumin and IVIG, respectively.

2. STUDY PURPOSE AND OBJECTIVES

Primary objective

The primary objective of this study is to evaluate the changes in the cognitive, functional, behavioral and global domains based on the different applicable psychometric batteries and scales.

Secondary objectives:

The secondary objectives of this study are:

- To determine the changes in the concentration of beta-amyloid peptide in plasma and cerebrospinal fluid (CSF) in the treatment group of patients with Alzheimer's disease (AD)
- To evaluate the structural changes in volume of the hippocampus, posterior cingulate area, and other associated areas based on neuroimaging studies with MRI (variations versus baseline).

- To determine functional brain changes through FDG-PET (fluordeoxyglucose-PET).
- To determine whether plasma exchange with human albumin combined with IVIG is safe, taking into account the following factors:
  - Type, severity and frequency of adverse reactions during and after the procedure and infusions.
  - Changes in vital signs and clinically relevant changes, according to the laboratory test findings.
  - Control of episodes of cerebrovascular accidents with MRI.

3. INVESTIGATIONAL PLAN

3.1 Global Study Design/Developmental Phase

The trial comprises a multicenter, randomized, controlled design. The subjects included in the study will be controlled from the first screening visit up to fourteen months after. Then, the maximum study duration for a given subject will be approximately one year and two months. There will be two weeks for screening and randomization of both groups (treatment and control). After these two weeks the treatment period will proceed as follows:

- One month and a half (six weeks) of intensive treatment with one full plasma exchange per week (all the three groups).
- Two of the three groups will follow 12 months of maintenance treatment with one low-volume plasma exchange every month combined with IVIG every 4 months administered at the end of the corresponding plasmapheresis instead of albumin (that is, 9 plasmaphereses with Albumin replacement and 3 plasmaphereses with IVIG replacement). During this year patients will follow one of the two different pre-allocated treatment arms: 1) with the doses of albumin and IVIG needed to replace those removed during the plasmapheresis, 2) with half of the doses of albumin and IVIG.
- One of the three groups will follow the same schedule as the above two groups but with half of the doses of albumin alone (without IVIG), that is, 12 plasmaphereses with half-dose albumin alone.

Patients in the control group will undergo sham procedures mimicking plasmaphereses but with neither fluid exchange nor albumin or IVIG administration (**See Appendix 8**).

There will be sites in Spain and USA.

The site of the Coordinating Investigator (Fundació ACE/Hospital Vall d'Hebron, Barcelona, SPAIN) will be the first site to be included. The rest of the sites will be incorporated progressively after the first site. Several months can elapse between the inclusion of the Spanish sites and the rest of sites.

3.2 Randomization and Treatment Assignment

After the screening period, the subjects will be randomized to one of the 3 treatment groups or the control group according to a [1:1:1:1] scheme.

3.3 Type of Control

Control group: the subjects will be AD patients involving the same inclusion and exclusion criteria as the treated subjects but will undergo sham procedures mimicking plasmaphereses but with neither fluid exchange nor albumin or IVIG administration.

3.4 Blinding Techniques

The control group will be subjected to simulated full plasma exchanges through a non-invasive procedure. Specifically, the tip of a cut catheter will be stitched to an adhesive gauze dressing (acting as a "second skin") which will be placed on the subclavicular or jugular region. Then, a second adhesive gauze will cover the one with the catheter tip stitched to it. The cut catheter will be of characteristics similar to the catheters used in the treatment group. Also simulated low-volume plasma exchanges will be performed. Likewise, these subjects will receive the same visits as the patients in the treatment group.

This study is blind for patients, caregivers and raters.

The study Sham manual guideline describes all the sham procedures throughout the study (Appendix 9).

3.5 Screening Period (Pre-Randomization or Pharmacological Washout)

There will be a two-week period for screening before the six weeks of intensive treatment. At the end of the screening period subjects will be randomized to one of the three treatment groups or to the sham group.

4. SELECTION OF STUDY POPULATION

4.1 Inclusion and Exclusion Criteria

4.1.1 Inclusion Criteria

In order to be eligible for participation in the trial, the patients must meet the following requirements:

1. Males or females between 55-85 years of age at the time of signing of the informed consent document.
2. A diagnosis of Alzheimer's disease (NINCDS-ADRDA criterion), and MMSE score between ≥18 and ≤26.
3. Current stable treatment with AchEIs and/or memantine for the previous three months.
4. The patient and a close relative or the legal representative must read the patient information sheet, agree to participation in the trial, and then sign the informed consent document (the patient personally and the close relative/legal representative).
5. The patient must be able to follow the study protocol, receive the treatment in the established time period, and continue during the follow-up interval.
6. A brain CAT or MRI study obtained in the 12 months prior to screening, showing the absence of cerebrovascular disease, should be available. Nevertheless, it is mandatory to use the MRI obtained during the screening period to rule out any cerebrovascular disease such as microhemorrages, infarction, hematoma, stroke, meningioma or other finding that could affect patient safety.
7. A stable care taker must be available, and must attend the patient study visits.

4.1.2 Exclusion Criteria

Patients meeting any of the following criteria will not be able to participate in the trial:

1. Any contraindication for plasma exchange due to behavioral disorders or abnormal coagulation parameters, such as for example:
   - Hypocalcemia (Ca^{++} < 8.7 mg/dL).
   - Thrombocytopenia (<100,000/µL).
   - Fibrinogen <1.5 g/L.
   - Prothrombin time (Quick) p<60% versus control (INR>1.5).
   - Beta-blocker treatment and bradycardia <55/min.
   - Treatment with ACEIs (increased risk of allergic reactions).
2. Hemoglobin < 10 g/dL
3. Difficult venous access precluding plasma exchange.
4. A history of frequent adverse reactions (serious or otherwise) to blood products.
5. Hypersensitivity to albumin or allergies to any of the components of Albutein® 5%.
6. History of immunoglobulin A (IgA) deficiency.
7. Known allergies to Flebogamma® DIF components such as sorbitol.
8. History of thromboembolic complications of intravenous immunoglobulins.
9. Plasma creatinine > 2 mg/dl.
10. Uncontrolled high blood pressure (systolic blood pressure of 160 mmHg or higher and/or diastolic blood pressure of 100 mmHg or higher despite regular treatment during the last 3 months).
11. Liver cirrhosis or any liver problem with GPT > 2.5 x ULN, or bilirubin > 2 mg/dL.
12. Heart diseases as evidenced by myocardial infarction, severe or unstable angina, or heart failure (New York Heart Association Class II, III or IV) in the past 12 months.
13. Participation in other clinical trials, or the receipt of any other investigational drug in the three months prior to the start of the study.
14. Any condition complicating adherence to the study protocol (illness with less than one year of expected survival, known drugs or alcohol abuse, etc.).
15. Pregnant or nursing women or women not using effective contraceptive methods for at least one month after plasma exchange.
16. Fewer than six years of education (exclusion criteria under medical criterion).
17. Less than three months with stable treatment for behavioral disorders including insomnia.
18. Patients being treated with anticoagulants or antiplatelet therapy (antiaggregants) should not be recruited in the study.

4.2 Disease Diagnostic Criteria

Probable mild to moderate Alzheimer's disease, according to NINCDS-ADRDA criteria 2, with stable AChEI treatment and an MMSE score of ≥18 and ≤26.

4.3 Planned Number of Subjects

A sample size of 312 subjects (78 per each of the 4 groups) will make it possible to detect with nearly 92% power for the first of the co-primary efficacy variable (the changes from baseline of the ADAS -Cog scores) a difference in the mean of 3 points between any of the treatment groups and the control group, assuming the common standard deviation (SD) to be 5.55 (according to the data obtained in the phase II study[65]), with a level of significance of 5%. This same sample size provides over 98% power for second of the co-primary endpoint variables (the changes from baseline of the ADCS-ADL inventory scores) a difference in the mean of 6.69 points between any of the treatment groups and the control group, assuming the common standard deviation (SD) to be 10.0 (according to the data obtained in the phase II study), with a level of significance of 5%.

The study will have joint power for these co-primary endpoints of at least 90% (0.92*0.98 = 0.90). The calculation makes the conservative assumption that these endpoints are independent. Since these endpoints may be positively correlated 90% should serve as a lower bound on power.

Assuming a global dropout rate of approximately 15%, the study would have to enroll 364 subjects to obtain 312 subjects for evaluation.

4.4 Withdrawal Criteria and Planned Analyses of Withdrawals and Dropouts

The subject will be withdrawn from the study if:

- Prior to the first plasma exchange session, no plasma sample has been collected for possible future assays.
- Any serious adverse event is recorded, advising removal of the subject.
- The subject or his/her legal representative wishes to abandon the study and continue follow-up and normal treatment in the center.
- The investigator is of the opinion that treatment continuation is contraindicated.
- The subject fails to adhere to the study protocol.

The reason for withdrawal of the subject will be documented, and a final visit will be scheduled to obtain blood samples and to complete the pertinent tests. Whenever possible, these tests will be made on the same day as the final visit. No subject can be re-enrolled into the study after having been withdrawn from the study.

4.5 Treatment of Pre-Randomization Losses, Entry Reclassification

Subjects withdrawn in the screening phase will not be able to participate in the trial. As discussed above, no subject will be re-enrolled into the study after having been withdrawn from the study.

4.6 Estimated Duration of the Recruitment Period

The planned trial recruitment period will last approximately 12 months.

5. TREATMENT OF SUBJECTS

5.1 Treatment Regimen

5.1.1 Study Treatment Procedure

After the screening visits, the subject will receive the following treatment:

- One month and a half (6 weeks) of intensive treatment with one full plasma exchange per week (all the three groups)
- Two of the three groups will follow 12 months of maintenance treatment with one low-volume plasma exchange every month combined with IVIG every 4 months administered at the end of the corresponding plasmapheresis instead of albumin (that is, 9 plasmaphereses with Albumin replacement and 3 plasmaphereses with IVIG replacement). During this year patients will follow one of the two different pre-allocated treatment arms: 1) with the doses of albumin and IVIG needed to replace those removed during the plasmapheresis, 2) with half of the doses of albumin and IVIG.(**See Appendix 8**).
- One of the three groups will follow the same schedule as the above two groups but with half of the doses of albumin alone (without IVIG), that is, 12 plasmaphereses with half-dose albumin alone.

Albutein® 5% (an approved medicinal product) will be used during the intensive period (full plasma exchanges).

Albutein® 20% (an approved medicinal product) will be used during the maintenance period (low-volume plasma exchanges), together with Flebogamma® DIF (an approved medicinal product).

5.1.2 Dosage and Treatment Regimen

The six full replacement sessions of the intensive treatment period will be completed in six weeks (one session per week)

The volume of each full plasma exchange will be approximately that of one plasma volume of the patient as calculated from body weight, height and hematocrit (approximately 35-45 mL/kg, corresponding to a volume of 2500-3000 mL).

The 12 low-volume plasma exchange sessions (9 with albumin 20% and 3 with IVIG) of the maintenance treatment period will be completed in12 months (one session per month).

The volume of each plasmapheresis during the low-volume plasma exchange period will be between 690 and 880 mL of plasma (depending on the patient body weight, following the nomogram for plasma donation used in the USA. (**See Appendix 3**).

5.1.3 Method of Application

Full plasma exchanges (Intensive treatment period)

Full plasma exchanges (FPE) can be carried out via peripheral access or centrally, according to the individual characteristics of the subject. In the latter case, and before the first replacement session, a double-lumen catheter must be placed in the subclavian or jugular vein. Implantation and maintenance will be carried out according to the standard procedures used in each center but it will be mandatory to perform a chest X-ray to confirm the correct placement of the catheter. Removed plasma volume will be replaced with the same volume of Albutein® 5% during the procedure.

The removed and replaced plasma volume will depend on patient's characteristics (sex, height, weight and hematocrit). It will be approximately 35-45 mL/kg, corresponding to a volume of 2500-3000 mL. This volume is calculated automatically by the device or manually by the operator depending on the device used.

For blinding the control group, adhesive gauze dressing will be placed on the subclavicular or jugular region, stitching a catheter of characteristics similar to the catheters used in the treatment group (see Appendix 9).

The central catheter has the advantage of increased convenience for the subject during the process, since it allows greater flows and thus shorter session times and obviates the need in each session to perform two peripheral venipunctures. The central line access also facilitates subject mobility during the procedure.

Each session is carried out at a rate of 60-100 mL/min., provided replacement is possible through a double-lumen catheter placed in the subclavian vein, with a continuous-flow cell separator. Each of these sessions will last approximately two to three hours. The control group will be subjected to simulated (sham) plasma exchanges during the same time as the subjects in the treatment group.

Each subject must remain in the facility throughout the procedure and then for as long as necessary to ensure a safe return home.

These details refer to the plasma exchange procedure performed through centrifugation (the most commonly used) although the procedure through filtration is also permitted.

Albutein® 5% will be used according to the instructions of the **Full Prescribing Information** (**Appendix 1**). The solution is normally clear to slightly opalescent. Do not use the product if the solution is cloudy or has precipitated. The product must be at body temperature before use. Do not dilute with water for injections. Once the container is open, the contents must be administered immediately. Unused contents must be discarded. No remaining product in an opened container can be stored in the refrigerator and used at a later time.

The vital signs of each subject (blood pressure, heart rate, respiratory rate, and body temperature) are to be monitored 15-30 minutes before replacement, during and again 15-30 minutes after the procedure, and as often as considered opportune by the investigator. The coagulation parameters must also be controlled, particularly fibrinogen concentration. In the case of any relevant alteration in the coagulation parameters, replacement must be suspended and no further sessions will be carried out until the parameters return to adequate levels (see sections 5.2.1 and 5.2.2).

<u>Low-volume plasma exchanges (Maintenance treatment period)</u>

In all sites where the low-volume replacement session will be performed, a device based on regular plasma donation (Auto-C, Fenwal, Illinois, USA with FDA PMA approval # BP850001 or Aurora, Fenwal, Illinois, USA with FDA 510(k) clearance # BK110072) will be tested (see **Appendix 3** for device protocol). The Auto-C (FDA PMA approval # BP850001) or Aurora (FDA 510(k) clearance # BK110072) based devices are used with the objective of offering the patient a procedure that is similar to a conventional plasma donation with the difference that albumin or IVIG will be infused at the end of the plasmapheresis.

Low volume plasma exchange (LVPE) will be carried out through a peripheral line. The removed plasma volume will be similar to that of a plasma donation (690 to 880 mL) and will depend on patient's weight.

The control group will undergo simulated (sham) low volume plasma exchange which will last approximately the same time as the subjects in the treatment group.

Albumin

Albutein® 20% will be infused at the end of the corresponding plasmapheresis using Auto-C (FDA PMA approval # BP850001) or Aurora (FDA 510(k) clearance # BK110072) based devices. During the Maintenance period the dosing will be as follows:

- 1st arm: 200 mL (40 g, 9 plasmaphereses)
- 2nd arm: 100 mL (20 g, 9 plasmaphereses)
- 3rd arm: 100 mL (20 g, 12 plasmaphereses)

Therefore, after the LVPE (690 to 880 mL of plasma removal), Albutein® 20% is infused depending also on patient's weight and treatment arm (200 to 80 mL). Due to the difference in volume (removed/infused), it is advised to infuse saline solution to avoid the risk of hypotension related events (see Appendix 10 for guidance).

The main characteristics of the product can be found in the **Full Prescribing Information** (**Appendix 1**). The solution is normally clear to slightly opalescent. Do not use the product if the solution is cloudy or has precipitated. The product must be at body temperature before use. Do not dilute with water for injections. Once the container is open, the contents must be administered immediately. Unused contents must be discarded. No remaining product in an opened container can be stored in the refrigerator and used at a later time.

Intravenous immunoglobulin infusions

Flebogamma® DIF will be infused at the end of the corresponding plasmapheresis during the Maintenance period following the instructions of the **Full Prescribing Information (Appendix 2).** At the discretion of the investigator, patients can be premedicated with paracetamol and antihistamines.

During the Maintenance period the dosing of IVIG will be as follows:

- 1st arm: 20 g
- 2nd arm: 10 g
- 3rd arm: None (only albumin)

5.2 Criteria for Treatment Modification or Interruption

5.2.1 Criteria for Modifying Regimens

In the case of any relevant alteration in the coagulation parameters, full plasma replacement (Intensive period) is to be suspended and no further sessions will be carried out until the parameters return to adequate levels.

Replacement is to be postponed 24 hours if fibrinogen <1 g/L or the prothrombin time (Quick) <60% of the control value.

Platelet depletion to below 100,000/µL is also an indication for replacement suspension.

Relevant alterations of coagulation parameters are not expected to occur with the low-volume plasma exchanges (Maintenance period).

If any adverse event occurs during the infusion of Flebogamma® DIF one of the following actions will be carried out taking into account the nature and severity of the event:

- To stop the infusion.
- To reduce progressively the infusion rate until the symptoms disappear.

After re-evaluation the adverse event, it can be proceed as follows:

- To restart the infusion or to increase as of a tolerated rate (once the symptoms have disappear) or
- To definitely stop the infusion.

If a patient presents an adverse event two times with the same infusion rate, the following doses will be administered at the maximum tolerated rate.

5.2.2 Special Warnings and Precautions for Use

Full plasma exchange is a safe technique that may induce very well-known and therefore preventable and controllable, adverse reactions, according to the guidelines provided below. In any case, and considering the special vulnerability of the patients studied, a number of precautions (in addition to the habitual measures) have been taken to minimize the risks of the procedure:

1. Plasma exchange will be carried out by specialized nursing personnel, under direct and continuous supervision by the specialists.
2. Subjects will be required to remain in the center before and after the procedure for longer periods of time than usual.
3. Vital signs and laboratory test parameters will be monitored more frequently than usual.
4. The treatment suspension / postponement criteria are clearly established.
5. The person accompanying the subject should be present and/or in proximity before, during and after the procedure but not in the same room in order to maintain the blind. Exceptionally, agitated patients could be accompanied by the caregiver during the procedure in the same room to ease patient's anxiety. In this case it is mandatory the subject is scheduled for the treatment a different day than the rest of the patients.

   The patient will have direct, 24-hour access to the specialist (by mobile phone) in both the replacement phase and during subsequent follow-up. In the case of any incident, the physician will decide the actions to take.

Adverse effects (AEs) may be observed during the replacement procedure, due to the process of apheresis, the replacement fluid used, or as a result of the patient disease or idiosyncrasy. These AEs include hypocalcemia, hypotension, allergic reaction, coagulation disorders, adverse events related to vascular access, and other AEs such as headache, nausea and anxiety.

The information contained in this and the following sections is also valid for plasma removal (plasmapheresis) during the low-volume plasma exchange period but the expected AE rate is much lower. In fact, the plasmaphereses performed within this period will be very similar to those performed for regular plasma donations (e.g., Grifols performs between 12,000 and 15,000 plasmaphereses for plasma collection daily in the US).

On the other hand, Albutein® and Flebogamma® DIF are safe products that are widely used worldwide. For special warnings and precautions see the corresponding **Full Prescribing Information (Appendix 1 and 2).**

5.2.2.1 Hypocalcemia

The great majority of side effects associated with plasma exchange apheresis is due to the administration of citrate and are related to diminish ionic calcium availability. Although some

adverse reactions not explainable in terms of hypocalcemia, or not resolved by calcium dosing, may be attributable to citrate interaction with magnesium (of characteristics similar to those of calcium).

When volume replacement is performed with 5% albumin, the risk of hypocalcemia is considerably lower than when plasma is used for replacement (seen historically in approximately 7.8% of sessions), and no important toxicity is detected.

The factors predisposing to hypocalcemia may be patient-related (hypoalbuminemia, vitamin D deficiency (malnutrition, malabsorption), hypomagnesemia, hyperphosphatemia, altered liver or kidney function or hypoparathyroidism) or related to the procedure (duration of citrate infusion >120 minutes, respiratory alkalosis generally induced by tachypnea).

All plasma exchanges produce transient hypocalcemia that is usually well tolerated by the patient. Occasionally, the decrease in ionic calcium levels can increase nerve cell membrane excitability, resulting in symptoms.

The symptoms and signs of hypocalcemia to be assessed are:

- paresthesias
- headache
- vision alterations (glinting)
- nausea
- cramps
- chest oppression

The degree of hypocalcemia is rated from 0 to 4 as follows:

Level	Symptoms	Treatment
0	None	None
1	Reported by patient, and tolerable	Calcium p.o. (Sandosten Calcium)
2	Cause discomfort	Infusion of CaCl2 i.v.
3	Important discomfort	Reduce flow 20% Infusion of CaCl2 i.v.
4	Unbearable	Stop procedure Infusion of CaCl2 i.v.

Treatment is with a calcium chloride infusion, which may cause arrhythmias. The calcium chloride formulation must be infused diluted in physiological saline solution, at a maximum concentration of 2 mg/mL.

As each infused milliliter of acid citrate-dextrose A (ACD-A) neutralizes 0.5 mg of calcium, the calcium chloride dose will be equal to the milliliters of ACD-A infused in the last hour multiplied by 0.5 and by the calcium chloride concentration of the dilution employed (2 mg/mL), using the following formula:

Volume of calcium chloride = Volume of ACD-A (last hour) / 4

The formulation is administered via drip through the return catheter, in 10-15 minutes. This rate may vary according to whether the symptoms improve or not.

If the patient shows **Level 2** symptoms from the start or before the first 120 minutes of replacement, prophylactic blood calcium treatment should be provided. A 0.2 mg/mL calcium chloride solution is prepared and infused through the return catheter. To calculate the infusion rate, the following must be applied:

- Correcting factor of the amount of ACD-A/ml citrated blood
- Proportion of ACD-A / blood
- Access flow

Table 1 below specifies the calcium chloride infusion rate (concentration 2 mg/mL) when administered prophylactically on a continuous basis according to the separator inlet flow, the proportion ACD-A / blood, and the correction factor.

Administration of $CaCl_2$

$CaCl_2$ infusion rate in ml/hour (concentration 2 mg/ml) according to inflow rate and ACD-A: blood ratio

	Flow (ml/min)																							
Ratio	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**	**100**	**105**	**110**	**115**	**120**	**125**	**130**
1:30	7	9	12	14	16	19	21	23	26	28	30	32	35	37	39	42	44	46	49	51	53	56	58	60
1:29	7	10	12	14	17	19	22	24	26	29	31	34	36	38	41	43	46	48	50	53	55	58	60	62
1:28	7	10	12	15	17	20	22	25	27	30	32	35	37	40	42	45	47	50	52	55	57	60	62	65
1:27	8	10	13	15	18	21	23	26	28	31	33	36	39	41	44	46	49	52	54	57	59	62	64	67
1:26	8	11	13	16	19	21	24	27	29	32	35	37	40	43	45	48	51	54	56	59	62	64	67	70
1:25	8	11	14	17	19	22	25	28	31	33	36	39	42	45	47	50	53	56	58	61	64	67	70	72
1:24	9	12	14	17	20	23	26	29	32	35	38	41	43	46	49	52	55	58	61	64	67	70	72	75
1:23	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	64	67	70	73	76	79
1:22	9	13	16	19	22	25	28	32	35	38	41	44	47	51	54	57	60	63	66	70	73	76	79	82
1:21	10	13	17	20	23	26	30	33	36	40	43	46	50	53	56	60	63	66	70	73	76	79	83	86
1:20	10	14	17	21	24	28	31	35	38	42	45	49	52	56	59	63	66	70	73	77	80	83	87	90
1:19	11	15	18	22	26	29	33	37	40	44	48	51	55	59	62	66	70	73	77	81	84	88	92	95
1:18	12	15	19	23	27	31	35	39	43	46	50	54	58	62	66	70	73	77	81	85	89	93	97	100
1:17	12	16	20	25	29	33	37	41	45	49	53	57	61	65	70	74	78	82	86	90	94	98	102	106
1:16	13	17	22	26	30	35	39	43	48	52	57	61	65	70	74	78	83	87	91	96	100	104	109	113
1:15	14	19	23	28	32	37	42	46	51	56	60	65	70	74	79	83	88	93	97	102	107	111	116	121
1:14	15	20	25	30	35	40	45	50	55	60	65	70	75	79	84	89	94	99	104	109	114	119	124	129
1:13	16	21	27	32	37	43	48	54	59	64	70	75	80	86	91	96	102	107	112	118	123	128	134	139
1:12	17	23	29	35	41	46	52	58	64	70	75	81	87	93	99	104	110	116	122	128	133	139	145	151
1:11	19	25	32	38	44	51	57	63	70	76	82	89	95	101	107	114	120	126	133	139	145	152	158	164

5.2.2.2 Hypotension

Hypotension, presents as paleness and perspiration, with lowered blood pressure and bradycardia. This AE appears as a result of a vasovagal response secondary to hypothalamic stimulation, and is very rarely attributable to volemia if the separator used is of the continuous flow type (seen historically in approximately 2.6% of all processes).

The predisposing factors may be patient-related (low body weight, a history of vasovagal reactions, or a low hematocrit) or related to the procedure (equipment fatigue or extracorporeal volume >20% of patient volemia).

The initial symptoms and signs include:

- perspiration
- bradycardia
- hypotension

The progressive manifestations comprise:

- nausea
- vomiting
- syncope
- involuntary defecation
- seizures

Treatment of hypotension consists of interrupting the process, opening the serum access, placing the patient horizontal or in the Trendelenburg position, recording blood pressure and pulse, and waiting for recovery while investigating the cause (disconnect in case of equipment leakage; if other cause is involved, assess continuation). Recommendations for preventing hypotension related events in low-volume plasma exchange procedures have been captured in a study guideline. Hypotension episodes can be avoided by infusing saline solution and adjusting the removed plasma volume in low-weight patients (see Appendix 10).

5.2.2.3 Allergic reaction

Allergic reactions are generally associated with the blood components and albumin, though they may also be due to ethylene oxide or other medication.

The signs and symptoms include:

- urticaria
- dyspnea
- wheezing
- hypotension
- tachycardia
- facial reddening
- palpebral edema

In case the patient suffers from an allergic reaction the site should follow their standard procedures to manage the situation. As a recommendation, mild allergic reactions could be controlled with antihistamines (diphenhydramine 25-50 mg i.v.). In case the reaction continues, or in the event of anaphylactic manifestations, it could be administered 0.3-0.5 mL of epinephrine 1:1000 s.c. This dose may be repeated after 10-15 minutes if no response is elicited. It is also possible to add methylprednisolone 100 mg i.v.

5.2.2.4 Coagulation disorders

When the extracted plasma is replaced with albumin, coagulation disorders may develop due to dilution of the coagulation factors, mainly lowered blood fibrinogen, since its half-life is 72 hours. During daily plasma exchange sessions the fibrinogen concentration may drop to below 1 g/L, requiring the infusion of plasma or fibrinogen. When replacement is performed every 72 hours, such measures are not necessary.

Minor bleeding associated with severe thrombopenia has been observed (seen historically in approximately 2.2%).

5.2.2.5 AEs Related to vascular access

The central catheter has the advantage of increased convenience for the subject during the process, since it allows greater flows and thus shorter session times, obviating the need in each session to perform two peripheral venipunctures.

As to the adverse effects if the catheter is placed in the subclavian vein, the risk of infection is minimal. Thus, while the incidence of infections with all the catheters is <5 per 1000 days (<3% of all catheters), this incidence decreases considerably if only those placed in the subclavian vein are considered[23,24]. Pneumothorax, hemothorax, bleeding at the time of placement and thrombosis in the case of indwelling catheters left in place for extended periods of time may be seen. The recommendations for the treatment and prevention of fever management and thromboembolic events have been captured in two study guidelines (see Appendix 11 and Appendix 12, respectively); nonetheless the sites should follow their local procedures.

As to adverse events related to peripheral access, the most commonly expected are: pain, phlebitis, infiltration and extravasation.

5.2.2.6 Other AEs and their treatment

Other potential AEs include headache, nausea and anxiety.

- Headache: administer paracetamol (acetaminophen) p.o. 1 g
- Nausea: administer Primperan p.o. (Reglan) 5 mL
- Anxiety: administer diazepam p.o. 5 mg (for body weight <55 kg) or 10 mg (weight > 55 kg), or alprazolam at doses of 0.5 mg and 1 mg, respectively
- IG levels decrease: A decrease in the IGs levels could occur during the FPE period. IG levels will recover their normal range at the end of the FPE period. Further investigations will be needed if the levels are not reestablished which may suggest an underlying pathology
- Anemia: Although not frequently, it is expected to observe anemia in patients with haematocrit in the low normal range after the first FPEs. In these cases, oral or intravenous iron therapy is recommended depending on the severity of the anemia and the investigator assessment

The recommendations to treat those AE are described above; nevertheless, the sites should follow their local procedures.

5.2.2.7 Incidence of adverse reactions and mortality

In a 1996 review of the adverse effects of plasma exchange in patients with Guillain-Barré syndrome, involving albumin replacements, a decrease in immediate adverse effects from

12% in 1986 to 7% in 1992 was recorded. Allergic reactions and hypotension were the most frequent problems. The estimated mortality was 1-2 deaths per 10,000 sessions, or two deaths per 2000 patients.

As stated earlier, the expected rate of AEs during the plasmaphereses of the low-volume plasma exchanges (Maintenance period) is much lower than that expected during the full plasma exchanges (Intensive period). For the expected adverse reactions relative to Albutein® and Flebogamma® DIF see **Section 9.1.3** and the corresponding **Full Prescribing Information (Appendix 1 and 2).**

5.3 Concomitant Treatment

Subjects may continue to receive his/her usual medication. In particular, the same acetylcholine esterase inhibitor (AChEI) and/or memantine treatment is to be continued, without modification during the study. If the subject develops adverse reactions related with the AChEI and/or memantine treatment, advising its withdrawal, the medication should be replaced by another drug of the same group at the end of all the plasma exchanges.

Regarding IVIG administrations, the subject can be premedicated with paracetamol and antihistamines or corticosteroids at the discretion of the investigator. There are some sites that include corticosteroids administration during immunoglobulin infusion.

Treatment with angiotensin-converting-enzyme inhibitors (ACEIs) is not allowed during the study. Principal Investigator may consider changing the medication to other antihypertensive drug at the screening visit and maintaining throughout the study.

Any medication administered during the study (including any blood products) will be recorded on the case report form (CRF).

5.4 Special Guidelines for Study Drug Handling

Albutein® and Flebogamma® DIF that are supplied for use in the trial are to be destined exclusively to the study. Any unused material must be returned to the sponsor or at the discretion of the sponsor, may be destroyed at the center according to their SOPs.

The investigator (or pharmacist) or designated team member is to keep all records of drug utilization. The sponsor will provide specific forms to be completed by the investigator, pharmacist or designated team member, at the time of dispensation. These forms can be replaced by proprietary forms of the study center, provided they meet the requirements of the sponsor.

Once the forms have been completed, they must be signed and dated by the monitor and by the investigator to confirm their accuracy.

5.4.1 Storage of Albutein® and Flebogamma® DIF

Albutein® is to be stored at a temperature below 30ºC. The shelf life is three years when maintained at this temperature.

The product should not be frozen. Once the container is open, the contents must be administered immediately. Unused contents must be discarded according to the Center's SOP. No remaining product in an opened container can be stored in the refrigerator and used at a later time. This product must not be used beyond the expiry date stated on the labeling.

The solution is normally clear to slightly opalescent. Do not use the IP if the solution is cloudy or has precipitated.

The product must be warmed to body temperature before use.

Access to the product must be strictly limited.

Flebogamma® DIF is a liquid product and then reconstitution is not necessary. Flebogamma® DIF should be stored below 25°C, should not be frozen and has a shelf-life of two years when stored at this temperature. It must not be frozen and the unused contents of the vial should be kept separately until drug accountability has been completed. Vials must be inspected for particles and discoloration before administration. Solutions with evidence of turbidity should not be used because of the risk of bacterial contamination. Flebogamma® DIF must not be mixed with other pharmaceutical products or intravenous solutions, and a dedicated sterile infusion line should be used to ensure proper administration. Flebogamma® DIF must be brought to room temperature before infusion and administered intravenously through a separate infusion line. Mixing and administration of any other drug (including normal saline) with Flebogamma® DIF is strictly prohibited.

5.5 Measures to Assess Compliance

Such measures are not applicable, since the procedure is carried out under the supervision of the principal investigator, and in the hospital setting.

5.6 Rescue Medication

No rescue medication is being provided under the clinical trial. The principal investigator is responsible for follow-up of the medical needs of the subject. During the treatment phase, the physician supervising the plasma exchanges will propose the adequate measures according to the condition of the subject.

6. TRIAL CONDUCT AND RESPONSE ASSESSMENT

6.1 Study Variables

6.1.1 Primary Efficacy Variable

Change from baseline in the cognitive scores as measured by ADAS-Cog (6 measurements: weeks -3, -2 or -1 and 7-8; months 6, 9, 12 and 14) within the 3 treatment arms.

Change from baseline in the ADCS-ADL inventory (6 measurements: weeks -3, -2 or -1 and 7-8; months 6, 9, 12 and 14) within the 3 treatment arms.

Since it has been suggested that systemic infections can have a detrimental effect on cognitive scores, monitoring of subjects for number and type of bacterial infections requiring antibiotics will be performed.

6.1.2 Secondary Efficacy Variables

a) Change from baseline in the cognitive, functional and neuropsychiatric scores and overall development as measured by MMSE, NPS battery, NPI, CDR-Sb, ADCS-CGIC, CSDD and C-SSRS. (6 measurements: week -3, -2 or -1 and 7-8; months 6, 9, 12 and 14) and QoL-AD, RUD-Lite® (5 measurements: weeks -3, -2 or -1; months 6, 9, 12 and 14). Moreover the optional questionnaires like OAS and ABS could be used at any visit in case any sign of aggression or restlessness.

b) Variation in levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in CSF in the period between baseline lumbar puncture (before the start of the Intensive treatment period) and lumbar puncture immediately after the end of the last low-volume plasmapheresis (whenever this may be)

c) Variation in the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in CSF between the finalization and beginning of each of the 2 treatment periods.

d) Levels of $A\beta_{1-40}$, $A\beta_{1-42,}$ T-tau and P-tau in CSF throughout the study.

e) Plasma levels of $A\beta_{1–40}$ and $A\beta_{1–42}$ before and after each plasma exchange for both treatment periods.

f) Structural changes in volume of the hippocampus, posterior cingulate area, and other associated areas by MRI. Five measurements will be made (weeks -3, -2 or -1 and 7-8; months 6, 9 and 14).

g) Variation in FDG-PET patterns (4 measurements: weeks -3, -2 or -1 and 7-8; months 9 and 14).

6.1.3 Safety Variables

The primary criterion of safety will be the percentage of plasma exchanges (full and low-volume exchanges, including the infusion of albumin and IVIG) associated with at least one adverse event that may be related to the study procedure (adverse reactions). In addition, consideration globally will be made of the percentage plasma exchanges (full and low-volume exchanges, including the infusion of albumin and IVIG) involving some adverse event, whether or not related to the procedure.

Vital signs (blood pressure, heart rate, respiration rate, and body temperature) will be recorded before, during and after each plasma exchange session, where required. Evaluation will also be made (where required) of the different laboratory test parameters (blood cell counts, platelet count, prothrombin time (Quick), aPPT, fibrinogen, total proteins, and calcium).

During the treatment periods (before each plasma exchange) and on the days when there is no replacement, anxiety and restlessness tests will be made based on the Overt Aggression Scale (OAS) and the Agitated Behavior Scale (ABS), whenever considered opportune.

According to the criterion of the investigator, all the clinically important changes in vital function, laboratory test parameters and neuroimaging findings will be evaluated.

6.2 Conduction of the Trial. Study Procedures.

See **Appendix 8** for the **Study Summary Table and for the Study Flow Chart.**

6.2.1 Screening Visits (Weeks -3, -2 and -1)

After obtaining informed consent, there will be screening period of up to 3 weeks which could consist of multiple visits. During the screening visit, all demographic data will be collected. Documentation will also include vital signs (axillary temperature, heart and respiration rate, and blood pressure) and physical examinations with ECG and a sample of blood for genetic markers determination.

Also at the screening visit, the subject's medical history, including AD, will be recorded together with the medication used in the preceding month. The CRF will also register a brief history of AD and the prior treatments received. However, on subsequent visits, recording will be limited to the abnormal conditions observed during the previous visit, along with the concomitant medication and any adverse event that may have been detected.

Sufficient blood will be collected for all the laboratory tests, and a plasma sample will be stored at -70ºC for any possible future analysis.

The following parameters will also be evaluated: biomarkers of AD (Aβ–40, Aβ–42, β-secretase, γ-secretase, cholesterol, LDL, HDL, VLDL, C-reactive protein, rheumatoid factor, IL-1b, IL-6, ferritin, TNF-α), coagulation factors (prothrombin time (Quick), aPPT, calcium

and fibrinogen), proteinogram (total proteins, albumin, α1-globulina, α2-globulina, β-globulina, γ-globulina and IgG), other liver or kidney biochemical markers (AST, ALT, bilirubin, LDH and creatinine), troponin and serology (anti-HIV and HCV antibodies, and HBsAg antigen). In addition, a blood cell count will be performed, including: hematocrit, hemoglobin and erythrocytes, platelets and leukocytes (if an abnormal leukocyte count is recorded, it will be obligate to specify neutrophils, basophils, eosinophils, lymphocytes and monocytes).

All the inclusion criteria are to be confirmed again after obtaining the laboratory test results. If the inclusion criteria are not met, or if some exclusion criterion proves applicable, the patient may not be entered into the study.

During the study period between week -3 and the first plasma exchange (week 0), MRI and FDG-PET will be performed.

At the same time, during this study period (from week -3 to week 0), the following tests will be administered to measure cognition, functionality, behavior, global evolution, impression and depression : MMSE, ADAS-Cog, NPS battery, ADCS-ADL, NPI, CDR-Sb, ADCS-CGIC, CSDD, CSSRS, QoL-AD and RUD-Lite® which are to be administered as indicated in the corresponding section. Likewise, anxiety and restlessness tests will be made based on the Overt Aggression Scale (OAS) and the Agitated Behavior Scale (ABS), whenever considered opportune.

If the subject is to receive full plasma exchanges through a central venous access, a double-lumen catheter will be placed in the subclavian or jugular vein before the full plasma exchange period. It is advisable to place the catheter a few days before the first plasma exchange session. For the low-volume exchanges peripheral access will be used.

Furthermore, it is obligatory to perform lumbar puncture before the first plasma exchange, to collect CSF samples and assess the levels of Aβ–40, Aβ-42, P-tau, T-tau, β-secretase, γ-secretase, cholesterol, LDL, HDL, VLDL, C-reactive protein, IL-1b, IL-6, ferritin, TNF-α) and to perform standard CSF tests (cell counts, glucose, proteins and albumin). Time collection for the CSF must be between 8am and 12am. If these procedures are not carried out in the days preceding plasma exchange, they will have to be done on the day of first plasma exchange with the subject remaining at rest until indication by the investigator that plasma exchange can be carried out. These evaluations are considered to be the baseline data.

6.2.2 Weeks 1 to 6 - Intensive plasma exchange period

Full Plasma Exchange (Window range +/- 1 day)

Plasma samples will be collected from the subject before and 15-30 minutes after each procedure, to allow the pertinent tests. Additional plasma samples will be collected each time for storage at -70ºC and possible future assays.

The following tests will be made before each procedure: biomarkers of AD (Aβ–40, Aβ–42, β-secretase, γ-secretase, cholesterol, LDL, HDL, VLDL, C-reactive protein, rheumatoid factor, IL-1b, IL-6, ferritin and TNF-α), coagulation factors (prothrombin time (Quick), aPPT, calcium and fibrinogen) and proteinogram (total proteins, albumin, α1-globulina, α2-globulina, β-globulina, γ-globulina and IgG). In addition, a cell count will be made including hematocrit, hemoglobin and erythrocytes, platelets and leukocytes (if an abnormal leukocyte count is recorded, it will be obligate to specify neutrophils, basophils, eosinophils, lymphocytes and monocytes). The samples obtained after plasma exchange will be used to evaluate the biomarkers of AD, cell count, platelets, coagulation factors (prothrombin time

(Quick), aPPT, calcium), proteinogram, fibrinogen and troponin, as well as other biochemistry parameters (AST, ALT, LDH and creatinine).

A maximum of six full plasma exchange sessions will be made during this period: one per week.

The subject will remain in the center throughout the procedure and then for as long as needed to ensure a safe return home, under conditions similar to before plasma exchange.

Before plasma exchange, a physical examination will be made, with the recording of any anomalies. Concomitant medication is also to be registered, together with any adverse event detected since the last visit or since the last plasma exchange session. The anxiety and restlessness tests, if necessary, will be performed before each plasma exchange process, and between 15-30 minutes after replacement. These tests will be carried out by the same patient supervisor.

Vital signs (axillary temperature, heart and respiration rate, and blood pressure) will be determined 15-30 minutes before replacement during and again 15-30 minutes after the procedure, and as often as considered opportune by the investigator.

Any adverse event during or after the procedure, will be recorded.

Control group

The control group will undergo simulated (sham) full plasma exchanges and will follow the same schedule of assessments including the lumbar puncture.

6.2.3 Weeks 7 to 8 – Intermediate visit (Window range +/- 2 days)

Plasma samples will be collected from the subjects (control and full plasma exchange group) to allow the pertinent tests. Additional plasma samples will be collected each time for storage at -70ºC and possible future assays.

The following tests will be made: biomarkers of AD (Aβ–40, Aβ–42, β-secretase, γ-secretase, cholesterol, LDL, HDL, VLDL, and C-reactive protein, rheumatoid factor, IL-1b, IL-6, ferritin, TNF-α) and plasma IgG.

During the visit medical history will be checked with the recording of any anomalies. Concomitant medication is also to be registered, together with any adverse event detected since the last visit or since the last plasma exchange session.

Central catheter will be also extracted during this intermediate visit.

After the intensive plasma exchange period (weeks 1 to 6) and before Maintenance period, lumbar puncture will be performed to assess the levels of Aβ–40, Aβ–42, P-tau, T-tau and other Alzheimer's disease biomarkers together with standard tests (cell counts, glucose, proteins and albumin). Time collection for the CSF must be between 8am and 12am.

During this intermediate visit (weeks 7 to 8) the following cognitive impairment and behavioral tests will be made: ADAS-Cog, MMSE, NPS battery, ADCS-ADL, NPI, CDR-Sb, ADCS-CGIC, CSDD and CSSRS. Likewise, anxiety and restlessness tests will be made based on the Overt Aggression Scale (OAS) and the Agitated Behavior Scale (ABS), if considered opportune.

During the study period between week 7 and 8, a FDG-PET and an MRI will be also carried out.

Control group

The control group will undergo simulated (sham) central catheter removal and will follow the same schedule of assessments as the treated groups

6.2.4 Months 3 to 14 - Maintenance Period

Treatment Groups: Maintenance Period (Plasmaphereses + albumin or IVIG) (Window range +/- 5 days)

At months 6, 9, 12 and 14, the following cognitive impairment and behavioral tests will be made: ADAS-Cog, MMSE, NPS battery, ADCS-ADL, NPI, CDR-Sb, ADCS-CGIC, CSDD, CSSRS, QoL-AD and RUD-Lite®. Likewise, anxiety and restlessness tests will be made based on the Overt Aggression Scale (OAS) and the Agitated Behavior Scale (ABS), whenever considered opportune.

The plasma samples of the patient will be collected before and 15-30 minutes after each procedure, to allow the pertinent tests. These plasma samples collected before and after the procedure will be stored at -70ºC for possible future assays.

The following tests will be made before each procedure: biomarkers of AD (Aβ–40, Aβ–42, β-secretase, γ-secretase, cholesterol, LDL, HDL, VLDL, C-reactive protein, rheumatoid factor, IL-1b, IL-6, ferritin, TNF-α), coagulation factors (prothrombin time (Quick), aPTT, calcium and fibrinogen), and proteinogram (total proteins, albumin, α_1-globulina, α_2-globulina, β-globulina, γ-globulina and IgG). In addition, a cell count will be made including hematocrit, hemoglobin and erythrocytes, platelets and leukocytes (if an abnormal leukocyte count is recorded, it will be obligate to specify neutrophils, basophils, eosinophils, lymphocytes and monocytes). The samples obtained after plasma exchange will only be used to evaluate the biomarkers of AD and, if necessary, to assess coagulation factors. A more intense monitoring is not considered mandatory since in this period the procedure is similar to a plasma donation plasmapheresis. However, the investigator can decide to assess other parameters for safety reasons. Other liver or kidney biochemical markers (AST, ALT, bilirubin, LDH, creatinine and troponin), and serology (anti-HIV and HCV antibodies, and HBsAg antigen) will be also done..

There will be 3 treatment groups. A maximum of 12 plasmaphereses will be performed, one plasmapheresis per month. In the first treatment group there will be 9 plasmaphereses with infusion of albumin 20% (200 mL, 40 g) at months 4, 5, 6, 8, 9, 10, 12, 13 and 14 and 3 plasmaphereses with infusion of IVIG (20 g) at months 3, 7 and 11. In the second treatment group, the schedule will be the same as previous but with half of the dose of albumin 20% (100 ml, 20 g) and IVIG (10 g). The third treatment group will follow the same schedule but only half-dose albumin (100 ml, 20 g) will be administered in each of the 12 plasmaphereses, that is, this group will not receive IVIG. The plasma removed and the albumin infused after the procedure depends on patient's weight (see Table 1, Appendix 10).

The subject will remain in the center throughout the procedure and then for as long as needed to ensure a safe return home, under conditions similar to before the procedure.

Before each plasmapheresis, a physical examination will be made, with the recording of any anomalies. Concomitant medication is also to be registered, together with any adverse event detected since the last visit or since the last plasma exchange session.

The anxiety and restlessness tests, if necessary, will be performed before each plasmapheresis, and between 15-30 minutes after the administration of albumin or IVIG. These tests will be carried out by the same patient supervisor.

Vital signs (axillary temperature, heart and respiratory rate, and blood pressure) will be determined 15-30 minutes before plasmapheresis, during and again 15-30 minutes after the administration of albumin or IVIG and as often as considered opportune by the investigator.

Any adverse event during or after the procedure, will be recorded.

A FDG-PET will be carried out at months 9 and an MRI also will be carried out at months 6, and 9.

Control group

The control group will undergo simulated (sham) low-volume plasma exchanges but will follow the same schedule of assessments.

6.2.5 Final Visit (Month 14)

During the final visit medical history will be checked with the recording of any anomalies. Concomitant medication is also to be registered, together with any adverse event detected since the last visit or since the last plasma exchange session.

Plasma samples will be collected from the subjects (control and treatment group) to allow the pertinent tests. Additional plasma samples will be collected each time for storage at -70ºC and possible future assays.

The following parameters will also be evaluated: biomarkers of AD (Aβ–40, Aβ–42, β-secretase, γ-secretase, cholesterol, LDL, HDL, VLDL, C-reactive protein, rheumatoid factor, IL-1b, IL-6, ferritin, TNF-α), coagulation factors (prothrombin time (Quick), aPTT, calcium and fibrinogen), proteinogram (total proteins, albumin, α_1-globulina, α_2-globulina, β-globulina, γ-globulina and IgG), other liver or kidney biochemical markers (AST, ALT, bilirubin, LDH and creatinine), troponin and serology (anti-HIV and HCV antibodies, and HBsAg antigen). In addition, a blood cell count will be performed, including: hematocrit, hemoglobin and erythrocytes, platelets and leukocytes (if an abnormal leukocyte count is recorded, it will be obligate to specify neutrophils, basophils, eosinophils, lymphocytes and monocytes).

During the final visit a MRI and a FDG-PET will also be performed.

The following tests will be performed to measure cognition, functionality, behavior, global evolution, impression and depression: ADAS-Cog, MMSE, NPS battery, ADCS-ADL, NPI, CDR-Sb, ADCS-CGIC, CSDD, CSSRS, QoL-AD and RUD-Lite® which are to be administered as indicated in the corresponding section. Likewise, anxiety and restlessness tests will be made based on the Overt Aggression Scale (OAS) and the Agitated Behavior Scale (ABS), whenever considered opportune.

Furthermore, it is obligatory to perform lumbar puncture, to collect CSF samples and assess the levels of Aβ–40, Aβ–42, P-tau, T-tau, β-secretase, γ-secretase, cholesterol, LDL, HDL, VLDL, C-reactive protein, IL-1b, IL-6, ferritin, TNF-α) and to perform standard CSF tests (cell counts, glucose, proteins and albumin).

These evaluations are considered to be the final data.

Control group

The control group will undergo the same schedule of assessments.

In case the final visit is conducted before completing the study, some assessments could be avoided. It will be decided case by case and depending on when the last assessments have been done.

6.2.6 Description of the Response Evaluation Methods

Medical history will be performed according to usual practice. Physical examination likewise will be performed according to the practice of the investigator. The investigator will carry out a general evaluation of the body systems, together with a full physical examination during the initial visit and again at the end of follow-up. The vital signs will be measured according to standard practice of the investigator.

Blood samples will be drawn according to standard practice of the center for the obtainment of blood, plasma and serum.

The amount of blood and CSF to be collected for all the tests will correspond to the amount needed for the assays in each visit or procedure. The precise amount of each sample will be established according to the requirements of the specific laboratory performing the analysis. As a recommendation, the minimum amounts are:

- Plasma samples for frozen back-up (-70^{o}C): 10 mL of blood which, once processed, will be divided into three equal plasma aliquots in three cryopreservation tubes.
- From the plasma bag corresponding to each plasma exchange (FPE and LVPE), six samples will be obtained, with a maximum volume of two ml each, at each of the following time points: 30 minutes after starting replacement, after one hour, and at the end of the procedure. Thus, 18 plasma samples will also be obtained in each replacement session. The removed plasma bag from the FPE and LVPE will also be collected. These samples will be collected in some selected sites.
- Aβ and P-tau: 10 mL of blood (to obtain 5 mL of plasma) with EDTA (collection in polypropylene tubes is needed, since Aβ adheres to glass). In addition, 10 mL of blood with EDTAK2 will be obtained separately to assess Aβ in blood.
- Genetic markers: 4 mL of blood (approximately 2 mL of plasma) with EDTA.
- Coagulation parameters: 5 mL of blood (collected according to standard practice of the laboratory).
- Hematological tests: 5 mL of blood (collected according to standard practice of the laboratory).
- Proteinogram, biochemistry (kidney and liver function), and serology: 10 mL of blood (collected according to standard practice of the laboratory).
- CSF: At least a minimum of 5 mL and a maximum of 10 mL of CSF will be obtained according to the standard technique employed in the center. The sample will be divided into three aliquots: 3 mL for general tests (cell count, glucose, proteins and albumin), 3 mL for Aβ and P-tau (collected in polypropylene tubes), and 4 mL for frozen back-up (-70^{o}C), stored in cryopreservation tubes.

  The CSF aliquot destined for general testing should not be frozen but kept at room temperature, with immediate analysis. The other two aliquots can be immediately frozen, however, future testing cannot be done if >10 erythrocytes are detected. To avoid rejection of the sample, these aliquots may be centrifuged and the clarified CSF saved to eliminate any possible residual cells.

In the event of insufficient blood or plasma sample volume, priority should center on the measurements of Aβ and P-tau, then reserving three mL for frozen back-up, and the rest for testing according to the course of the study and investigator criterion (e.g., during the Intensive treatment phase the coagulation parameters are a greater priority than the

proteinogram; in contrast, during screening or the final visit, serology is the most important aspect).

In the event of insufficient CSF volume, priority is to center on the Aβ and P-tau measurements, then reserving two mL for frozen back-up, and the rest for general testing (particularly cell counts).

During the visits, and after blood collection, processing of the samples to obtain the plasma and/or serum is to be made within two hours. The blood and plasma and/or serum samples will be collected and taken immediately to the site laboratory for processing.

The serum and/or plasma samples can be analyzed on the same day (depending on the standard practice of the laboratory and test involved), or stored at -20ºC for analysis within no more than two months after collection.

The routine hematological tests are to be made within a maximum of 12 hours after blood or CSF collection. Prior to analysis, the samples can be kept at 2-8ºC, with the exception of the CSF samples, which must be kept at room temperature.

The plasma and CSF samples collected for possible future assays will be stored at -70ºC.

Hematological, coagulation, and kidney and liver function tests will be performed by the standard practices of the laboratory.

7. SPECIFIC METHODOLOGY

7.1 Behavioral and cognitive impairment scales and tests

The cognitive measurement instruments used are mainly those sensitive to change, in regard to the nature of the study (pre-post condition). Specifically, the following tests are will be performed (see **Appendix 7**):

7.1.1 Gold standards for the study of dementia

Screening: Mini-Mental Status Examination (MMSE)[36]. This test is widely used to assess cognitive alterations, and is the most commonly used brief screening test. The score ranges from 0 to 30, and is obtained by summing the points corresponding to each answer. Results below 26 points may indicate cognitive impairment. The test performed on occasion of the selection visit will serve as criterion for inclusion in the study. The subject is required to yield a score of ≥18 and ≤26 points.

For the evaluation of cognitive function in AD: Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog)[37]. This is an instrument specifically designed to evaluate the severity of the fundamental alterations in cognitive and behavioral function that are characteristic of patients with AD. The instrument consists of two parts (ADAS-Cog and ADAS-no Cog): the first measures cognitive function and the second (not to be applied in the present study) assesses non-cognitive (behavioral) function. The first part comprises 11 subtests that explore different areas: spoken language skills, test instructions recall, difficulty finding words, following instructions, naming objects, construction of figures, ideation praxis, orientation, recall and recognition of words. Scoring is by errors, and the total score ranges from 0 (no impairment) to 70 (maximum impairment).

7.1.2 Specific neuropsychological battery (NPS battery)

a) **Processing speed**

i. *Symbol Digit Modalities Test (SDMT)[38]:* developed principally for examining visual attention and tracking, concentration and psychomotor speed. The correct symbol-number pairs during 90 seconds are counted. Maximum score = 110.

b) **Language (global measures)**

i. *Semantic and phonetic verbal fluency (animals per minute)[39,40]:* allowing voluntary access to a certain vocabulary assessing reduction in verbal spontaneity and fluency difficulties.

ii. *Boston Naming Test (BNT)[41] reduced to 15 words:* This is an essential test for the assessment of semantic memory in dementia evaluation protocols. The total score is the sum of the correct answers given spontaneously, together with the correct answers after a semantic cue. The first incorrect item is also reflected in the last section.

c) **Verbal memory (immediate/delayed)** - *Rey Auditory Verbal Learning Test (RAVLT)[42]:* This is a test of 5 learning series of 15 words presented in the same order that the subject listens and must remember and repeat at the end of each series. Next, a new series of 15 different words is administered as interference. Again, the subject is asked to remember the initial series. Finally, after 30 minutes, the subject is asked to the words from the initial list (deferred memory). Scoring is based on the number of words recognized.

In addition, the following instruments are included for the measurement of other variables:

Functional ability

Activities of daily living: Alzheimer's Disease Cooperative Study - Activities of Daily Living (ADCS-ADL)[43]: This offers detailed descriptions of each activity and requests the informer to describe the actions or behaviors observed. The evaluator describes ability to carry out a series of activities of daily life (e.g., personal hygiene, using the phone, leaving home, etc.). The total sum is not calculated, though increased autonomy is associated with higher scores.

Neuropsychiatric disorders

Neuropsychiatric Inventory (NPI)[44]: This evaluates the most frequent neuropsychiatric manifestations of dementia and also determines their frequency and intensity. Generally, the NPI is used to evaluate the changes in patient behavior that have appeared in the course of a given period of time. The NPI comprises 12 domains: delusions, hallucinations, dysphoria, apathy, euphoria, disinhibition, aggressivity and restlessness, irritability, anxiety, aberrant motor behavior, appetite and eating disorders, and nocturnal behavior. Each domain comprises an exploratory question, where an affirmative answer is followed by a series of seven to nine follow-up questions relating to symptoms present or not in that particular domain.

Frequency is scored as follows: 0 = none; 1 = occasional [less than once a week]; 2 = often [more or less once a week]; 3 = frequent [several times a week]; 4 = very frequent [daily or continuously].

Severity is scored as 1 = mild; 2 = moderate [if possible to reconduct by care taker]; 3 = severe [if not possible to reconduct].

The NPI thus evaluates response to therapy; the total score of the NPI is obtained by adding the scores (frequency x severity) of all the inventory domains. The score on the stress scale is not included in the total NPI score.

Measurement of depression in dementia: Cornell Scale for Depression in Dementia (CSDD)[45]. This scale evaluates the signs and symptoms of major depression in patients with dementia. The information is obtained from two semistructured interviews - one with the caretaker and the other with the patient. Each item is scored for intensity (0 = none; 1 = mild or intermittent; 2 = severe).

Clinical grading of dementia, global evolution: Clinical Dementia Rating – Sum of boxes (CDR-Sb)[46,47]. This is a clinical test validated in patients with AD. Impairment is scored as follows: 0 = normal; 0.5 = slight cognitive impairment or questionable dementia; 1 = slight dementia; 2 = moderate dementia; 3 = severe dementia. Six categories are contemplated: memory, orientation, judgment and problem solving, social and occupational activities, domestic activities and hobbies, and personal care.

Clinical global impression of change: Alzheimer's Disease Cooperative Study – Clinical Global Impression of Change (ADCS-CGIC)[48]. This instrument offers a method for the reliable assessment of global change in a clinical trial from baseline, using a 7-point Likert scale. It is administered to the caretaker and to the patient.

7.2 Method of Behavioral and Cognitive Testing

The established order in each visit will be as follows:

With the patient:

Rater 1

a) MMSE (when stated by the protocol)
b) ADAS-Cog
c) NPS battery
    i. RAVLT (Parts 1, 2, 3, 4 and 5)
    ii. RAVLT (Part 6: interference)
    iii. RAVLT (Part 6B: evocation)
    iv. BNT (15 items)
    v. SDMT
    vi. Semantic and phonetic verbal fluency
    vii. CSDD (includes the 30 minutes for differed memory)
    viii. RAVLT (Part 7: differed memory 30 minutes after part 6B)
    ix. RAVLT (Part 8: differed recognition)
d) QoL-AD
e) C-SSR (only once rater 1 has administered scales to caretaker)

Rater 2

Next, the global impairment and clinical impression scales are administered by the second evaluator:

a) CDR-Sb
b) ADCS-CGIC

With the care taker:

The second evaluator, while the evaluator 1 is administering the above mentioned battery, will administer the global impairment and clinical impression scale to the care taker in the following order:

a) CDR-Sb

b) ADCS-CGIC

The first evaluator, once he has finished the NPS battery, will interview the care taker to evaluate the subject functionality and behavior:

- NPI
- ADCS-ADL
- CSDD
- QoL-AD
- RUD-Lite

Evaluators are blinded to patient treatment. Importantly, the evaluators of the neuropsychological tests will not have access to any kind of information allowing them to identify patient assignment to treatment.

An evaluator will administer all the neuropsychological battery scales: MMSE, ADAS-Cog, NPS Battery, behavior scales NPI and CSDD and the functional scale ADCS-ADL.

The second evaluator, also on a blind basis, will administer the CDR-Sb and ADCS-CGIC. Results from previous evaluations will remain unknown for both evaluators and they must not comment any result to each other or to other study members and they will be considered blinded evaluators and will sign a document to the effect.

Anxiety and restlessness tests (during the replacement phase):

In case any sign of aggression or restlessness during the procedure, the following instruments will be used:

Measurement of overt aggression*:* Overt Aggression Scale (OAS)[49]. This instrument measures patient aggression and provides a global score as the sum of the scores assigned to each of the different items. Increased severity is associated with higher scores.

Measurement of restlessness: Agitated Behavior Scale (ABS)[50]. This includes 14 items grouped into three factors (disinhibition, and liability). The global score is obtained from the sum of the scores assigned to the different items. Increased severity is associated with higher scores.

Columbia-Suicide Severity Rating Scale (C-SSRS):

The scale is currently being used in a variety of settings from clinical trials to clinical practice. The Columbia–Suicide Severity Rating Scale (C-SSRS) was designed to quantify the severity of suicidal ideation and behaviour.

The primary outcomes to evaluate are: suicidal behaviour categorical rating, suicidal ideation categorical rating and to estimate the rate of suicidality.

We are going to use two versions of the scale that are currently being used in clinical trials:

- The **Baseline** version of the scale assesses lifetime suicidal ideation and behavior. This version is suitable as part of a patient's *first* interview in a private practice or as part of a clinical study.

- The **Since Last Visit** version of the scale assesses suicidality since the patient's last visit. This version is meant to assess patients who have completed at least one initial C-SSRS assessment, and should be used in every subsequent visit. The 'Since Last Visit' version of the C-SSRS is asking about any suicidal thoughts or behaviors the patient/participant may have had since the last time you have administered the C-SSRS.

Quality of Life AD Measure (QoL-AD):

The QoL-AD is a brief, 13-item measure designed specifically to obtain a rating of the patient's Quality of Life from both the patient and the caregiver. It was developed for individuals with dementia, based on patient, caregiver, and expert input, to maximize construct validity, and to ensure that the measure focuses on quality of life domains thought to be important in cognitively impaired older adults. It uses simple and straightforward language and responses & includes assessments of the individual's relationships with friends and family, concerns about finances, physical condition, mood, and an overall assessment of life quality.

Caregivers complete the measure as a questionnaire about their patients' QoL, while patients complete it in interview format about their own QoL. The measure consists of 13 items, rated on a four point scale, with 1 being poor and 4 being excellent. Total scores range from 13 to 52. It generally takes caregivers about 5 minutes to complete the measure about their patients; for patients, the interview takes about 10 to 15 minutes to administer. Detailed instructions for interviewer administration are available.

Scoring is straightforward - the sum of all items; patient and caregiver reports can be evaluated separately and/or combined into a single score if desired. Patients with MMSE scores of 10 or higher can usually complete it with no problem; below that caregivers can continue to complete it as proxies indefinitely.

Resource Utilization in Dementia (RUD-Lite©) Questionnaire:

The Resource Utilization in Dementia (RUD) Questionnaire was developed as a comprehensive tool to assess the amount of resource use among demented patients, which in a further step can be calculated into costs. It has been used in pharmacoeconomical studies and it has proven to be a useful tool in the evaluation of dementia care. RUD Lite© has been developed as a new shorter version of the instrument RUD.

RUD assesses both formal and informal resource use of patients and the primary caregiver, making it possible to calculate costs from a societal perspective. It consists of two parts with a similar content, but the wording is adapted to a baseline situation or follow-up situation(s). The questionnaire is divided in two parts, the first one is about the patients' and the second one is about the caregivers' health status and resource use.

7.3 Method of Biochemical Marker Testing

Aβ in plasma, CSF and withdrawn plasma and P-tau and T-tau in CSF: These determinations will be carried out using ELISA as detailed below:

- Determination of plasma and CSF $A\beta_{1-40}$ (The Genetics Company) and $A\beta_{1-42}$ (Innogenetics and The Genetics Company)
- Determination of P-tau and T-tau in CSF using the Innotest P-tau and Innotest T-tau (Innogenetics)
- Determination of $A\beta_{1-40}$ and $A\beta_{1-42}$ pool in blood (Araclon Biotech).

- Determination of other biomarkers will be assessed from plasma removed samples.

The plasma-EDTA samples will be collected in the course of the study and will be stored frozen. The assays will be performed at the same time and in parallel with pre-treatment (baseline) samples and post-treatment samples (coded), to avoid modifications related to day variations.

7.4 Method of Neuroimaging Studies

FDG-PET

This study will be carried out according to the standard operation procedures of this study. The images will be sent to a central vendor for interpretation.

Four FDG-PET studies per patient will be made. A comparison between control and treatment groups will be performed. Qualitative and quantitative evaluation will be made by an experienced specialist.

MRI

MRI will be performed with a 1.5 Tesla magnet with a real-time acquisition system (Real-time fMRI). LX 9.1 M4 software will be used. The gradient amplitude will be 40 mT/m, with a slew rate of 150 T/m/s. A schematic representation of the process is provided.

The MRI scans will be examined by the central vendor clinical neuroradiologist, who in turn will issue a safety report for each subject. Any imaging anomaly will be communicated to the investigating team.

Once all the study MRI scans have been obtained, they will be analyzed using a visual scale with the CVHS standards, based on adequate software (3D-SPGR), including:

- Brain reconstruction in the axial plane, aligning with a standard reference image (MNT T1 matrix)
- Normal tracing of the hippocampus and of the anterior cingulate region using standard software
- Analysis of the volume of the hippocampus and of the anterior cingulate region using the LOWI system[51].

Series 1 - Sagittal T1 Scout

Plane	Mode	PSD	TE	TR	FOV	Slice/Gap	#sli	Matrix	NEX	Freq Dir	Auto Shim	Time
Sagittal	2D	SE	MF	400	20	5/inter L50-R50	21	256 x 192	1	SI	Y	2:46

Series 2 – Axial T1

Plane	Mode	PSD	TE	TR	FOV	Options	Slice/Gap	#sli	Matrix	NEX	Freq Dir	Auto Shim	Time
Axial	2D	SE	MF	400	20	VBw=15.63	5/1	24	256 x 192	1	AP	Y	2:49

Series 3 – Axial fse PD

Plane	Mode	PSD	TE	TR	ETL	FOV	Options	Slice/Gap	#sli	Matrix	NEX	Freq Dir	Auto Shim	Time
Axial	2D	fse	17	2000	4	20	VBw=15 .63 FC	5/1	24	256 x 192	1	AP	Y	1:40

Series 4– Axial fse T2

Plane	Mode	PSD	TE	TR	ETL	FOV	Options	Slice/Gap	#sli	Matrix	NEX	Freq Dir	Auto Shim	Time
Axial	2D	fse	102	2500	8	20	Vbw=15.63 FC	5/1	24	256 x 192	1	AP	Y	2:10

Series 5 – Coronal 3D SPGR

Plane	Mode	PSD	TE	TR	Flip	FOV	Slice	Matrix	NEX	Freq Dir	Auto Shim	Time
Cor	3D	SPGR	5	25	40	24x18 3/4 PFOV	124	256 x 192	1	SI	Y	7:44

Series 6 - Axial FLAIR

Plane	Mode	PSD	TE	TR	TI	FOV	Options	Slice/Gap	#sli	Matrix	NEX	Freq Dir	Auto Shim	CV's	Time
Ax	2D	FLAIR ir	156	10000	2200	20	Vbw=3 1.25	5/1	24	256 x192	1	AP	Y	Min Acq =1	3:20

8. ASSESSMENT OF EFFICACY

The first co-primary efficacy endpoint is to compare the changes to baseline of the cognitive scores as measured by ADAS-Cog between each of the three treatment arms and the control group and between the three treatment arms themselves. The second of the co-primary efficacy endpoints is to compare the changes to baseline of the ADCS-ADL inventory scores between each of the three treatment arms and the control group and between the three treatment arms themselves.

The secondary efficacy endpoint is to assess the variations in other cognitive scores, AD biomarkers and other biochemical parameters.

8.1 Efficacy variables

Change from baseline in the cognitive scores as measured by ADAS-Cog (6 measurements: weeks -3, -2 or -1, and 7-8, months 6, 9, 12 and 14).

Change from baseline in the ADCS-ADL inventory (6 measurements: weeks -3, -2 or -1 and 7-8; month 6, 9, 12 and 14).

8.2 Secondary efficacy variables

- Change from baseline in the cognitive, functional and neuropsychiatric scores and overall development as measured by MMSE, NPS battery, NPI, CDR-Sb, ADCS-CGIC, CSDD, C-SSRS. (6 measurements: weeks -3, -2 or -1 and 7-8, month 6, 9, 12 and 14) and QoL-AD, RUD-Lite® (5 measurements: weeks -3, -2 or -1; months 6, 9, 12 and 14). Moreover the optional questionnaires like OAS and ABS could be used at any visit in case any sign of aggression or restlessness.
- Variation in levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in CSF in the period between baseline lumbar puncture (before the start of the intensive treatment period) and lumbar puncture immediately after the end of the last plasma exchange (whenever this may be).
- Variation in the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in CSF between the finalization and beginning of each of the 2 treatment periods.
- Levels of $A\beta_{1-40}$, $A\beta_{1-42,}$ T-tau and P-tau in CSF throughout the study.
- Plasma levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ before and after each plasma exchange for both treatment periods.
- Structural changes in volume of the hippocampus, posterior cingulate area, and other associated areas by MRI. Five measurements will be made (weeks -3, -2 or -1 and 7-8; months 6, 9 and 14).
- Variation in FDG-PET patterns (4 measurements: weeks -3, -2 or -1 and 7-8; months 9, and 14).

8.3 Safety variables

The primary criterion of safety will be the percentage of plasma exchanges (full and low-volume exchanges, including the infusion of albumin and IVIG) associated with at least one adverse event that may be related to the study procedure (adverse reactions) within 72 hours after infusion completion (or after the infusion stops). In addition, consideration globally will be made of the percentage plasma exchanges (full and low-volume exchanges, including the infusion of albumin and IVIG) involving some adverse event, whether or not related to the procedure.

Vital signs (blood pressure, heart rate, respiration rate and body temperature) will be recorded before, during and after each plasma exchange session, where required. Evaluation will also be made (where required) of the different laboratory test parameters (blood cell counts, platelet count, troponin, prothrombin time (Quick), aPPT, fibrinogen, total proteins, and calcium).

During the treatment periods (before each plasma exchange) and on the days when there is no replacement, anxiety and restlessness tests will be made based on the Overt Aggression Scale (OAS) and the Agitated Behavior Scale (ABS), whenever considered opportune.

According to the criterion of the investigator, all the clinically important changes in vital function, laboratory test parameters and neuroimaging findings will be evaluated.

9. ASSESSMENT OF SAFETY

9.1 Adverse Events

9.1.1 Information to specify

Descriptions/Definitions:

- *Adverse Event (AE)* is any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

- *Adverse Reaction (AR)* is all untoward and unintended responses to an investigational medicinal product related to any does administered.

- *Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)* is any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.

Likewise, medical criterion will decide whether an event is serious in other situations such as those requiring intervention to prevent some of the aforementioned consequences.

Medical and scientific criteria will decide whether other situations that have not led to the outcomes listed in the above definitions should be notified as SAE.

By life-threatening event, it is understood a situation which according to the investigator opinion, would have led to the patient death in the absence of a timely therapeutic intervention.

- *Unexpected Adverse Event*: any adverse experience of a nature or severity that does not correspond to the information available in reference to the investigational procedure or study drug. Once the adverse event has been evaluated and reasonable bases are established for suspecting a causal relation to the study procedure and the SPC for the study drugs, the event is to be classified as an **adverse reaction** to the procedure or to the drug**.**

Detection and recording:

- *Method of Detection:* The AEs will be daily collected from a careful clinical observation of the patient, laboratory analyses, spontaneous communication from the patient and also through and open questioning from the investigator

- *Adverse events recording:* All adverse events and serious adverse events will be recorded in the patient's medical records and in the CRF (Adverse Events Form). In addition, the adverse

events related to the Auto-C (FDA PMA approval # BP850001) or Aurora (FDA 510(k) clearance # BK110072) based devices used in the low-volume plasma exchanges (Maintenance period) will be recorded in the specific form attached to the Auto-C (FDA PMA approval # BP850001) or Aurora (FDA 510(k) clearance # BK110072) clinical protocols **(Appendix 3)**.

In the context of this clinical trial, all incidents clearly related to progression of the disease will not be considered as adverse events, and will not be necessary to include them in the CRF.

All adverse events occurring during the study are should be included in the CRF of the corresponding patient. The minimum information to be supplied comprises the nature of the event, its time of appearance in relation to the administration, its duration, dose, seriousness, intensity, causality, the actions taken, and the course. If it is a preexisting AE that gets worse regarding intensity or frequency it should be indicated.

Adverse events will be classified according to severity, depending on the intensity of the event, as follows:

Mild, when well tolerated by the patient, causing only minimal discomfort, and without interfering with the activities of daily living (ADL)

Moderate, when interfering with activities of daily living

Intense or severe, when impeding activities of daily living

Severity must be distinguished from seriousness, which is based on the consequence of the adverse event. For example, a headache may be mild, moderate or severe, though rarely is it serious.

9.1.2 Imputability criteria

To analyze the possible relation cause-effect, it will be considered the temporal relationship between drug administration and the AE, possible alternative causes, evolution (complete remission, partial recovery, death and sequelae), persistence or not after drug discontinuation, reappearance with drug re-administration, or the previous knowledge of the event matching up with the known or expected response pattern of the study drug.

The following definitions will be used to establish cause-effect relationships:

Definitive: the adverse event occurs after a reasonable time sequence related to the study drug administration or study procedure, with a known response model, but cannot be explained by the patient clinical condition and other treatments provided, confirmed by improvement after interrupting or diminishing treatment dose, and with relapse after reintroducing treatment.

Probable: the adverse event occurs after a reasonable time sequence related to the study drug administration or study procedure, with a known response model, confirmed by improvement after interrupting treatment, and that cannot be explained by the patient clinical condition or other treatments provided.

Possible: the adverse event occurs after a reasonable time sequence related to the study drug administration or study procedure, with a known response model, but can be explained by the patient clinical condition and other treatments provided.

Unlikely: the adverse event occurs after a reasonable time sequence related to the study drug administration or study procedure, not following a known response model, and that cannot be explained by the patient clinical condition or other treatments provided.

Unrelated: Any other adverse event.

9.1.3 Expected adverse events

There are a series of **expected adverse events** attributable to the plasma exchange procedure, with others that are inherent to the replacement fluid used (human albumin). Lastly, other events are a consequence of patient disease or idiosyncrasy.

The expected adverse events of plasma exchange are the following: hypocalcaemia (manifesting as paresthesias, headache, vision alterations, nausea, cramps and chest oppression), anaemia and coagulation disorders secondary to coagulation factor dilution (mainly hypofibrinogenemia, with possible minor bleeding associated to severe thrombopenia). Hypotension may occur (accompanied by paleness, perspiration, bradycardia, nausea, vomiting, syncope, sphincter relaxation and seizures); though the risk is minimal, since the infusion amount is equivalent to the extracted amount. Allergic reactions are also possible, with urticaria, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema. Other events may be of psychological origin or may be inherent to the process, such as for example pain after remaining immobile for several hours.

As indicated by the **Full Prescribing Information** (**Appendix 1**), the Albutein® specific adverse events are mild reactions such as reddening, urticaria, fever and nausea. Such reactions are rare and normally subside when the perfusion rate is reduced or perfusion is suspended. Anaphylactic shock may be observed in isolated cases. In such situations, perfusion is to be suspended immediately with the provision of adequate treatment.

Albutein® is obtained from human plasma. The controls applied in the centers where the plasma is obtained, and in the fractionation plant, as well as the validated infectious agent inactivation/elimination procedures included in the production process, are all designed to minimize the risk of transmission of infectious disease. In any case, when dealing with blood products it is not possible to fully rule out possible transmission of infectious agents. This also refers to the possible transmission of unknown pathogens.

In a 1996 review of the adverse effects of plasma exchange in patients with Guillain-Barré syndrome involving albumin replacements, a decrease in immediate adverse effects from 12% in 1986 to 7% in 1992 was recorded. Allergic reactions and hypotension were the most frequent problems. The estimated mortality was 1-2 deaths per 10,000 sessions, or two deaths per 2000 patients.

With regard to Flebogamma®DIF (see **Full Prescribing Information, Appendix 2**) Adverse reactions such as chills, headache, fever, vomiting, allergic reactions, nausea, arthralgia, low blood pressure and moderate low back pain may occur occasionally. Rarely human normal immunoglobulins may cause a sudden fall in blood pressure and, in isolated cases, anaphylactic shock, even when the patient has shown no hypersensitivity to previous administration. Cases of reversible aseptic meningitis, isolated cases of reversible haemolytic anaemia/haemolysis and rare cases of transient cutaneous reactions, have been observed with human normal immunoglobulin. Increase in serum creatinine level and/or acute renal failure have been observed. Very rarely: Thromboembolic reactions such as myocardial infarction, stroke, pulmonary embolism, deep vein thromboses.

In addition, there may be adverse events related to the concomitant medication provided. Thus, acetilcolinesterase inhibitors have been associated with diarrhea, muscle cramps, fatigue, nausea, vomiting, insomnia, headache, pain, accidents, common cold, gastrointestinal problems and dizziness. There have also been sporadic reports of syncope, bradycardia, sinoatrial block, atrioventricular block, seizures, liver dysfunction, psychiatric problems

(hallucinations, restlessness and aggressive behavior), anorexia, gastric and duodenal ulcers, gastrointestinal bleeding, extrapyramidal symptoms, and a small increase in serum muscle creatine kinase (CK).

9.2 Procedures for the immediate notification of serious adverse events

Immediate notification of **serious** adverse events (expected or unexpected) by the investigator:

The Principal Investigator must report **immediately** to United BioSource Corporation Safety Team (CRO) all **serious adverse events (SAEs)** regardless of the study drug causality. The notification deadline (whether expected or unexpected) will be 24h from the moment the investigator first receives news of the event..

The initial submission of the SAE (whether expected or unexpected) must be done in less than 24h by email to United BioSource Corporation Safety Team (Contact email: AMBARSAFETY@unitedbiosource.com, fax number: 0800 24 25 26 27).

Moreover, this information must be subsequently completed within a maximum of 3 days using the Serious Adverse Event Notification form. The form will be preferably sent via email or by fax to the above mentioned numbers.

In case of SAE (expected or unexpected), the investigator should provide to United BioSource Corporation Safety Team all the information related to the event (additional laboratory tests, discharge report, etc).

In case of death, the investigator should provide to United BioSource Corporation Safety Team and to the Ethics Committee/Institutional Review Board involved, all the additional information requested.

Serious and unexpected adverse events potentially related to the study drug will be promptly reported to the Ethics Committee/Institutional Review Board involved and to the Health Authorities in accordance with the applicable legislation. All adverse events will be reported tabulated in the final clinical trial report.

10. STATISTICAL METHODS

10.1.1 Analytical Populations

All subjects included in the study and subjected to at least one plasma exchange session (i.e., randomized and treated) during the intensive treatment phase (the six first weeks of treatment) will form part of the efficacy population.

Control group subjects will also be included (without the plasma exchange) if they attended at least 1 of the 6 intensive treatment phase visits.

A second efficacy analysis will also be carried out (per protocol analysis) with the patients who complete the treatment without major breaches in the study protocol.

10.1.2 Missing Data

Subjects with missing values for a given efficacy value at baseline of at the time point analyzed will be treated as missing rather than using imputed values. More details regarding handling of missing data will be provided in the statistical analysis plan (SAP).

10.1.3 Demographic and Baseline Characteristics

The demographic and baseline characteristics of the study patients will be presented in table form by treatment group and center. The results will also be expressed in total.

All continuous variables will be reported using standard statistical measures, i.e. number of observations, mean, standard deviation, minimum and maximum, median, and first and third quartiles.

All categorical variables will be summarized by frequency tables.

Equality of the baseline characteristics between the treatment groups will be assessed by descriptive methods.

10.2 Assessment of Efficacy

10.2.1 General

In general, the statistical tests will be performed with a 5% significance level and will be two-sided. In addition to the tests, two-sided 95% confidence intervals (95% CI) will be reported.

Specifically, the null hypothesis for all inferential analyses, including the primary endpoint, is that all three treatment groups are equal to placebo. Specific doses will be considered different from placebo if statistically significant following the Hochberg procedure described below. The study is considered positive if at least one dose group differs from placebo in the primary analysis. Subsequently, secondary endpoints are considered positive if at least one dose group differs from placebo.

More details will be provided in the statistical analysis plan (SAP).

10.2.2 Primary Endpoint

The co-primary endpoints, changes from baseline of the ADAS-Cog scores and change from baseline in the ADCS-ADL inventory, will be assessed with the principal analytical objective being to determine whether there are differences between each of the 3 treatment groups and the control group. In addition, a comparison between the 3 treatment groups will be performed.

Success of the study is determined by the change from baseline in the Total ADAS-Cog score at 14 months and the change from baseline in the ADCS-ADL inventory score ADAS-Cog and ADCS-ADL scores at remaining time points are considered to be secondary to the scores at month 14. Missing

The mean change from baseline in both the ADAS-Cog and ADCS-ADL scores will be analyzed using analysis of covariance (ANCOVA) with adjustment for respective baseline as the covariate to compare the difference between each treatment group and the placebo group in the efficacy population.

If the data distribution assumptions are significantly violated, a non-parametric analysis will be used. If the test for either normality of error terms or equality of error variances fails at the significance level of 0.01, the rank ANCOVA procedure will be applied. The standardized ranks for both covariate and the response variable will be produced for each stratum. Then linear regression models will be performed on ranked data by stratum to generate the residuals. Finally the stratified mean score test using the value of the residuals as scores will compare the treatment groups using the Cochran-Mantel-Haenszel procedure.

Because the endpoints are co-primaries, both must be statistically significant for the study to provide evidence of efficacy. Therefore, no multiplicity adjustment is needed to adjust for the co-primary endpoints. However, to account for the three dose group comparisons to placebo and to maintain the overall significance level of 0.05, adjustment for α will be made for multiple dose groups according to the Hochberg procedure. The Hochberg procedure will be implemented as follows: For each endpoint the three dose group comparisons versus placebo will be ordered according to the p-value from the largest to the smallest. If the largest p-value is < 0.05 then all comparisons to placebo will be declared significant. Otherwise, if the second largest p-value is < 0.025 (0.05/2) then this dose and all doses with smaller p-values will be declared significant. Otherwise if the smallest p-value is < 0.0167 (0.05/3) then this dose will be declared significant. Otherwise, no dose is considered significantly different from placebo. This procedure has been demonstrated to control the overall Type I error at 0.05. Both co-primary endpoints must be significant at a given dose to provide evidence of efficacy for that dose.

A supportive analysis will be performed in per protocol analysis set for the primary efficacy endpoint applying the same respective methodologies, but without adjusting for Type I error.

10.2.3 Secondary Endpoints

All continuous secondary endpoints (Section 8.2) will be tested in a manner identical to the primary analysis, with the corresponding baseline used as the covariate.

10.3 Evaluation of Tolerability

The analysis of tolerability will be based on description of the safety variables according to their nature. In addition, a comparison of Adverse Drug Reactions between the treatment and the control groups will be performed.

The adverse reactions will be coded according to the adverse events classification of the World Health Organization (WHO) (MeDRA current version), and will be described by a synonym (Lowest Level Term) and the affected organ / system, the intensity, causality and seriousness.

10.4 Intermediate Analysis and Stopping Rules

A descriptive analysis is planned once half of the subjects are undergoing low-volume plasma exchanges.

Serious adverse events related to full plasma exchanges (performed during the first six weeks), AEs leading to withdrawal of the study and additional safety measures if required will be monitored by the independent DMC on a regular basis. Special focus will be put on those SAEs considered as related to the therapeutic procedure or Investigational Product(s) by the Principal Investigator as defined in the protocol. Additional common side effects might also be analyzed if significantly higher proportions are found in any of the treatment arms.

Under the following circumstances the independent DMC will recommend to halt the trial partly (stopping a specific treatment arm) temporarily (until modifications to the protocol are completed) or wholly (abandon all study activities):

- If more than 30% of patients undergoing full plasma exchanges experience an SAE as defined by the protocol labeled “Definitively related to the therapeutic procedure” or “Probably related to the therapeutic procedure” or “Possibly related to the therapeutic procedure” and not attributable to the Investigational Products, as judged by the principal investigator.

- If more than 30% of patients undergoing full plasma exchanges experience an SAE as defined by the protocol labeled "Definitively related to the Investigational Product(s)" or "Probably related to the Investigational Product(s)" or "Possibly related to the Investigational Product(s)" and not attributable to the therapeutic procedure or technique of application, as judged by the principal investigator.

10.5 Conduction of the Statistical Analysis

The statistical analysis will be carried out by the Biometrics Department of UBC (United Biosource Corporation).

11. CHANGES TO THE PROTOCOL

Any attached document or document related to the present study protocol must be considered as part of the latter.

After protocol review and signing, neither the investigators nor the sponsor may introduce modifications or alterations without written approval from both parties.

Any modification or alteration of the protocol after signing of the latter must be jointly discussed and approved by the principal investigator and sponsor, and must be signed by both. Protocol amendments are to be incorporated as part of the original protocol. The Institutional Review Board (IRB) must be informed of all protocol amendments that may affect the safety of the participating subjects or conduction of the trial. Amendment approval must be requested if considered necessary.

12. ETHICS

12.1 General Considerations

The ethical standards adopted by the XVIII World Medical Assembly **Declaration of Helsinki** (and subsequent revisions) will be strictly observed (**Appendix 5**). The trial likewise will be performed in compliance with European Union standards of Good Clinical Practice (GCP) relating to trials involving drug products[64] (see **Appendix 6** for the responsibilities of the sponsor, monitor and investigator).

The study cannot begin until an Institutional Review Board (IRB) or Ethics Committee and the health authorities (if necessary) have approved the protocol, the informed consent document, and the patient information sheets. The study file is to include a letter of approval of the Institutional Review Board or Ethics Committee before the trial is started. The Institutional Review Board or Ethics Committee must be informed of all protocol amendments that may affect the safety of the participating subjects or conduction of the trial. All serious or unexpected adverse reactions and other information that may alter the study design or entail patient risk must be reported to the Committee. The study file must also contain a list of the members of the Institutional Review Board or Ethics Committee, indicating those who participated in the discussion.

An individual code will be used for the identification of patients. A subject information sheet is to be elaborated and filed at the center.

Lastly, and considering the particular vulnerability of the subjects to be included in the trial, mention should be made of some of the ethical particulars specifically related to this study.

The informed consent document takes into account the opinion of the relative or legal representative, who ultimately has the capacity to decide patient participation in the study. The subject will not be able to participate unless both he/she and the relative or legal representative sign the consent form.

The informed consent document is elaborated in such a way that at least the relative / care taker is able to understand all the implications of patient participation in the study.

According to the experts, since only mild-moderate disease cases are involved in the study, it is very likely that the patients themselves will have no difficulties understanding the implications of participation in the study.

If the study hypothesis is confirmed, direct clinical benefit for the subject may be expected.

The method explored is warranted by extensive experience in other applications.

12.2 Written Informed Consent and Information

The study characteristics will be duly described to all subjects amenable to participation in the trial (or to the legal representatives in the case the patient is unable) - followed by the request for free and voluntary authorization. The subject and the accepted legal representative of the subject will be informed of the nature, purpose and procedures of the study, with a description of the possible risks involved.

The subject (and representative) will be informed of the voluntary nature of participation, and of the right to withdraw from the study at any time, without this implying any negative consequences for the patient. They must also be informed that the sponsor, its representatives, and the supervising authorities may have access to the subject's information.

If the subject (and relative/legal representative) agrees to participation in the study, the consent document must be signed accordingly, and filed by the investigator in the study archives. If the subject is unable, the legal representative must sign the consent form. The subject should also do so as far as possible. The subject will not be able to participate until he/she (and/or the legal representative) sign the consent form.

Informed consent must also be signed by one patient relative or by one legal representative (when patient's relative is not available), who will serve as witness. This relative must receive the same information as the subject.

The investigator or the study personnel obtaining consent (if different from the investigator) will also sign and date the consent form, thus reflecting that informed consent has been obtained, and that the subject (and his/her representative) has had the opportunity to ask questions, and has received adequate answers.

The subject and relative/legal representative will receive a copy of the informed consent form and of the subject information sheet. The original will be filed along with the study documentation.

12.3 Confidentiality of Subject Records

All data related to the procedure, medications, patents, scientific information and other data on materials will be considered confidential, and are the property of the sponsor.

The study protocol and other important documents must be submitted to the Institutional Review Board or Clinical Research Ethics Committee and regulatory authorities to obtain approval for conducting the study.

There will be a file for each subject participating in the trial, where the investigator is to include all the information relating to the patient and the treatment. Data will be collected using specifically designed case report forms (CRF) that will be in an electronic version. The personal information of each subject needed for the study (age, sex, health data, etc.) is confidential, and the identity of the subject will not be disclosed except for the purposes of the study and in the event of a medical emergency or if required by law. The personal information obtained will be kept and processed by a computer system ensuring confidentiality.

The investigator accepts that the sponsor may use the results of the clinical trial, including CRF database or their copies, or reports with or without comments, and with or without analyses, to submit them to the authorities in charge of granting the license, and may reveal them if need be to other investigators. To allow use of the information obtained in the clinical trial, the investigator understands that he/she is obliged to supply the sponsor with full results of the tests and all the information developed during the study.

12.4 Institutional Review Board and Data Monitoring Committee (DMC)

The present protocol and all the required documentation will be subject to evaluation by the corresponding Institutional Review Board or Clinical Research Ethics Committee, in order to obtain the required authorization before starting the study.

An independent Data Monitoring Committee (DMC) will be established for this trial in order to monitor its progress and enhance the safety of trial participants. Review of unblinded interim data will include comparison of adverse event rates occurring in each treatment arm and the control group with special attention given to serious events in order to perform a risk/benefit assessment. The committee will be responsible for making recommendations to the sponsor regarding continuation, early termination or modification of study activities based on the analysis of accumulating outcome data. Aspects of study conduct such as protocol adherence, patient withdrawal, and timeliness of data submission, eligibility rates and reasons for ineligibility will also be monitored by the DMC.

12.5 Responsibilities of the Participants in the Clinical Trial, and Applicable Regulations

The regulations applicable to the clinical trial will be the following, together with any other applicable norms in accordance to national law in the participating countries:

Declaration of Helsinki (Helsinki, 1964) and subsequent revisions

ICH. Note for guidance on good clinical practice. CPMP/ICH/135/95, 1996.

Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.

Spanish Royal Decree 223/2004, of February 6, regulating clinical trials with drugs.

United States Food and Drug Administration (FDA) Code of Federal Regulations.

13. QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Audits and Inspections by the Health Authorities

A person designated by the sponsor will monitor the study to ensure that all the required documentation is available, and that the collected data precisely reflect the data of the CRF. Access to the history and clinical course of the subjects will be required.

When the study is completed, or at some other time, a trial quality assurance audit may be conducted.

The health authorities and/or authorities from the Food and Drug Administration may inspect the centers where the study is carried out, as well as any analytical laboratory involved.

14. DATA HANDLING AND RECORD KEEPING

14.1 Handling of the Principal Study File

14.1.1 Documents Required Before the Start of the Study

- Investigator acceptance to participate in the clinical trial following the duly signed protocol
- Updated curriculum vitae of the investigators implicated in the study
- Institutional Review Board or Ethics Committee approval
- Sample of the informed consent approved by the Institutional Review Board or Ethics Committee
- Approval from the authorities, if required
- Normal laboratory values/ranges for all tests required in the study and laboratory certifications

14.1.2 Study File

The essential documents are to be filed for at least two years from the last authorization of the procedure and until there is no pending or contemplated registry process in any country, or until two years have elapsed from formal discontinuation of the clinical development of the investigational procedure. If so required by the corresponding regulatory authorities, these documents are to be retained for a longer period of time. Before the investigator destroys material related to the clinical trial, written approval must be obtained from the Sponsor.

The investigator is to keep a file including the full name and address of each subject, and all the signed informed consent documents, for no less than 15 years after conclusion of the study. Any original information related to the study allowing verification of the inclusion and exclusion criteria, including the case history, a copy of all CRFs database and the documents on the use of the investigational product are to be filed for the maximum period of time allowed by the center.

At the request of the monitor, auditor, Institutional Review Board or Ethics Committee or regulatory authorities, the investigator will provide direct access to all requested documents related to the trial.

The following study documents are to be filed:

- Signed final version of the protocol and any subsequent amendments
- Institutional Review Board or Ethics Committee approval
- Approval from the authorities, if required

- Informed consent of each subject
- A full copy of the case report form and of the distribution of the product
- Normal values/ranges for medical, laboratory or technical procedures
- Correspondence between the investigator and the Institutional Review Board or Ethics Committee or sponsor
- Reports from the monitoring visits
- Clinical trial report
- Audit certificate (if available)
- Reporting of serious adverse events to the authorities or regulatory authorities

14.1.3 Data Handling, Processing and Correction

All case report forms will be completed electronically.

The investigator must sign and date electronically each of the eCRF forms once completed.

All the original documentation (laboratory test results, treatment forms, etc.) will be filed by the investigator. The investigator also must file subject's medical records, together with the informed consent and the other study documents, for possible future auditing.

14.1.4 Identification of the Clinical Research Samples and Persons Responsible for Their Supply and Storage. Labeling of Samples

The albumin and IVIG destined for use in the trial will come from batches - the largest of which is to be available on the market. Each vial and corresponding box will have a label stating the protocol number, and specifying that the product is only to be used in the clinical trial.

15. PUBLICATION POLICY

The sponsor will prepare a report summarizing the results of the study, based on the statistical analysis of the results, and on any other relevant additional information. The study will include a description of the methods, materials and plans. The investigator will express conformity by signing it.

The investigators are free to publish the results after signing the final report, reflecting as co-authors all persons who have significantly participated in the project. In the event several papers are published, each will be prepared mainly by the investigator with most experience in the field, which moreover will appear as first signing author. The rest of co-authors will appear in the order considered opportune by the principal investigators. The sponsor will receive a copy of the manuscript for review at least 30 days prior to submission for publication or presentation of the Abstract at some scientific meeting.

16. LIABILITIES AND INSURANCE

16.1 Trial Budget Contents

A contract will be developed by common agreement with the investigators and the respective centers, detailing the costs and economical funding for conduction of the study procedures, for each participating subject and for each visit. The Institutional Review Board or Ethics Committee may review and approve the economical memoranda associated to these contracts.

16.2 Insurance / Compensation

The sponsor will contract an insurance to cover possible damage to the patient resulting from application of the study procedure, in accordance with applicable legislation; such coverage will be renewed periodically for the full duration of the study.

LITERATURE REFERENCES

1. Andreasen N. Biomarkers for Alzheimer disease in the cerebrospinal fluid: tau, hyperphosphorilated tau and amyloid beta protein. *Brain Aging.* 2003;3:7-14.
2. Andreasen N, Sjogren M, Blennow K. CSF markers for Alzheimer's disease: total tau, phospho-tau and Abeta42. *World J Biol Psychiatry.* Oct 2003;4(4):147-155.
3. Tapiola T. *Biological narkers for Alzheimer's disease, with special emphasis on cerebrospinal fluid beta-amyloid and tau* [Doctoral dissertation]. Kuopio, Finland: Deparment of Neurology, University of Kuopio; 2001.
4. Kuo YM, Kokjohn TA, Kalback W, et al. Amyloid-beta peptides interact with plasma proteins and erythrocytes: implications for their quantitation in plasma. *Biochem Biophys Res Commun.* Feb 24 2000;268(3):750-756.
5. Knopman D. The necessity of human trials of putative therapies for Alzheimer's disease. *Neurobiol Aging.* Sep-Oct 1989;10(5):633-634; discussion 648-650.
6. Miller CC, Anderton BH. Alzheimer's disease: transgenic models to test new chemicals and pharmaceuticals. *Curr Opin Biotechnol.* Dec 1992;3(6):683-686.
7. Lemere C, Spooner E, LaFrancois J, et al. A-beta immunization of PS/APP mice leads to decreased cerebral A-beta and a corresponding increase in serum A-beta. *Soc Neurosci Abstr.* 2002;27:687.610.
8. DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A.* Jul 17 2001;98(15):8850-8855.
9. Sigurdsson E, Frangione B, Wisniewski T. Immunization for Alzheimer's disease. *Drug Dev Res.* 2002;56:135-142.
10. Cleary JP, Walsh DM, Hofmeister JJ, et al. Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci.* Jan 2005;8(1):79-84.
11. Ghersi-Egea JF, Gorevic PD, Ghiso J, Frangione B, Patlak CS, Fenstermacher JD. Fate of cerebrospinal fluid-borne amyloid beta-peptide: rapid clearance into blood and appreciable accumulation by cerebral arteries. *J Neurochem.* Aug 1996;67(2):880-883.
12. Ji Y, Permanne B, Sigurdsson EM, Holtzman DM, Wisniewski T. Amyloid beta40/42 clearance across the blood-brain barrier following intra-ventricular injections in wild-type, apoE knock-out and human apoE3 or E4 expressing transgenic mice. *J Alzheimers Dis.* Feb 2001;3(1):23-30.
13. Mackic JB, Weiss MH, Miao W, et al. Cerebrovascular accumulation and increased blood-brain barrier permeability to circulating Alzheimer's amyloid beta peptide in aged squirrel monkey with cerebral amyloid angiopathy. *J Neurochem.* Jan 1998;70(1):210-215.
14. Maness LM, Banks WA, Podlisny MB, Selkoe DJ, Kastin AJ. Passage of human amyloid beta-protein 1-40 across the murine blood-brain barrier. *Life Sci.* 1994;55(21):1643-1650.
15. Martel CL, Mackic JB, McComb JG, Ghiso J, Zlokovic BV. Blood-brain barrier uptake of the 40 and 42 amino acid sequences of circulating Alzheimer's amyloid beta in guinea pigs. *Neurosci Lett.* Mar 15 1996;206(2-3):157-160.
16. Poduslo JF, Curran GL, Haggard JJ, Biere AL, Selkoe DJ. Permeability and residual plasma volume of human, Dutch variant, and rat amyloid beta-protein 1-40 at the blood-brain barrier. *Neurobiol Dis.* 1997;4(1):27-34.

17. Poduslo JF, Curran GL, Sanyal B, Selkoe DJ. Receptor-mediated transport of human amyloid beta-protein 1-40 and 1-42 at the blood-brain barrier. *Neurobiol Dis.* Jun 1999;6(3):190-199.
18. Shibata M, Yamada S, Kumar SR, et al. Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest.* Dec 2000;106(12):1489-1499.
19. Zlokovic BV, Ghiso J, Mackic JB, McComb JG, Weiss MH, Frangione B. Blood-brain barrier transport of circulating Alzheimer's amyloid beta. *Biochem Biophys Res Commun.* Dec 30 1993;197(3):1034-1040.
20. Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG. Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *J Neurosci.* Jan 15 2001;21(2):372-381.
21. Kuo YM, Crawford F, Mullan M, et al. Elevated A beta and apolipoprotein E in A betaPP transgenic mice and its relationship to amyloid accumulation in Alzheimer's disease. *Mol Med.* May 2000;6(5):430-439.
22. DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM. Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science.* Mar 22 2002;295(5563):2264-2267.
23. Gandy S, Walker L. Toward modeling hemorrhagic and encephalitic complications of Alzheimer amyloid-beta vaccination in nonhuman primates. *Curr Opin Immunol.* Oct 2004;16(5):607-615.
24. Goni F, Sigurdsson EM. New directions towards safer and effective vaccines for Alzheimer's disease. *Curr Opin Mol Ther.* Feb 2005;7(1):17-23.
25. Matsuoka Y, Saito M, LaFrancois J, et al. Novel therapeutic approach for the treatment of Alzheimer's disease by peripheral administration of agents with an affinity to beta-amyloid. *J Neurosci.* Jan 1 2003;23(1):29-33.
26. Galeazzi L, Galeazzi R, Valli MB, Corder EH, Giunta S. Albumin protects human red blood cells against Abeta25-35-induced lysis more effectively than ApoE. *Neuroreport.* Nov 15 2002;13(16):2149-2154.
27. Biere AL, Ostaszewski B, Stimson ER, Hyman BT, Maggio JE, Selkoe DJ. Amyloid beta-peptide is transported on lipoproteins and albumin in human plasma. *J Biol Chem.* Dec 20 1996;271(51):32916-32922.
28. Meca-Lallana JE, Rodriguez-Hilario H, Martinez-Vidal S, et al. [Plasmapheresis: its use in multiple sclerosis and other demyelinating processes of the central nervous system. An observation study]. *Rev Neurol.* Nov 16-30 2003;37(10):917-926.
29. Suarez JI, Shannon L, Zaidat OO, et al. Effect of human albumin administration on clinical outcome and hospital cost in patients with subarachnoid hemorrhage. *J Neurosurg.* Apr 2004;100(4):585-590.
30. Bambauer R, Arnold A. Plasmapheresis with a substitution solution of human serum protein (5%) versus plasmapheresis with a substitution solution of human albumin (5%) in patients suffering from autoimmune diseases. *Artif Organs.* Dec 1999;23(12):1079-1087.
31. Dyck PJ, Litchy WJ, Kratz KM, et al. A plasma exchange versus immune globulin infusion trial in chronic inflammatory demyelinating polyradiculoneuropathy. *Ann Neurol.* Dec 1994;36(6):838-845.
32. Weinshenker BG, O'Brien PC, Petterson TM, et al. A randomized trial of plasma exchange in acute central nervous system inflammatory demyelinating disease. *Ann Neurol.* Dec 1999;46(6):878-886.

33. Mazzi G, Raineri A, Zucco M, Passadore P, Pomes A, Orazi BM. Plasma-exchange in chronic peripheral neurological disorders. *Int J Artif Organs.* Jan 1999;22(1):40-46.
34. Pratico D YY, Rokach J, Mayo M, Silverberg GG, McGuire D. . Reduction of brain lipid peroxidation by CSF drainage in Alzheimer's disease patients. *J Alzheimers Dis.* . 2004 6(4):385-389; discussion 443-389.
35. Silverberg GD, Levinthal E, Sullivan EV, et al. Assessment of low-flow CSF drainage as a treatment for AD: results of a randomized pilot study. *Neurology.* Oct 22 2002;59(8):1139-1145.
36. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* Nov 1975;12(3):189-198.
37. Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. *Am J Psychiatry.* Nov 1984;141(11):1356-1364.
38. Smith A. *Symbol Digits Modality Test*. Los Angeles: Western Psychological Services; 1973.
39. Benton A, Hamsher K. *Multilingual aphasia examination*. 2nd ed ed. Iowa City: ASA Associates; 1983.
40. Morris JC, Heyman A, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology.* Sep 1989;39(9):1159-1165.
41. Kaplan E, Goodglass H, Weintraub S. *The Boston naming test*. 2nd ed ed. Philadelphia: Lea & Febiger; 1983.
42. Rey A. *L'examen clinique en psychologie*. Paris, France: Presses Universitaires de France; 1964.
43. Galasko D, Bennett D, Sano M, et al. An inventory to assess activities of daily living for clinical trials in Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *Alzheimer Dis Assoc Disord.* 1997;11 Suppl 2:S33-39.
44. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology.* Dec 1994;44(12):2308-2314.
45. Alexopoulos GS AR, Young RC, Shamoian CA. Cornell Scale for Depression in Dementia. *Biol Psychiatry.* . 1988;23(3):271-284.
46. Berg L. Clinical Dementia Rating (CDR). *Psychopharmacol Bull.* 1988;24(4):637-639.
47. Morris JC MDJ, Fulling K, Torack RM, Berg L. . Validation of clinical diagnostic criteria for Alzheimer's disease. *Ann Neurol.* 1988;24(1):17-22.
48. Schneider LS OJ, Doody RS, Clark CM, Morris JC, Reisberg B, Schmitt FA, Grundman M, Thomas RG, Ferris SH. . Validity and reliability of the Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change. The Alzheimer's Disease Cooperative Study. *Alzheimer Dis Assoc Disord.* 1997;11(Suppl 2):22-32.
49. Yudofsky SC, Silver JM, Jackson W, Endicott J, Williams D. The Overt Aggression Scale for the objective rating of verbal and physical aggression. *Am J Psychiatry.* Jan 1986;143(1):35-39.
50. Corrigan JD. Development of a scale for assessment of agitation following traumatic brain injury. *J Clin Exp Neuropsychol.* Mar 1989;11(2):261-277.
51. Thompson PM, Hayashi KM, De Zubicaray GI, et al. Mapping hippocampal and ventricular change in Alzheimer disease. *Neuroimage.* Aug 2004;22(4):1754-1766.

52. Directive 2001/20/EC of the European Pariliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Members States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. In: Union TEPatCotE, ed. Vol 2001/20/EC: Official Journal of the European Communities; 2001.
53. Real Decreto 223/2004, de 6 de febrero, por el que se regulan los ensayos clínicos con medicamentos. Vol BOE 233: Ministerio de Sanidad y Consumo; 2004:5429-5443.
54. Karch FE, Lasagna L. Adverse drug reactions. A critical review. *Jama.* Dec 22 1975;234(12):1236-1241.
55. Simon T. Coagulation Disorders with plasma exchange. *Plasma Ther Transfus Technol.* 1982;3:147-152.
56. Straves R, McLeold B. Adverse reactions to therapeutic apheresis. In: Popousky M, ed. *Transfusion reactions*. Bethesda: AABB Press; 1996:281-300.
57. Rodnitzky RL, Goeken JA. Complications of plasma exchange in neurological patients. *Arch Neurol.* Jun 1982;39(6):350-354.
58. Domen RE, Kennedy MS, Jones LL, Senhauser DA. Hemostatic imbalances produced by plasma exchange. *Transfusion.* Jul-Aug 1984;24(4):336-339.
59. Hazards of apheresis. *Lancet.* Nov 6 1982;2(8306):1025-1026.
60. Burgstaler EA, Pineda AA. Therapeutic cytapheresis: continuous flow versus intermittent flow apheresis systems. *J Clin Apher.* 1994;9(4):205-209.
61. Huestis DW. Risks and safety practices in hemapheresis procedures. *Arch Pathol Lab Med.* Mar 1989;113(3):273-278.
62. Uldall P, Dyck R, Woods F, Merchant N, Martin G, Cardella C. A subclavian cannula for temporary vascular access for hemodialysis or plasmapheresis. *Dial Transplant* 1979;8:963-968
63. Ficha técnica de Aricept® (donepezilo clorhidrato), comprimidos de 5 y 10 mg (números de la autorización de comercialización: 61869 y 61870). Pfizer; 2003.
64. Note for guidance. Good Clinical Practice. CPMP/ICH/135/95. In: ICH, ed; 1996.
65. Boada M, Ortiz P, Anaya F et al. Use of human Albumin in plasma exchange as a novel approach for Abeta mobilization. *Drug News Perpect.* 2009; 22(6):325-339
66. Dodel, R.C., Depboylu, C. et al. Intravenous immunoglogulins containing antibodies against beta-amyloid for the treatment of Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 2004; **75**: 1472-1474
67. Relkin, N.R., Szabo, P. et al. 18-month study of intravenous immunoglobulin for treatment of mild Alzheimer disease. *Neurobiol Aging* 2009;30(11):1728-1736
68. U.S. National Institutes of Health (2011) [on line]. Phase II study of intravenous immunoglobulin (IVIg) for Alzheimer's disease. Available from : http://clinicaltrials.gov/ct2/show/NCT00299988?term=baxter+AND+alzheimer&rank=2
69. U.S. National Institutes of Health (2011) [on line]. A Phase 3 Study Evaluating Safety and Effectiveness of Immune Globulin Intravenous (IGIV 10%) for the Treatment of Mild to Moderate Alzheimer´s Disease. Available from : http://clinicaltrials.gov/ct2/show/NCT00818662?term=baxter+AND+alzheimer&rank=1
70. Lehmann HC, Hartung HP. Plasma exchange and intravenous immunoglobulins: Mechanism of action in immune-mediated neuropathies. *J Neuroimmunol*. 2011; 231(1-2):61-69

71. Ludwig KP, Thiesset HF, Gayowski TJ et al. Plasmapheresis and Intravenous Immune Globulin Improve Neurologic Outcome of Central Pontine Myelinolysis Occurring Post Orthotopic Liver Transplant. *Ann. Pharmacother.* 2011; 45 (Feb 8, Epub ahead of print).

APPENDIX 1

FULL PRESCRIBING INFORMATION

(Albutein®)

3043519

GRIFOLS

Albumin (Human) U.S.P.
Albutein® 5% Solution

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use ALBUTEIN 5% safely and effectively. See full prescribing information for ALBUTEIN 5%.

ALBUTEIN 5% (albumin [human] U.S.P.)
5% solution
Initial U.S. Approval: 1978

INDICATIONS AND USAGE

ALBUTEIN 5% is an albumin solution indicated for:

- Hypovolemia. (1.1)
- Cardiopulmonary bypass procedures. (1.2)
- Hypoalbuminemia. (1.3)
- Plasma exchange. (1.4)

DOSAGE AND ADMINISTRATION

For Intravenous Use Only

Dosage and infusion rate should be adjusted to the patient's individual requirements.

Indication	Dose
Hypovolemia	Adults: Initial dose of 20 g (including renal dialysis). For acute liver failure: initial dose of 12 to 25 g. (2.1)
Cardiopulmonary bypass procedures	Adults: Initial dose of 25 g. (2.1)
Hypoalbuminemia	Adults: 50 to 75 g For pre- and post-operative hypoproteinemia: 50 to 75 g. For burn therapy after the first 24 h: initial dose of 25 g and dose adjustment to maintain plasma protein concentration of 2.5 g per 100 mL. Third space protein loss due to infection: initial dose of 50 to 100 g. (2.1)
Plasma exchange	The dose required depends on the volume of plasma removed during the procedure.

Do not dilute with sterile water for injection as this may cause hemolysis in recipients. (5.6)

DOSAGE FORMS AND STRENGTHS

ALBUTEIN 5% is a solution containing 50 g per L of total protein of which at least 95% is human albumin.

CONTRAINDICATIONS

- Hypersensitivity to albumin preparations or to any of the excipients.
- Severe anemia or cardiac failure with normal or increased intravascular volume. (4)

WARNINGS AND PRECAUTIONS

- Suspicion of allergic or anaphylactic reactions requires immediate discontinuation of the injection and implementation of appropriate medical treatment. (5.1)
- Hypervolemia may occur if the dosage and rate of infusion are not adjusted to the patient's volume status. Use with caution in conditions where hypervolemia and its consequences or hemodilution could represent a special risk to the patient. (5.2)
- Monitor electrolytes, coagulation and hematology parameters, and hemodynamic status when albumin is given. (5.3, 5.4, 5.5)
- Do not dilute with sterile water for injection. (5.6)
- This product is made from human plasma and may contain infectious agents, e.g., viruses and, theoretically, the Creutzfeldt-Jakob disease agent. (5.7)

ADVERSE REACTIONS

The most common adverse reactions are anaphylactoid type reactions. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Grifols Biologicals Inc. at 1-888-GRIFOLS (1-888-474-3657) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

USE IN SPECIFIC POPULATIONS

- Pregnancy: No human or animal data. Use only if clearly needed. (8.1)

See 17 for PATIENT COUNSELING INFORMATION

Revised: 08/2015

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Hypovolemia

For restoration and maintenance of circulating blood volume where hypovolemia is demonstrated and colloid use is appropriate. When hypovolemia is long standing and hypoalbuminemia exists accompanied by adequate hydration or edema, 20-25% albumin solutions should be used.[1,2,3]

Acute liver failure is a special situation in which both hypovolemia and hypoalbuminemia can be present. ALBUTEIN 5% can be used in such cases.[1]

ALBUTEIN 5% may be of value in the treatment of shock or hypotension in renal dialysis patients.[1]

1.2 Cardiopulmonary Bypass Procedures (Treatment Adjunct)

Preoperative dilution of blood using albumin and crystalloid can be used in cardiopulmonary bypass procedures. Albumin also may be used in the priming fluid.[4,5,6]

1.3 Hypoalbuminemia

ALBUTEIN 5% may be indicated for subjects with hypoalbuminemia who are critically ill and/or actively bleeding. When albumin deficit is the result of excessive protein loss, the effect of ALBUTEIN 5% administration will be temporary unless the underlying disorder is reversed.[7,8,9] Septic patients and patients undergoing major surgery may lose more than half of their circulating plasma volume.[1,10] Treatment with ALBUTEIN 5% may be of value in such cases, especially when plasma colloid oncotic pressure is abnormally low.[1]

In the first 24 hours after thermal injury, large volumes of crystalloids are infused to restore the depleted extracellular fluid volume. Beyond 24 hours, ALBUTEIN 5% can be used to maintain plasma colloid osmotic pressure.[2,11,12] Protein loss from the third space due to infection (acute peritonitis, pancreatitis, mediastinitis or extensive cellulitis) may require treatment with an infusion of albumin.[13,14]

1.4 Plasma Exchange

ALBUTEIN 5% may be used as a replacement fluid during therapeutic Plasma Exchange treatments.[15]

2 DOSAGE AND ADMINISTRATION

For Intravenous Use Only

2.1 Dosage

Adjust the concentration, dosage and infusion rate of the albumin preparation to the patient's individual requirements.

The dose required depends on the patient's body weight, severity of injury/illness and on continuing fluid and protein losses. Use adequacy of circulating blood volume, not plasma albumin levels, to determine the dose required.

Indication	Dose
Hypovolemia	Adults: Initial dose of 20 g. If hemodynamic stability is not achieved within 15 to 30 minutes, an additional dose may be given. Hemodilution may follow administration of ALBUTEIN 5%. Anemia resulting from hemorrhage should be corrected by administration of compatible red blood cells or compatible whole blood. For acute liver failure: initial dose of 12 to 25 g. An infusion rate of 1-2 mL per minute is usually indicated. For renal dialysis, the initial dose should not exceed 20 g and patients should be carefully observed for signs of fluid overload.
Cardiopulmonary bypass procedures	Adults: Initial dose of 25 g. Additional amounts may be administered as clinically indicated.
Hypoalbuminemia	Adults: 50 to 75 g For pre- and post-operative hypoproteinemia: 50 to 75 g. In burns, therapy usually starts with administration of large volumes of crystalloid solution to maintain plasma volume. After 24 hours: initial dose of 25 g and dose adjustment to maintain plasma protein concentration of 2.5 g per 100 mL or a serum protein concentration of 5.2 g per 100 mL. Third space protein loss due to infection: initial dose of 50 to 100 g. An infusion rate of 1-2 mL per minute is usually indicated in the absence of shock. Treatment should always be guided by hemodynamic response.
Plasma exchange	The dosage and infusion rate of ALBUTEIN 5% infused should be titrated to the volume of plasma removed during the procedure.

2.2 Administration

Intravenous use only

- ALBUTEIN 5% is a clear and slightly viscous solution. Visually inspect parenteral drug products for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if the solution is turbid or if there is sediment in the bottle.
- Do not freeze.
- Warm product to room temperature before use if large volumes are administered.
- ALBUTEIN 5% contains no preservatives. Do not begin administration more than 4 hours after the container has been entered. Discard unused portion.
- Do not dilute with sterile water for injection [see *Warnings and Precautions (5.6)*].
- Adjust the infusion rate to the individual circumstances and the indication. In plasma exchange, adjust the infusion rate to the rate of plasma removal.

3 DOSAGE FORMS AND STRENGTHS

ALBUTEIN 5% is a solution containing 50 g per L of total protein of which at least 95% is human albumin.

4 CONTRAINDICATIONS

- Hypersensitivity to albumin preparations or to any of the excipients.
- Severe anemia or cardiac failure with normal or increased intravascular volume.

5 WARNINGS AND PRECAUTIONS

5.1 Hypersensitivity

Suspicion of allergic or anaphylactic reactions requires immediate discontinuation of the infusion and implementation of appropriate medical treatment.

5.2 Hypervolemia/Hemodilution

Hypervolemia may occur if the dosage and rate of infusion are not adjusted to the patient's volume status. At the first clinical signs of fluid overload (headache, dyspnea, jugular venous distention, increased blood pressure), the infusion must be slowed or stopped immediately.

Use albumin with caution in conditions where hypervolemia and its consequences or hemodilution could represent a special risk to the patient. Examples of such conditions are:

- Decompensated heart failure
- Hypertension
- Esophageal varices
- Pulmonary edema
- Hemorrhagic diathesis
- Severe anemia
- Renal and post-renal anuria

5.3 Electrolyte Imbalance

Monitor regularly the electrolyte status of the patient and take appropriate steps to restore or maintain the electrolyte balance when albumin is administered.

5.4 Coagulation Abnormalities

Regular monitoring of coagulation and hematology parameters is necessary if comparatively large volumes are to be replaced. Care must be taken to ensure adequate substitution of other blood constituents (coagulation factors, electrolytes, platelets, and erythrocytes).

5.5 Laboratory Monitoring

Monitor regularly hemodynamic parameters during administration of ALBUTEIN 5%; this may include:

- Arterial blood pressure and pulse rate
- Central venous pressure
- Pulmonary artery occlusion pressure
- Urine output
- Electrolytes
- Hematocrit/hemoglobin

5.6 Application Precautions

ALBUTEIN 5% must not be diluted with sterile water for injection as this may cause hemolysis in recipients [see *Dosage and Administration (2.2)*].

5.7 Transmissible Infectious Agents

Albumin is a derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases. A theoretical risk for transmission of Creutzfeldt-Jakob disease (CJD) is also considered extremely remote. No cases of transmission of viral diseases or CJD have been identified for ALBUTEIN 5%.

6 ADVERSE REACTIONS

The most serious adverse reactions are anaphylactic shock, heart failure and pulmonary edema. The most common adverse reactions are anaphylactoid type reactions.

Adverse reactions to ALBUTEIN 5% normally resolve when the infusion rate is slowed or the infusion is stopped. In case of severe reactions, the infusion is stopped and appropriate treatment initiated.

6.1 Clinical Trials Experience

No clinical studies were done using ALBUTEIN 5%.

6.2 Post-Marketing Experience

Because adverse reactions are reported voluntarily post-approval from a population of uncertain size, it is not always possible to reliably estimate their frequency or to establish a causal relationship to product exposure. The following adverse reactions have been identified during post approval use of human albumin, including ALBUTEIN (all strengths) in decreasing order of significance:

- Anaphylactic shock
- Heart failure
- Pulmonary edema
- Hypotension
- Tachycardia
- Vomiting
- Urticaria
- Rash
- Headache
- Chills
- Fever
- Flushing
- Nausea

7 DRUG INTERACTIONS

ALBUTEIN 5% must not be mixed with other medicinal products.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C. Animal reproduction studies have not been conducted with ALBUTEIN 5%. It is also not known whether ALBUTEIN 5% can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. ALBUTEIN 5% should be given to a pregnant woman only if clearly needed.

8.2 Labor and Delivery

No human or animal data. Use only if clearly needed.

8.3 Nursing Mothers

No human or animal data. Use only if clearly needed.

8.4 Pediatric Use

No human or animal data. Use only if clearly needed.

8.5 Geriatric Use

No human or animal data. Use only if clearly needed.

11 DESCRIPTION

ALBUTEIN 5% is a sterile, aqueous solution for single dose intravenous administration containing 5% human albumin (weight/volume). ALBUTEIN 5% is prepared by a cold alcohol fractionation method from pooled human plasma obtained from venous blood. The product is stabilized with 0.08 millimole sodium caprylate and 0.08 millimole sodium acetyltryptophanate per gram of protein.

ALBUTEIN 5% is osmotically and isotonically equivalent to an equal volume of normal human plasma.

A liter of ALBUTEIN 5% solution contains 130-160 milliequivalents of sodium ion. The aluminum content of the solution is not more than 200 micrograms per liter during the shelf life of the product. The product contains no preservatives.

ALBUTEIN 5% is manufactured from Source Plasma collected from FDA approved plasmapheresis centers in the United States. ALBUTEIN 5% is heated at 60 °C for ten hours against the possibility of transmitting viruses.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Human Albumin accounts for more than half of the total protein in the plasma and represents about 10% of protein synthesis activity by the liver.

Human Albumin 5% is almost isooncotic to normal plasma.

The primary physiological function of albumin results from its contribution to plasma colloid oncotic pressure and transport function. Albumin stabilizes circulating blood volume and is a carrier of hormones, enzymes, medicinal products and toxins. Other physiological functions include antioxidant properties, free radical scavenging and capillary membrane integrity.

12.3 Pharmacokinetics

Albumin is distributed throughout the extracellular space and more than 60% of the body albumin pool is located in the extravascular fluid compartment. Albumin has a circulating life span of 15-20 days, with a turnover of approximately 15 g per day.

The balance between synthesis and breakdown is normally achieved by feedback regulation.

Elimination is predominantly intracellular and due to lysosome proteases.

In healthy subjects, less than 10% of infused albumin leaves the intravascular compartment during the first 2 hours following infusion. There is considerable individual variation in the effect of albumin on plasma volume. In some patients, plasma volume can remain elevated for several hours. In critically ill patients, however, albumin can leak out of the vascular space in substantial amounts at an unpredictable rate.

15 REFERENCES

1. Tullis JL. Albumin: 1. Background and Use. 2. Guidelines for Clinical Use. JAMA. 1977;237:355-360, 460-463.
2. Vermeulen LC, et al. A Paradigm for Consensus. Arch Intern Med. 1995;155:373-379.
3. SAFE Study investigators. A comparison of albumin and saline for fluid resuscitation in the intensive care unit. N Engl J Med. 2004;350:2247-2256.
4. Sedrakyan A, Gondek K, Paltiel D, et al. Volume expansion with albumin decreases mortality after coronary artery bypass graft surgery. Chest. 2003;123:1853-1857.
5. Russell JA, Navickis RJ, Wilkes MM. Albumin versus crystalloid for pump priming in cardiac surgery: meta-analysis of controlled trials. J Cardiothorac Vasc Anesth. 2004;18:429-37.
6. American Thoracic Society. Evidence-based colloid use in the critically ill: American Thoracic Society consensus statement. Am J Respir Crit Care Med. 2004;170:1247-59.
7. Mendez CM, McClain CJ, Marsano LS. Albumin Therapy in Clinical Practice. Nutrition in Clinical Practice. 2005;20:314-320.
8. Haynes GR, Navickis RJ, Wilkes MM. Albumin administration-what is the evidence of clinical benefit? A systematic review of randomized controlled trials. Eur J Anaesthesiol. 2003 Oct;20(10):771-93.
9. Vincent JL, Navickis RJ, Wilkes MM. Morbidity in hospitalized patients receiving human albumin: a meta-analysis of randomized, controlled trials. Crit Care Med. 2004;32:2029-38.
10. Skillman JJ, Tanenbaum BJ. Current Topics in Surgical Research. Vol. 2. New York: Academic Press. 1970;523.
11. Muir IA, Barclay TL. Burns and their treatment. Chicago: Year Book Medical Publishers. 1974.
12. Pruitt BA Jr, Goodwin CW Jr. Current treatment of the extensively burned patient. Surg Annu. 1983;15:331-64.
13. Clowes GHA Jr, Vucinic M, Weidner MG. Circulatory and metabolic alterations associated with survival or death in peritonitis: clinical analysis of 25 cases. Ann Surg. 1966;166:866-85.
14. Sort P, Navasa M, Arroyo V, et al. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. N Engl J Med. 1999;341:403-409.
15. Szczepiorkowski ZM, Bandarenko N, Kim HC, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Apheresis applications committee of the American Society for Apheresis. J Clin Apheresis. 2007; 22:106-175.

16 HOW SUPPLIED/STORAGE AND HANDLING

ALBUTEIN 5% is supplied in single-use, individually laser etched vials.

The following vial sizes of ALBUTEIN 5% are available:

NDC Number	Fill Size	Grams Protein
68516-5214-5	50 mL	2.5 g
68516-5214-1	250 mL	12.5 g
68516-5214-2	500 mL	25.0 g

Each vial size label incorporates integrated hangers. Each label has a peel-off strip showing the product name and lot number.

ALBUTEIN 5% is stable for three years provided the storage temperature does not exceed 30 °C. Protect from freezing.

17 PATIENT COUNSELING INFORMATION

This product is usually given in a hospital setting.

Inform patients being treated with ALBUTEIN 5% about the risks and benefits of its use [see *Adverse Reactions (6)*].

Inform patients to immediately report the following signs and symptoms to their physician:

- Allergic or anaphylactic type reactions [see *Warnings and Precautions (5.1)*].
- Cardiovascular overload (e.g., headache, dyspnea and jugular venous distention) [see *Warnings and Precautions (5.2)*].
- Increased blood pressure, raised venous pressure and pulmonary edema [see *Warnings and Precautions (5.2)*].

Inform patients that ALBUTEIN 5% is a derivative of human plasma and may contain infectious agents that cause disease (e.g., viruses, and theoretically, the CJD agent). Inform patients that the risk that ALBUTEIN 5% may transmit an infectious agent has been reduced by screening plasma donors for prior exposure to certain viruses, by testing the donated plasma for certain viral agents and by the inactivation and/or removal of certain viruses during the manufacturing process [see *Warnings and Precautions (5.7)*].

Manufactured by:

Grifols Biologicals Inc.
5555 Valley Boulevard
Los Angeles, CA 90032, U.S.A.
U. S. License No. 1694

3043519

3043520

GRIFOLS

Albumin (Human) U.S.P.
Albutein® 20% Solution

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use ALBUTEIN 20% safely and effectively. See full prescribing information for ALBUTEIN 20%.

ALBUTEIN 20% (albumin [human] U.S.P.)
20% solution
Initial U.S. Approval: 1978

INDICATIONS AND USAGE

ALBUTEIN 20% is an albumin solution indicated for:

- Hypovolemia. (1.1)
- Cardiopulmonary bypass procedures. (1.2)
- Acute nephrosis. (1.3)
- Hypoalbuminemia. (1.4)
- Ovarian hyperstimulation syndrome. (1.5)
- Neonatal hyperbilirubinemia. (1.6)
- Adult respiratory distress syndrome (ARDS). (1.7)
- Prevention of central volume depletion after paracentesis due to cirrhotic ascites. (1.8)

DOSAGE AND ADMINISTRATION

For Intravenous Use Only

Dosage and infusion rate should be adjusted to the patient's individual requirements.

Indication	Dose
Hypovolemia	Adults: Initial dose of 20 g (including renal dialysis). For acute liver failure: initial dose of 12 to 25 g. (2.1)
Cardiopulmonary bypass procedures	Adults: Initial dose of 25 g. (2.1)
Acute nephrosis	Adults: 25 g together with diuretic once a day for 7-10 days. (2.1)
Hypoalbuminemia	Adults: 50 to 75 g For pre- and post-operative hypoproteinemia: 50 to 75 g. For burn therapy after the first 24 h: initial dose of 25 g and dose adjustment to maintain plasma protein concentration of 2.5 g per 100 mL. Third space protein loss due to infection: initial dose of 50 to 100 g. (2.1)
Ovarian hyperstimulation syndrome	Adults: 50 g to 100 g over 4 hours and repeated at 4–12 hour intervals as necessary. (2.1)
Neonatal hyperbilirubinemia	1 g per kilogram body weight prior to or during exchange transfusion. (2.1)
Adult respiratory distress syndrome (ARDS)	Adults: 25 g over 30 minutes and repeated at 8 hours for 3 days, if necessary. (2.1)
Prevention of central volume depletion after paracentesis due to cirrhotic ascites	Adults: 8 g for every 1000 mL of ascitic fluid removed. (2.1)

Do not dilute with sterile water for injection as this may cause hemolysis in recipients. (5.7)

DOSAGE FORMS AND STRENGTHS

ALBUTEIN 20% is a solution containing 200 g per L of total protein of which at least 95% is human albumin.

CONTRAINDICATIONS

- Hypersensitivity to albumin preparations or to any of the excipients.
- Severe anemia or cardiac failure with normal or increased intravascular volume. (4)

WARNINGS AND PRECAUTIONS

- Suspicion of allergic or anaphylactic reactions requires immediate discontinuation of the injection and implementation of appropriate medical treatment. (5.1)
- Hypervolemia may occur if the dosage and rate of infusion are not adjusted to the patient's volume status. Use with caution in conditions where hypervolemia and its consequences or hemodilution could represent a special risk to the patient. (5.2)
- When concentrated albumin is administered, care must be taken to assure adequate hydration of the patient. (5.3)
- Monitor electrolytes, coagulation and hematology parameters, and hemodynamic status when albumin is administered. (5.4, 5.5, 5.6)
- Do not dilute with sterile water for injection. (5.7)
- This product is made from human plasma and may contain infectious agents, e.g., viruses and, theoretically, the Creutzfeldt-Jakob disease agent. (5.8)

ADVERSE REACTIONS

The most common adverse reactions are anaphylactoid type reactions. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Grifols Biologicals Inc. at 1-888-GRIFOLS (1-888-474-3657) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

USE IN SPECIFIC POPULATIONS

- Pregnancy: No human or animal data. Use only if clearly needed. (8.1)

See 17 for PATIENT COUNSELING INFORMATION

Revised: 08/2015

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Hypovolemia

For restoration and maintenance of circulating blood volume where hypovolemia is demonstrated and colloid use is appropriate. When hypovolemia is long standing and hypoalbuminemia exists accompanied by adequate hydration or edema, 20-25% albumin solutions should be used.[1,2,3]

Acute liver failure is a special situation in which both hypovolemia and hypoalbuminemia can be present. ALBUTEIN® 20% can be used in such cases.[1]

ALBUTEIN 20% may be of value in the treatment of shock or hypotension in renal dialysis patients.[1]

1.2 Cardiopulmonary Bypass Procedures (Treatment Adjunct)

Preoperative dilution of blood using albumin and crystalloid can be used in cardiopulmonary bypass procedures. Albumin also may be used in the priming fluid.[4,5,6]

1.3 Acute Nephrosis (Treatment Adjunct)

ALBUTEIN 20% may be used to treat peripheral edema in patients with acute nephrosis who are refractory to cyclophosphamide, corticosteroid therapy or diuretics.[1,2,7]

1.4 Hypoalbuminemia

ALBUTEIN 20% may be indicated for subjects with hypoalbuminemia who are critically ill and/or actively bleeding. When albumin deficit is the result of excessive protein loss, the effect of ALBUTEIN 20% administration will be temporary unless the underlying disorder is reversed. [8,9,10] Septic patients and patients undergoing major surgery may lose more than half of their circulating plasma volume.[1,11] Treatment with ALBUTEIN 20% may be of value in such cases, especially when plasma colloid oncotic pressure is abnormally low.[1]

In the first 24 hours after thermal injury, large volumes of crystalloids are infused to restore the depleted extracellular fluid volume. Beyond 24 hours, ALBUTEIN 20% can be used to maintain plasma colloid osmotic pressure.[2,12,13] Protein loss from the third space due to infection (acute peritonitis, pancreatitis, mediastinitis or extensive cellulitis) may require treatment with an infusion of albumin.[14,15]

1.5 Ovarian Hyperstimulation Syndrome

ALBUTEIN 20% may be used as a plasma volume expander in fluid management relating to severe forms of ovarian hyperstimulation syndrome.[16,17]

1.6 Neonatal Hyperbilirubinemia

ALBUTEIN 20% is indicated for the treatment of neonatal hyperbilirubinemia. It may be used prior to or during an exchange procedure in an attempt to bind free bilirubin and enhance its excretion.[18,19,20]

1.7 Adult Respiratory Distress Syndrome (ARDS) (Treatment Adjunct)

ALBUTEIN 20% infusions may be indicated in conjunction with diuretics to correct fluid overload and hypoproteinemia associated with ARDS.[6,21]

1.8 Prevention of Central Volume Depletion after Paracentesis due to Cirrhotic Ascites (Treatment Adjunct)

ALBUTEIN 20% may be used to maintain cardiovascular function following removal of large volumes of ascitic fluid after paracentesis due to cirrhotic ascites.[2,22,23,24]

2 DOSAGE AND ADMINISTRATION

For Intravenous Use Only

2.1 Dosage

Adjust the concentration, dosage and infusion rate of the albumin preparation to the patient's individual requirements.

The dose required depends on the patient's body weight, severity of injury/illness and on continuing fluid and protein losses. Use adequacy of circulating blood volume, not plasma albumin levels, to determine the dose required.

Indication	Dose
Hypovolemia	Adults: Initial dose of 20 g. If hemodynamic stability is not achieved within 15 to 30 minutes, an additional dose may be given. Hemodilution may follow administration of ALBUTEIN 20%. Anemia resulting from hemorrhage should be corrected by administration of compatible red blood cells or compatible whole blood. For acute liver failure: initial dose of 12 to 25 g. An infusion rate of 1-2 mL per minute is usually indicated. For renal dialysis, the initial dose should not exceed 20 g and patients should be carefully observed for signs of fluid overload.
Cardiopulmonary bypass procedures	Adults: Initial dose of 25 g. Additional amounts may be administered as clinically indicated.
Acute nephrosis	Adults: 25 g together with diuretic once a day for 7 - 10 days.
Hypoalbuminemia	Adults: 50 to 75 g For pre- and post-operative hypoproteinemia: 50 to 75 g. In burns, therapy usually starts with administration of large volumes of crystalloid solution to maintain plasma volume. After 24 hours: initial dose of 25 g and dose adjustment to maintain plasma protein concentration of 2.5 g per 100 mL or a serum protein concentration of 5.2 g per 100 mL. Third space protein loss due to infection: initial dose of 50 to 100 g. An infusion rate of 1-2 mL per minute is usually indicated in the absence of shock. Treatment should always be guided by hemodynamic response.

Ovarian hyperstimulation syndrome	Adults: 50 g to 100 g over 4 hours and repeated at 4-12 hour intervals as necessary, when infusion of normal saline fails to achieve or maintain hemodynamic stability and urine output.
Neonatal hyperbilirubinemia	1 g per kilogram body weight prior to or during exchange transfusion.
Adult respiratory distress syndrome (ARDS)	Adults: 25 g over 30 minutes and repeated at 8 hours for 3 days, if necessary.
Prevention of central volume depletion after paracentesis due to cirrhotic ascites	Adults: 8 g for every 1000 mL of ascitic fluid removed.

2.2 Administration

Intravenous use only

- ALBUTEIN 20% is a clear and slightly viscous solution. Visually inspect parenteral drug products for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if the solution is turbid or if there is sediment in the bottle.
- Do not freeze.
- Warm product to room temperature before use if large volumes are administered.
- ALBUTEIN 20% contains no preservatives. Do not begin administration more than 4 hours after the container has been entered. Discard unused portion.
- Do not dilute with sterile water for injection. The product can be diluted in an isotonic solution (e.g., 5% dextrose in water or 0.9% sodium chloride) [see *Warnings and Precautions (5.7)*].
- Adjust the infusion rate to the individual circumstances and the indication.

3 DOSAGE FORMS AND STRENGTHS

ALBUTEIN 20% is a solution containing 200 g per L of total protein of which at least 95% is human albumin.

4 CONTRAINDICATIONS

- Hypersensitivity to albumin preparations or to any of the excipients.
- Severe anemia or cardiac failure with normal or increased intravascular volume.

5 WARNINGS AND PRECAUTIONS

5.1 Hypersensitivity

Suspicion of allergic or anaphylactic reactions requires immediate discontinuation of the infusion and implementation of appropriate medical treatment.

5.2 Hypervolemia/Hemodilution

Hypervolemia may occur if the dosage and rate of infusion are not adjusted to the patient's volume status. At the first clinical signs of cardiovascular overload (headache, dyspnea, jugular venous distention, increased blood pressure), the infusion must be slowed or stopped immediately.

Use albumin with caution in conditions where hypervolemia and its consequences or hemodilution could represent a special risk to the patient. Examples of such conditions are:

- Decompensated heart failure
- Hypertension
- Esophageal varices
- Pulmonary edema
- Hemorrhagic diathesis
- Severe anemia
- Renal and post-renal anuria

5.3 Dehydration

The colloid-osmotic effect of human albumin 20% is approximately four times that of blood plasma. Therefore, when concentrated albumin is administered, care must be taken to assure adequate hydration of the patient. Patients should be monitored carefully to guard against circulatory overload and hyperhydration. Patients with marked dehydration require administration of additional fluids.

5.4 Electrolyte Imbalance

20% - 25% human albumin solutions are relatively low in electrolytes compared to 4% - 5% human albumin solutions. Monitor regularly the electrolyte status of the patient and take appropriate steps to restore or maintain the electrolyte balance when albumin is administered.

5.5 Coagulation Abnormalities

Regular monitoring of coagulation and hematology parameters is necessary if comparatively large volumes are to be replaced. Care must be taken to ensure adequate substitution of other blood constituents (coagulation factors, electrolytes, platelets and erythrocytes).

5.6 Laboratory Monitoring

Monitor regularly hemodynamic parameters during administration of ALBUTEIN 20%; this may include:

- Arterial blood pressure and pulse rate
- Central venous pressure
- Pulmonary artery occlusion pressure
- Urine output
- Electrolytes
- Hematocrit/hemoglobin

5.7 Application Precautions

ALBUTEIN 20% must not be diluted with sterile water for injection as this may cause hemolysis in recipients. The product can be diluted in an isotonic solution (e.g., 5% dextrose in water or 0.9% sodium chloride) [see *Dosage and Administration (2.2)*].

5.8 Transmissible Infectious Agents

Albumin is a derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases. A theoretical risk for transmission of Creutzfeldt-Jakob disease (CJD) is also considered extremely remote. No cases of transmission of viral diseases or CJD have been identified for ALBUTEIN 20%.

6 ADVERSE REACTIONS

The most serious adverse reactions are anaphylactic shock, heart failure and pulmonary edema.

The most common adverse reactions are anaphylactoid type reactions.

Adverse reactions to ALBUTEIN 20% normally resolve when the infusion rate is slowed or the infusion is stopped. In case of severe reactions, the infusion is stopped and appropriate treatment initiated.

6.1 Clinical Trials Experience

No clinical studies were done using ALBUTEIN 20%.

6.2 Post-Marketing Experience

Because adverse reactions are reported voluntarily post-approval from a population of uncertain size, it is not always possible to reliably estimate their frequency or to establish a causal relationship to product exposure. The following adverse reactions have been identified during post approval use of human albumin, including ALBUTEIN (all strengths) in decreasing order of significance:

- Anaphylactic shock
- Heart failure
- Pulmonary edema
- Hypotension
- Tachycardia
- Vomiting
- Urticaria
- Rash
- Headache
- Chills
- Fever
- Flushing
- Nausea

7 DRUG INTERACTIONS

ALBUTEIN 20% must not be mixed with other medicinal products.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C. Animal reproduction studies have not been conducted with ALBUTEIN 20%. It is also not known whether ALBUTEIN 20% can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. ALBUTEIN 20% should be given to a pregnant woman only if clearly needed.

8.2 Labor and Delivery

No human or animal data. Use only if clearly needed.

8.3 Nursing Mothers

No human or animal data. Use only if clearly needed.

8.4 Pediatric Use

No human or animal data. Use only if clearly needed.

8.5 Geriatric Use

No human or animal data. Use only if clearly needed.

11 DESCRIPTION

ALBUTEIN 20% is a sterile, aqueous solution for single dose intravenous administration containing 20% human albumin (weight/volume). ALBUTEIN 20% is prepared by a cold alcohol fractionation method from pooled human plasma obtained from venous blood. The product is stabilized with 0.08 millimole sodium caprylate and 0.08 millimole sodium acetyltryptophanate per gram of protein. The colloid osmotic effect of human albumin 20% is approximately four times that of normal human plasma. A liter of ALBUTEIN 20% solution contains 130-160 milliequivalents of sodium ion. The aluminum content of the solution is not more than 200 micrograms per liter during the shelf life of the product. The product contains no preservatives.

ALBUTEIN 20% is manufactured from Source Plasma collected from FDA approved plasmapheresis centers in the United States. ALBUTEIN 20% is heated at 60 °C for ten hours against the possibility of transmitting viruses.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Human Albumin accounts for more than half of the total protein in the plasma and represents about 10% of protein synthesis activity by the liver. Human Albumin 20% has a corresponding hyperoncotic effect.

The primary physiological function of albumin results from its contribution to plasma colloid oncotic pressure and transport function. Albumin stabilizes circulating blood volume and is a carrier of hormones, enzymes, medicinal products and toxins. Other physiological functions include antioxidant properties, free radical scavenging, and capillary membrane integrity.

12.3 Pharmacokinetics

Albumin is distributed throughout the extracellular space and more than 60% of the body albumin pool is located in the extravascular fluid compartment. Albumin has a circulating life span of 15-20 days, with a turnover of approximately 15 g per day.

The balance between synthesis and breakdown is normally achieved by feedback regulation. Elimination is predominantly intracellular and due to lysosome proteases.

In healthy subjects, less than 10% of infused albumin leaves the intravascular compartment during the first 2 hours following infusion. There is considerable individual variation in the effect of albumin on plasma volume. In some patients, the plasma volume can remain elevated for several hours. In critically ill patients, however, albumin can leak out of the vascular space in substantial amounts at an unpredictable rate.

15 REFERENCES

1. Tullis JL. Albumin: 1. Background and Use. 2. Guidelines for Clinical Use. JAMA. 1977;237:355-360, 460-463.
2. Vermeulen LC, et al. A Paradigm for Consensus. Arch Intern Med. 1995;155:373-379.
3. SAFE Study investigators. A comparison of albumin and saline for fluid resuscitation in the intensive care unit. N Engl J Med. 2004;350:2247-2256.
4. Sedrakyan A, Gondek K, Paltiel D, et al. Volume expansion with albumin decreases mortality after coronary artery bypass graft surgery. Chest. 2003;123:1853-1857.
5. Russell JA, Navickis RJ, Wilkes MM. Albumin versus crystalloid for pump priming in cardiac surgery: meta-analysis of controlled trials. J Cardiothorac Vasc Anesth. 2004;18:429-37.
6. American Thoracic Society. Evidence-based colloid use in the critically ill: American Thoracic Society consensus statement. Am J Respir Crit Care Med. 2004;170:1247-59.
7. Fliser D, Zurbrüggen I, Mutschler E, et al. Coadministration of albumin and furosemide in patients with nephrotic syndrome. Kidney Int. 1999;55:629-34.
8. Mendez CM, McClain CJ, Marsano LS. Albumin Therapy in Clinical Practice. Nutrition in Clinical Practice. 2005;20:314-320.
9. Haynes GR, Navickis RJ, Wilkes MM. Albumin administration-what is the evidence of clinical benefit? A systematic review of randomized controlled trials. Eur J Anaesthesiol. 2003 Oct;20(10):771-93.
10. Vincent JL, Navickis RJ, Wilkes MM. Morbidity in hospitalized patients receiving human albumin: a meta-analysis of randomized, controlled trials. Crit Care Med. 2004;32:2029-38.
11. Skillman JJ, Tanenbaum BJ. Current Topics in Surgical Research. Vol. 2. New York: Academic Press. 1970;523.
12. Muir IA, Barclay TL. Burns and their treatment. Chicago: Year Book Medical Publishers. 1974.
13. Pruitt BA Jr, Goodwin CW Jr. Current treatment of the extensively burned patient. Surg Annu. 1983;15:331-64.
14. Clowes GHA Jr, Vucinic M, Weidner MG. Circulatory and metabolic alterations associated with survival or death in peritonitis: clinical analysis of 25 cases. Ann Surg. 1966;166:866-85.
15. Sort P, Navasa M, Arroyo V, et al. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. N Engl J Med. 1999;341:403-409.
16. Aboulghar M, Evers JH, Al Inany H. Intravenous albumin for preventing severe ovarian hyperstimulation syndrome: a Cochrane review. Hum Reprod. 2002;17:3027-3032.
17. Practice Committee of the American Society for Reproductive Medicine. Ovarian hyperstimulation syndrome. Fertil Steril. 2006;86:S178-S183.
18. Tsao YC, Yu VY. Albumin in management of neonatal hyperbilirubinaemia. Arch Dis Child. 1972;47:250-256.
19. Practice parameter: management of hyperbilirubinemia in the healthy term newborn. Pediatrics. 1994;94(4 pt 1):558-62.
20. Dennery PA, Seidman DS, Stevenson DK. Neonatal hyperbilirubinemia. N Eng J Med. 2001;344:581-90.
21. Martin GS, et al. A randomized, controlled trail of furosemide with or without albumin in hypoproteinemic patients with acute lung injury. Crit Care Med. 2005;33:1681-1687.
22. Ginés P, Cárdenas A, Arroyo V, et al. Management of cirrhosis and ascites. N Engl J Med. 2004;350:1646-54.
23. Runyon BA. AASLD Practice Guidelines. Management of adult patients with ascites due to cirrhosis. Hepatology. 2009;49(6):2087-107.
24. Moore KP, Wong F, Ginés P, et al. The management of ascites in cirrhosis: report on the consensus conference of the International Ascites Club. Hepatology. 2003;38:258-66.

16 HOW SUPPLIED/STORAGE AND HANDLING

ALBUTEIN 20% is supplied in single-use, individually laser etched vials.
The following vial sizes of ALBUTEIN 20% are available:

NDC Number	Fill Size	Grams Protein
68516-5215-1	50 mL	10 g
68516-5215-2	100 mL	20 g

Each vial size label incorporates integrated hangers. Each label has a peel-off strip showing the product name and lot number.

ALBUTEIN 20% is stable for three years provided the storage temperature does not exceed 30 °C. Protect from freezing.

17 PATIENT COUNSELING INFORMATION

This product is usually given in a hospital setting.

Inform patients being treated with ALBUTEIN 20% about the risks and benefits of its use [see *Adverse Reactions (6)*].

Inform patients to immediately report the following signs and symptoms to their physician:

- Allergic or anaphylactic type reactions [see *Warnings and Precautions (5.1)*].
- Cardiovascular overload (e.g., headache, dyspnea and jugular venous distention) [see *Warnings and Precautions (5.2)*].
- Increased blood pressure, raised venous pressure and pulmonary edema [see *Warnings and Precautions (5.2)*].

Inform patients that ALBUTEIN 20% is a derivative of human plasma and may contain infectious agents that cause disease (e.g., viruses, and theoretically, the CJD agent). Inform patients that the risk that ALBUTEIN 20% may transmit an infectious agent has been reduced by screening plasma donors for prior exposure to certain viruses, by testing the donated plasma for certain viral agents and by the inactivation and/or removal of certain viruses during the manufacturing process [see *Warnings and Precautions (5.8)*].

Manufactured by:

Grifols Biologicals Inc.
5555 Valley Boulevard
Los Angeles, CA 90032, U.S.A.
U. S. License No. 1694

APPENDIX 2

FULL PRESCRIBING INFORMATION

(Flebogamma® DIF)

GRIFOLS

Flebogamma® 5% DIF

Immune Globulin Intravenous (Human)
For intravenous administration, 5% Liquid Preparation

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use FLEBOGAMMA 5% DIF safely and effectively. See full prescribing information for FLEBOGAMMA 5% DIF.
FLEBOGAMMA 5% DIF (immune globulin intravenous [human]), solution for intravenous administration
Initial U.S. Approval: 2006

WARNING: THROMBOSIS, RENAL DYSFUNCTION, and ACUTE RENAL FAILURE
See full prescribing information for complete boxed warning.

- **Thrombosis may occur with immune globulin products, including FLEBOGAMMA 5% DIF. Risk factors may include: advanced age, prolonged immobilization, hypercoagulable conditions, history of venous or arterial thrombosis, use of estrogens, indwelling vascular catheters, hyperviscosity, and cardiovascular risk factors.**
- **For patients at risk of thrombosis administer FLEBOGAMMA 5% DIF at the minimum dose and infusion rate practicable. Ensure adequate hydration in patients before administration. Monitor for signs and symptoms of thrombosis and assess blood viscosity in patients at risk of hyperviscosity.**
- **Renal dysfunction, acute renal failure, osmotic nephrosis and death may occur with the administration of human immune globulin intravenous (IGIV) products, particularly those products that contain sucrose. FLEBOGAMMA 5% DIF does not contain sucrose.**
- **For patients at risk of renal dysfunction or failure, administer FLEBOGAMMA 5% DIF at the minimum dose and infusion rate practicable. (5.2)**

RECENT MAJOR CHANGES

Warnings and Precautions: Hereditary Fructose Intolerance (5.12) 7/2017

INDICATIONS AND USAGE

Flebogamma 5% DIF is an immune globulin intravenous (human), indicated for treatment of primary (inherited) immunodeficiency (PI) in adults and pediatric patients 2 years of age and older. (1)

DOSAGE AND ADMINISTRATION

For Intravenous Use Only

Indication	Dose	Initial Infusion Rate	Maintenance Dose Rate (if tolerated)
PI	300-600 mg per kg every 3-4 weeks	0.01 mL per kg per minute (0.5 mg per kg per min)	Increase to 0.10 mL per kg per minute (5 mg per kg per min)

- For patients at risk of renal dysfunction or thrombosis, administer Flebogamma 5% DIF at the minimum dose and infusion rate practicable. (5.2, 5.4)
- Ensure that patients with pre-existing renal insufficiency are not volume-depleted and discontinue Flebogamma 5% DIF if renal function deteriorates. (5.2)

DOSAGE FORMS AND STRENGTHS

Solution for intravenous injection containing 5% IgG (50 mg per mL). (3)

CONTRAINDICATIONS

- History of anaphylactic or severe systemic reactions to human immunoglobulin. (4)
- IgA-deficient patients with antibodies against IgA and a history of hypersensitivity. (4)

WARNINGS AND PRECAUTIONS

- IgA-deficient patients with antibodies to IgA are at greater risk of developing severe hypersensitivity and anaphylactic reactions. (5.1)
- Monitor renal function, including blood urea nitrogen, serum creatinine, and urine output in patients at risk of developing acute renal failure. (5.2)
- Hyperproteinemia, increased serum viscosity, and hyponatremia may occur in patients receiving Flebogamma 5% DIF therapy. (5.3)
- Thrombosis may occur. Monitor patients with known risk factors for thrombosis and consider baseline assessment of blood viscosity for those at risk of hyperviscosity. (5.4)
- Aseptic meningitis syndrome (AMS) may occur in patients receiving Flebogamma 5% DIF therapy, especially with high doses or rapid infusion. (5.5)
- Hemolysis, either intravascular or due to enhanced red blood cell sequestration, can develop subsequent to Flebogamma 5% DIF treatments. Risk factors include high doses and non-O blood group. Monitor patients for hemolysis and hemolytic anemia. (5.6)
- Monitor patients for pulmonary adverse reactions (transfusion-related acute lung injury, TRALI). (5.7)
- Patients receiving Flebogamma 5% DIF for the first time or being restarted on the product after a treatment hiatus of more than 8 weeks may be at a higher risk for development of fever, chills, nausea, and vomiting. (5.8)
- Flebogamma 5% DIF is made from human plasma and may contain infectious agents, e.g., viruses, the variant Creutzfeldt-Jakob disease (vCJD) agent and, theoretically, the Creutzfeldt-Jakob disease (CJD) agent. (5.9)
- Passive transfer of antibodies may confound serologic testing. (5.11)
- Flebogamma 5% DIF contains sorbitol. The presence of sorbitol presents a risk to those with hereditary fructose intolerance (HFI). (5.12)

ADVERSE REACTIONS

The most common adverse reactions (reported in at least 5% of clinical trial adult subjects) were headache, pyrexia/fever, pain, infusion site reactions, diarrhea, rigors or chills, urticaria, and infusion site inflammation. (6)
The most common adverse reactions (reported in at least 5% of clinical trial pediatric subjects) were headache, pyrexia, hypotension, tachycardia, diastolic hypotension, nausea, abdominal pain, diarrhea, pain, and vomiting. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Grifols Biologicals at 1-888-GRIFOLS (1-888-474-3657) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

DRUG INTERACTIONS

Passive transfer of antibodies may transiently interfere with the immune response to live virus vaccines, such as measles, mumps, and rubella. (7)

USE IN SPECIFIC POPULATIONS

- Pregnancy: No human or animal data. Use only if clearly needed. (8.1)
- Geriatric: In patients over age 65 or in any patient at risk of developing renal insufficiency, do not exceed the recommended dose, and infuse Flebogamma 5% DIF at the minimum dose and infusion rate practicable and at less than 0.06 mL per kg per minute (3 mg per kg per min). (8.5)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 7/2017

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

WARNING: THROMBOSIS, RENAL DYSFUNCTION, and ACUTE RENAL FAILURE

- **Thrombosis may occur with immune globulin products, including FLEBOGAMMA 5% DIF. Risk factors may include: advanced age, prolonged immobilization, hypercoagulable conditions, history of venous or arterial thrombosis, use of estrogens, indwelling central vascular catheters, hyperviscosity, and cardiovascular risk factors. Thrombosis may occur in the absence of known risk factors. *(see Warnings and Precautions [5.4] and Patient Counseling Information [17])***
- **For patients at risk of thrombosis, administer FLEBOGAMMA 5% DIF at the minimum dose and infusion rate practicable. Ensure adequate hydration in patients before administration. Monitor for signs and symptoms of thrombosis and assess blood viscosity in patient at risk for hyperviscosity. *(see Dosage and Administration [2.3] and Warnings and Precautions [5.4])***
- **Renal dysfunction, acute renal failure, osmotic nephrosis, and death[1] have been related to intravenous immune globulin (IGIV) products. Patients predisposed to acute renal failure include patients with any degree of pre-existing renal insufficiency, diabetes mellitus, age greater than 65, volume depletion, sepsis, paraproteinemia, or patients receiving known nephrotoxic drugs.**
- **Administer FLEBOGAMMA 5% DIF at the minimum dose and rate of infusion practicable in patients at risk for renal dysfunction or failure.**
- **Reports of renal dysfunction and acute renal failure occur more commonly in patients receiving IGIV products containing sucrose as a stabilizer. They account for a disproportionate share of the total number of reported cases of renal dysfunction and acute renal failure. FLEBOGAMMA 5% DIF does not contain sucrose. *(see Dosage and Administration [2.3] and Warnings and Precautions [5.2])***

1 INDICATIONS AND USAGE

Flebogamma 5% DIF is an immune globulin intravenous (human) solution indicated in adults and pediatric patients 2 years of age and older for the treatment of primary immunodeficiency (PI), including the humoral immune defects in common variable immunodeficiency, x-linked agammaglobulinemia, severe combined immunodeficiency, and Wiskott-Aldrich syndrome.

2 DOSAGE AND ADMINISTRATION

For Intravenous Use Only

2.1 Dosage

Treatment of Primary Immunodeficiency (PI)

Dose	Initial Infusion Rate	Maintenance Dose Rate (if tolerated)
300-600 mg per kg body weight (6.0-12.0 mL per kg) administered every 3-4 weeks	0.01 mL per kg per minute (0.5 mg per kg per min)	Increase to 0.10 mL per kg per minute (5 mg per kg per min)

As there are significant differences in the half-life of IgG among patients with PI, the frequency and amount of immunoglobulin therapy may vary from patient to patient. Adjust the dose according to the clinical response. Adjust the dosage over time to achieve the desired trough IgG levels and clinical responses. No randomized controlled trial data are available to determine an optimum target trough serum IgG level.

2.2 Preparation and Handling

- Inspect Flebogamma 5% DIF visually for particulate matter and color prior to administration. Do not use the vial if particles are detected. Do not use if turbid.
- Several vials of Flebogamma 5% DIF may be pooled into an empty sterile solution container by using aseptic technique, if large doses are to be administered.
- Do not dilute with intravenous fluids. Do not inject other medications into intravenous tubing being used for Flebogamma 5% DIF.
- Infuse Flebogamma 5% DIF through a separate intravenous line. Do not add any medications or intravenous fluids to the Flebogamma 5% DIF infusion container. Do not mix IGIV products of different formulations or from different manufacturers.
- Discard unused contents and administration devices after use.
- Use promptly any vial that has been entered.
- Discard partially used vials. Do not save for future use because the solution contains no preservative.
- Do not use solution that has been frozen.

2.3 Administration

The recommended initial infusion rate of Flebogamma 5% DIF is 0.01 mL per kg body weight per minute (0.5 mg per kg per min). If the infusion is well-tolerated during the first 30 minutes, the rate may be gradually increased to a maximum of 0.10 mL per kg per minute (5 mg per kg per min).
Monitor patient vital signs throughout the infusion. Slow or stop infusion if adverse reactions occur. If symptoms subside promptly, the infusion may be resumed at a lower rate that is comfortable for the patient.

3 DOSAGE FORMS AND STRENGTHS

Flebogamma 5% DIF is a liquid preparation containing 5% IgG (50 mg per mL).

4 CONTRAINDICATIONS

- Flebogamma 5% DIF is contraindicated in patients who have had a history of anaphylactic or severe systemic hypersensitivity reactions to the administration of human immune globulin.
- Flebogamma 5% DIF is contraindicated in IgA-deficient patients with antibodies to IgA and a history of hypersensitivity. *(see Warnings and Precautions [5.1])*

5 WARNINGS AND PRECAUTIONS

5.1 Hypersensitivity

Severe hypersensitivity reactions and anaphylactic reactions with a fall in blood pressure may occur, even in patients who had tolerated previous treatment with IGIV. *(see Contraindications [4])* If hypersensitivity reaction develops, discontinue Flebogamma 5% DIF infusion immediately and institute appropriate treatment.
Flebogamma 5% DIF contains trace amounts of IgA (less than 50 µg/mL). *(see Description [11])* Patients with antibodies to IgA have a greater risk of developing potentially severe hypersensitivity and anaphylactic reactions. Flebogamma 5% DIF is contraindicated in patients with antibodies against IgA and a history of hypersensitivity reaction. *(see Contraindications [4])*

5.2 Renal Dysfunction/Failure

Acute renal dysfunction/failure, acute tubular necrosis, proximal tubular nephropathy, osmotic nephrosis, and death have been reported in patients receiving IGIV, particularly those products containing sucrose[2]. Flebogamma 5% DIF does not contain sucrose.
Ensure that patients are not volume-depleted before administering Flebogamma 5% DIF. For patients judged to be at risk for developing renal dysfunction, including patients with any degree of pre-existing renal insufficiency, diabetes mellitus, age greater than 65, volume depletion, sepsis, paraproteinemia, or patients receiving known nephrotoxic drugs, administer Flebogamma 5% DIF at the minimum dose and rate of infusion practicable[3]. *(see Boxed Warning, Dosing and Administration [2.3])*
Periodic monitoring of renal function and urine output is particularly important in patients judged to be at increased risk of developing acute renal failure[1]. Assess renal function, including measurement of blood urea nitrogen (BUN) and serum creatinine, before the initial infusion of Flebogamma 5% DIF and at appropriate intervals thereafter. If renal function deteriorates, consider discontinuation of the product.

5.3 Hyperproteinemia, Increased Serum Viscosity, and Hyponatremia

Hyperproteinemia, increased serum viscosity, and hyponatremia may occur in patients receiving Flebogamma 5% DIF therapy. It is clinically critical to distinguish true hyponatremia from a pseudohyponatremia that is temporally or causally related to hyperproteinemia with concomitant decreased calculated serum osmolarity or elevated osmolar gap, because treatment aimed at decreasing serum free water in patients with pseudohyponatremia may lead to volume depletion, a further increase in serum viscosity, and a higher risk of thrombosis.

5.4 Thrombosis

Thrombosis may occur following treatment with immune globulin products, including FLEBOGAMMA 5% DIF. Risk factors may include advanced age, prolonged immobilization, hypercoagulable conditions, history of venous or arterial thrombosis, use of estrogens, indwelling central vascular catheters, hyperviscosity, and cardiovascular risk factors. Thrombosis may occur in the absence of known risk factors.
Consider baseline assessment of blood viscosity in patients at risk for hyperviscosity, including those with cryoglobulins, fasting chylomicronemia/markedly high triacylglycerols (triglycerides), or monoclonal gammopathies. *(see Warnings and Precautions [5.10])* For patients at risk of thrombosis, administer FLEBOGAMMA 5% DIF at the minimum dose and infusion rate practicable. Ensure adequate hydration in patients before administration. Monitor for signs and symptoms of thrombosis and assess blood viscosity in patients at risk for hyperviscosity. *(see Boxed Warning, Dosage and Administration [2.3], and Patient Counseling Information [17])*

5.5 Aseptic Meningitis Syndrome (AMS)

AMS has been reported to occur following IGIV treatment. Discontinuation of IGIV treatment has resulted in remission of AMS within several days without sequelae[4-7]. The symptoms of AMS usually begin within several hours to 2 days following IGIV treatment.
AMS is characterized by the following signs and symptoms: severe headache, nuchal rigidity, drowsiness, fever, photophobia, painful eye movements, nausea, and vomiting. *(see Patient Counseling Information [17])* Cerebrospinal fluid (CSF) studies frequently reveal pleocytosis up to several thousand cells per cubic millimeter, predominantly from the granulocytic series, and elevated protein levels up to several hundred mg/dL, but negative culture results. Conduct a thorough neurological examination to patients exhibiting such signs and symptoms, including CSF studies, to rule out other causes of meningitis. AMS may occur more frequently following high-dose (e.g. over 1.0 g per kg body weight) or rapid infusion of IGIV.

5.6 Hemolysis

Flebogamma 5% DIF may contain blood group antibodies that may act as hemolysins and induce *in vivo* coating of red blood cells (RBCs) with immunoglobulin, causing a positive direct antiglobulin test (DAT) (Coombs' test) result and hemolysis[8-11]. Delayed hemolytic anemia can develop subsequent to IGIV therapy due to enhanced RBC sequestration and acute hemolysis, consistent with intravascular hemolysis, has been reported[12]. Cases of severe hemolysis-related renal dysfunction/failure or disseminated intravascular coagulation have occurred following infusion of IGIV.
The following risk factors may be associated with the development of hemolysis following IGIV administration: high doses (e.g., at least 2 g per kg), given either as a single administration or divided over several days, and non-O blood group[13]. Other individual patient factors, such as an underlying inflammatory state (as may be reflected by, for example, elevated C-reactive protein or erythrocyte sedimentation rate), have been hypothesized to increase the risk of hemolysis following administration of IGIV[14], but their role is uncertain. Hemolysis has been reported following administration of IGIV for a variety of indications, including ITP and PI[11]. Monitor patients for clinical signs and symptoms of hemolysis, particularly patients with risk factors noted above. Consider appropriate laboratory testing in higher risk patients, including measurement of hemoglobin or hematocrit prior to infusion and within 36 to 96 hours post infusion. If clinical signs and symptoms of hemolysis or a significant drop in hemoglobin or hematocrit have been observed, perform appropriate confirmatory laboratory testing. If transfusion is indicated for patients who develop hemolysis with clinically compromising anemia after receiving IGIV, perform adequate cross-matching to avoid exacerbating on-going hemolysis. *(see Patient Counseling Information [17])*

5.7 Transfusion-Related Acute Lung Injury (TRALI)

Non-cardiogenic pulmonary edema has been reported in patients following IGIV treatment[15]. TRALI is characterized by severe respiratory distress, pulmonary edema, hypoxemia, normal left ventricular function, and fever. Symptoms typically appear within 1 to 6 hours after transfusion.
Monitor patients for pulmonary adverse reactions. *(see Patient Counseling Information [17])* If TRALI is suspected, perform appropriate tests for the presence of antineutrophil antibodies and anti-HLA antibodies in both the product and patient serum. TRALI may be managed by using oxygen therapy with adequate ventilatory support.

5.8 Infusion Reactions

Individuals receiving Flebogamma 5% DIF for the first time, or being restarted on the product after a treatment hiatus of more than 8 weeks, may be at a higher risk for the development of fever, chills, nausea, and vomiting. Careful monitoring of recipients and adherence to recommendations regarding dosage and administration may reduce the risk of these types of events. *(see Dosage and Administration [2.3])*

5.9 Transmissible Infectious Agents

Because Flebogamma 5% DIF is made from human plasma, it may carry a risk of transmitting infectious agents, e.g., viruses, the variant Creutzfeldt-Jakob disease (vCJD) agent and, theoretically, the Creutzfeldt-Jakob disease (CJD) agent. This also applies to unknown or emerging viruses and other pathogens. No cases of transmission of viral diseases or CJD have been associated with the use of Flebogamma 5% DIF. All infections suspected by a physician possibly to have been transmitted by this product should be reported by the physician or other healthcare provider to Grifols Biologicals at 1-888-474-3657.
Before prescribing or administering Flebogamma 5% DIF, the physician should discuss the risks and benefits of its use with the patient. *(see Patient Counseling Information [17])*

5.10 Monitoring: Laboratory Tests

- Periodic monitoring of renal function and urine output is particularly important in patients judged to be at increased risk of developing acute renal failure. Assess renal function, including measurement of BUN and serum creatinine, before the initial infusion of Flebogamma 5% DIF and at appropriate intervals thereafter.
- Consider baseline assessment of blood viscosity in patients at risk for hyperviscosity, including those with cryoglobulins, fasting chylomicronemia/markedly high triacylglycerols (triglycerides), or monoclonal gammopathies because of the potentially increased risk of thrombosis..
- If signs and/or symptoms of hemolysis are present after an infusion of Flebogamma 5% DIF, perform appropriate laboratory testing for confirmation.
- If TRALI is suspected, perform appropriate tests for the presence of antineutrophil antibodies and anti-HLA antibodies in both the product and patient serum.

5.11 Interference with Laboratory Tests

After infusion of IgG, the transitory rise of the various passively transferred antibodies in the patient's blood may yield positive serological testing results, with the potential for misleading interpretation. Passive transmission of antibodies to erythrocyte antigens (e.g. A, B, and D) may cause a positive direct or indirect antiglobulin (Coombs') test.

5.12 Hereditary Fructose Intolerance

Flebogamma 5% DIF contains sorbitol. The presence of sorbitol presents a risk to those with hereditary fructose intolerance (HFI). The incidence of HFI is estimated at 1 in 20,000 births and is usually diagnosed at the time of weaning when fructose or sucrose is introduced into the diet. Clinical symptoms include recurrent vomiting, abdominal pain and hypoglycemia. Flebogamma 5% DIF must not be administered to subjects with HFI.

6 ADVERSE REACTIONS

The most common adverse reactions (reported in at least 5% of clinical trial adult subjects) were headache, pyrexia/fever, pain, infusion site reactions, diarrhea, rigors or chills, urticaria, and infusion site inflammation.
The most common adverse reactions (reported in at least 5% of clinical trial pediatric subjects) were headache, pyrexia, hypotension, tachycardia, diastolic hypotension, nausea, abdominal pain, diarrhea, pain, and vomiting.
To report SUSPECTED ADVERSE REACTIONS, contact Grifols Biologicals at 1-888-GRIFOLS (1-888-474-3657) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in clinical practice.
Adverse reactions were reported in a study of 46 individuals with PI receiving infusions every 3 to 4 weeks of 300-600 mg per kg body weight. Thirty-one subjects (67.4%) had at least one adverse reaction at some time during the study that was considered product-related. None of the 46 subjects who participated in this study discontinued the study prematurely due to an adverse drug reaction.
Adverse reactions that occurred with an incidence of at least 5% on a per-subject basis are summarized in Table 1.

Table 1. Adverse Reactions Occurring in at Least 5% of Subjects

Adverse Reaction	Subjects (%) N=46	Number of Events
Headache	**10 (21.7)**	**24**
Pyrexia[a]	**9 (19.6)**	**12**
Pain[b]	**7 (15.2)**	**11**
Injection site reaction	**6 (13.0)**	**10**
Diarrhea	**4 (8.7)**	**5**
Rigors	**4 (8.7)**	**7**
Urticaria	**3 (6.5)**	**3**
Infusion site inflammation[c]	**3 (6.6)**	**3**

a. include combined reported terms of pyrexia and body temperature increase.
b. include combined reported terms of pain such as pain (not otherwise specified), injection site pain, back pain, abdominal pain (not otherwise specified), and abdominal pain upper.
c. include combined reported terms of infusion site inflammation and others such as injection site oedema and injection site swelling.

Other common adverse drug reactions reported in fewer than 5% of the subjects included hypertension, sinusitis, nausea/vomiting, positive Coombs test, arthralgia, myalgia, dizziness, bronchitis, and hypotension.
The total number of adverse reactions reported whose onset were within 72 hours after the end of an infusion of Flebogamma 5% DIF was 94. There were a total of 709 infusions, resulting in a ratio of 0.13 temporally associated adverse reactions per infusion (the upper bound of the 1-sided 95% confidence interval = 0.18). Of the 709 total infusions, 70 (9.7%, 1-sided 95% upper bound CI = 12.4%) were associated with at least one adverse reaction that began within 72 hours after the completion of an infusion. In this analysis, each infusion is only counted once, regardless of the number of adverse reactions that occurred during the infusion, when during the 72-hour period after the infusion the adverse reaction started, or the intensity of those adverse reactions.
Factoring adverse reaction intensity into the analysis of the 709 infusions shows that there were 58 infusions with at least one mild adverse reaction (8.2% [upper bound 95% CI=10.5%]), 25 infusions with at least one moderate adverse reaction (3.5% [upper bound 95% CI=5.2%]), and 1 infusion with a severe adverse reaction (0.1% [upper bound 95% CI=0.8%]). In this analysis, if a subject reported multiple events with different intensities during the same infusion (e.g., mild headache and moderate pyrexia), that infusion would be counted in all relevant categories. Therefore, the number of infusions counted is 84.
Three subjects (6.5%) experienced a treatment-emergent rise in AST (> 3x the upper-limit of normal), and 1 subject (2.2%) experienced a treatment-emergent rise in ALT (> 3x the upper-limit of normal). None of these abnormal lab values were long-lasting (i.e. they occurred at 1 or 2 infusions), and none of these subjects had a concomitant treatment-emergent rise in total bilirubin.

A clinical study with Flebogamma 5% DIF for the treatment of PI was conducted in 24 pediatric subjects aged 2-16 years to determine whether they respond differently from adult subjects. Pediatric subjects received intravenous infusions of 262-625 mg per kg body weight every 3-4 weeks. Twenty subjects (83.3%) had at least one adverse reaction at some time during the study that was considered product-related. There were no deaths or serious adverse reactions.
In 317 infusions, 20 pediatric subjects reported 159 treatment-related adverse drug reactions (ADR). Treatment-related adverse reactions that occurred with an incidence of at least 5% on a per-subject basis included headache (42%), pyrexia (29%), hypotension (25%), tachycardia (25%), diastolic hypotension (21%), nausea (8%), abdominal pain (8%), diarrhea (8%), pain (8%), and vomiting (8%). Of these, 99 ADRs were mild, 54 were moderate, and 6 were severe in intensity. The most common severe ADR was headache. Tachycardia was defined as mild, moderate, or severe by ratio of heart rate over baseline of 1.2-1.4, 1.41-1.6, or >1.6, respectively. Two episodes of tachycardia were moderate and 14 were mild, 1 mild case had the infusion interrupted and the rest had no action taken, and all resolved. Hypotension was defined as mild, moderate, or severe by the decrease in pressure below baseline of 10-15%, 16-25%, and >25%, respectively. Ten episodes of hypotension/diastolic hypotension were moderate and 29 were mild, one moderate case had the infusion interrupted and the rest had no action taken, and all resolved. None of the ADRs related to fluctuation in vital signs were severe, all resolved without sequelae, and none were considered clinically significant.
One subject (6.5%) experienced a treatment-emergent rise in ALT (over 2.5x the upper-limit of normal). This was considered a mild treatment-related AE and resolved without sequelae. All other lab values were within normal limits. One subject experienced one positive Coombs' test result after baseline (experienced 14 days after the final infusion). No subjects experienced clinically significant abnormal lab values for LDH, bilirubin, serum creatinine. In addition, no subjects experienced positive test results for HBsAg, HCV, or HIV.

6.2 Post-marketing Experience

Because adverse reactions are reported voluntarily post-approval from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to product exposure.
The following adverse reactions have been identified during the post-approval use of IGIV products[16, 17], including Flebogamma 5% DIF.

Infusion Reactions	Hypersensitivity (e.g. anaphylaxis), headache, diarrhea, tachycardia, fever, fatigue, dizziness, malaise, chills, flushing, urticaria or other skin reactions, wheezing or other chest discomfort, nausea, vomiting, rigors, back pain, myalgia, arthralgia, and changes in blood pressure
Renal	Acute renal dysfunction/failure, osmotic nephropathy
Respiratory	Apnea, Acute Respiratory Distress Syndrome (ARDS), Transfusion-Related Acute Lung Injury (TRALI), cyanosis, hypoxemia, pulmonary edema, dyspnea, bronchospasm
Cardiovascular	Cardiac arrest, thromboembolism, vascular collapse, hypotension
Neurological	Coma, loss of consciousness, seizures, tremor, aseptic meningitis syndrome
Integumentary	Stevens-Johnson Syndrome, epidermolysis, erythema multiformae, dermatitis (e.g. bullous dermatitis)
Hematologic	Pancytopenia, leukopenia, hemolysis, positive direct antiglobulin (Coombs) test
Musculoskeletal	Back pain
Gastrointestinal	Hepatic dysfunction, abdominal pain
General/Body as a Whole	Pyrexia, rigors

7 DRUG INTERACTIONS

Passive transfer of antibodies may transiently impair the immune response to live attenuated virus vaccines, such as measles, mumps, and rubella. Inform the immunizing physician of recent therapy with Flebogamma 5% DIF so that appropriate measures can be taken *(see Patient Counseling Information [17])*

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary
There are no studies of Flebogamma 5% DIF use in pregnant women. Animal reproduction studies have not been performed with Flebogamma 5% DIF. It is also not known whether Flebogamma 5% DIF can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Immunoglobulins cross the placenta from maternal circulation increasingly after 30 weeks of gestation. Flebogamma 5% DIF should be given to a pregnant woman only if clearly needed.

8.2 Lactation

Risk Summary
There is no information regarding the presence of Flebogamma 5% DIF in human milk, its effects on the breastfed infant, or its effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Flebogamma 5% DIF and any potential adverse effects on the breastfed infant from Flebogamma 5% DIF or from the underlying maternal condition.

8.4 Pediatric Use

Flebogamma 5% DIF was studied in a multicenter clinical trial for the treatment of PI in 24 subjects aged 2-16 years (seven were 2-5 years of age, seven were 6-11 years, and ten were 12-16 years), and found to be efficacious for the prevention of acute serious bacterial infections. No pediatric-specific dose requirements were necessary to achieve the desired serum IgG levels.
Twenty subjects (83.3%) had at least one adverse reaction at some time during the study that was considered product-related. There were no deaths or serious adverse reactions. Treatment-related adverse reactions that occurred with an incidence of at least 5% on a per-subject basis included headache (42%), pyrexia (29%), hypotension (25%), tachycardia (25%), diastolic hypotension (21%), nausea (8%), abdominal pain (8%), diarrhea (8%), pain (8%), and vomiting (8%).
Safety and efficacy of Flebogamma 5% DIF in pediatric patients below the age of 2 years have not been established.

8.5 Geriatric Use

Limited information is available for the geriatric use of Flebogamma 5% DIF. Clinical studies of Flebogamma 5% DIF did not include sufficient numbers of subjects over the age of 65 to determine whether they respond differently from younger subjects. Use caution when administering Flebogamma 5% DIF to patients age 65 and over who are judged to be at increased risk for developing thrombosis or renal insufficiency. Do not exceed recommended dose, and administer Flebogamma 5% DIF at the minimum dose and infusion rate practicable, and at less than 0.06 mL per kg per minute (3 mg per kg per min). *(see Boxed Warning, Warning and Precautions [5.2, 5.4], and Dosage and Administration [2.3])*

10 OVERDOSAGE

Overdose may lead to fluid overload and hyperviscosity. Patients at particular risk of complications of fluid overload and hyperviscosity include elderly patients and patients with cardiac or renal impairment.

11 DESCRIPTION

Flebogamma 5% DIF is a ready to use, sterile, clear or slightly opalescent and colorless to pale yellow, liquid preparation of purified immunoglobulin (IgG) obtained from human plasma pools. The purification process includes cold ethanol fractionation, polyethylene glycol precipitation, ion exchange chromatography, low pH treatment, pasteurization, solvent detergent treatment, and Planova nanofiltration using 20 nanometer (nm) filters.
Flebogamma 5% DIF is a purified (at least 97% IgG), unmodified, human IgG. The distribution of the four IgG subclasses is approximately 66.6% IgG_1, 28.5% IgG_2, 2.7% IgG_3, and 2.2% IgG_4. Flebogamma 5% DIF contains trace amounts of IgA (typically less than 50 µg/mL) and trace amounts of sodium and IgM.
Flebogamma 5% DIF contains 5 g human normal immunoglobulin and 5 g D-sorbitol (as stabilizer) in 100 mL of water for injection, and ≤ 3 mg/mL polyethylene glycol. There is no preservative in the formulation. The pH of the solution ranges from 5 to 6 and the osmolality from 240 to 370 mOsm/kg, which is within the normal physiological range.
Screening against potentially infectious agents begins with the donor selection process and continues throughout plasma collections and plasma preparation. Each individual plasma donation used in the manufacture of Flebogamma 5% DIF is collected only at FDA-approved blood establishments and is tested by FDA-licensed serological test for hepatitis B virus (HBV) surface antigen (HBsAg), and for antibodies to human immunodeficiency virus (HIV-1/HIV-2) and hepatitis C virus (HCV) in accordance with U.S. regulatory requirements. As an additional safety measure, mini-pools of plasma are tested for the presence of HBV, HIV-1 and HCV by FDA-licensed nucleic acid testing (NAT) and found to be negative. In addition, plasma is tested by in-process NAT for hepatitis A virus (HAV) and parvovirus B19 (B19) on mini-pools and the viral load limit for B19 in the manufacturing pool is set not to exceed 10^4 IU/mL. NAT for the presence of HCV and HIV in the manufacturing plasma pool is also performed and found to be negative.
To further improve the margin of safety, three dedicated, independent virus inactivation/removal steps have been integrated into the manufacturing and formulation processes, namely pasteurization at 60 ºC, 10 hours, solvent-detergent treatment for 6 hours, and nanofiltration down to 20 nm Planova filters.
In vitro virus spiking studies have been used to validate the capability of the manufacturing process to inactivate and remove viruses. To establish the minimum applicable virus clearance capacity of the manufacturing process, these virus clearance studies were performed on seven steps of the production process (pasteurization, solvent-detergent treatment, nanofiltration, Fraction I precipitation, Fraction II+III precipitation, 4% PEG precipitation, and pH treatment for 4 hours at 37 ºC).
The viral reduction data (in $\log_{10}$) from these experiments are summarized in Table 2.

Table 2. Flebogamma 5% DIF: Viral Reduction Capacity of Combined Steps ($\log_{10}$)

Target virus	HIV-1, HIV-2 (env. RNA)	HBV Herpesvirus (env. DNA)		HCV (env. RNA)		WNV (env. RNA)	HAV (non-env. RNA)	Virus B19 (non-env. DNA)
Model virus	**HIV-1**	**PRV**	**IBR**	**BVDV**	**SINDBIS**	**WNV**	**EMC**	**PPV**
Fraction I precipitation	**< 1.00***	**nd**	**nd**	**nd**	**nd**	**2.78**	**nd**	**< 1.00***
Ethanol incubation (Fraction II+III)	**1.48**	**nd**	**nd**	**nd**	**nd**	**< 1.00***	**nd**	**nd**
PEG precipitation	**≥ 6.10**	**≥ 5.92**	**nd**	**≥ 5.78**	**nd**	**nd**	**≥ 6.41**	**6.35**
Acid pH treatment	**2.47**	**≥ 5.32**	**nd**	**< 1.00***	**nd**	**nd**	**1.36**	**na**
Pasteurization	**≥ 5.64**	**≥ 4.96**	**≥ 6.33**	**≥ 4.69**	**≥ 6.49**	**≥ 5.42**	**≥ 5.56**	**4.08**
Solvent Detergent	**≥ 4.61**	**≥ 6.95**	**nd**	**≥ 6.14**	**nd**	**≥ 5.59**	**na**	**na**
Nanofiltration 20 nanometer	**≥ 4.81**	**≥ 4.63**	**nd**	**≥ 4.67**	**nd**	**≥ 3.63**	**≥ 5.92**	**4.61**
Overall Reduction Capacity	**≥ 25.11**	**≥ 27.78**	**≥ 6.33**	**≥ 21.28**	**≥ 6.49**	**≥ 17.42**	**≥ 19.25**	**15.04**

* When the RF is < 1 $\log_{10}$, it is not taken into account for the calculation of the overall reduction capacity.
≥ is no residual infectivity detected; nd = not done; na = non-applicable, since the virus is theoretically resistant to this treatment.
Abbreviations: HIV = Human immunodeficiency virus; PRV = Pseudorabies virus; IBR = Infectious bovine rhinotracheitis virus; BVDV = Bovine viral diarrhea virus; SINDBIS = Sindbis virus; WNV = West Nile virus; EMC = Encephalomyocarditis virus; PPV = Porcine parvovirus.

Additionally, the manufacturing process was investigated for its capacity to decrease infectivity of an experimental agent of transmissible spongiform encephalopathy (TSE), considered as a model for the vCJD and CJD agents. Several individual production steps in the Flebogamma 5% DIF manufacturing process have been shown to decrease TSE infectivity of an experimental model agent. TSE reduction steps include 4% polyethylene glycol precipitation [at least 6.19 $\log_{10}$] and Planova nanofiltration using a 20 nanometer filter [at least 5.45 $\log_{10}$]. These studies provide reasonable assurance that low levels of CJD/vCJD agent infectivity, if present in the starting material, would be removed.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Flebogamma 5% DIF, immune globulin intravenous (human), is a replacement therapy for PI. It supplies a broad spectrum of opsonizing and neutralizing IgG antibodies against a wide variety of bacterial and viral agents. Flebogamma 5% DIF also contains a spectrum of antibodies capable of reacting with cells such as erythrocytes. The role of these antibodies and the mechanisms of action of IgG in Flebogamma 5% DIF have not been fully elucidated.

12.3 Pharmacokinetics

In the clinical study assessing safety and efficacy in PI, Flebogamma 5% DIF was administered as an IV infusion (300-600 mg per kg) to subjects every 3 (n = 8) or 4 (n = 12) weeks for 12 months. The pharmacokinetics of total IgG was determined after the 7th infusion for the 3-week dosing interval and after the 5th infusion for the 4-week dosing interval (Table 3).

Table 3. Pharmacokinetic Variables of Total IgG in Subjects with PI

Variable	3-Week Dosing Interval (n = 8)		4-Week Dosing Interval (n = 12)	
	Mean [Range]	SD	Mean [Range]	SD
Cmax (mg/dL)	**1,929 [1,300-2,420]**	**441**	**2,069 [1,590-2,800]**	**338**
AUC_{0-wk} (day·mg/dL)	**31,159 [20,458-40,104]**	**6,572**	**32,894 [27,650-41,814]**	**3,886**
Clearance (mL/day)	**139 [81-243]**	**57**	**109 [59-161]**	**33**
Half-life (days)[a]	**30 [19-41]**	**9**	**32 [25-39]**	**5**
Trough IgG level (mg/dL)[b]	**951.38 [773.17-1143.15]**	**132.42**	**899.89 [776.70-1,137.14]**	**92.03**

a. This half-life is an apparent value derived from a period of measurement of 28 days.
b. For subjects on the 3-week schedule, the average of the trough levels from Infusion 7 to the end of the study was calculated; for those on a 4-week schedule, the average of the trough levels from Infusion 5 to the end of the study was calculated. The means of the subject means are presented in this table.

There were 3 adolescent (up to 16 years of age) subjects who underwent pharmacokinetic testing, all of whom were on the 3-week infusion schedule. There were no clinically relevant differences among the adults and adolescents that were tested.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenicity, Mutagenesis, Impairment of Fertility

No animal studies were conducted to evaluate the carcinogenic or mutagenic effect of Flebogamma 5% DIF or its effects on fertility.

13.2 Animal Toxicology and/or Pharmacology

Acute toxicity studies were performed in mice and rats at doses up to 2.5 g per kg body weight with infusion rates 6-37 times higher than the maximum rates recommended for humans. The most common clinical observations in mice studies were piloerection, ptosis, ataxia, and increase in respiration all lasting 90 minutes or less. No relevant adverse effects could be confirmed affecting respiratory, circulatory, renal, autonomic and central nervous systems, somatomotor activity, and behavior of the treated mice and rats.
Five of the 25 rats treated with the highest dose, approximately 8 times the maximum infusion rate recommended for humans, showed a transient "reddish urine" sign which was not confirmed as a relevant toxicity-causing phenomenon after renal macroscopic and microscopic analysis. This phenomenon was ascribed to hemolysis when serum was analyzed, suggesting a possible relation to cross reactivity of rodent red cells with human antibodies. No "reddish urine" was detected in any mouse, a much smaller animal where the rate of infusion was comparatively much higher than in rats. The macroscopic inspection of all treated mice did not show any renal alteration.

14 CLINICAL STUDIES

A multicenter, open-label, historically controlled study was conducted in the United States to assess the efficacy, safety, and pharmacokinetics of Flebogamma 5% DIF in adult and pediatric subjects with PI. A total of 46 subjects aged 15-75 years (63% male, 37% female) were enrolled and treated with Flebogamma 5% DIF at a dose of 300-600 mg per kg per infusion every 3-4 weeks for 12 months.
Since the subjects in the clinical study were assigned to two different treatment intervals (3-week vs. 4-week infusion schedules), the dosage had to be adjusted to ensure that the subjects received approximately the same dosage on an annualized basis. Therefore, subjects in the 3-week schedule received 75% of the monthly (4-week) dosage per infusion. This resulted in a mean annualized dosage of 451 mg per kg per month for subjects in the 3-week schedule (n=13, range 288-588 mg per kg per month) and 448 mg per kg per month for subjects in the 4-week schedule (n=33, range 298-591 mg per kg per month).
During the study period, the annual rate of acute serious bacterial infection, defined as bacterial pneumonia, bacteremia or sepsis, osteomyelitis/septic arthritis, visceral abscess, and bacterial meningitis per subject per year, was 0.021 (with an upper 1-sided 98% confidence interval of 0.112). One subject had one episode of bacterial pneumonia and there were no other episodes of serious bacterial infections reported (Table 4).

Table 4. Summary of Bacterial Infections (Intention-to-Treat Population, N = 46)

Infections	Subjects (N=46) N (%)	Episodes	Estimates[a]	98% CI[b]
Bacterial pneumonia	1 (2.2)	1		
Bacteremia or sepsis	0 (0.0)	0		
Osteomyelitis/septic arthritis	0 (0.0)	0		
Bacterial meningitis	0 (0.0)	0		
Total subjects	**1 (2.2)**	**1**	**0.021**	**(0.001-0.112)**

a. Estimate = Total episodes/Total subject years.
b. The confidence interval is obtained by using a generalized linear model procedure for Poisson distribution.

The number of days of work/school missed, hospitalizations and days of each hospitalization, the number of visits to physicians or emergency rooms, other infections documented by positive radiographic findings and fever, and days on therapeutic and prophylactic oral/parenteral antibiotic use were also evaluated. These variables were annualized by using the subject-years exposure data of those subjects experiencing the events, but not the entire study cohort. With regard to the number of other validated infections, the mean rate was less than 2 days per subject per year (this calculation used all subjects, including those who had no infections). (Table 5)

Table 5. Summary of Annualized Efficacy Variables

Variable	Subjects		Mean number of events, days or visits per subject per year[a]
	N	%	
Work/school days missed	**23**	**50.0**	**12.95**
Days of normal activities missed	**18**	**39.1**	**7.28**
Days in hospital	**4**	**8.7**	**0.77**
Visits to physician/ER	**29**	**63.0**	**4.31**
Number of other documented infectious episodes	**33**	**71.7**	**1.96**
Days of therapeutic oral antibiotic use	**35**	**76.1**	**55.52**
Days of therapeutic parenteral antibiotic use	**2**	**4.3**	**0.14**
Days of other therapeutic antibiotic use	**16**	**34.8**	**44.30**
Days of prophylactic oral antibiotic use	**19**	**41.3**	**81.08**
Days of prophylactic parenteral antibiotic use	**1**	**2.3**	**0.02**
Days of other prophylactic antibiotic use	**0**	**0.0**	**0.00**

a. Days of work/school missed per subject year are derived as total days of work/school missed divided by total days in study multiplied by 365. If data are missing for a period (e.g. between Infusion 2 and Infusion 3), then number of days in this period is not counted in the denominator. All other endpoints are derived similarly.

A multicenter, open-label, historically controlled study was conducted in the United States to assess the efficacy of Flebogamma 5% DIF in pediatric subjects with PI. A total of 24 subjects aged 2-16 years (79% male, 21% female) were enrolled and treated with Flebogamma 5% DIF at a dose of 262-625 mg per kg per infusion every 3-4 weeks for 12 months.
The annual rate of acute serious bacterial infections, defined as bacterial pneumonia, bacteremia or sepsis, osteomyelitis/septic arthritis, visceral abscess, and bacterial meningitis per subject per year, was 0.051 (with an upper 1-sided 99% confidence limit of 0.53). One subject had one episode of bacterial pneumonia and there were no other episodes of serious bacterial infections reported.

15 REFERENCES

1. Cayco AV, Perazella MA, Hayslett JP. Renal insufficiency after intravenous immune globulin therapy: a report of two cases and an analysis of the literature. *J Am Soc Nephrol* 1997; 8:1788-94.
2. Winward DB, Brophy MT. Acute renal failure after administration of intravenous immunoglobulin: review of the literature and case report. *Pharmacotherapy* 1995; 15:765-72.
3. Tan E, Hajinazarian M, Bay W, et al. Acute renal failure resulting from intravenous immunoglobulin therapy. *Arch Neurol* 1993; 50:137-9.
4. Sekul EA, Cupler EJ, Dalakas MC. Aseptic meningitis associated with high-dose intravenous immunoglobulin therapy: frequency and risk factors. *Ann Intern Med* 1994; 121:259-62.
5. Kato E, Shindo S, Eto Y, et al. Administration of immune globulin associated with aseptic meningitis. *JAMA* 1988; 259:3269-71.
6. Casteels-Van Daele M, Wijndaele L, Hanninck K, et al. Intravenous immune globulin and acute aseptic meningitis. *N Engl J Med* 1990; 323:614-5.
7. Scribner CL, Kapit RM, Phillips ET, et al. Aseptic meningitis and intravenous immunoglobulin therapy. *Ann Intern Med* 1994; 121:305-6.
8. Copelan EA, Strohm PL, Kennedy MS, et al. Hemolysis following intravenous immune globulin therapy. *Transfusion* 1986; 26:410-2.
9. Thomas MJ, Misbah SA, Chapel HM, et al. Hemolysis after high-dose intravenous Ig. *Blood* 1993; 15:3789.
10. Reinhart WH, Berchtold PE. Effect of high-dose intravenous immunoglobulin therapy on blood rheology. *Lancet* 1992; 339:662-4.
11. Wilson JR, Bhoopalam N, Fisher M. Hemolytic anemia associated with intravenous immunoglobulin. *Muscle Nerve* 1997; 20:1142-1145.
12. Kessary-Shoham H, Levy Y, Shoenfeld Y, et al. In vivo administration of intravenous immunoglobulin (IVIG) can lead to enhanced erythrocyte sequestration. *J Autoimmune* 1999; 13:129-35.
13. Kahwaji J, Barker E, Pepkowitz S, et al. Acute hemolysis after high-dose intravenous immunoglobulin therapy in highly HLA sensitized patients. *Clin J Am Soc Nephrol* 2009; 4:1993-1997.
14. Daw Z, Padmore R, Neurath D, et al. Hemolytic transfusion reactions after administration of intravenous immune (gamma) globulin: A case series analysis. *Transfusion* 2008; 48:1598-1601.
15. Rizk A, Gorson KC, Kenney L, et al. Transfusion-related acute lung injury after the infusion of IVIG. *Transfusion* 2001; 41:264-8.
16. Pierce LR, Jain N. Risks associated with the use of intravenous immunoglobulin. *Transfus Med Rev* 2003; 17:241-51.
17. Orbach H, Katz U, Sherer Y, Shoenfeld Y. Intravenous immunoglobulin: adverse effects and safe administration. *Clin Rev Allergy Immunol*, 2005; 29:173-184.

16 HOW SUPPLIED/STORAGE AND HANDLING

Flebogamma 5% DIF is supplied in single-use, individually laser-etched vials containing the labeled amount of functionally active IgG. The following presentations of Flebogamma 5% DIF are available:

NDC Number	Size	Grams Protein
61953-0004-1	10 mL	0.5 g
61953-0004-2	50 mL	2.5 g
61953-0004-3	100 mL	5.0 g
61953-0004-4	200 mL	10.0 g
61953-0004-5	400 mL	20.0 g

Each vial has an integral suspension band and a label with two peel-off strips showing the product name and lot number.
Flebogamma 5% DIF may be stored at room temperature at 2 to 25 ºC (36 to 77 ºF) for 24 months, as indicated by the expiration date printed on the outer carton and container label. Discard after expiration date. **Do not freeze.**
Keep Flebogamma 5% DIF in its original carton to protect it from light.
Not made with natural rubber latex.

17 PATIENT COUNSELING INFORMATION

Instruct patients to immediately report the following signs and symptoms to their physician:
- Decreased urine output, sudden weight gain, fluid retention/edema, and/or shortness of breath *(see Renal Failure [5.2])*
- Symptoms of thrombosis which may include: pain and/or swelling of an arm or leg with warmth over the affected area, discoloration of an arm or leg, unexplained shortness of breath, chest pain or discomfort that worsens on deep breathing, unexplained rapid pulse, numbness or weakness on one side of the body *(see Thrombosis [5.4])*
- Severe headache, neck stiffness, drowsiness, fever, sensitivity to light, painful eye movements, nausea, and vomiting *(see Aseptic Meningitis Syndrome [5.5])*
- Fatigue, increased heart rate, yellowing of the skin or eyes, and dark-colored urine *(see Hemolysis [5.6])*
- Trouble breathing, chest pain, blue lips or extremities, fever *(see TRALI [5.7])*

Inform patients that Flebogamma 5% DIF is made from human plasma and may contain infectious agents that can cause disease (e.g., viruses, the vCJD agent and, theoretically, the CJD agent). The risk of Flebogamma 5% DIF transmitting an infectious agent has been reduced by screening plasma donors for prior exposure, testing the donated plasma, and inactivating and/or removing certain viruses during manufacturing. *(see Warnings and Precautions [5.8])*
Instruct patients to report any symptoms that concern them and might be caused by infections.
Inform patients that Flebogamma 5% DIF may interfere with their immune response to live viral vaccines such as measles, mumps, and rubella. Inform patients to notify their health care professional of this potential interaction when they are receiving vaccinations. *(see Drug Interactions [7])*

Manufactured by:
INSTITUTO GRIFOLS, S.A.
BARCELONA - SPAIN
U.S. License No. 1181

Instituto Grifols, S.A. Can Guasc, 2 - Parets del Vallès 08150 Barcelona - SPAIN 3048714

APPENDIX 3a

AUTO-C CLINICAL PROTOCOL

The Auto-C device mentioned in this protocol corresponds to FDA PMA approval # BP850001

Document Detail

Type: Protocol
Document No.: 931-PRO-010255[7.0]
Title: Evaluation of the Autopheresis-C Plasma Exchange Protocol within a Clinical Trial
Owner: [redacted]
Status: [redacted]
Effective Date: 08-Aug-2012 Expiration Date:

Reference

Document No.	Content Type	Relation	Fixed Rev	Status
PKG-000933 [A]	DOCUMENT	Master Document	No	CURRENT

Title:Auto-C Plasma Exchange DHF

Activities

Document Build No.	Access Activity	Accessed By	Accessed Date
1	Check In	979414	08-Aug-2012

Note:

Review

Build No.: 1 Closed Date:
Review: Document Approval
Review Purpose:
Review Note:

Level	Owner Role	Actor	Sign-off By
1	Document Owner Document Owner	[redacted]	

Note To Approver:
Note From Approver:

2	Document Control Document Control	[redacted]	

Note To Approver:
Note From Approver:

1	Quality Approver Quality Approver	[redacted]	

Note To Approver:
Note From Approver:

1	Clinical Affairs Approver Clinical Affairs Approver	[redacted]	

Note To Approver:
Note From Approver:

[redacted]

Protocol Number: [redacted]	
Title: Evaluation of the Autopheresis-C Plasma Exchange Procedure within a Clinical Trial	
Proposed Completion Date: December 31, 2013	
Project: Auto-C Plasma Exchange	Requesting Individual/Department: [redacted] Project Management
Additional Distribution (Original to Quality System Archives): [redacted]	Global Plasma Business Regulatory Affairs Regulatory Affairs Plasma Services

APPROVALS: Entire Document

Name	Department	Signature	Date
[redacted]	**Author** **R&D Systems Engineering**	See Electronic Signature	
[redacted]	**Clinical Affairs** **Technical Approver**	See Electronic Signature	
[redacted]	**Quality Engineering**	See Electronic Signature	

Study must not begin until all approval signatures are obtained.

1. Purpose

This protocol defines system evaluation for the Autopheresis-C Plasma Exchange Protocol, running complete procedures as part of a clinical trial conducted by Grifols SA. The tests defined herein are intended to satisfy system performance requirements that cannot be fully tested on bagged blood procedures. Additionally, this document will provide the data sheet to be used during the procedures outlined herein.

The following are the purposes for this protocol:

- To observe typical return rates for a mixture of blood and replacement fluid in Alzheimer's patients,
- To compare replacement fluid volume delivered to the programmed volume to show that the delivered volume of replacement fluid is within +/-10% of the programmed target volume for 90% of completed procedures, and
- To provide a method for Grifols SA to communicate this data to Fenwal, Inc.

The results of execution of this protocol are intended to satisfy, in part, 931-PLN-010004.

2. Background

The AUTOPHERESIS-C® (Auto-C™) system (Fenwal, Inc.) is an automated plasma collection system that has been in use in the United States since its Food and Drug Administration (FDA) clearance in March, 1986. It was classified at that time as a Class III PMA device (BP850001). In February 2003, automated blood cell separator devices operating by filtration principles intended for the routine collection of blood and blood components, including the Autopheresis-C, were re-classified by the FDA as Class II devices. This modified their regulatory review pathway from PMA (pre-market approval) to 510(k) (pre-market notification).

The Grifols SA company is a Spanish holding company that performs research, development, manufacturing and marketing of plasma derivatives, IV therapy, enteral nutrition, diagnostic systems and medical materials. It is currently in Phase II clinical trials using albumin as a plasma replacement fluid for the treatment of Alzheimer's disease. Grifols has requested that Fenwal design and develop a plasma exchange procedure and disposable set for the Auto-C plasmapheresis platform that could use albumin replacement with IVIG for treatment of patients with Alzheimer's for use in their clinical trials.

The testing described in this document will utilize Autopheresis-C instruments, investigational human-use software, along with investigational disposables to perform procedures on Alzheimer's patients. Testing is conducted under the clinical investigation conducted by Grifols SA.

Features of the Autopheresis-C Plasma Exchange Protocol investigational human use software include:

- Operator selection of a replacement fluid target of 160, 190, or 200 mL to correspond with a plasma collection volume of 690, 825, and 880 mL of collected plasma, respectively. An editable option is also available.
- Return of the programmed volume of replacement fluid after collection of the target plasma volume via a series of phases in which patient blood is mixed with replacement fluid and then the mixture is returned to the patient.

The purpose of this protocol is to investigate the use of the Autopheresis-C Plasma Exchange Protocol to return a 20% human serum albumin (HSA) solution as the replacement fluid. The disposable sets for use during this study are investigational (Fenwal Product Code FTX-3900); they include a fistula needle set attached at a luer, an attached 1000 mL plasma collection container, a spike for connection to an anticoagulant solution, and a luer for connection to a transfer set supplied by Grifols SA that allows for connection of two bottles of replacement fluid.

3. Materials and Equipment

Materials and equipment that may be used in this study include, but are not limited to:

- Plasmacell-C Kit[redacted]
- AUTOPHERESIS-C Instrument with investigational plasma exchange protocol software, Code R4R4585 or 4R4550
- Grifols Replacement Fluid Transfer Set, *Grifill 3.0 Plastic 2-to-1 Set*
- Sodium Citrate Solution, USP, 500 mL, Code 4B7889Q or equivalent
- Human Serum Albumin 20%, 50 or 100 mL
- Tubing sealer
- Weight scale, if available

4. Methods

4.1. Software Installation and Removal

Installation of the software onto clinical instruments shall be through chipset install as described in TSB-TSB12560043. Software tracking and removal is also managed in the TSB.

4.2. Training

All Autopheresis-C Plasma Exchange Protocol Operators and support team members will be trained on the Autopheresis-C Plasma Exchange Protocol per the applicable operating instructions, 07-19-54-188 and REC-003672. Operators, prescribers, and support team members will also be trained on this protocol. All training will be documented and recorded per FQA-01-007.

4.3. General Considerations

4.3.1. Multiple Companies

Multiple companies (Fenwal, Inc. and Grifols SA) will contribute to this study. Each group may have their own original data forms, standard operating procedures, etc. It is neither required nor expected that every form or procedure be included or referenced in this protocol. It is required, however, that all original data be preserved as detailed in section 7.2.

4.3.2. Procedure Data

The data sheet in Appendix B will be completed as the procedure progresses. Data will be reported to Fenwal, reviewed, and analyzed per the statistical equations indicated in this protocol.

4.4. Set Up

4.4.1. Plasma Collection Volumes

The target Plasma Collection Volume is chosen at the discretion of a physician in accordance with the clinical protocol. It is recorded in Appendix B. The nomogram that has been tested is indicated below.

Patient Weight lbs (kg)	Maximum Plasma Collection Volume (mL)
110-149 (50-67)	690
150-174 (68-79)	825
175-999 (80-454)	880

4.4.2. Replacement Fluid Infusion Volumes

The target Replacement Fluid Infusion Volume is chosen at the discretion of a physician in accordance with the clinical protocol. It is recorded in Appendix B. The nomogram that has been tested is indicated below.

Patient Weight lbs (kg)	Maximum Plasma Collection Volume (mL)	Replacement Fluid Infusion Volume (mL)		
		Full Dose	1/2 Dose	1/3 Dose
110-149 (50-67)	690	160	80	55
150-174 (68-79)	825	190	95	65
175-999 (80-999)	880	200	100	70*

*This setting has not been tested, but is recommended for use in the clinical trial

4.5. Patient Assessment

The physician requesting the plasma exchange protocol may provide guidelines under which a patient may be excluded from the study. The Autopheresis-C plasma exchange protocol is recommended and designed for patients weighing at least 110 lbs (50 kg) with a hematocrit of 38% or greater.

4.6. Sampling and Processing

4.6.1. Pre-Procedure Samples

Before the procedure, the patient's blood is sampled and hematocrit recorded. Additionally, the weight of the replacement fluid containers is measured and recorded. See Appendix B.

4.6.2. In-Procedure Samples

No samples are taken during the procedure, but the following information may be recorded:

- Patient Adverse Reactions
- Alarm Information (name of alarm and approximate time or state in which it occurred)
- Other Notes

4.6.3. Samples after Patient Disconnect

After patient disconnect, the following measurements and observations are recorded:

- The weights of the following, if a weight scale is available:
  - The plasma product
  - The replacement fluid containers
  - The disposable set, excluding AC spike, needle set and luer, and replacement fluid transfer set and luer
- The presence of fluid and/or air in the replacement fluid line.

5. Product Performance

If the Autopheresis-C system does not perform as expected, see Appendix C.

6. Evaluation of Data and Acceptance Criteria

6.1. Definitions and Abbreviations

Term	Definition
Hct	Hematocrit
HSA	Human serum albumin
Kit	The Plasmacell-C disposable set after the AC spike, plasma bag, replacement fluid luer, and fistula needle set (including the luer) have been sealed and removed
Platelet-Poor Plasma (PPP)	The collected, platelet-poor waste plasma at Day 0
RF	Replacement Fluid
VP Time	Venipuncture time; the amount of time between the operator acknowledging that venipuncture is complete and the instrument instructing the operator to disconnect the patient

6.2. Equations

Kit $_{\text{PRE-WEIGHT}}$[6] = 165.8 g

Kit Residual Volume

= [(Kit $_{\text{POST-WEIGHT}}$ – Kit $_{\text{PRE-WEIGHT}}$) $_{g}$ ÷ 1.05 $_{g/mL}$ [8]] *mL*

Disposable Set and Transfer Set Plasma/RF/AC Line Volumes

= AC Line Length[7] x AC Tubing Volume per Length[7] + (Plasma Line Length[7] + RF Line Length[7] + RF Transfer Set Length) x Blood Tubing Volume per Length[7]
= 84" x 0.0196 mL/inch + (25" + 27" + 12") x 0.2043 mL/inch
= 14.72 mL

Kit RF Volume

= [(1/4) * (Kit Residual Volume - Disposable Set and Transfer Set Plasma/RF/AC Line Volumes)] *mL*

Plasma Volume

= [(PPP $_{\text{POST-WEIGHT}}$ – PPP $_{\text{BAG-EMPTY}}$) $_{g}$ ÷ 1.027 $_{g/mL}$] *mL*

PPP $_{\text{BAG-EMPTY}}$[6] = 41.87 g

Replacement Fluid Line and Transfer Set Volume

(if filled with fluid) = [39" x 0.2043 mL/inch] = 7.97 *mL*
(if filled with air) = 0 *mL*
(if filled with both) = [19.5" x 0.2043 mL/inch] = 3.98 *mL*

RF Cap Weight[6] = 3.60 g

Replacement Fluid Volume

= [(RF $_{\text{PRE-WEIGHT}}$ – RF $_{\text{POST-WEIGHT}}$ – RF Cap Weight) $_{g}$ ÷ 1.06 $_{g/mL}$ [9] - Replacement Fluid Line and Transfer Set Volume – Kit RF Volume] *mL*

6.3. Statistical Analysis

6.3.1. Statistical Equations

Interval, Mean Confidence

$= \bar{x} \pm \left[t_{(\alpha/2, df)} \times s \div \sqrt{n} \right]$ where: $\bar{x}$ = Sample Mean (or Average)

α = Risk of Type I error (here 0.05, for 95% confidence)
n = Sample size
df = Degrees of freedom = (Sample size – 1)
s = Sample Standard Deviation
$t_{(\alpha/2, df)}$ = t-value of t-Distribution (to be determined by study sample sizes)

Interval, Statistical Tolerance

$$= \bar{x} \pm \left[k_{(1-\alpha, R, n)} \times s \right]$$

where: $\bar{x}$ = Sample Mean (or Average)

$1 - \alpha$ = Confidence Level (here 0.95, for 95% confidence)

n = Sample size

s = Sample Standard Deviation

$k_{(1-\alpha, R, n)}$ = k-factor based on confidence level, reliability, and sample size (to be determined for specific confidence level and reliability)

6.3.2. Sample Size

This study will utilize a minimum sample size of 29 complete Auto-C plasma exchange procedures, including post-procedure weight data. This sample size, with zero failures, will provide 95% confidence that the delivered volume of replacement fluid is within ± 10% of programmed volume 90% of the time. If fewer than 29 samples are available, the study lacks the statistical power required to demonstrate that the acceptance criterion is met.

6.3.3. Acceptance Criteria

The acceptance criterion for this portion of Grifols' clinical trial is that the delivered volume of replacement fluid (shown as **Replacement Fluid Volume** in the Equations section) is within +/-10% of the programmed target volume 90% of the time.

7. Post Study Disposition

7.1. Disposition of Samples

Upon completion of a procedure, the disposable set and replacement fluid should be discarded. Collected plasma may be retained for sampling and testing. When testing of the plasma is complete, the plasma should also be discarded. All biological material must be discarded as biohazardous waste.

7.2. Original Data

Original data will be recorded during the execution of the protocol on worksheets provided in Appendix B and other data forms, as appropriate. This protocol, all original data, the final report, and all amendments will be archived in the appropriate Fenwal, Inc. archives.

7.3. Hardware and Software

The instruments will be retained by Grifols after study completion, but the experimental software must be returned to Fenwal, Inc. See TSB-TSB-12560043 for tracking of the return of experimental software.

8. References

1. 931-PLN-01004, Auto-C Plasma Exchange Product Validation Plan
2. TSB-TSB12560043, Install, Test and Configure an Auto-C Instrument with Dory Project Software
3. FQA-01-007, Fenwal Training Process
4. REC-003672, Autopheresis-C Plasma Exchange Protocol Supplemental Operating Instructions
5. 07-19-54-188, Autopheresis-C™ Plasmapheresis System Operator's Manual
6. NB00016-134. "Measurement of Auto-C Plasma Exchange Protocol Kit Tare Weights, Revisited".
7. DWG-0312581824, AUTO-C Plasma Exchange FTX-3900 Disposable Set
8. Trudnowski, RJ and Rico, RC. "Specific Gravity of Blood and Plasma at 4 and 37°C". *Clin Chem* 20 (1974): 615-616.
9. Nugent, RL and Towle, LW. "The Specific Gravity of Synthetic Solutions of Serum Albumin and Serum Globulin". *J Biol Chem* (1933).

9. List of Appendices

A. Steps for Recording Auto-C Plasma Exchange Procedure Data
B. Auto-C Plasma Exchange Procedure Data Record
C. Product Performance Flow Chart
D. Product Performance Report
E. Revision History

A. Steps for Recording Auto-C Plasma Exchange Procedure Data

Pre-Procedure:

Before the procedure, record the following on the data sheet in Appendix B:

- Run ID
- Patient Weight

Based on the patient's weight and physician's orders, identify and record the following on the data sheet in Appendix B:

- Plasma Target
- Replacement Fluid (RF) Target
- Type of RF Used

Record the weight of the replacement fluid containers on the data sheet as "RF Weight - Pre" in Appendix B.

Take a fingerstick hematocrit of the patient and record the hematocrit on the data sheet in Appendix B.

During the Procedure:

When entering the replacement fluid infusion states, record any changes to the infusion rate under "Flow Rate during RF Infusion". If no changes are made, the RF Infusion rate is 20 mL/min.

During the procedure, record the following (as applicable) on the data sheet in Appendix B. If there are no notes for the section, write "N/A".

- Patient Adverse Reactions
- Alarm Information (name of alarm and approximate time or state in which it occurred)
- Other Notes

If any product performance issues are observed, see Appendix C.

After the Procedure:

At the end of the procedure, record the following from the display:

- RF Infused
- VP Time

Disconnect the patient.

Note whether the replacement fluid line is filled with fluid, air, or both under "Air/Fluid in the RF Line" on the data sheet in Appendix B.

Seal the disposable set in the following locations:

- On the apheresis needle set near the luer
- On both sides of the replacement fluid transfer set luer
- On both pressure transducer connector lines
- Near the AC spike
- Below the separation device on the plasma line
- Above the sampling site on the plasma line

These locations are shown in the diagram below.

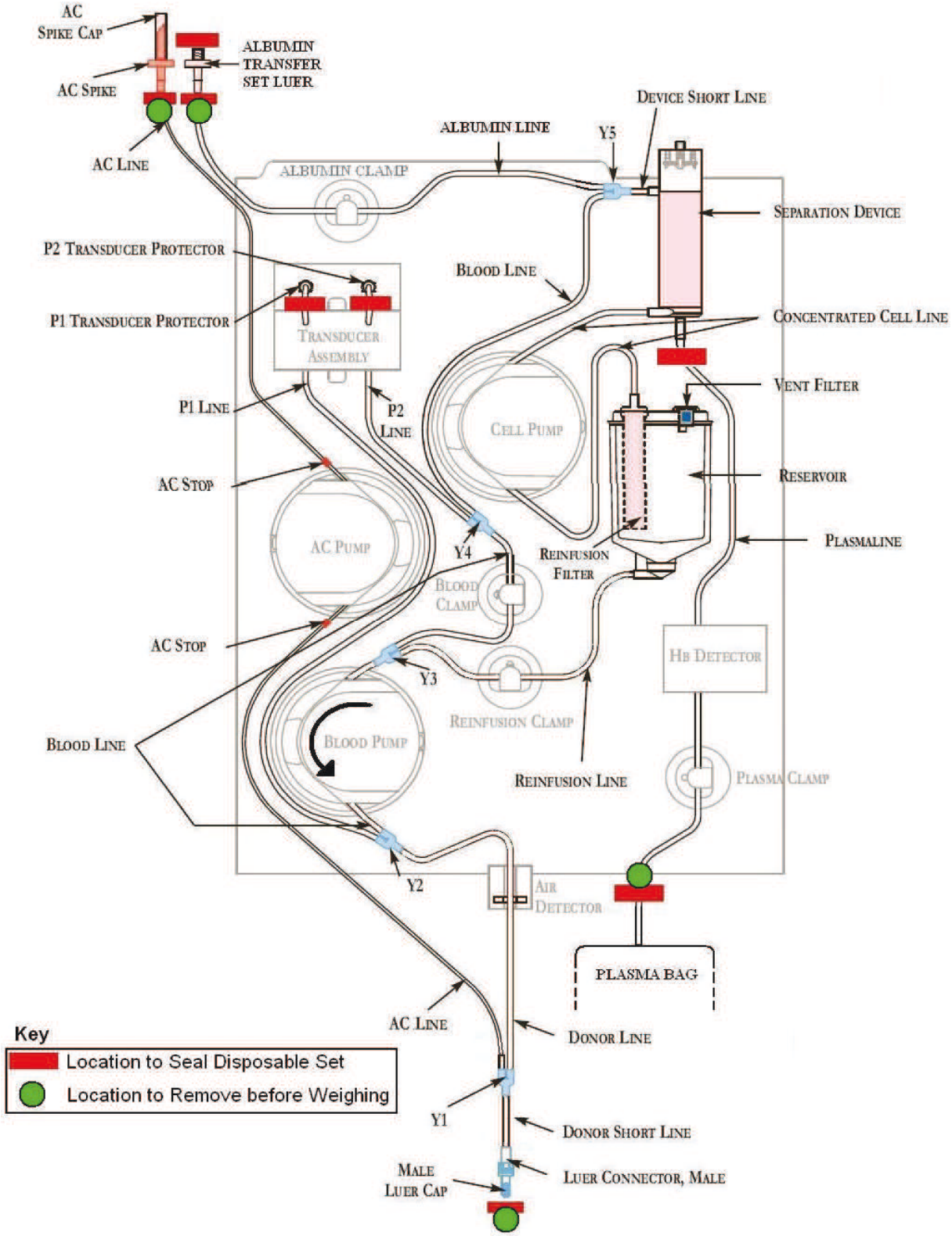


Figure 1 Locations where the disposable set should be sealed before removing the set from the instrument and where the seals should be separated to weigh the kit.

Remove the disposable set from the instrument.

Remove the replacement fluid transfer set from the replacement fluid containers and the remainder of the disposable set. Ensure that the luer is removed. Also remove the fistula needle set including the luer. Discard appropriately.

If possible, weigh the replacement fluid containers and record their weight under “RF Weight - Post” on the data sheet in Appendix B.

Remove the plasma container. If possible, weigh it and record the weight under “Plasma Bag Weight - Post” on the data sheet in Appendix B.

If possible, weigh the remainder of the disposable set and record the weight under “Kit Weight - Post” on the data sheet in Appendix B.

If weights are not recorded, write “NR” in the appropriate space on the data sheet in Appendix B.

Discard the disposable set appropriately.

B. Auto-C Plasma Exchange Procedure Data Record

Pre Procedure		Post Procedure	
Run ID		RF Infused	mL
Patient Weight	kg	VP Time	:
Plasma Target	mL	Air/Fluid in RF Line (Circle one)	Air Fluid Both
RF Target	mL		
Type of RF Used	[ ] 20% HSA [ ] Other: _____	RF Weight - Post	g
Patient Hct -OR- Hb	_____ % -OR- _____ g/dL	Kit Weight - Post	g
		Plasma Bag Weight - Post	g
RF Weight - Pre	g		

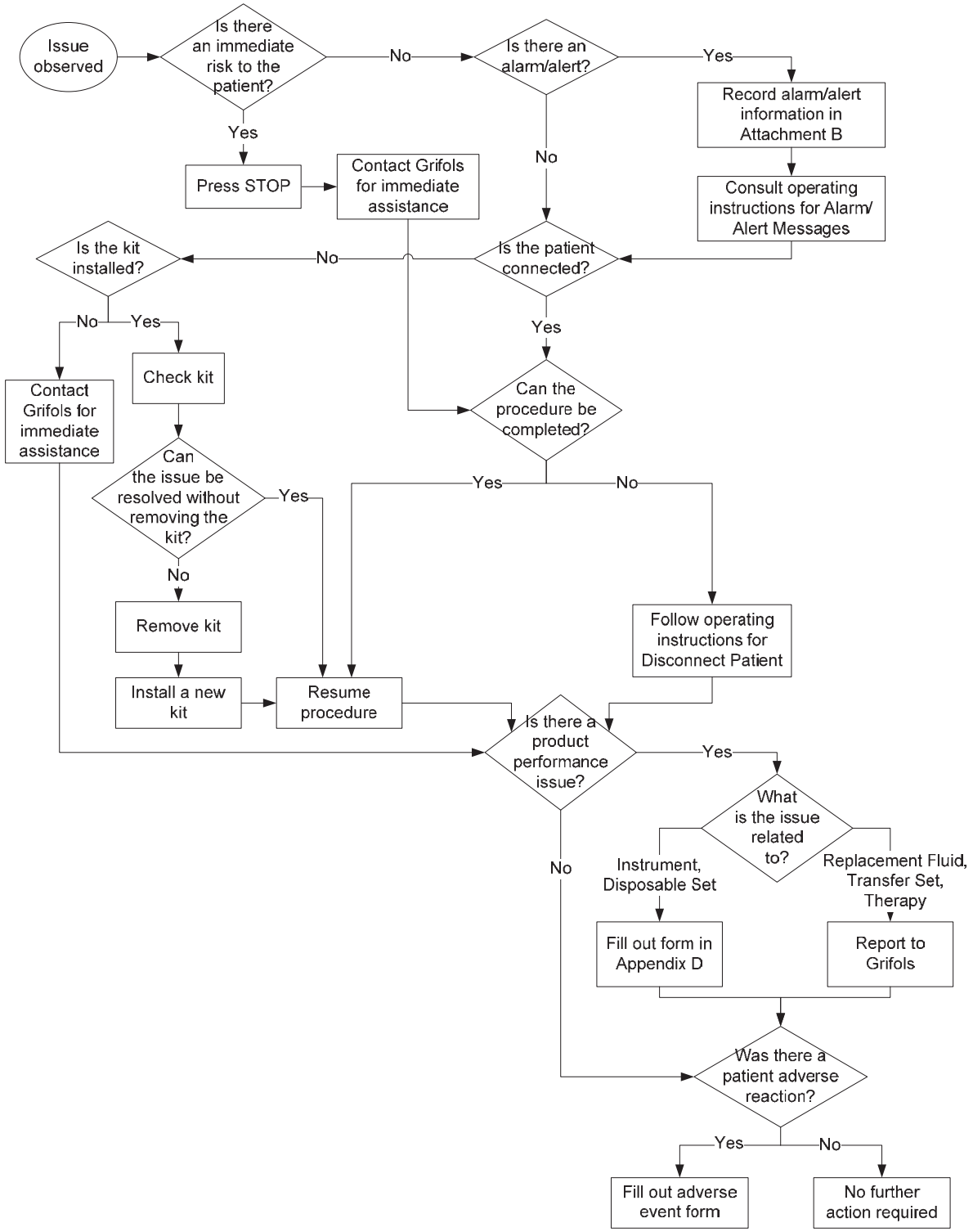
During Procedure	
Patient Reaction(s)	[ ] None [ ] Yes (please reference adverse event reporting data): _____
Flow Rate during RF Infusion (20-60 mL/min)	Cycle 1: _____ mL/min Cycle 2: _____ mL/min Cycle 3: _____ mL/min Cycle 4: _____ mL/min Cycle 5: _____ mL/min
Alarm/Alert/Help Code Information (Please include cause if known)	
Other Notes	

Entered by:_____Signature:_____ Date:_____

Reviewed by:_____Signature:_____Date:_____

C. Product Performance Flow Chart

If a product performance issue is observed, follow the steps below.



D. Product Performance Report

The product performance report below will be sent by the center to [redacted] at [redacted] UBS will forward product performance report and relevant adverse event data to [redacted]

Important: If adverse reaction or injury has occurred, contact Grifols.

[redacted]™

Plasmacell-C™ Product Performance Report

Was the donation successfully completed? Yes ☐ No ☐
If No, was the donation stopped due to a soft goods incident? Yes ☐ No ☐

Incident Date: _____ Instrument Serial Number: _____
Product Code: _____ Lot Number: _____
Batch Tag Info Time: ____:____:____ Number_____ Video Jet Number: _____
Patient Bleed Number: _____ Volume of Plasma Collected: _____mL

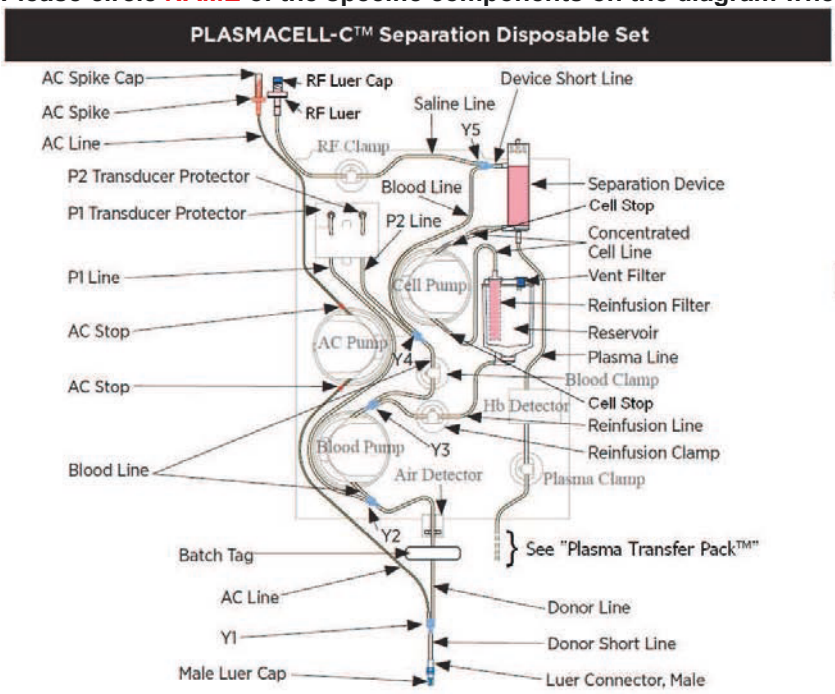
When Was The Incident Detected?

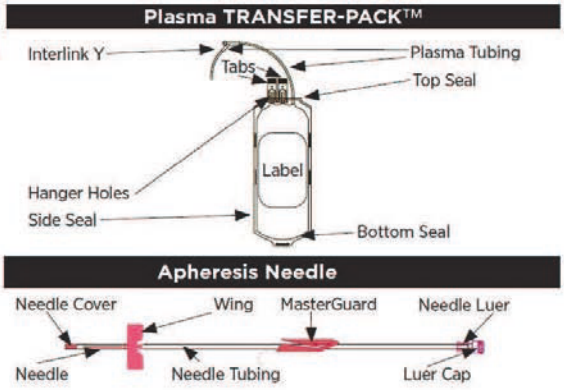
☐ Before Use ☐ Set-Up ☐ Install Check ☐ Solution Prime ☐ Blood Prime ☐ Collection (specify cycle)_____
☐ During Venipuncture ☐ Reinfusion (specify cycle)_____ ☐ RF Priming ☐ RF Mixing (specify cycle)_____
☐ RF Infusion (specify cycle)_____ ☐ Procedure Completion ☐ After Collection ☐ Freezing/Processing

Incident Type (Mark all applicable)

☐ Alarm/Alert/Help Code_____ ☐ Detached/Separated ☐ Noise ☐ Kinked ☐ Blood/Plasma Leak ☐ Low/No Plasma Flow ☐ Cut/Slice ☐ Red Plasma (Visual) ☐ Illegible Label ☐ Installation Check ☐ Dented
☐ Missing (not in box) ☐ Hole ☐ Other (Specify)_____

Please circle NAME of the specific components on the diagram where incident occurred





Additional Incident Description / Explanation

_____
_____
_____
_____
_____
_____

Kit Return To Fenwal

1. Sample available for evaluation? Yes ☐ No ☐
2. Sample return box needed? Yes ☐ No ☐ Label only needed ☐
3. Do you request a letter regarding the investigation results? Yes ☐ No ☐
4. Picture taken of defective kit? Yes ☐ No ☐
   (If yes, send picture to [redacted])

Center Authorized Signature/Date: **Fenwal Reviewed By/Date:**

Please Print

Account #: _____
Site Name: _____
Contact Person: _____
Operator Name: _____
Street Address: _____
City/State/Zip: _____
Phone Number: _____
E-Mail: _____
Fax Number: _____

Notification No. ***(Fenwal Personnel Only)***

E-mail this report to AMBARsafety@unitedbiosource.com along with an adverse event report, if applicable. Include a copy of this form when returning a kit.

E. Revision History

Revision	Date	Author	Description of Change
1.0	January 31, 2011	[redacted]	New document created
2.0	March 04, 2011	[redacted]	• Updated kit sealing location on fistula needle set and corrected kit pre-weight accordingly • Updated kit residual and replacement fluid densities to 1.05 and 1.06 g/mL, respectively; included references • Clarified wording in pre-procedure steps • Updated infusion rates to 20 to 80 mL/min with a default of 24 mL/min
3.0	August 02, 2011	[redacted]	• Changed kit tare weight to correspond to kits with tubing keepers on the concentrated cell line • Changed the Kit RF Volume formula to correlate with the 3:1 mixture of blood and replacement fluid • Corrected statistical equations to 95% confidence that the acceptance criteria will be met 90% of the time, to align with purpose of study • Updated infusion rates to 20 to 60 mL/min with a default of 20 mL/min • Added Cell Stop components to disposable set diagram on the product performance report
4.0	September 06, 2011	[redacted]	• Revised the Training section to indicate that prescribers and operators will be trained on this protocol, in its entirety • Added sealing location below separation device on plasma line (to facilitate sampling of any observed issues with plasma quality)
5.0	October 19, 2011	[redacted]	• Revised Software Installation section to include software installation and removal • Revised Hardware and Software section to reference the TSB for software tracking
6.0	March 12, 2012	S[redacted]	Updated to indicate that weighing of components is optional
7.0	August 8, 2012	[redacted]	Updated contact information on the product performance report

APPENDIX 3b

AURORA CLINICAL PROTOCOL

The Aurora device mentioned in this protocol corresponds to FDA 510(k) clearance # BK110072

[redacted]

Protocol Number: 938-PRO-039808	
Title: Evaluation of the Aurora Plasma Exchange Procedure within the AMBAR Clinical Trial	
Proposed Completion Date: December 31, 2016	
Project: Aurora Plasma Exchange	Requesting Individual/Department: [redacted] Project Management
Additional Distribution (Original to Quality System Archives): [redacted] [redacted] Plasma Services	

APPROVALS:

Name	Department	Signature	Date
[redacted]	**Author Systems Engineering**	See Electronic Signature	
[redacted]	**Clinical Affairs**	See Electronic Signature	
[redacted]	**Quality Engineering**	See Electronic Signature	
[redacted]	**Project Management**	See Electronic Signature	
[redacted]	**Clinical Project Manager Grifols, S.A**	See Electronic Signature	

Study must not begin until all approval signatures are obtained.

1. Purpose

This protocol defines system evaluation for the Aurora Plasma Exchange Procedure, and applies to completed procedures performed as part of the AMBAR clinical trial conducted by Grifols S.A. The intent of this protocol is to outline how data and information will be obtained during the clinical trial to ensure that relevant systems level requirements are satisfied. Additionally, this document will provide the data sheet to be completed for all the procedures performed using the Aurora Plasma Exchange System during the clinical trial.

The following are the primary purposes for this protocol:

- To document typical return rates during replacement fluid infusion in Alzheimer's patients,
- To compare replacement fluid volume delivered to the programmed volume to confirm the accuracy of volume delivery. Specifically, the following requirements from 938-REQ-032960:
    - SYRS-567: Upon completion of a successful procedure, the system shall have infused to the patient at an actual replacement fluid (i.e. 20% albumin solution) infusion volume that is within +/- 15% of the Target RF Infusion Volume.
    - SYRS-1142: If selected protocol is REPLACEMENT_FLUID, the displayed replacement fluid (i.e. 20% albumin solution) volume infused to the patient shall be within +/- 15% of the actual volume of replacement fluid infused.
    - SYRS-336: If the selected protocol is a REPLACEMENT_FLUID procedure, the system shall display, via the operator visual/input interface, the infused volume of replacement fluid (i.e. 20% albumin solution) that is within +/- 15% of the Target RF Infusion Volume upon completion of a successful procedure.
- To document any issues/observations related to Y-Junction Site (aka Injection-Y Site) when performing offline Intravenous Immunoglobulin (IVIG) infusion
- To document specific alerts/alarms/issues observed during execution of the Aurora Plasma Exchange Procedure
- To provide a method for Grifols, S.A. to communicate this information/data to Fresenius Kabi USA, LLC

2. Background

The Aurora Plasmapheresis System (Fresenius Kabi USA, LLC) is an automated plasma collection system based on the Autopheresis-C® system (Auto-C). Aurora uses the same separation technology and many of the same components of the Auto-C but also incorporates a touch screen and GUI interface, and updates to replace obsolete components. The Aurora Plasmapheresis System was cleared under 510(k) BK110072, as a design update to the pre-existing Auto-C System. The Aurora Plasmapheresis System became the foundation for the development of the Aurora Plasma Exchange System.

Grifols, S.A. is a Spanish holding company that performs research, development, manufacturing and marketing of plasma derivatives, IV therapy, enteral nutrition, diagnostic systems and medical materials. Grifols has requested that Fresenius Kabi USA, LLC design and develop a plasma exchange procedure and disposable set for the Aurora platform to facilitate direct infusion of albumin as a replacement fluid, as well as to provide for infusion of IVIG through the venipuncture site when performing non-albumin protocols (accomplished by providing a disposable set interface for connection to an external infusion pump) for treatment of patients with Alzheimer's for use in their clinical trial.

The procedure described in this document will utilize Aurora instruments, investigational human-use software, along with investigational disposables to perform procedures on Alzheimer's patients. This protocol is to be conducted under the clinical trial investigation conducted by Grifols, S.A.

Features of the investigational Aurora Plasma Exchange System with investigational human use software include:

- Configurable target replacement fluid infusion volume with presets of half doses of 80, 95, 100, and full doses of 160, 190, or 200 mL to correspond with a plasma collection volume of 690, 825, and 880 mL, respectively. An editable option is also available.
- Infusion of programmed volume of replacement fluid via direct infusion of replacement fluid at programmable target infusion rates from 5 to 20 mL/min after collection of the target plasma volume.

- Ability to infuse IVIG offline from the Aurora instrument using the disposable set interface (Injection Y-Site) for connection to an external infusion pump.

The purpose of this protocol is to investigate the use of the Aurora Plasma Exchange System to return a 20% Albutein® (human serum albumin or HSA) solution as the replacement fluid to a patient (if configured for the Albumin protocol). Connection of the Aurora Plasma Exchange Disposable Set to the external infusion pump disposable set via the Injection-Y Site is also a subject of investigation (if configured for the No Albumin protocol). The disposable sets for use during this study are investigational (Product Code FTX3920); they include a fistula needle set attached at a luer, an Injection Y-Site on the patient short line for connection to an external infusion pump, an attached 1000 mL plasma collection container, a spike for connection to an anticoagulant solution, and a luer for connection to a transfer set (aka Fleboset), supplied by Grifols, S.A., that allows for connection of two bottles of 20% Albutein.

3. Materials and Equipment

Materials and equipment that may be used in this study include, but are not limited to:

- SMARTCONNECT Plasma Exchange Disposable Set, Code FTX3920
- Aurora Instrument (Code: 6R4601) with investigational plasma exchange procedure software (software kit product code: 6S9820)
- Grifols Replacement Fluid Transfer Set, *Fleboset® Double*
- Sodium Citrate Solution, USP, 500 mL, Code 4B7889Q or equivalent (if configured for 6:100 ratio of Anticoagulant-to-Whole Blood)
- Anticoagulant Citrate Dextrose Solution A (ACD-A), USP, 500mL, Code 4B7898Q or equivalent (Grifols, S.A. branded ACD-A USP 500 mL, REF# 721781) (if configured for 8:100 ratio of Anticoagulant-to-Whole Blood)
- 20% Albutein® (Human Serum Albumin, or HSA) , 50 or 100 mL
- RF tubing sealer
- Weight scale

Materials for Optional IVIG infusion (if configured for the No Albumin Protocol):

- Flebogamma® solution
- External infusion pump & infusion pump disposable set
- Appropriate adapter to establish connection between infusion pump disposable set to Injection Y-Site of FTX3920 SMARTCONNECT Plasma Exchange Disposable Set (e.g. lever-lock luer),

4. Methods

4.1. Software Installation and Removal

Installation of the software onto clinical instruments is to be performed as described in TSB12570019. Software tracking and removal is also managed in the Technical Service Bulletin (TSB).

4.2. Training

All Aurora Plasma Exchange Procedure operators, prescribers and support team members will be trained on the Aurora Plasma Exchange Procedure per the applicable operating instructions: REC-011226 Aurora Plasma Exchange System Operator's Manual, REC-011227 Aurora Plasma Exchange System Administrator's Guide, and REC-012040 Aurora Plasma Exchange System Release Notes. All training, including any additional required per this protocol, will be documented and recorded per Fresenius Kabi SOP-FQA01007, Fenwal Training Process, or applicable facility or clinical trial training procedures.

4.3. General Considerations

4.3.1. Patient Assessment

The physician requesting the Plasma Exchange Procedure may provide guidelines under which a patient may be excluded from the study**. The Aurora Plasma Exchange Procedure is intended for use with Alzheimer's patients having a hematocrit of 38% or greater and a weight of 50 kg (110 lbs) or greater.**

4.3.2. Multiple Companies

Multiple companies, including Fresenius Kabi USA, LLC and Grifols S.A., will contribute to this study. Each group may have their own original data forms, standard operating procedures, etc. It is neither required nor expected that every form or procedure be included or referenced in this protocol. It is required, however, that all original data be preserved as detailed in section 7.2.

4.3.3. Procedure Data

The data sheet in Appendix B will be completed as the procedure progresses. Data for each procedure is expected to be submitted to Fresenius Kabi for review. Any statistical methods used in data analysis will be documented in the final report.

4.4. Set Up

4.4.1. Plasma Collection Volumes

The target Plasma Collection Volume is chosen at the discretion of a physician in accordance with the clinical protocol. The recommended nomogram is indicated below. Note that plasma collection volumes (plasma plus anticoagulant) shall comply with local regulatory requirements as applicable.

Patient Weight lbs (kg)	Maximum Plasma Collection Volume (Plasma + Anticoagulant) (mL)
110-149 (50-67)	690
150-174 (68-79)	825
≥175 (≥80)	880

4.4.2. Replacement Fluid Infusion Volumes

The target Replacement Fluid Infusion Volume is chosen at the discretion of a physician in accordance with the clinical protocol. The recommended nomogram is indicated below.

Patient Weight lbs (kg)	Replacement Fluid Infusion Volume (mL)	
	Full Dose	1/2 Dose
110-149 (50-67)	160	80
150-174 (68-79)	190	95
≥175 (≥80)	200	100

4.5. Data Collection

Collected data shall be recorded in Appendix B, Aurora Plasma Exchange Procedure Data Record per instructions provided in Appendix A, Instructions for Recording Aurora Plasma Exchange Procedure Data.

5. Product Performance

If the Aurora system does not perform as expected, follow the steps outlined in Appendix D, Product Performance Flow Chart, and fill out Appendix E, Product Performance Report.

6. Evaluation of Data and Acceptance Criteria

6.1. Acceptance Criteria

The acceptance criterion for this portion of Grifols' clinical trial is confirmation of the following system-level requirements:

- SYRS-567: Upon completion of a successful procedure, the system shall have infused to the patient at an actual replacement fluid (i.e. 20% albumin solution) infusion volume that is within +/- 15% of the Target RF Infusion Volume.
- SYRS-1142: If selected protocol is REPLACEMENT_FLUID, the displayed replacement fluid (i.e. 20% albumin solution) volume infused to the patient shall be within +/- 15% of the actual volume of replacement fluid infused.
- SYRS-336: If the selected protocol is a REPLACEMENT_FLUID procedure, the system shall display, via the operator visual/input interface, the infused volume of replacement fluid (i.e. 20% albumin solution) that is within +/- 15% of the Target RF Infusion Volume upon completion of a successful procedure.

Any sample size calculation/statistical analyses used to analyze the data will be included in the final report.

Note that only completed procedures will be included in the data analysis.

Instructions for calculating the residual albumin volume in the disposable set following a completed procedure is outlined in Attachment A, Calculating $W_{RESIDUAL}$. The remaining equations for determining total volume of albumin infused are included in Section 7.2.1.

7. Post Study Disposition

7.1. Disposition of Samples

Upon completion of a procedure, the disposable set and the 20% Albutein container should be discarded. Collected plasma may be retained for sampling and testing, but eventually when testing of the plasma is complete, the plasma should also be discarded. All biological materials must be discarded as biohazardous waste.

7.2. Original Data

Original data will be recorded during the execution of the protocol on worksheets provided in Appendix B, Appendix E, and other data forms, as appropriate. For every procedure, Appendix B must be filled out. Appendix E shall be filled out when the Aurora system does not perform as expected. This protocol, all original data, the final report, and all amendments will be archived in the appropriate Fresenius Kabi archives. The Aurora Plasma Exchange Procedure Data Record (Appendix B) and the Product Performance Report (Appendix E) should be sent by the medical facility to United Bio Source (UBS) at AMBARsafety@unitedbiosource.com. UBS will forward the appendices and relevant adverse event data to Fresenius Kabi Quality at Alicia.Gorecki@fresenius-kabi.com. If further procedural data are required for analysis, an Authorized Service Personnel may manually retrieve the instrument data at the clinical site (see page 1-3 of the Operator's Manual for description of an Authorized Service Personnel).

7.2.1. Calculating Volume of Total Infused Albumin to the Patient

The following sequences of equations outline the method for calculating the volume of infused albumin ($V_{INFUSED}$) in an Albumin Protocol.

To calculate the volume, various weight measurements of the disposable set and Albutein containers are taken at different points in the procedure. These measurements allows for derivation of the weight of residual albumin inside the disposable set after a completed procedure, and the weight of total albumin infused following completion of the procedure. The weights are converted to a volume using the known density of albumin (1.06 g/mL).

The variables/parameters of interest, necessary for this calculation, are outlined below:

- W_{TOTAL} = weight of total albumin used for a procedure
- W_{PRE} = weight of Albutein bottles before procedure
- W_{POST} = weight of Albutein bottles after procedure

- $W_{INFUSED}$ = weight of albumin infused to the patient
- $W_{SET, ALBUMIN\ RESIDUAL}$ = weight of residual albumin in the disposable set
- W_{CAP} = weight of protective cap of the albumin container
- $V_{INFUSED}$ = volume of total albumin used for a procedure

W_{TOTAL} can be calculated by finding the difference between W_{PRE} and W_{POST}:

1) $W_{TOTAL} = W_{PRE} - W_{POST} - W_{CAP}$

W_{TOTAL} can also be expressed in terms of $W_{INFUSED}$ and $W_{SET, ALBUMIN\ RESIDUAL}$.

2) $W_{TOTAL} = W_{INFUSED} + W_{SET, ALBUMIN\ RESIDUAL}$

Combining 1) and 2) together, the following equation can be derived:

3) $W_{PRE} - W_{POST} - W_{CAP} = W_{INFUSED} + W_{SET, ALBUMIN\ RESIDUAL}$

which can then be rearranged to the equation for calculating $W_{INFUSED}$

4) $W_{INFUSED} = W_{PRE} - W_{POST} - W_{CAP} - W_{SET, ALBUMIN\ RESIDUAL}$

W_{PRE} and W_{POST} are directly measured by the operators before and after the procedure, and the values recorded as Albutein® container weight (Pre-Procedure) and Albutein® container weight (Post-Procedure), respectively, in Appendix B. Steps for estimating $W_{SET, ALBUMIN\ RESIDUAL}$ is provided in Attachment A, Calculating $W_{SET, ALBUMIN\ RESIDUAL}$.

Once $W_{INFUSED}$(g) is calculated, the calculated weight can be divided by the density of albumin (1.06 g/mL) to convert the weight into a volume (i.e. $V_{INFUSED}$).

$$V_{INFUSED}(mL) = (W_{INFUSED}) \div (1.06\ g/mL)$$

7.3. Hardware and Software

The instruments and the investigational software will be retained by Grifols after study completion under the agreement that the software is for the clinical trial/investigation only and not for commercial use. Disposition of clinical inventory, including the clinical software, will be documented by Grifols according to their clinical protocol(s).

8. References

1. TSB12570019, Conversion of an Aurora to Aurora Plasma Exchange System (Aurora PES)
2. FQA01007, Fenwal Training Process
3. REC-011226, Aurora Plasma Exchange System Operator's Manual
4. REC-011227, Aurora Plasma Exchange System Administrator's Guide
5. 931-PRO-010255, Evaluation of the Autopheresis-C Plasma Exchange Procedure within a Clinical Trial
6. Trudnowski, RJ and Rico, RC. "Specific Gravity of Blood and Plasma at 4 and 37°C". *Clin Chem* 20 (1974): 615-616.
7. Nugent, RL and Towle, LW. "The Specific Gravity of Synthetic Solutions of Serum Albumin and Serum Globulin". *J Biol Chem* (1933).

9. List of Appendices

A. Instructions for Recording Aurora Plasma Exchange Procedure Data in Appendix B
B. Aurora Plasma Exchange Procedure Data Record
C. Instructions for Post-Procedure Disposable Set Weighing
D. Product Performance Flow Chart
E. Product Performance Report

10. List of Attachments

A. Calculating $W_{SET, ALBUMIN\ RESIDUAL}$

Appendix
A. Instructions for Recording Aurora Plasma Exchange Procedure Data in Appendix B

For every procedure, Appendix B, Aurora Plasma Exchange Procedure Data Record, must be filled out. Follow the instructions provided below to complete the appendix. Completed Appendix B must be sent to AMBARsafety@unitedbiosource.com. UBS will forward the appendices and relevant adverse event data to Fresenius Kabi at Alicia.Gorecki@fresenius-kabi.com. If further procedural data are required for analysis, an Authorized Service Personnel may manually retrieve the instrument data at the clinical facility.

10.1.1. Pre-Procedure Data

- Procedure ID: identifier for every procedure. All data sheets must be labeled and identified with Procedure ID, and configured per instructions in Section 4.5, Entering Patient and Procedure Information in the Operator's Manual.
- Albutein® container size and weight: the protective cap on the bottle shall NOT be removed before weighing the containers. Measure the weight of the full container before spiking to the Fleboset.
- Patient's weight and hematocrit (Hct)
- Selected protocol: IVIG infusion is to be performed under the No Albumin Protocol.

10.1.2. Intra-Procedure Data

No samples are taken during the procedure, but the following information is to be recorded:

- Patient Adverse Reactions
- Alarm/Alert Information (number and name of alarm/alert and approximate time or procedural phase in which it occurred)
- Flow Rate(s) during RF Infusion: if the RF infusion rate is modified at any point during RF infusion, the new modified rate shall be recorded also. The progress of the infusion (i.e. the volume of replacement fluid infused) at the point of modification shall also be recorded.
- Other Observations and Notes. Any issues/observations related to the injection Y-site for IVIG infusion shall also be recorded in this section. Notable observations may include, but not be limited to, integrity of the component and the presence of air bubbles.

10.1.3. Post-Procedure Data

Before sealing the disposable set, check and record the presence of fluid in the Replacement Fluid Line. Follow instructions in Appendix C for sealing points in the disposable set. The disposable set must be sealed at these locations to ensure accurate data analysis. After patient disconnect, the following measurements and observations are to be recorded. The section **Recording Procedure Information** of the Aurora Plasma Exchange System Operator's Manual provides a description of many of the elements of Appendix B:

- Presence of fluid in Replacement Fluid Line: note whether the line is filled with fluid, half-full, or empty. See **Section 2.4, Plasma Exchange Disposable Set Components**, of the Operator's Manual for illustration of the disposable set.
- Replacement Fluid Infused/Target Replacement Fluid Volume
- Collected Plasma Volume/Target Plasma Collection Volume
- Weight scale measurements of the following:
    - The collected plasma
    - 20% Albutein® containers (excluding Fleboset)
- Needle-in, Needle-out Time (NiNo Time, aka VP Time)
- Disposable set weight (after sealed in locations identified in Appendix C)
- If IVIG infusion was performed, indicate whether any issues were observed by circling "Yes" or "No". If "Yes", provide a description of the issue under the "Other Notes" section.

B. Aurora Plasma Exchange Procedure Data Record

Procedure (Run) ID				RF Infused/Target RF Infusion Volume	mL / mL		
				NiNo (VP) Time (min : sec)	:		
				Post-procedure Disposable Set Weight (see Appendix C)	g		
Patient Weight (circle lbs/kg)	lbs / kg			Collected Plasma/ Target Plasma Collection Volume	mL / mL		
Selected Protocol	[ ] Albumin [ ] No Albumin			Albutein® container weight (Post-Procedure)	g		
Patient Hct -OR- Hb	_____ % - OR- _____ g/dL			Plasma Bag Weight – post-procedure	g		
Albutein® container weight (Pre-Procedure)	g			Air/Fluid in RF Line (circle one)	Air	Fluid	Both
Number of air purges during RF infusion phase	0	1	>1	If infusing IVIG, were there issues observed with injection Y-site? If Yes, document in "Other Notes"	Yes	No	
Patient Reaction(s)	[ ] None [ ] Yes (please reference adverse event reporting data): _____						
Flow Rate during RF Infusion (5-20 mL/min, Albumin Protocol Only)	Initial rate: _____ mL/min If the RF infusion rate is modified at any point during RF infusion, record the modified rate and the progress of the RF infusion at the time of modification below. Modified rate: _____ mL/min, Infusion progress: _____mL/_____mL Modified rate: _____ mL/min, Infusion progress: _____mL/_____mL Modified rate: _____ mL/min, Infusion progress: _____mL/_____mL						
Alarm/Alert Information (Please include cause/procedural phase if known)							
Other Notes							

Entered by:_____Signature:_____ Date:_____

Reviewed by:_____Signature:_____Date:_____

C.Instructions for Post-Procedure Disposable Set Weighing

Step 1. Sealing the Disposable Set

1.1) Prior to sealing the disposable set at the locations designated in 1.2), also ensure that the Robert's Clamp on the Fleboset Double is closed.

1.2) Using the RF sealer, Seal the disposable set in the following locations per Figure 1:

- On the apheresis needle set near the luer
- On both sides of the replacement fluid transfer set luer
- On both pressure transducer connector lines
- Near the AC spike
- Below the separation device on the plasma line
- Above the sampling site on the plasma line

Note: Only seal the disposable set at the specified locations. DO NOT disconnect any components until indicated in *Step 2.*

Step 2. Disconnect the Plasma Bag, the Plasma Line, AC Line, and the Needle Assembly (beneath the Donor/Patient Short Line) from the rest of the disposable set

At the locations designated in **Figure 1** (see *Locations to Remove before Weighing*), disconnect the Plasma Line and the Plasma Bag from the disposable set.

Step 3. Remove Fleboset spike from the albumin bottles

Disconnect the Fleboset from the albumin bottles by removing both of the spikes from the silicone septum.

Step 4. Weigh the remaining disposable set INCLUDING the Fleboset, then record the weight

Do not disconnect the Fleboset from the disposable set. Weigh the disposable set and record the weight in Appendix B. If weights are not recordable, write "NR" in the appropriate location in the data sheet in Appendix B with reason for not recording the weight.

Step 5. Discard the disposable set appropriately

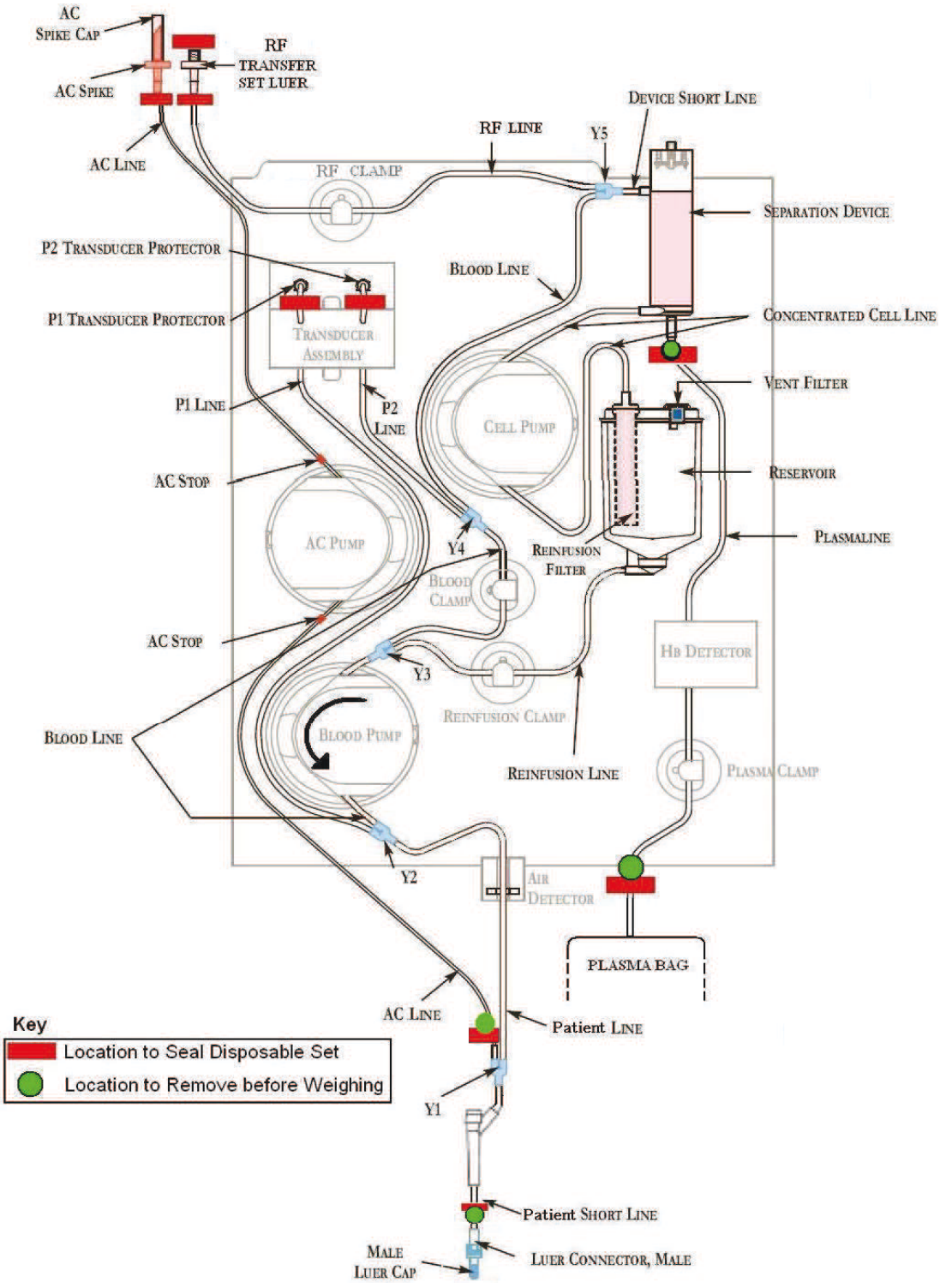
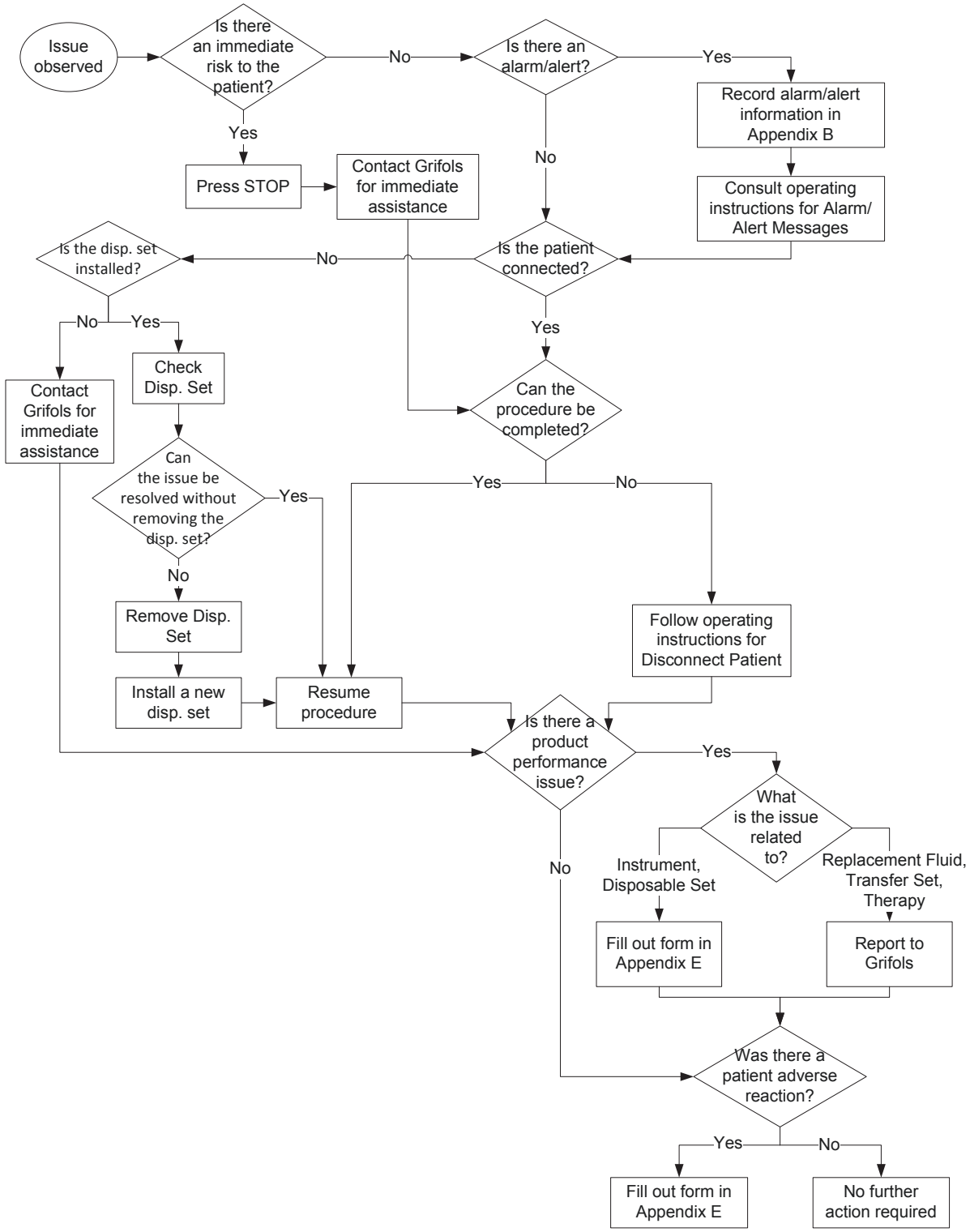


Figure 1. Locations where the disposable set should be sealed before removing the set from the instrument and where the seals should be separated to weigh the kit.

D. Product Performance Flow Chart

If a product performance issue is observed, follow the steps below.



E. Product Performance Report

[redacted]
Plasma Exchange Performance Report

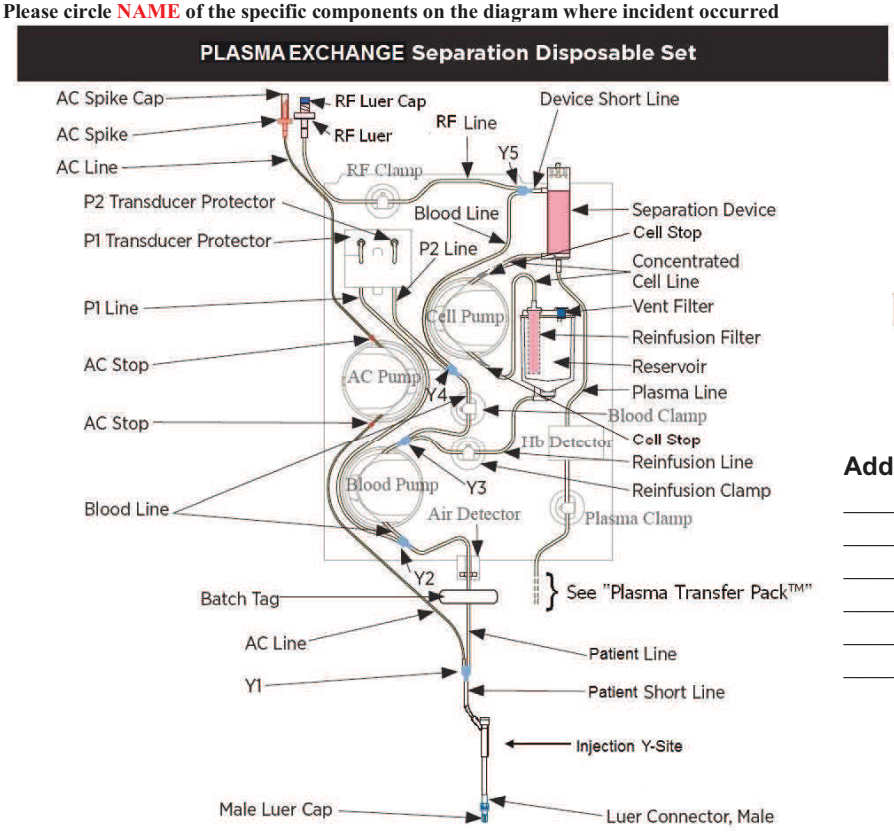
Important: If adverse reaction or injury has occurred, contact Grifols.
Was the procedure successfully completed? Yes ☐ No ☐
If No, was the procedure stopped due to a disposable set incident? Yes ☐ No ☐

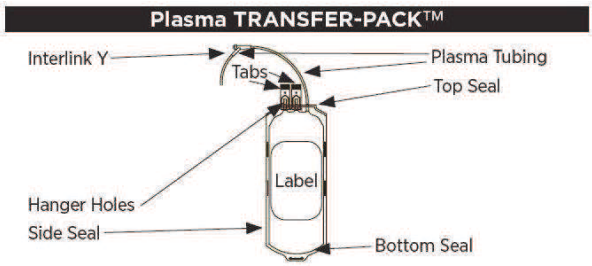
Incident Date: _____ Instrument Serial Number: _____
Product Code: _____ Lot Number: _____
Batch Tag Info Time: ____:____:____ Number_____ Video Jet Number: _____
Patient Bleed Number: _____ Volume of Plasma Collected: _____mL

When Was The Incident Detected?
☐ Before Use ☐ Set-Up ☐ Install Check ☐ Solution Prime ☐ Blood Prime ☐ Collection (specify cycle)_____
☐ During Venipuncture ☐ Reinfusion (specify cycle)_____ ☐ RF Priming ☐ RF Infusion _____
☐ Procedure Completion ☐ During IVIG Infusion ☐ Post-Procedure Processing

Incident Type (Mark all applicable)
☐ Alarm/Alert_____ ☐ Detached/Separated ☐ Noise ☐ Kinked ☐ Blood/Plasma Leak ☐ Low/No Plasma Flow ☐ Cut/Slice ☐ Red Plasma (Visual)
☐ Illegible Label ☐ Installation Check ☐ Dented
☐ Missing (not in box) ☐ Hole ☐ Other (Specify)_____

Please circle NAME of the specific components on the diagram where incident occurred





Additional Incident Description / Explanation

_____
_____
_____
_____
_____
_____

Disposable Set Return To Fresenius Kabi
1. Sample available for evaluation? Yes ☐ No ☐
2. Sample return box needed? Yes ☐ No ☐ Label only needed ☐
3. Picture taken of defective kit? Yes ☐ No ☐
   (If yes, send picture to AMBARsafety@unitedbiosource.com)

Facility Authorized Signature/Date:	Fresenius Kabi Reviewed By/Date:

Please Print
Account #: _____
Site Name: _____
Contact Person: _____
Operator Name: _____
Street Address: _____
City/State/Zip: _____
Phone Number: _____
E-Mail: _____
Fax Number: _____

E-mail this report to AMBARsafety@unitedbiosource.com along with an adverse event report, if applicable.
Include a copy of this form when returning a disposable set.

F. Revision History

Revision	Date	Author	Description of Change
A	See Pilgrim SmartSolve	[redacted]	Initial Release

APPENDIX 4

REPORTING OF SERIOUS ADVERSE EVENTS

GRIFOLS	SERIOUS ADVERSE EVENT FORM	Study: IG1002 (AMBAR)
Patient ID: __ __ __ - __ __ __	❑ Initial ❑Follow-up # ___	

. PATIENT INFORMATION

1. Country	2. Date of Birth (DD/MMM/YYYY)	3. Gender	4. Weight	5. Race	6. Ethnicity
	__ __/__ __ __/__ __ __ __	☐ Male ☐ Female	☐ lb ☐ kg	☐ White or Caucasian ☐ Black or African American ☐ Asian ☐ American Indian or Alaskan Native ☐ Native Hawaiian / Other Pacific Islander ☐ Other:_____	☐ Hispanic / Latino ☐ Not Hispanic / Latino

II. EVENT INFORMATION

7. Event Term/Diagnosis

8. Reason for Seriousness *(check all that apply):*

☐ Resulted in DEATH *(if yes, complete section 10)* .

☐ LIFE-THREATENING

☐ Required/prolonged HOSPITALIZATION on

__ __/__ __ __/__ __ __ __ (DD/MMM/YYYY)

☐ Persistent or significant DISABILITY/INCAPACITY

☐ CONGENITAL anomaly/birth defect

☐ OTHER (Important medical event):

9. Describe patient status, details of event and complications *(attach additional sheet, if extra space is needed)*

10. Death Details

Date of death:

__ __/__ __ __/__ __ __ __ (DD/MMM/YYYY)

Autopsy ☐ Yes ☐ No

Is death certificate attached? ☐ Yes ☐ No

11. Event Onset/Start Date (DD/MMM/YYYY)

__ __/__ __ __/__ __ __ __ **Start Time**:__ __:__ __ (24 hrs)

12. Event Stop Date (DD/MMM/YYYY) ☐ Ongoing

__ __/__ __ __/__ __ __ __ **Stop Time**:__ __:__ __ (24 hrs)

Hospital discharge date (if applicable): __ __/__ __ __/__ __ __ __

13. Relationship to Study Drug	14. Relationship to Medical Condition	15. Relationship to Other Drugs or Procedures	16. Event Outcome	17. Severity
☐ UNRELATED ☐ RELATED ○ Unlikely ○ Possibly ○ Probably ○ Definitely	☐ UNRELATED ☐ RELATED ○ Unlikely ○ Possibly ○ Probably ○ Definitely	☐ UNRELATED ☐ RELATED ○ Unlikely ○ Possibly ○ Probably ○ Definitely	☐ RECOVERED/RESOLVED ☐ RECOVERING/RESOLVING ☐ RECOVERED/RESOLVED WITH SEQUELAE ☐ NOT RECOVERED/NOT RESOLVED ☐ FATAL	☐ MILD ☐ MODERATE ☐ SEVERE

III. STUDY MEDICATION

18. Treatment arm:

☐ **Treatment A** (Full dose, Albutein+Flebogamma DIF)

☐ **Treatment B** (Half dose, Albutein+Flebogamma DIF)

☐ **Treatment C** (Half dose, Albutein only)

☐ **Sham group** (No therapy)

19. Product* (*last administered prior event):

_____

20. Study period:

☐ Screening ☐ FPE # …….. ☐ IV LVPE # …….. ☐ FV

21. Product* started on (DD/MMM/YYYY)	22. Last date of Study Treatment (prior event) (DD/MMM/YYYY)	23. Dose	24. Frequency	25. Route of administration
__ __/__ __ __/__ __ __ __	__ __/__ __ __/__ __ __ __			

26. Lot number	27. Action taken with study drug due to the event	28. Did event abate after stopping or reducing study treatment?	29. Did event reappear after reintroduction of study treatment?
30. Expiration Date	☐ No change/None ☐ Dose reduced ☐ Interrupted ☐ Discontinued ☐ Other, specify: _____	☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable

GRIFOLS	SERIOUS ADVERSE EVENT FORM	Study: IG1002 (AMBAR)
Patient ID: __ __ __ - __ __ __	❑ Initial	❑Follow-up # ___

IV. OTHER MEDICAL INFORMATION

31. Patient's relevant Medical History	32. Relevant Lab/Confirmatory tests/Imaging reports: Please include units and reference ranges/imaging reports ☐ *Check if reports are attached*

33. Treatment Medications: *Drug(s) used to treat the adverse event.*

Trade and Generic name	Dose	Route of administration	Start Date (DD/MMM/YYYY)	Stop Date (DD/MMM/YYYY)	Indication

34. Other non-drug treatments for the adverse event (e.g. surgery, etc.)

35. Concomitant drug(s): *Only those drugs the subject received at onset or up to 1 month prior to the event; exclude those to treat event.*

Trade and Generic name	Dose	Route of administration	Start Date (DD/MMM/YYYY)	Stop Date (DD/MMM/YYYY)	Indication

V. REPORTER/INVESTIGATOR INFORMATION

36. Reporter's name	37. Reporter's Phone #	38. Reporter's e-mail
39. Principal Investigator name	40. Principal Investigator Phone #	41. Principal Investigator e-mail
42. Hospital Name and Address		
43. Signature	44. Date of the notification (DD/MMM/YYYY) __ __/__ __ __/__ __ __ __	

Please send by e-mail within **24h** to [redacted] PV Department [redacted]

Serious Adverse Event (SAE) Report
Completion Guidelines

General Guidelines:

- The ***Subject No***. (Which includes the site and subject number) must be completed on both pages of the form.
- The SAE report must be signed and dated by the Investigator. However, if for some reason the investigator is not available to sign the report, DO NOT hold up sending in the report. The report must be completed and sent to Grifols within **24 hours** of being informed of the event. As soon as possible, the Investigator must send in a signed copy of the original that was previously sent to Grifols and annotate next to the checked "Initial" or "Follow-up" box "signature" to indicate that the only thing changed was an added signature.
- Send the completed Serious Adverse Event Report via E-mail to [redacted].

Report Status (Initial or Follow-up):

Check on the form header whether the report is an initial or a follow-up:

- Initial Report –If any information is not available at the time of the Initial Report, leave the field blank.
- Follow-up Report – Must be submitted when new information becomes available or previously reported information has changed.

You can use a copy of the initial report and make the applicable corrections/updates or use a blank form. In the second case, DO NOT rewrite all the information from the initial report; provide only new information.

A follow-up Report **must** always have identifying information (Subject No.) completed on each page, event name (7), event onset date (11), Investigator's signature and date (43 & 44).

SAE Form boxes:

1. **Country of Origin:** Record the country where the event occurred.
2. **Date of Birth:** Record the subject's Date of Birth.
3. **Gender:** Check Male or Female.
4. **Weight:** Record weight in kilograms or pounds and check the appropriate box for the units.
5. **Race:** Record the subject's race.
6. **Ethnicity:** Record the subject's ethnicity. Please note that as per FDA guidelines *Hispanic/Latino* is described as *"a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race."*
7. **Event Term/Diagnosis:** Record the clinical diagnosis (verbatim of the event). Do not include concurrent diagnoses or symptoms associated with this event. If a diagnosis is unknown, record the main symptom(s) of the event. If the event is an exacerbation of a chronic condition present prior to study, include the wording "Worsening of …" to the event name.
8. **Reason for Seriousness:** Check ALL serious criteria that apply for this event. There must be at least one criteria checked. Note: "Resulted in Death" should only be checked if death was the outcome of the event reported.
9. **Describe details of event:** Describe the event details including a description of what occurred and a summary of all relevant clinical information (medical status prior to the event, signs, symptoms, diagnoses, clinical course, labs or diagnostic testing, treatment, and outcome). If relevant, include synopses of any office visit notes or the hospital discharge summary. You may record only the basic information and attach copies of hospital discharge summaries with any confidential information blacked out. Use an additional page if more space is needed.
10. **Death Details** (Only for the event which resulted in death)**:** Enter the date of death, check if autopsy and/or death certificated are attached, or leave blank if the subject did not die from THIS event.
11. **Event Onset/Start Date and time:** Record the date and time the first symptoms of the event presented or when the event satisfied one of the serious event criteria, whichever date was earlier.
12. **Event Stop Date and time:** Record the date and time the event resolved. If event is continuing, check the "Ongoing" box.
    Event Stop Date is required for all events except cases where subject was lost to follow-up, permanently disabling/incapacitating events, or in cases of death not related to the event. If the event led to the subject's death, then Outcome of this event should be marked "Fatal" and date of death should be entered as the Event Stop Date. If the subject died of another cause while experiencing this adverse event, the Event Stop Date should be left blank, "Ongoing" checked, and Event Outcome of this event should be marked as "Not Recovered/Not Resolved".
    If the patient was hospitalized as result of the event, please also complete discharge date in this box.
13. **Relationship to Study Drug:** Check the event's relationship to study drug. This MUST be completed. *Note that "Unlikely" is classified as potentially related to study drug.*

Serious Adverse Event (SAE) Report
Completion Guidelines

14. **Relationship to Medical Condition:** Check the event's relationship to patient's medical condition.
15. **Relationship to Other Drugs or Procedures:** Check the event's relationship to other drugs or study procedures.
16. **Event Outcome:** Check the appropriate outcome of this event. If the subject's death was related to the event, then Outcome should be marked "Fatal." If the subject died from another cause while experiencing this event, DO NOT mark "Fatal" as an outcome, but check "Not Recovered/Not Resolved".
17. **Severity:** Check the appropriate severity for this event.
18. **Treatment arm:** please indicate randomization arm for the subject.
19. **Product* (*last administered prior event):** please indicate the study product that the patient last received prior presenting the serious adverse event.
20. **Study period:** please indicate in which study phase the patient presented the serious adverse event, it can either be the screening phase, while undergoing weekly Full Plasma Exchange (please specify visit number), intermediate visit, while undergoing monthly Low Volume Plasma Exchange (please specify visit number) or Follow-up visit.
21. **Product* started on:** Record the date the patient first received the study product indicated in box #19.
22. **Last date of Study Treatment (prior event):** Record the date the study product indicated in box #19 was last administered before the patient presented the serious adverse event.
23. **Dose:** Record infused dose or volume of the study product indicated on box #19.
24. **Frequency:** Record the frequency of dosing for the study product indicated on box #19.
25. **Route of administration:** Record the route of administration for the study product indicated on box #19.
26. **Lot number:** Record the lot number for the study product indicated on box #19.
27. **Action taken with study drug due to the event (check all that apply):** Check the appropriate action(s) taken with study product indicated on box #19 due to THIS serious adverse event. Check "No Change" if action was taken due to some other event, and NOT to THIS event.
28. **Did event abate after stopping or reducing study treatment?** Check the appropriate response.
29. **Did event reappear after reintroduction of study treatment?** Check the appropriate response.
30. **Expiration date:** Record the expiration date for the study product indicated on box #19.
31. **Medical History:** Record only relevant medical history and include allergies, smoking and alcohol use, or drug abuse (if applicable). If preferred, attach a copy of the Medical History eCRF and record "see attached".
32. **Relevant Lab/Confirmatory tests/Imaging reports:** Record relevant laboratory data including date collected, test name, test results, units, and reference ranges. If preferred, attach copies of any reports (all confidential information blacked out) and record "see attached." If the event is a lab abnormality, include lab data prior to the event (baseline). Include all laboratory data used in diagnosing the event and lab data at the time of resolution. Include a synopsis of any relevant autopsy or pathology reports, if applicable.
33. **Treatment Medications:** Record all medications used to treat the event *or* if attaching Concomitant Medication electronic case report forms, circle the treatment medications.
34. **Other non-drug treatments for the adverse event (e.g. surgery, etc.):** Record any procedures and surgeries used to treat the event.
35. **Concomitant drug(s):** Record only relevant medications taken within 1 month prior to the onset of the event. If preferred, attach a copy of the Concomitant Medication eCRF and record "see attached".
36. **Reporter's name:** Print the name and title of the person who is reporting the event to UBC.
37. **Reporter's Phone** #: Record the Reporter's phone number.
38. **Reporter's e-mail**: Record the Reporter's e-mail address.
39. **Principal Investigator name:** Record the Principal Investigator's name.
40. **Principal Investigator Phone #:** Record the Investigator's phone number.
41. **Principal Investigator e-mail:** Record the Investigator's e-mail.
42. **Hospital Name and Address:** Record the hospital name and address where the study is performed.
43. **Signature:** The Investigator MUST sign and date this form prior to sending it. If the investigator cannot sign and date prior to the form meeting the 24 hour submission deadline, a copy must be signed as soon as possible annotating block #1 Report status with "signature."
44. **Date of the notification:** Record the Date of Investigator's Signature.

APPENDIX 5

DECLARATION OF HELSINKI

WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words,

“The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by

individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and

standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain

for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made

publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

APPENDIX 6

E6 GCP GUIDELINES

Draft

COMMISSION DIRECTIVE ../…/EC

of […]

laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorisation of manufacturing or importation of such products

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use[1], and in particular Article 1(3), Article 13(1) and Article 15(5) thereof,

Whereas:

(1) Article 1(3) of Directive 2001/20/EC requires that principles of good clinical practice and detailed guidelines in line with those principles shall be adopted. Article 13(1) of Directive 2001/20/EC requires that minimum requirements for authorisation of manufacture and import of investigational medicinal products shall be adopted. Article 15(5) of directive 2001/20/EC requires that detailed guidelines on the documentation relating to the clinical trial to verify the compliance of the clinical trial in question with Directive 2001/20/EC shall be adopted.

(2) The principles and guidelines for good clinical practice should ensure that the conduct of clinical trials on investigational medicinal products, as defined in Article 2(d) of Directive 2001/20/EC, is founded in the protection of human rights and the dignity of the human being, .

(3) Manufacturing requirements to be applied to investigational medicinal products are provided for by Commission Directive 2003/94/EC of 8 October 2003 laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use[2]. Title IV of Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use[3] contains the provisions applied for the authorisation for the manufacture of medicinal products as part of the requirements needed for the application for a marketing authorisation. Article 3(3) of this

[1] OJ L 121, 1.5.2001, p. 34
[2] OJ L 262, 14.10.2003, p. 22.
[3] OJ L 311, 28.11.2003, p. 67.

Directive establishes that these requirements are not applicable for medicinal products intended for research and development trials. It is therefore necessary to lay down minimal requirements regarding applications for and management of authorisations to manufacture and/or import investigational medicinal products, as well as for the granting and the content of the authorisations to guarantee the quality of the investigational medicinal product used in the clinical trial.

(4) With regard to the protection of trial subjects and to ensure that unnecessary clinical trials will not be conducted, it is important to define principles and detailed guidelines of good clinical practice allowing that the results of the trials are documented for use in a later phase.

(5) To ensure that all experts and individuals involved in the designing, initiating, conducting and recording of clinical trials will apply the same standards of good clinical practice, principles and detailed guidelines of good clinical practice have to be defined.

(6) Provisions for the functioning of the Ethics Committees should be established in each Member State based on common detailed guidelines in order to ensure the protection of the trial subject while at the same time allowing a harmonised application in the different Member States of the procedures to be used by Ethics Committees.

(7) To secure the compliance of clinical trials with the provisions on good clinical practice, inspectors shall ensure the practical effectiveness of such provisions. It is essential therefore to provide detailed guidelines on the minimum standards for their qualification, in particular as regards their education and training. For the same reason detailed guidelines on inspection procedures, in particular on the co-operation of the various agencies, and the follow-up to the inspections, should be laid down.

(8) The International Conference on Harmonisation (ICH) reached a consensus in 1995 to provide a harmonised approach for Good Clinical Practice. The consensus paper should be taken into account as agreed upon by the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMEA) and published by the EMEA.

(9) Sponsors, investigators and other participants shall take into account the scientific guidelines relating to the quality, safety and efficacy of medicinal products for human use as agreed upon by the CHMP and published by the EMEA and the other pharmaceutical Community guidelines published by the Commission in the different volumes of *The rules governing medicinal products in the European Community*.

(10) In conducting clinical trials on investigational medicinal products for human use the safety and the protection of the rights of trial subjects should be ensured. Article 3(1) of Directive 2001/20/EC provides for the Member States to adopt detailed rules to protect from abuse individuals who are incapable of giving their informed consent; this should also include individuals temporarily incapable of giving their informed consent, i.e. in emergency situations.

(11) Non-commercial clinical trials conducted by researchers without the participation of the pharmaceutical industry may be of great benefit to the patients concerned. Directive 2001/20/EC recognises the specificity of these non-commercial clinical trials. In particular, when trials are conducted with authorised medicinal products and on patients with the same characteristics as those covered by the authorised indication, requirements already fulfilled by these authorised medicinal products, as far as manufacturing or importation are concerned,

should be taken into consideration. However, it could also be necessary, due to the specific conditions under which non-commercial trials are conducted, that Member States foresee specific modalities to be applied to these trials not only when conducted with authorised medicinal products and on patients with the same characteristics, in order to comply with the principles imposed by this Directive, in particular as far as the manufacturing or import requirements for authorisation and the documentation to be submitted and archived for the trial master file are concerned. The conditions under which the non-commercial research is conducted by public researchers and the places where this research takes place, make the application of certain of the principles of good clinical practice unnecessary or guaranteed by other means. Member States will ensure in these cases, when providing for specific modalities, that the objectives of the protection of the rights of the patients, which participate to the trial, as well as, in general, the correct application of the good clinical practice principles are achieved.

(12) The measures provided for in this Directive are in accordance with the opinion of the Standing Committee on Medicinal Products for Human Use, set up by Article 121 of Directive 2001/83/EC,

HAS ADOPTED THIS DIRECTIVE:

Chapter 1

Scope

Article 1

1. This Directive lays down the following provisions to be applied to investigational medicinal products for human use:

(a) the principles of good clinical practice and detailed guidelines in line with those principles, as referred to in Article 1(3) of Directive 2001/20/EC, for the design, conduct and reporting of clinical trials on human subjects involving such products;

(b) the requirements for the authorisation of the manufacture or importation of such products as foreseen in Article 13(1) of Directive 2001/20/EC;

(c) the detailed guidelines on the documentation relating to clinical trials, archiving, qualifications of inspectors and inspection procedures in accordance with Article 15(5) of Directive 2001/20/EC.

2. When applying these principles, detailed guidelines and requirements Member States shall take into account, the technical implementing modalities provided for in the detailed guidance published by the Commission in *The Rules governing medicinal products in the European Union*.

3. When applying these principles, detailed guidelines and requirements on non-commercial clinical trials conducted by researchers without the participation of the pharmaceutical

industry, Member States may introduce specific modalities in order to take into account the specificity of these trials as far as Chapter 3 and Chapter 4 of this Directive are concerned.

Member States may also take into account the special position of the trials whose planning do not require particular manufacturing or packaging processes, carried out with medicinal products with marketing authorisations within the meaning of Directive 2001/83/EC, manufactured or imported in accordance with the same Directive and conducted on patients with the same characteristics as those covered by the indication specified in this marketing authorisation. Labelling of investigational medicinal products intended for trials of this nature may be subject to simplified provisions laid down in the good manufacturing practice guidelines on investigational medicinal products. Member States shall inform the Commission as well as the other Member States of any specific modalities implemented in accordance with this paragraph. These modalities will be published by the Commission

Chapter 2

Good clinical practice, for the design, conduct, record and reporting of clinical trials

GOOD CLINICAL PRACTICE

Article 2

(a) The rights, safety and interests of the trial subjects shall prevail over those of science and society.

(b) Each individual involved in conducting a trial shall be qualified by education, training, and experience to perform [his/her] respective tasks.

(c) Clinical trials shall be scientifically sound and ethical well defined in all aspects of the trials.

(d) Member States shall ensure that necessary procedures to secure that the quality of every aspect of the trials are complied with.

Article 3

The available non-clinical and clinical information on an investigational medicinal product shall be adequate to support the proposed clinical trial.

Clinical trials shall be conducted in accordance with the ethical principles laid down in the Ethical Principles for Medical Research Involving Human Subjects that are reflected in World Medical Association Declaration of Helsinki (1996).

Article 4

The clinical trials protocol referred to in Article 2 of Directive 2002/20/EC shall provide for the definition of inclusion and exclusion of subjects participating in a trial, monitoring and publication policy.

The investigator and sponsor shall consider all relevant guidance with respect to commencing and conducting of a clinical trial.

Article 5

All clinical trial information shall be recorded, handled, and stored in such a way that it can be accurately reported, interpreted and verified while protecting the confidentiality of records of the trials subjects.

THE ETHICS COMMITTEE

Article 6

1. Each Ethics Committee shall adopt the relevant rules of procedure necessary to implement the requirements set out in Directive 2001/20/EC and, in particular, in Articles 6 and 7 thereof.

2. The Ethics Committees shall, in every case, retain the essential documents, as defined in Article 16 of Directive 2001/20/EC, relating to a clinical trial for at least 3 years after its completion. They shall retain the documents for a longer period, where required by other applicable regulatory requirements.

3. Communication of information between the Ethics Committee(s) and the competent authorities of the Member States shall be ensured through appropriate and efficient systems.

THE SPONSORS

Article 7

1. A sponsor may delegate any or all of his trial-related functions to an individual, a company, an institution or an organisation.

However, in such cases the sponsor shall remain responsible for ensuring that the conduct of the trials and the final data generated by those trials comply with the requirements of Directive 2001/20/EC as well as of this Directive.

2. The investigator and the sponsor may be the same person.

INVESTIGATOR'S BROCHURE

Article 8

1. The information in the investigator's brochure, referred to in Article 2(g) of Directive 2001/20/EC, shall be presented in a concise, simple, objective, balanced and non-promotional form that enables a clinician or potential investigator to understand it and make an unbiased risk-benefit assessment of the appropriateness of the proposed clinical trial. This summarised style is also required for any update of this brochure.

2. If the investigational medicinal product has a marketing authorisation, the Summary of Product Characteristics can be used instead of the investigator's brochure.

3. The investigator's brochure shall be validated and updated on a regular basis by the sponsor, at least annually.

Chapter 3

Manufacturing or import authorisation

Article 9

1. The authorisation to manufacture or import investigational medicinal products, referred to in Article 13(1) of Directive 2001/20/EC, shall be required for both total and partial manufacture, and for the various processes of dividing up, packaging or presentation.

   The authorisation shall be required notwithstanding that the products manufactured are intended for export. The authorisation shall also be required for imports from third countries into a Member State.

2. However, such authorisation shall not be required for preparation, dividing up, changes in packaging or presentation where these processes are carried out solely for retail supply, by pharmacists in dispensing pharmacies or by persons legally authorised in the Member States to carry out such processes.

Article 10

1. In order to obtain the authorisation the applicant must meet at least the following requirements:

(a) specify the medicinal products and pharmaceutical forms to be manufactured or imported;

(b) specify the relevant manufacture or import operations;

(c) specify, where relevant as in the case of viral or non conventional agents' inactivation, the manufacturing process;

(d) specify the place where the products are to be manufactured orhave at his disposal, for the manufacture or import of the above, suitable and sufficient premises, technical equipment and control facilities complying with the legal requirements of Commission Directive 2003/94/EC as regards the manufacture, control and storage of the products;

(e) have permanently and continuously at his disposal the services of at least one qualified person as referred to in Article 13(2) of Directive 2001/20/EC.

2. The applicant must provide with his application documentary evidence that he complies with the first paragraph.

Article 11

1. The competent authority shall issue the authorisation only after verifying the accuracy of the particulars provided by the applicant in accordance with Article 10 by the means of an inquiry carried out by its agents.

2. Member States shall take all appropriate measures to ensure that the procedure for granting an authorisation is completed within 90 days of the submission of a valid application from the day on which the competent authority receives the application.

3. The competent authority of the Member State may require from the applicant further information concerning the particulars supplied pursuant to Article 10 and concerning the qualified person referred to in Article 10; where the competent authority concerned exercises this right, the application of the time-limits referred to in this Article shall be suspended until the additional data required have been supplied.

Article 12

In order to ensure that the requirements referred to in Article 10 are complied with, authorisation may be made conditional on the carrying out of certain obligations imposed either when authorisation is granted or at a later date

The authorisation shall apply only to the premises specified in the application and to the medicinal products and pharmaceutical forms specified in that same application.

Article 13

The holder of the authorisation shall at least be obliged:

(a) to have at his disposal the services of staff that comply with the legal requirements existing in the Member State concerned both as regards manufacture and controls;

(b) to dispose of the investigational/authorised medicinal products only in accordance with the legislation of the Member States concerned;

(c) to give prior notice to the competent authority of any changes the holder may wish to make to any of the particulars supplied pursuant Article 10; in particular, the competent authority shall be informed immediately if the qualified person referred to in Article 13(2) of Directive 2001/20/EC is replaced unexpectedly;

(d) to allow agents of the competent authority of the Member State concerned access to his premises at any time;

(e) to enable the qualified person referred to in Article 13(2) of Directive 2001/20/EC to carry out his duties, for example by placing at his disposal all the necessary facilities;

f) to comply with the principles and guidelines for good manufacturing practice for medicinal products as laid down by Community law. Detailed guidelines in line with those principles will be published by the Commission and revised necessary to take account of technical and scientific progress.

Article 14

If the holder of the manufacturing authorisation requests a change in any of the particulars referred to in points (a) and (e) of the first paragraph of Article 13, the time taken for the procedure relating to this request shall not exceed 30 days. In exceptional cases this period of time may be extended to 90 days.

Article 15

The competent authority shall suspend or revoke the authorisation, as a whole or in part, if the holder of the authorisation fails at any time to comply with the relevant requirements.

Chapter 4

Documentation constituting the Trial Master File and archiving

Article 16

The documentation referred to Article 15(5) of Directive 2001/20/EC as the Trial Master File, shall consist of essential documents, which enable both the conduct of a clinical trial and the quality of the data produced to be evaluated. Those documents have to show whether the investigator and the sponsor have complied with the principles and guidelines of good clinical practice and with the applicable regulatory requirements and, in particular, with Annex I to Directive 2001/83/EC.

The Trial Master File shall provide the basis for the audit by the sponsor's independent auditor and for the inspection by the competent authority.

The content of the essential documents referred to above shall be in accordance with the specificities of each phase of the clinical trial.

Article 17

The sponsor and the investigator shall, in every case, retain the essential documents relating to a clinical trial for at least 5 years after its completion.

They shall retain the documents for a longer period, where required by other applicable regulatory requirements or by an agreement between the sponsor and the investigator.

Essential documents shall be archived in a way that ensures that they are readily available, upon request, to the competent authorities.

The trials subject's medical files should be retained in accordance with national legislation and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

Article 18

Any transfer of ownership of the data or of documents shall be documented. The new owner shall assume responsibility for data retention and archiving.

Article 19

The sponsor shall appoint individuals within its organisation who are responsible for archives.

Access to archives shall be restricted to the named individuals responsible for the archive.

Article 20

The media used to store essential documents shall be such that those documents remain complete and legible throughout the required period of retention and can be made available to the competent authorities upon request.

Any alteration to records shall be traceable.

Chapter 5

Qualifications of inspectors

Article 21

1. The inspectors, appointed by the Member States in conformity with Article 15(1) of Directive 2001/20/EC, shall be made aware of and maintain confidentiality whenever they gain access to confidential information as a result of good clinical practice inspections according to applicable Community requirements, national laws or international agreements.

2. Member States shall ensure that inspectors have completed education at university level, or have equivalent experience, in medicine, pharmacy, pharmacology, toxicology or other relevant fields.

3. Member States shall ensure that inspectors receive appropriate training, that their training needs are assessed regularly and that appropriate action is taken to maintain and improve their skills.

   Member States shall guarantee that the inspectors have knowledge of the principles and processes that apply to the development of medicinal products and clinical research. Inspectors shall also have knowledge of applicable Community and national legislation and guidelines applicable to the conduct of clinical trials and the granting of marketing authorisations.

   The inspectors shall be familiar with the procedures and systems for recording clinical data, and with the organisation and regulation of the health care system in the relevant Member State(s) and, where appropriate, in third countries.

4. Member States shall maintain up-to-date records of the qualifications, training and experience of each inspector.

5. Each inspector shall be provided with a document setting out standard operating procedures and giving details of the duties, responsibilities and ongoing training requirements. Those procedures shall be maintained up to date.

6 Inspectors shall be provided with suitable means of identification.

7. Each inspector shall sign a statement declaring any financial or other links to the parties to be inspected. That statement shall be taken into consideration when inspectors are to be assigned to a specific inspection.

Article 22

In order to ensure the presence of skills necessary for specific inspections, Member State may appoint teams of inspectors and experts with appropriate qualifications and experience to fulfil collectively the requirements necessary for conducting the inspection.

Chapter 6

Inspection procedures

Article 23

1. Good clinical practice inspections may take place before, during or after the conduct of clinical trials and/or as part of the verification of applications for marketing authorisations or as follow-up to these.

2. In accordance with Article 15(1) and (2) of Directive 2001/20/EC, inspections may be requested and co-ordinated by the European Medicines Agency (EMEA), within the scope of Regulation (EC) No726/2004 of the European Parliament and of the Council of 31 March 2004, laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency[4], especially in connection with clinical trials relating to applications through the procedure established by this Regulation.

3. Inspections shall be conducted in accordance with the inspections guidance documents developed to support the mutual recognition of inspection findings within the Community.

4. Improvement and harmonisation of inspection guidance shall be achieved by the Member States, in collaboration with the Commission and theEMEA, through joint inspections, agreed processes and procedures and sharing of experience and training.

Article 24

Member States shall make publicly available within their territories the documents relating to the adoption of good clinical practice principles.

[4] OJ L 136, 30.4.2004, p.1

They shall establish the legal and administrative framework within which their good clinical practice inspections operate, with definition of the powers of inspectors for entry into clinical trial sites and access to data. They shall ensure that inspectors of the competent authority of the other Member States have also access to the clinical trial sites and data at any time.

Article 25

Member States shall provide for sufficient resources and in particular shall appoint an adequate number of inspectors to ensure effective verification of compliance with good clinical practice.

Article 26

Member States shall establish the relevant procedures for verification of good clinical practice compliance.

The procedures shall include the modalities which will be used to examine both the study management procedures and the conditions under which clinical trials are planned, performed, monitored and recorded and follow-up measures.

Article 27

Member States shall establish the relevant procedures for:

(1) appointing experts for accompanying inspectors in case of need;

(2) requesting inspections/assistance from other Member States, in line with Article 15(1) of Directive 2001/20/EC and for co-operating in inspections sites in another Member State;

(3) arranging inspections in third countries.

Article 28

Member States shall maintain records of national and if applicable international inspections including the good clinical practice compliance status, and of their follow-up.

Article 29

1. In order to harmonise the conduction of inspections by the competent authorities of the different Member States, guidance documents containing the common provisions on the conduction of these inspections shall be published by the Commission after consultation with the Member States.

2. Member States shall ensure that national inspections procedures are in compliance with the guidance documents referred in paragraph 1.

3. The guidance documents referred to in paragraph 1 may be updated regularly according to scientific and technical development.

Article 30

1. Member States shall lay down all necessary rules to ensure confidentiality by inspectors and other experts. With regard to personal data, the requirements of Directive 95/46/EEC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data[5] shall be respected.

2. Inspection reports shall be made available by the Member States only to the recipients referred to in Article 15(2) of Directive 2001/20/EC, in accordance with national regulations of the Member States and subject to any arrangements concluded between the Community and third countries.

Chapter 7

Final provisions

Article 31

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive by [6 months after entry into force] at the latest. They shall communicate to the Commission the text of the provisions and correlation table between those provisions and the provisions of this Directive.

When Member States adopt these provisions, they shall contain a reference to this Directive or be accompanied by such a reference on the occasion of their official publication. The Member States shall determine how such reference is to be made.

Article 32

This Directive shall enter into force on the twentieth day following that of its publication in the *Official Journal of the European Union*.

Article 33

This Directive is addressed to the Member States.

[5] OJ L 281, 23.11.1995, p.31.

Done at Brussels, […]

For the Commission
[…]
Member of the Commission

APPENDIX 7

BEHAVIORAL AND COGNITIVE IMPAIRMENT SCALES AND TESTS

APPENDIX 8

STUDY SUMMARY TABLE AND STUDY FLOW CHART

Visit	Screening		Randomization	FPE1	FPE2	FPE3	FPE4	FPE5	FPE6	IV	LVP E1	LVP E2	LVP E3	LVP E4	LVP E5	LVP E6	LVP E7	LVP E8	LVP E9	LVP E10	LVP E11	LVP E12	Final
Time / Weeks	-3	-2	-1	1	2	3	4	5	6	7-8	9	13	17	21	25	29	33	37	41	45	49	53	54-55
Window Range (days)				+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 2	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 2
Months	-1			1				2			3	4	5	6	7	8	9	10	11	12	13	14	14
Study Schedule																							
Informed Consent	X																						
Inclusion/Exclusion criteria	X																						
Demographic data	X																						
Medical History	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X			X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	
ECG	X																						
Genetic Markers	X																						
Vital signs	X			B+D+A	B+D+A	B+D+A	B+D+A	B+D+A	B+D+A		B+D+A	B+D+A	B+D+A	B+D+A	B+D+A	B+D+A	B+D+A	B+D+A	B+D+A	B+D+A	B+D+A	B+D+A	
Adverse Events				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medication	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Central catheter implantation	X																						
Central catheter extraction										X													
Plasmapheresis				FPE	FPE	FPE	FPE	FPE	FPE		LVPE	LVPE	LVPE	LVPE	LVPE	LVPE	LVPE	LVPE	LVPE	LVPE	LVPE	LVPE	
Albumin 5%				35-45 ml/kg	35-45 ml/kg	35-45 ml/kg	35-45 ml/kg	35-45 ml/kg	35-45 ml/kg														
1st and 2nd Arm: Albumin 20%											-	200 100mL	200 100 mL	200 100 mL	-	200 100 mL	200 100 mL	200 100 mL	-	200 100mL	200 100 mL	200 100mL	
1st and 2nd Arm: IgIV											20 g 10 g	-	-	-	20 g 10 g	-	-	-	20 g 10 g	-	-	-	
3rd Arm: Albumin 20%											100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	

Visit	Screening		Randomization	FPE1	FPE2	FPE3	FPE4	FPE5	FPE6	IV	LVP E1	LVP E2	LVP E3	LVP E4	LVP E5	LVP E6	LVP E7	LVP E8	LVP E9	LVP E10	LVP E11	LVP E12	Final
Time / Weeks	-3	-2	-1	1	2	3	4	5	6	7-8	9	13	17	21	25	29	33	37	41	45	49	53	54-55
Window Range (days)				+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 2	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 2
Months	-1			1				2			3	4	5	6	7	8	9	10	11	12	13	14	14
Study Schedule																							
Neuropsychological Tests[A]	X			O	O	O	O	O	O	X	O	O	O	X	O	O	X	O	O	X	O	O	X
Lumbar Puncture	X									X													X
AD Biomarkers[B]	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Laboratory Tests[C]	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Neuroimaging																							
MRI	X									X				X			X						X
FDG-PET	X									X							X						X

FPE:Full Plasma Exchange LVPE: Low-Volume Plasma Exchange X: Mandatory for all groups (Treatment Group and Sham Group) B: Before D: During A: After O: ABS & OAS just if it's necessary IV: Intermediate Visit

[A] See separate breakout of Neuropsychologial Tests

[B] See separate breakout of AD Biomarkers

[C] See separate breakout of Laboratory Tests

Visit	Screening		Randomization	FPE 1	FPE 2	FPE 3	FPE 4	FPE 5	FPE 6	IV	LVP E1	LVP E2	LVP E3	LVP E4	LVP E5	LVP E6	LVP E7	LVP E8	LVP E9	LVP E10	LVP E11	LVP E12	Final
Time / Weeks	-3	-2	-1	1	2	3	4	5	6	7-8	9	13	17	21	25	29	33	37	41	45	49	53	54-55
Window Range (days)				+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 2	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 2
Months	-1			1				2			3	4	5	6	7	8	9	10	11	12	13	14	14
Neuropsychologial Tests																							
ADAS-Cog	X									X				X			X			X			X
MMSE	X									X				X			X			X			X
NPS battery	X									X				X			X			X			X
ADCS- ADL	X									X				X			X			X			X
NPI	X									X				X			X			X			X
CDR-Sb	X									X				X			X			X			X
ADCS-CGIC	X									X				X			X			X			X
CSDD	X									X				X			X			X			X
C-SSRS	X									X				X			X			X			X
QoL-AD	X													X			X			X			X
RUD-Lite®	X													X			X			X			X
OAS	O			O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
ABS	O			O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O

FPE:Full Plasma Exchange LVPE: Low-Volume Plasma Exchange X: Mandatory for all groups (Treatment Group and Sham Group) B: Before D: During A: After O: Just if it's necessary IV: Intermediate Visit

Visit	Screening		Randomization	FPE 1	FPE 2	FPE 3	FPE 4	FPE 5	FPE 6	IV	LVP E1	LVP E2	LVP E3	LVP E4	LVP E5	LVP E6	LVP E7	LVP E8	LVP E9	LVP E10	LVP E11	LVP E12	Final
Time / Weeks	-3	-2	-1	1	2	3	4	5	6	7-8	9	13	17	21	25	29	33	37	41	45	49	53	54-55
Window Range (days)				+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 2	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 2
Months	-1			1				2			3	4	5	6	7	8	9	10	11	12	13	14	14
AD Biomarkers																							
CSF Aβ40	X									X													X
CSF Aβ42	X									X													X
CSF T-Tau	X									X													X
CSF P-Tau	X									X													X
CSF β-secretase	X									X													X
CSF γ-secretase	X									X													X
CSF cholesterol	X									X													X
CSF LDL	X									X													X
CSF HDL	X									X													X
CSF VLDL	X									X													X
CSF CRP	X									X													X
CSF Rheumatoid factor	X									X													X
CSF IL-1b	X									X													X
CSF IL-6	X									X													X
CSF Ferritin	X									X													X
CSF TNF-α	X									X													X
CSF Cell counts	X									X													X
CSF Glucosa	X									X													X
CSF Proteins	X									X													X
CSF Albumin	X									X													X

Visit	Screening		Randomization	FPE 1	FPE 2	FPE 3	FPE 4	FPE 5	FPE 6	IV	LVP E1	LVP E2	LVP E3	LVP E4	LVP E5	LVP E6	LVP E7	LVP E8	LVP E9	LVP E10	LVP E11	LVP E12	Final
Time / Weeks	-3	-2	-1	1	2	3	4	5	6	7-8	9	13	17	21	25	29	33	37	41	45	49	53	54-55
Window Range (days)				+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 2	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 2
Months	-1			1				2			3	4	5	6	7	8	9	10	11	12	13	14	14
AD Biomarkers																							
Plasma Aβ40	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma Aβ42	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma β-secretase	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma γ-secretase	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma cholesterol	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma LDL	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma HDL	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma VLDL	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma CRP	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma Rheumatoid factor	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma IL-1b	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma IL-6	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma Ferritin	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma TNF-α	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X
Plasma Sample Collection	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	B+A	X

FPE:Full Plasma Exchange LVPE: Low-Volume Plasma Exchange X: Mandatory for all groups (Treatment Group and Sham Group) B: Before D: During A: After O: Just if it's necessary IV: Intermediate Visit

Visit	Screening		Randomization	FPE 1	FPE 2	FPE 3	FPE 4	FPE 5	FPE 6	IV	LVP E1	LVP E2	LVP E3	LVP E4	LVP E5	LVP E6	LVP E7	LVP E8	LVP E9	LVP E10	LVP E11	LVP E12	Final
Time / Weeks	-3	-2	-1	1	2	3	4	5	6	7-8	9	13	17	21	25	29	33	37	41	45	49	53	54-55
Window Range (days)				+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 1	+/- 2	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 5	+/- 2
Months	-1			1				2			3	4	5	6	7	8	9	10	11	12	13	14	14
Laboratory Tests																							
Blood cell counts	X			B+A	B+A	B+A	B+A	B+A	B+A		B	B	B	B	B	B	B	B	B	B	B	B	X
Platelet counts	X			B+A	B+A	B+A	B+A	B+A	B+A		B	B	B	B	B	B	B	B	B	B	B	B	X
Prothrombin time (Quick)	X			B+A	B+A	B+A	B+A	B+A	B+A		B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	X
aPTT	X			B+A	B+A	B+A	B+A	B+A	B+A		B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	X
Fibrinogen	X			B+A	B+A	B+A	B+A	B+A	B+A		B	B	B	B	B	B	B	B	B	B	B	B	X
Proteinogram	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B	B	B	B	B	B	B	B	B	B	B	B	X
Plasma IgG	X			B+A	B+A	B+A	B+A	B+A	B+A	X	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	X
Calcium	X			B+A	B+A	B+A	B+A	B+A	B+A		B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	B(X) + A(O)	X
AST	X			A	A	A	A	A	A														X
ALT	X			A	A	A	A	A	A														X
Bilirubin	X																						X
LDH	X			A	A	A	A	A	A														X
Creatinine	X			A	A	A	A	A	A														X
Troponin *	X			A	A	A	A	A	A														X
Anti-HIV	X																						X
HCV antibodies	X																						X
HBsV Ag	X																						X

FPE:Full Plasma Exchange

LVPE: Low-Volume Plasma Exchange

X: Mandatory for all groups (Treatment Group and Sham Group)

B: Before

D: During

A: After

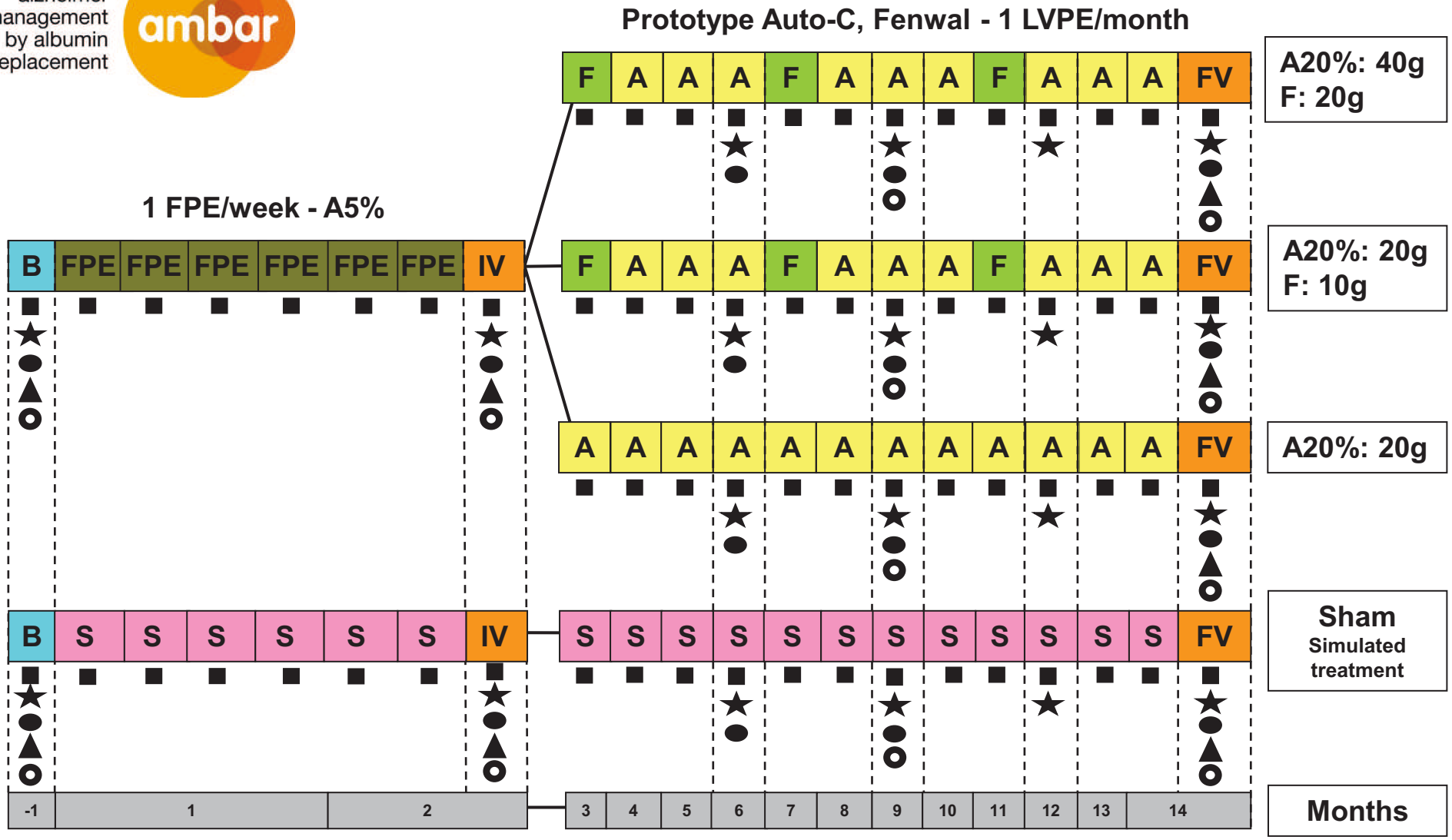
O: Just if it's necessary

IV: Intermediate Visit

*** Troponin will be assessed in a central laboratory.**

alzheimer management by albumin replacement

ambar



FPE: Full Plasma Exchange
LVPE: Low Volume Plasma Exchange
F: Flebogamma DIF 5% (Fixed dose)
A: Albutein 5% - 20% (Weight-dependent dose)

■ : AD Biomarkers
★ : Neuropsychological Tests
● : MRI
▲ : Lumbar Puncture
○ : FDG-PET

APPENDIX 9

SHAM MANUAL GUIDELINE

IG1002 Clinical Trial: Sham Procedure Instructions

Very important: this manual must be accompanied by a DVD with the videos showing the procedures described in it. Please refer to those videos to visually follow the details according to the indications given in these instructions.

If you are to use a peripheral catheter for Full Plasma Exchange (FPE) you will receive a DVD with 3 short videos for the sham procedures:

1) ***Sham procedures FPE + Albutein 5%, peripheral access***
2) ***Sham procedures Low Volume Plasma Exchange (LVPE) + Albutein 20%***
3) ***Sham procedures LVPE + Flebogamma DIF 5%***

If you are to use a central catheter for FPE you will receive a DVD with 4 short videos:

1) ***Simulated jugular central catheter implantation***
2) ***Sham procedures FPE + Albutein 5%, central access***
3) ***Sham procedures Low Volume Plasma Exchange (LVPE) + Albutein 20%***
4) ***Sham procedures LVPE + Flebogamma DIF 5%***

Introduction

A sham procedure will be used in the control group of the multicenter, randomized, controlled study IG1002 to evaluate the efficacy and safety of short-term plasma exchange followed by long-term plasmapheresis with infusion of human albumin combined with intravenous immunoglobulin (IVIG) in patients with mild-moderate Alzheimer's disease. The sham procedure will be applied to both, full plasma exchange and low volume plasma exchange periods:

- Full plasma exchange (FPE) period: one month and half (6 weeks) with one full plasma exchange per week.
- Low volume plasma exchange (LVPE) period: one low volume plasma exchange per month for 12 months.

The study groups will be divided into 4 arms: 3 treatment arms and 1 control (sham) arm. The treatment arms consist of 2 arms that combine plasmapheresis together with albumin and IVIG administration in 2 different doses. The third treatment arm will receive plasmapheresis with albumin only infusions (see protocol section 5 for dosing and scheduling details).

The purpose of the sham procedure is to mimic those two types of plasmaphereses (FPE and LVPE) but with neither fluid exchange nor albumin or IVIG administration.

The following instructions describe how to proceed with the sham procedure in the two different exchange periods. As a general reminder, the control (sham) group will undergo simulated plasma exchanges under similar conditions as the patients in the treatment groups. It is important to make the sham procedure look as 'real' as possible for the benefit of the patients.

Patients undergoing actual and sham procedures must not coincide in the same treatment facility at the same time. That is, if there is more than one patient scheduled for an exchange session, only treatment patients or only control (sham) patients must coincide. This can be achieved, for instance, by scheduling treatment and control patients in

different days. At the same time, family members and caregivers will not be allowed to enter and accompany the patient during the procedure, for both the treatment and control (sham) groups. Exceptionally, agitated patients could be accompanied by the caregiver during the procedure to ease patient's anxiety. In this case it is mandatory the subject to b scheduled for the treatment a different day than the rest of the patients.

1. Central Catheter Implantation

If you are to perform FPE through a peripheral catheter please go directly to section 2, Full Plasma Exchange Procedure.

If you are to perform FPE through a central catheter please follow this section.

Before the first sham FPE replacement session, a simulated implantation of a central catheter will be performed (Please also watch the video entitled *"Simulated jugular central catheter implantation"*).

1.1. Specific materials

1.1.1. Catheter characteristics

A double lumen central catheter type Evenmore®, from Angiodynamics®, with 14.5-15.5 Fr (1 Fr =0.33 mm) and surgical wings joined to the catheter will be used **(Figure 1)**. The catheter will be cut for the sham procedure (see Implantation below).

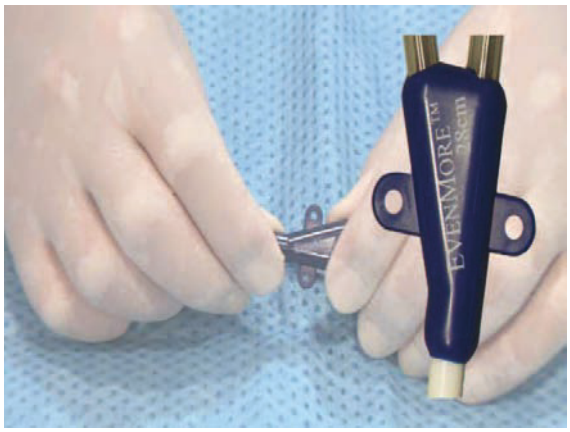


Figure 1: Catheter with surgical wings joined

At clinical sites where a different type of double lumen catheter is used for the actual procedure, the same type of catheter can be used for the sham procedure if the surgical wings are also joined to the catheter.

1.1.2. Round hydrocolloid patch characteristics

A urostomy bag hydrocolloid patch, Assura® 1-piece pediatric urostomy clear bag 10-35 mm, product reference 2135, from the company Coloplast Ltd. will be used to attach it to the catheter **(Figure 2)**. The patch will act as a kind of "second skin" and the cut catheter will be stitched to the patch, not to the skin. The patch is normally bound to the urostomy bag, so it will need to be cut in order to free the round hydrocolloid patch.

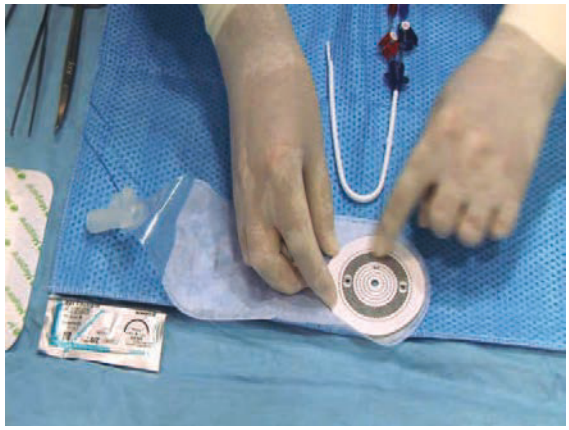


Figure 2: Urostomy bag with hydrocolloid patch

1.1.3. Adhesive gauze dressing characteristics

External adhesive gauzes type Mepore® from the company Mölnlycke Health Care, a water-based polyacrylate adhesive, size 9x15 cm. (3.6 x 6 in) will be used.

1.1.4. Surgical silk characteristics

Surgical silk with curved suture needle size 2/0 will be used.

1.2. Implantation procedure

Patients will be sent to the surgical area following the standard procedure at each site including the pre-medication used at each site for the real procedure.

1.2.1. Central catheter assembly to hydrocolloid patch

First, cut the hydrocolloid patch from the urostomy bag and then discard the bag **(Figure 3)**.

Next, a bevel cut will be made to the catheter (to avoid its insertion into the patient's skin) right before the polyester cuff **(Figure 4)**.

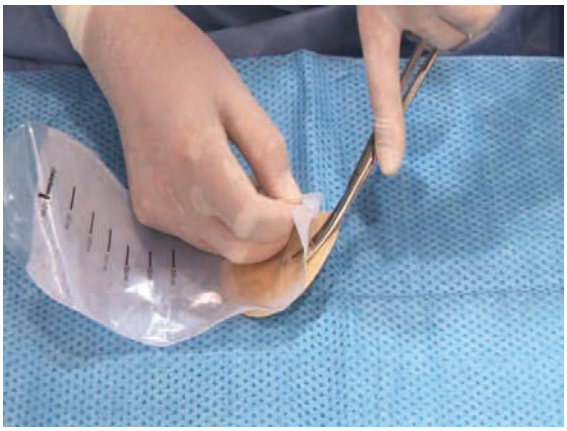


Figure 3: Cut the hydrocolloid patch from the urostomy bag

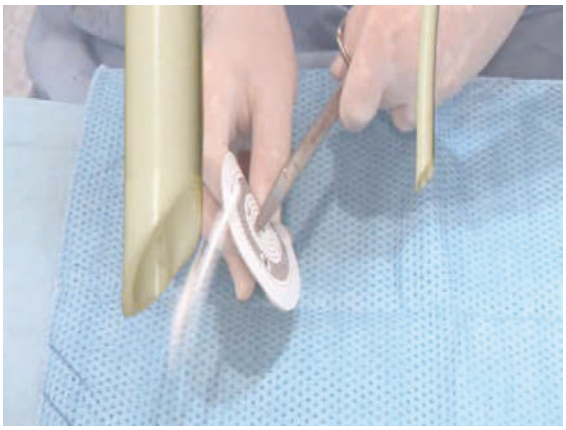


Figure 4: Make a bevel just before catheter's cuff

The cut catheter will be introduced across the central hole of the round hydrocolloid patch allowing the distal part of the catheter to remain slightly under the patch (between the patch and the skin). Then, the catheter will be secured to the hydrocolloid patch with surgical silk (2/0) stitches on both sides of hydrocolloid patch: two stitches at the bottom base where the arterial and venous connection joins (in each of the surgical wings), fixing the catheter onto the front side of the hydrocolloid patch **(Figure 5)**; and two more stitches to secure the distal part of the catheter to the central hole of the patch from the reverse side of hydrocolloid patch **(Figure 6)**.

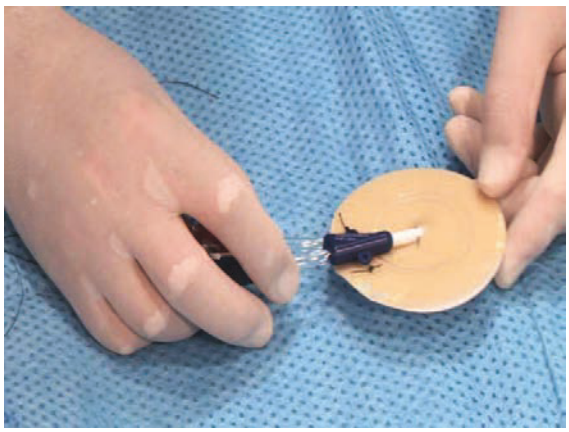


Figure 5: Front side of simulated central catheter

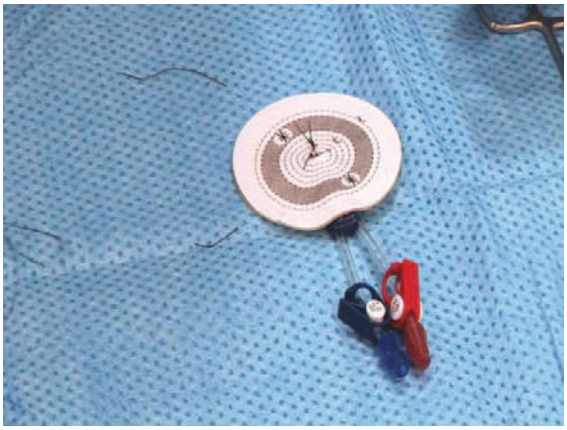


Figure 6: Reverse side of simulated central catheter

1.2.2. Simulated catheter implantation

The anatomical jugular region where the simulated catheter should be implanted will be shaved and cleaned with a standard antiseptic procedure (clean with saline solution and 70% alcohol).

When the implantation region is completely dried, the hydrocolloid patch assembled to the catheter will be adhered on patient's skin.

To further secure the hydrocolloid patch, a plastic skin dressing spray will be applied on the implantation area. After that, an adhesive gauze dressing will cover the implantation area in the same manner as the actual central catheter to avoid visual contact and physical access to the catheter, according standard procedures of each center **(Figure 7)**. Then, catheter Luer locks will be placed and the visible portion of the double catheter will be covered with adhesive gauze. Finally, gauze will cover the apparent catheter's insertion point in the upper part of implantation area.

The patient will be instructed not to remove the catheter or scratch the surrounding area, and how to proceed in case an adverse event related to the catheter occurs.

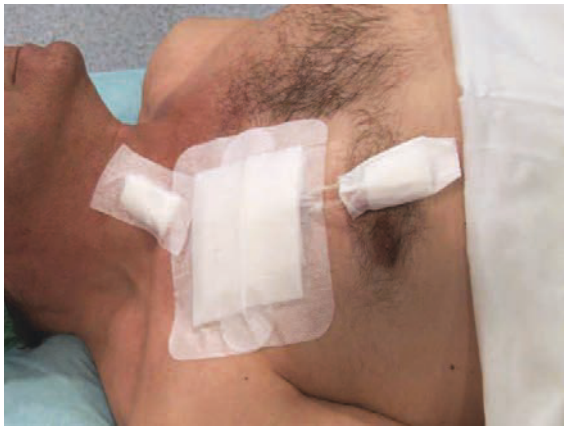


Figure 7: Simulated central catheter dressing

1.3. Removal of simulated catheter's insertion point

In the actual procedure of catheter implantation, two stitches are placed in the catheter insertion point to close the wound and the area is covered with gauze. After 1-2 weeks, these stitches are to be removed and the area will be visible.

In both the treated group and the control (sham) group, some precautions will be taken to assure that the rater maintains his/her blind during the subsequent neuropsychological tests.

Therefore, following the actual procedures, 1-2 weeks after the catheter implantation in treated groups, the stitches will be removed, but one extra step will be needed. Iodine solution will be apply around the insertion point (and wrapped with gauze or plaster) for the length of 2 weeks. It can be removed before the next FPE.

To mimic this situation in the control (sham) patients, at the same time without actually adding any stitches, it also is recommended to apply some iodine solution around the apparent catheter's insertion point and wrap it with gauze for 2 weeks until the next FPE.

Meaning, after 2 weeks more, the central catheter insertion wound is completely healed and the area is uncovered in both groups.

2. Full plasma exchange procedure

(If you are to perform FPE through a peripheral catheter please watch the video entitled *"Sham procedures FPE + Albutein 5%, peripheral access"*. If you are to perform FPE through a central catheter please watch the video entitled *"Sham procedures FPE + Albutein 5%, central access"*).

Before the patient enters the treatment room, some procedures must be prepared beforehand.

First, the saline solution with intravenous iron (Fe) or betadine used as a sham solution will be prepared. To prepare it, 2 or 3 intravenous Fe vials (depending on the commercial brand) will be added to the saline solution until a dark, almost black-color solution is obtained **(Figure 8)**. Otherwise, betadine solution can be used to get the black-color solution. This

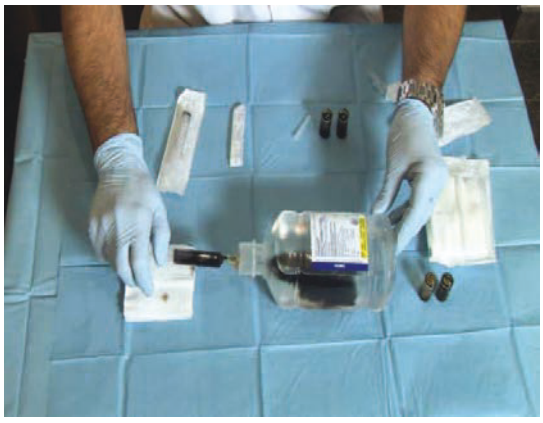


Figure 8: Preparation of the saline solution with intravenous Fe or betadine

will give a realistic plasma-blood color once the sham solution is processed by the plasmapheresis device.

Second, the plasmapheresis device will be setup, and the plasmapheresis kit will be installed. Each site will perform the sham procedure using its standard FPE device. Therefore, the standard procedure of each site will guide the setup of the FPE device.

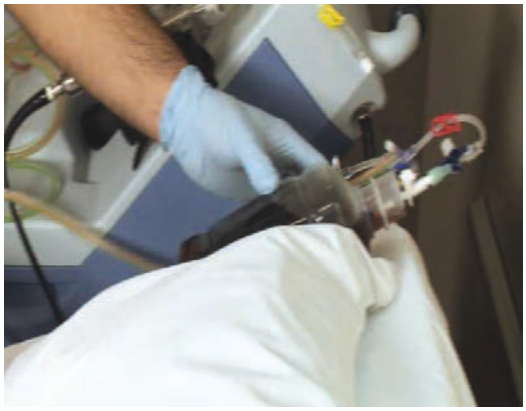


Figure 9: Place the saline solution with intravenous Fe or betadine hidden under the pillow

Third, the saline solution with intravenous Fe or betadine will be connected to the plasmapheresis kit. In case the treatment is done in a bed, the bag will be placed hidden under the patient bed pillow **(Figure 9).**

The bed will be inclined at 45º. In case the treatment is done in a chair the bag will be placed hidden hanging behind the device and will be covered with a cloth to avoid any visual contact.

At this point, the patient will enter the room (family members and caregivers must remain outside of the room). During the sham procedure, blood samples extraction will be real and will be performed according to the Lab Manual. An actual peripheral venous line implantation will be required since blood samples also will be needed pre- and post-plasmapheresis. Vital signs (heart rate, blood pressure, body temperature) will be measured 15-30 minutes before, during and 15-30 minutes after the plasmapheresis procedure.

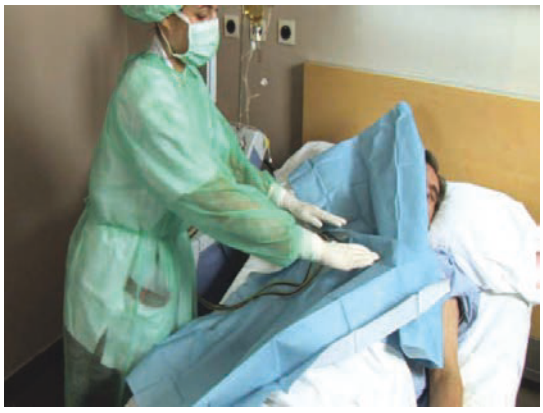


Figure 10: Sham FPE under sterile conditions

In case the sham procedure for FPE is performed by using a central catheter, it

requires the use of a simulated central catheter (as described in section 1) under sterile conditions (**Figure 10**). Therefore, the nurse will be dressed with appropriate clothing, and will work to maintain a sterile environment (for example, cleaning the simulated central catheter).

2.1. Sham connection to peripheral venous line.

(If you are to perform FPE through a peripheral catheter please follow this section. If you are to perform FPE through a central catheter please follow section 2.2, Sham connection to simulated central catheter).

Control (sham) patients will appear to be connected to the FPE device. To simulate the connection, the plasmapheresis kit tubing from the device will be placed next to the peripheral venous line. The plasmapheresis kit tubing will be secured onto the patient's skin with surgical tape and then covered with a sterile cloth to avoid any visual contact from the patient **(Figure 11)**.

Patients will be told that such measures are needed to avoid line contamination. During the simulated connection the patients will be instructed to turn their head in the opposite direction, and it will be covered with a sterile cloth.

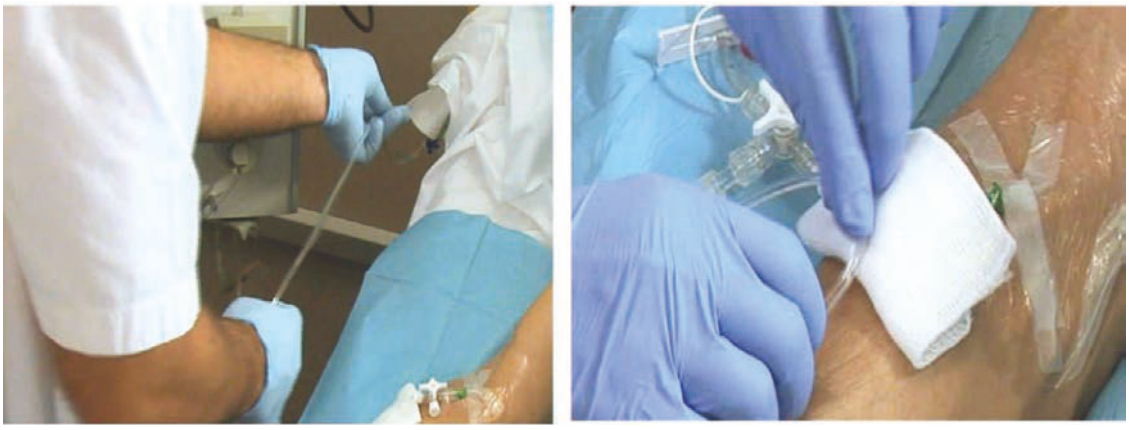


Figure 11: Sham connection to peripheral venous line

2.2. Sham connection to simulated central catheter

(If you are to perform FPE through a central catheter please follow this section. If you are to perform FPE through a peripheral catheter please follow section 2.1, Sham connection to simulated peripheral venous line).

Control (sham) patients will appear to be connected to the FPE device. To simulate the connection, the plasmapheresis kit tubing from the device will be placed next to the visible portion of simulated central catheter and will be secured using surgical tape to the simulated central catheter and covered with a gauze..

The sham connection then will be covered by a sterile cloth to avoid any visual contact from the patient. Patients will be told that such measures are needed to avoid catheter contamination. During the simulated connection the patients will be instructed to turn their head in the opposite direction, and it will be covered with a sterile cloth.

2.3. Sham full plasma exchange procedure

Each site will perform the sham procedure using its standard FPE device. In all cases, two bottles of Albutein® 5% will be placed at the top of the FPE device (same as in the treatment groups) but no albumin infusion will be carried out. In parallel, the saline solution with intravenous iron or betadine will be circulating in a closed-circuit manner through the plasmapheresis kit line. The FPE device will be running for approximately 50-70 minutes; which will be enough time to simulate the real procedure (70-80minutes).

After stopping the FPE device, the disconnection from the plasmapheresis kit will be simulated. Again, the simulation of catheter cleaning will be performed and new clean adhesive gauzes will be placed. The staff that disconnects the patient from the device does an evaluation of the catheter's fastening. If any of the sides is unfastened a plastic skin dressing spray will be applied to seal the sides. After that, an adhesive gauze dressing will cover the implantation area in the same manner as described in 1.2.2.
During the simulated disconnection, the patients will be instructed again to turn their head in the opposite direction, and it will be covered with a sterile cloth.

Post-plasmapheresis blood samples will be obtained and the peripheral venous line will be removed. Vital signs will be measured once again, and the patient will be let to rest and recover from the plasmapheresis procedure for approximately 45 minutes.

Some adhesive gauze dressings will be given to the caregiver. They will be instructed that in case the dressing is unfastened they must cover the old dressing with new adhesive gauze. The catheter must not be manipulated either taken off under any circumstances.

3. Low volume plasma exchange

3.1. Low volume plasma exchange with Albutein® 20%

(Please watch the video entitled *"Sham procedures LVPE + Albutein 20%"*).

A prototype device based on the Autopheresis-C from Fenwal will be used for the LVPE in all sites.

Before the patient enters the treatment room, some procedures must be prepared beforehand.

First, the Fenwal device will be setup and calibrated, the plasmapheresis kit will be installed, and anticoagulant and albumin bottles (two bottles connected through a Fleboset® kit from Grifols) will be hanged on top of the device. The Fenwal device will be programmed by the nurse to obtain 200 mL of plasma, and infuse 50 mL of replacement fluid.

Second, human blood bags (expired or with non-standard volume) will be used as sham solution for this procedure. In some cases, the blood bag for the simulation will be prepared by discarding the donation kit coupled to the blood bag. A spike will be inserted into the blood bag. Then, the blood bag will be connected to the plasmapheresis kit **(Figure 12).** In case the treatment is done in a bed, the blood bag will be placed hidden under the sheet (**Figure 13)**, covered with the patient bed pillow, and the bed inclined at 45º.

In case the treatment is done in a chair, the blood bag will be placed hidden hanging behind the device and covered by a gauze

Figure 12: Blood bag connection to the plasmapheresis kit

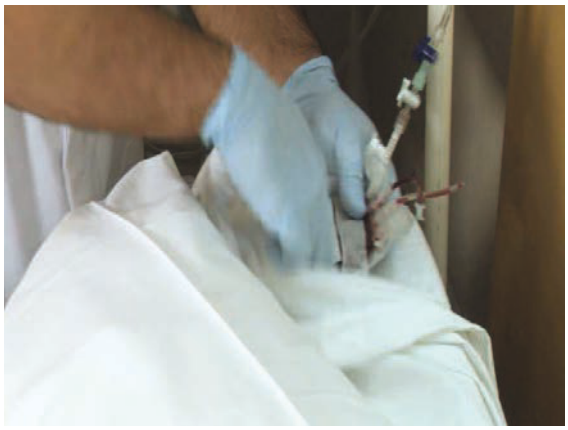


Figure 13: Blood bag hidden under the patient pillow

At this point the patient will enter the treatment room (family members and caregivers must remain outside of the room). During the sham procedure, blood samples extraction will be real according to the Lab Manual. An actual peripheral venous line implantation will be required, since blood samples will be also needed pre- and post-plasmapheresis. Vital signs (heart rate, blood pressure, body temperature) will be measured 15-30 minutes before, during and 15-30 minutes after the plasmapheresis procedure

3.1.1. Sham connection to peripheral venous line.

Control (sham) patients will appear to be connected to the Fenwal device. To simulate the connection, the plasmapheresis kit tubing from the device will be placed next to the peripheral venous line. The plasmapheresis kit tubing will be secured onto the patient's skin with surgical tape and then covered with a sterile cloth to avoid any visual contact from the patient **(Figure 14)**. Patients will be told that such measures are needed to avoid line contamination. During the simulated connection the patients will be instructed to turn their head in the opposite direction, and it will be covered with a sterile cloth.

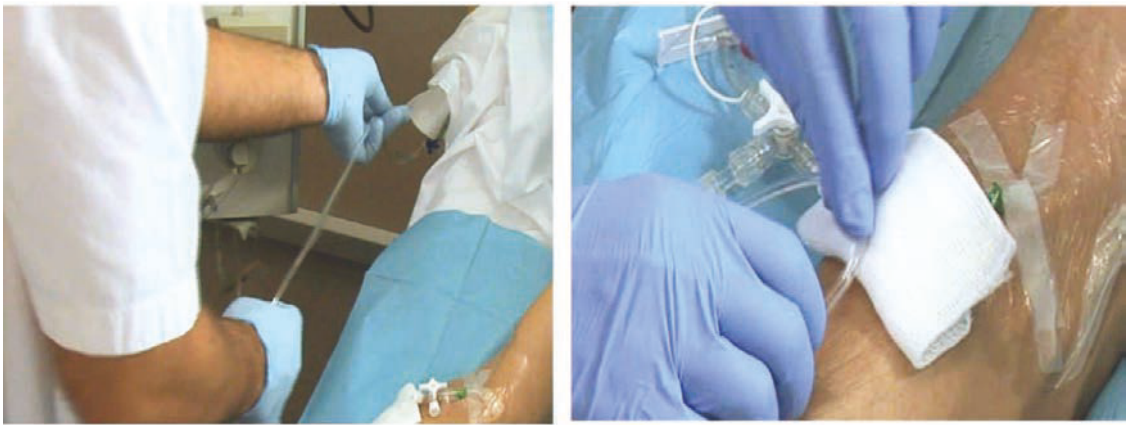


Figure 14: Sham connection to peripheral venous line

3.1.2. Sham low volume plasma exchange procedure with albumin

The Fenwal Autopheresis-C prototype will provide an apparently and realistic working status, in which human blood will be circulating in a closed-circuit manner. Each session will last one cycle that is approximately 60-80 minutes in duration, which will be enough time to simulate the real procedure.(90-120 minutes)

In the case of the sham LVPE, 2 bottles of Albutein® 20% will be placed at the top of the Fenwal device. As defined by the protocol, at the end of the actual LVPE, albumin will be infused in the treatment groups. For this reason in the control (sham) group, after stopping the Fenwal device, the albumin line will be removed. This will allow, simply by gravity, the albumin bottles to empty and the liquid will be collected in the reservoir, maintaining the closed-circuit of the whole procedure (**Figure 15)**.

Disconnection from the plasmapheresis kit will be simulated **(Figure 16)**. During the simulated disconnection, the patients again will be instructed to turn their head in the opposite direction, and it will be covered with a sterile cloth.

Post-plasmapheresis blood samples will be obtained and then the peripheral venous line will be removed. Vital signs will be measured once again, and the patient will be let to rest and recover from the plasmapheresis procedure for approximately 45-60 minutes.

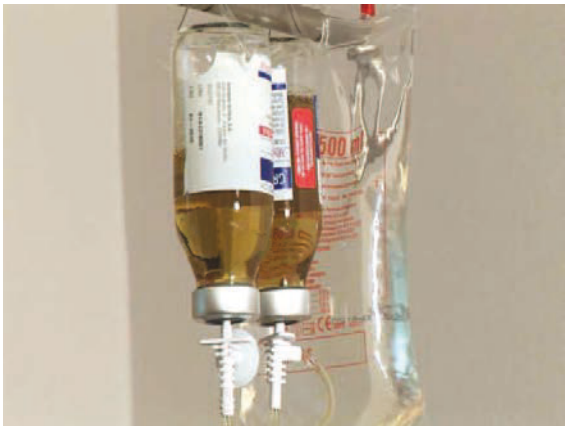


Figure 15: Sham albumin infusion

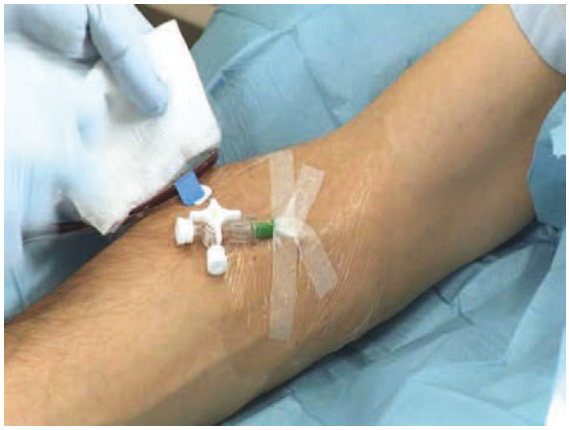


Figure 16: No real connection is performed in sham LVPE

3.2. Low volume plasma exchange with IVIG

(Please watch the video entitled *"Sham procedures LVPE + Flebogamma DIF 5%"*).

The LVPE with IVIG infusion is very similar to the LVPE with albumin infusion. Therefore, the exact same setup of the Fenwal device and simulated connection will be used as it was explained in the section above.

The main difference occurs that after one running cycle of the Fenwal device, some extra steps will be added since a perfusion pump is needed to infuse IVIG.

3.2.1. Sham low volume plasma exchange procedure with IVIG

First, after stopping the Fenwal device, the disconnection from the plasmapheresis kit will be simulated. During the simulated disconnection the patients will be instructed to turn their head in the opposite direction, and it will be covered with a sterile cloth.

Second, a masked saline solution bottle or bag (used as sham IVIG vial) will be placed at the top of the IVIG perfusion pump. The IVIG perfusion pump will be setup and an

empty bag will be connected to the perfusion kit to collect the saline solution coming out from the perfusion pump.

Third, a simulated connection of the IVIG perfusion pump to the peripheral venous line will be performed. As before, the perfusion kit tubing will be placed next to peripheral venous line and it will be secured with surgical tape. Additional adhesive gauze will be placed and a sterile cloth will cover the patient's arm (**Figure 17)**. During the simulated connection, the patients will be instructed to turn their head in the opposite direction and it will be covered with a sterile cloth.

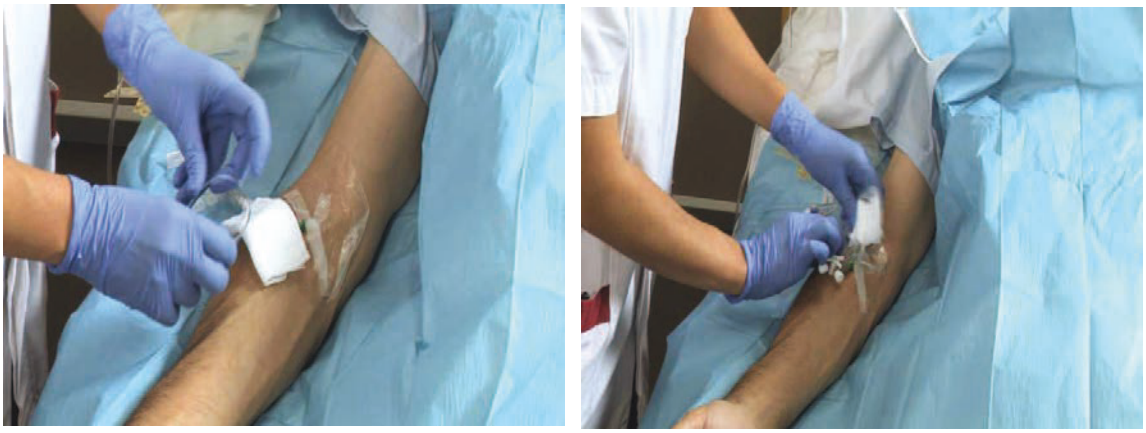


Figure 17: Sham IVIG perfusion kit connection to peripheral venous line

Fourth, the IVIG perfusion pump will be programmed by the nurse to proceed with a realistic working status with the appropriate rate for intravenous infusion. The IVIG perfusion pump will be running for approximately 60 minutes.

After stopping the IVIG perfusion pump, the disconnection from the perfusion kit will be simulated. During the simulated disconnection the patients will be instructed to turn their head in the opposite direction, and it will be covered with a sterile cloth.

Post-plasmapheresis blood samples will be obtained and then the peripheral venous line will be removed. Vital signs will be measured once again and the patient will be let to rest and recover from the plasmapheresis procedure for approximately 15 minutes.

Very important: this manual must be accompanied by a DVD with the videos showing the procedures described in it. Please refer to those videos to visually follow the details according to the indications given in these instructions.

If you are to use a peripheral catheter for Full Plasma Exchange (FPE) you will receive a DVD with 3 short videos for the sham procedures:

1) ***Sham procedures FPE + Albutein 5%, peripheral access***
2) ***Sham procedures Low Volume Plasma Exchange (LVPE) + Albutein 20%***
3) ***Sham procedures LVPE + Flebogamma DIF 5%***

If you are to use a central catheter for FPE you will receive a DVD with 4 short videos:

1) ***Simulated jugular central catheter implantation***
2) ***Sham procedures FPE + Albutein 5%, central access***
3) ***Sham procedures Low Volume Plasma Exchange (LVPE) + Albutein 20%***
4) ***Sham procedures LVPE + Flebogamma DIF 5%***

APPENDIX 10

HYPOVOLEMIA GUIDELINE

GRIFOLS

[redacted]

alzheimer management by albumin replacement

ambar

AMBAR
Protocol Number IG1002

Recommendations for preventing hypotension related events in Low Volume Plasma Exchange procedures: Infusion of saline solution and adjustment of removed plasma volume in low-weight patients.

Date: 24SEP2014

Site: All Sites (Spain and US)

1. Infusion of saline solution

This recommendation is only applicable to the LVPE procedures with Albutein®.

In LVPE with Albutein® infusion, a volume of 690-880ml of plasma is extracted during the procedure, depending on the patient's weight. However, based on the patient's weight and/or treatment arm, only a volume of 80-200ml of Albumin 20% is infused during the procedure. Due to the difference in volume, saline solution can be infused to reduce the risk of hypotension related events.

How to proceed for the LVPE with Albutein® infusion:

1- Perform plasma collection regularly
2- Infuse Albutein® regularly
3- Measure vital signs after infusion
4- Collect blood samples for laboratory tests
5- Infuse saline solution as recommended in table 1 (see below).
6- Measure vital signs after saline infusion

2. Adjustment of removed plasma volume in patients who weight less than 110 lbs.

This recommendation is applicable to ALL LVPE procedures (Albutein® and Flebogamma 5% DIF®).

In those patients whose weigth is less than 110 lbs., the volume of plasma to remove will be proportional to the actual weight and calculated according to the following formula:

$$Plasma\,volume\,to\,collect\,(mL) = \frac{Weight\,(lbs)\,x\,690\,mL}{110\,lbs}$$

(see table 1 below as well).

For example, for a patient weighting 100 lbs:

$$Plasma\,volume\,to\,collect\,(mL) = \frac{100\,lbs\,x\,690\,mL}{110\,lbs} \approx 630\,mL$$

Table1. Removal and replacement volume during LVPE

REMOVAL AND REPLACEMENT VOLUME DURING LVPE					
Patient weight	**Plasma collected**	**Albutein® to infuse depending on the treatment arm**		**+**	**Saline Solution to infuse (arm 1, 2 &3)**
		Full dose (arm 1)	**½ dose (arm 2 &3)**		
< 110 lbs	**Adjusted Volume†**	**160 ml**	**80 ml**	**+**	**350 ml**
110-149 lbs	**690 ml**	**160 ml**	**80 ml**	**+**	**350 ml**
150-174 lbs	**825 ml**	**190 ml**	**95 ml**	**+**	**400 ml**
175-999 lbs	**880 ml**	**200 ml**	**100 ml**	**+**	**500 ml**

†: Plasma volume to collect (ml) = (Patient weight in lbs X 690 ml) / 110 lbs

These procedures are not listed in the protocol and they are recommended to be performed at the discretion of the Investigators and Plasmapheresis Teams.

Finally, as in regular apheresis practice, it is recommended that patients be placed in Trendelemburg position for ALL LVPE procedures.

Signature: _____
Name of Person:
Job title:

Signature: _____
Name of Person:
Job title:

Signature: _____
Name of Person:
Job title:

APPENDIX 11

FEVER MANAGEMENT GUIDELINE

GRIFOLS

AMBAR
Protocol Number IG1002

alzheimer management by albumin replacement — ambar

Guideline to manage fever during full plasma exchange (FPE) period in patients with a central line

1- Upon the detection of fever, the patient or the caregiver will call the Investigator and will go to a nearby Emergency Room (ER) hospital (depending on each site the reference hospital will be different).

2- In the ER an anamnesis and a complete physical examination will be done focused on investigating any potential infection focus. As a routine, an electrocardiogram, thoracic X-ray, blood test with blood count, basic biochemistry and acute phase reactants, urine analysis and urine culture should be done.

3- In case patient's temperature is higher than 37.5ºC (99.5ºF) blood culture from the central line and peripheral blood will be collected. The microbiologist should be asked to inform as quick as possible about any microorganism growth from both, the peripheral and central samples, and the time difference, if any, between both.

4- With the information obtained during the anamnesis, the physical examination and the additional tests done in the ER, 3 situations can be considered:

   a. There are infection signs of soft tissues in the **catheter insertion area but no other apparent infectious foci:** In this case the intervention will be according to the following systemic involvement grade:

      i. **If there are no clinical or analytical data of systemic involvement** the patient can be discharged and **the cutaneous infection** can be treated empirically with amoxicillin and clavulanic acid at a dose of:
      875/125mg 3 times per day during one week.
      A different antibiotic active against the main microorganisms responsible of cutaneous around-catheter bacterial infections can be used. The Investigator will contact the patient by phone frequently. The patient will

be given a Temperature Log Form which will be completed by the caregiver.

ii. **If there is high fever (>38ºC, >100ºF) and systemic involvement** (unconsciousness, tachycardia, hypotension) the patient should be admitted to the hospital. The catheter should be removed and the patient will be treated with a broad-spectrum empiric antibiotic while waiting the culture results. The treatment regimen should cover the most frequent bacteria related to catheter infections; for example, Meropenem (ultra-broad-spectrum injectable antibiotic).
Meropenem 1g I.V. every 8 hours + linezolid 600mg I.V. every 12 hours*
Afterwards the treatment will be according to the antibiogram results. The catheter removal involves subject's study withdrawn.

b. There is the existence of **clinical and analytical data of an infectious focus other than the catheter:** An UTI (urinary tract infection), a respiratory infection or other infectious focus is diagnosed. In this case the patient will be treated according to the involvement grade:

i. **If the patient is in good general condition**, does not have high fever, there is no unconsciousness, and the infection can be treated out of the hospital in safe conditions, patient can be discharge with an empiric antibiotic treatment. It is mandatory to daily follow the patient by phone and to be in contact with the microbiologist, who will inform about the culture results. If after 48-72 hours the patient condition has improved, the patient should continue with the treatment regimen; if there is no improvement or there is a worsening of the patient condition, it should go again to the reference ER hospital to get a reevaluation.

ii. **If the patient is in bad general condition**, has high fever, hemodynamic effects, unconsciousness or the infection is severe (for example, pneumonia), the patient should be admitted to the hospital and be treated. Treatment with empiric antibiotics is started while waiting the culture results.

c. The patient has fever and there is **not clinical or analytical data that suggest a different infectious focus other than the catheter:** In this case the patient always should be admitted to the hospital and remain under observation.
There are 2 possible scenarios:

i. **If the patient is in good general condition** the treatment can wait until the culture results are obtained. A negative blood and catheter culture

results, rules out a catheter infection as a focus. If the good general condition remains the patient could be discharged and be controlled by phone. If the blood and catheter culture results are positive the catheter should be removed and the bacterial infection should be treated.
In any case, both situations require patient's admission until the culture results are obtained.
If the removal of the catheter is required, this involves subject's study withdrawn.

ii. **If the patient is in bad general condition**, even if there is no local infection around the catheter's insertion point; the catheter should be removed and broad-spectrum antibiotic therapy should be started.
The recommended treatment is:
Meropenem 1g I.V. every 8 hours + linezolid 600mg I.V. every 12 hours*
while waiting the culture results.
The catheter removal involves subject's study withdrawn.

* The antibiotic protocol is a recommendation. You can follow other protocols taking into account that:

- We should include treatment for all skin bacteria usually involved in cutaneous infections.
- We should also include treatment for anaerobic bacteria.
- The catheter of these patients are manipulated weekly, therefore we should include treatment for nosocomial bacteria.

Meropenem is recommended because it is a carbapenem and includes a high number of gram negative, anaerobic and nosocomial bacteria coverage.

To include the rest of the gram positive bacteria coverage, Linezolid is recommended because it is easier to handle than vancomycin.

Meropenem's adverse events are: diarrhea, gastrointestinal discomfort and transaminase increase. Regarding Linezolid the most frequent adverse events are: cutaneous rash, diarrhea and gastrointestinal discomfort and in rare occasion thrombocytopenia.

GRIFOLS

Bibliography

Bambauer, R. et al., 2012. Surface-Treated versus Untreated Large-Bore Catheters as Vascular Access in Hemodialysis and Apheresis Treatments. *International journal of nephrology*, 2012, p.956136.
Available at:
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3332189&tool=pmcentrez&rendertype=abstract [Accessed February 7, 2013].

Maki, D.G., Kluger, D.M. & Crnich, C.J., 2006. The risk of bloodstream infection in adults with different intravascular devices: a systematic review of 200 published prospective studies. *Mayo Clinic proceedings Mayo Clinic*, 81(9), pp.1159–1171.
Available at: http://www.mayoclinicproceedings.com/cgi/doi/10.4065/81.9.1159.

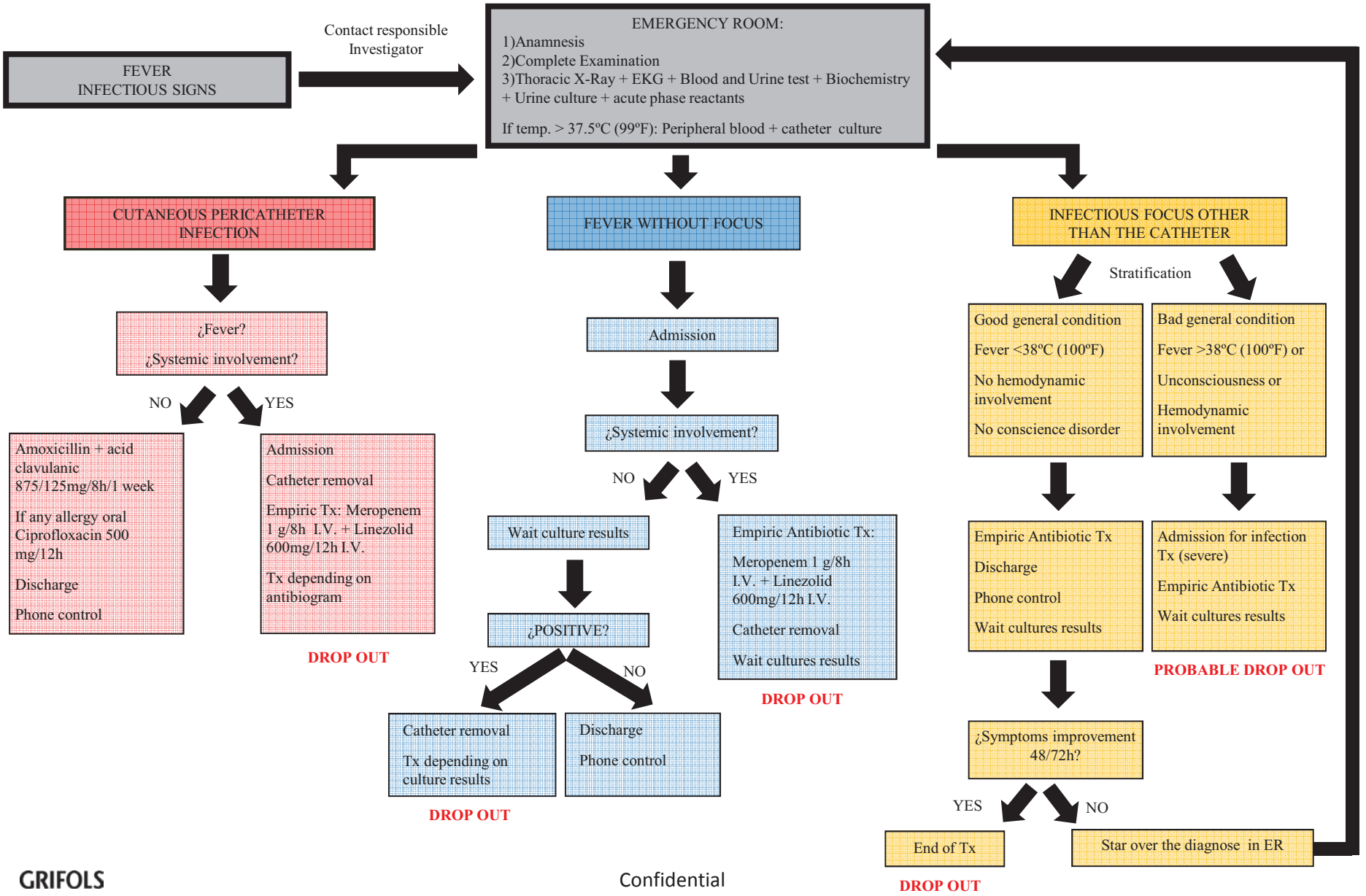
Mermel, L. a et al., 2009. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 49(1), pp.1–45.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/19489710 [Accessed January 28, 2013].

O'Grady, N.P. et al., 2002. Guidelines for the prevention of intravascular catheter-related infections. *Clinical Infectious Diseases*, 35(9), pp.1281–1307.
Available at:
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3106269&tool=pmcentrez&rendertype=abstract.

alzheimer management by albumin replacement

ambar

Appendix 11 – Guideline for Fever Management



APPENDIX 12

THROMBOEMBOLIC EVENTS GUIDELINE

GRIFOLS

AMBAR
Protocol Number IG1002

alzheimer management by albumin replacement — ambar

Guideline for thromboembolic events during the full plasma exchange (FPE)

A) Suspicion of venous thrombosis related to the catheter

1- In case of cervical pain, neck or superior limb edema, odynophagia or pulsating cephalea, the caregiver will call the Investigator and will go to a nearby Emergency Room (ER) hospital (depending on each site the reference hospital will be different).

2- In the ER an anamnesis and a complete physical examination, an electrocardiogram, thoracic X-ray, blood test with blood count, basic biochemistry and coagulation will be done.

3- In case the clinical evaluation of the patient shows a specific cause not related to the catheter as the source of the symptoms (pharyngitis for example) it will be specifically treated.

4- In case the clinical evaluation points out towards a **thrombotic complication** related to the catheter a **CT Angiography with Venous-Phase** should be done. It will allow identifying the thoracic and neck veins. In case this angiography cannot be performed, a **Doppler echocardiography** made by expert staff will be done.

   a. If the result is **NEGATIVE** and the general condition is good the patient is discharged and will be controlled by phone and treated symptomatically if needed.

   b. If the result is **POSITIVE** there are two scenarios:

      i. If the patient **has fever and infection signs** in addition to a proved thrombosis, the catheter should be removed and the patient admitted to the ER. Perform blood cultures, start anticoagulation treatment and broad-spectrum antibiotic therapy:
      Meropenem 1 g/8h I.V. + Linezolid 600 mg/12h I.V. or similar*
      If a bacteremia is confirmed an **echocardiogram** should be done to rule out infectious endocarditis.

ii. If the **CT Angiography** with Venous-Phase **shows thrombosis** shows thrombosis, the patient has no fever and the general condition is good, the catheter will be removed and treatment will be started with:
Enoxaparin 1 mg/Kg/12h S.C. or 1.5 mg/Kg/24h.
The patient will be discharged and controlled by phone. It will be sent as soon as possible to a Clinical Hematologist who will advise the patient about the long or short-term anticoagulant therapy.

5- If there is clinical suspicious of **pulmonary embolism** (dyspnea, O_2 desaturation, tachycardia, syncope) it should be complemented with a **CT Angiography of the Pulmonary Arteries.** A Pulmonary Thromboembolism (PTE) confirmation always needs anticoagulation treatment and admission to the ER (see Section B).

6- The appearance of any thromboembolic complication always means the subject is withdrawn from the study.

B) Pulmonary thromboembolism suspicion

1- If a subject has a sudden onset of shortness of breath (dyspnea) and/or syncope, tachycardia or flank pain the caregiver will call the Investigator and will go to a nearby Emergency Room (ER) hospital (depending on each site the reference hospital will be different).

2- In the ER an anamnesis and a complete physical examination, an electrocardiogram, thoracic X-ray, blood test with blood count, basic biochemistry, coagulation and arterial blood gas will be done.

3- In case the clinical evaluation of the patient shows a specific cause not related to the catheter and as the source of the symptoms it will be specifically treated.

4- In case the clinical evaluation points out towards a **PTE** related to the catheter a **CT Angiography with Venous-Phase** (allowing the identification of thoracic and neck veins) and also a **CT Angiography of the Pulmonary Arteries** should be done. In case this angiography cannot be performed, a **Doppler echocardiography** made by expert staff will be done. Depending on the results of the tests there are 3 scenarios:

a. If both results are **NEGATIVE** it will be reevaluated to look for other causes. If any other causes are found and the patient is in good general condition the patient is discharged and controlled by phone.

b. In case a **PTE is diagnosed**, the patient should be admitted to the ER and the catheter removed. Treatment will be started with:
Sodium Heparin or low-molecular-weight heparin.
When the subject is discharged it should follow low-molecular-weight heparin or oral anticoagulation therapy and visit a Clinical Hematologist.

c. In case a **venous thrombosis (but not a PTE)** is diagnosed the subject will be treated as a PTE: the catheter will be removed and the patient admitted to the ER. Anticoagulation treatment will be started. A Pulmonary ventilation/perfusion scan will be done because a diagnose of PTE is highly probable.

5- The appearance of any thromboembolic complication always means the subject is withdrawn from the study.

6- The catheter will be always removed except in case of an intra-auricular thrombus. If it is suspected, an **Echocardiogram** should be done to rule it out. It is a highly infrequent complication but potentially severe. The best management of this situation is unclear but could require urgent cardiac surgery.
In this situation anticoagulation treatment with sodium heparin will be started. The catheter will not be removed due to the high risk of embolism and the subject will be urgently sent to a hospital where cardiac surgery can be assessed.

* The antibiotic protocol is a recommendation. You can follow other protocols taking into account that:

- We should include treatment for all skin bacteria usually involved in cutaneous infections.
- We should also include treatment for anaerobic bacteria.
- The catheter of these patients are manipulated weekly, therefore we should include treatment for nosocomial bacteria.

Meropenem is recommended because it is a carbapenem and includes a high number of gram negative, anaerobic and nosocomial bacteria coverage.

To include the rest of the gram positive bacteria coverage, Linezolid is recommended because it is easier to handle than vancomycin.

Meropenem's adverse events are: diarrhea, gastrointestinal discomfort and transaminase increase. Regarding Linezolid the most frequent adverse events are: cutaneous rash, diarrhea and gastrointestinal discomfort and in rare occasion thrombocytopenia.

GRIFOLS

Bibliography

Bambauer, R. et al., 2012. Surface-Treated versus Untreated Large-Bore Catheters as Vascular Access in Hemodialysis and Apheresis Treatments. *International journal of nephrology*, 2012, p.956136.
Available at:
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3332189&tool=pmcentrez&rendertype=abstract [Accessed February 7, 2013].

Burns, K. & McLaren, A., 2009. Catheter-related right atrial thrombus and pulmonary embolism: A case report and systematic review of the literature. *Journal of the Canadian Thoracic*, 16(5), pp.163–165.
Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2779172/ [Accessed February 7, 2013].

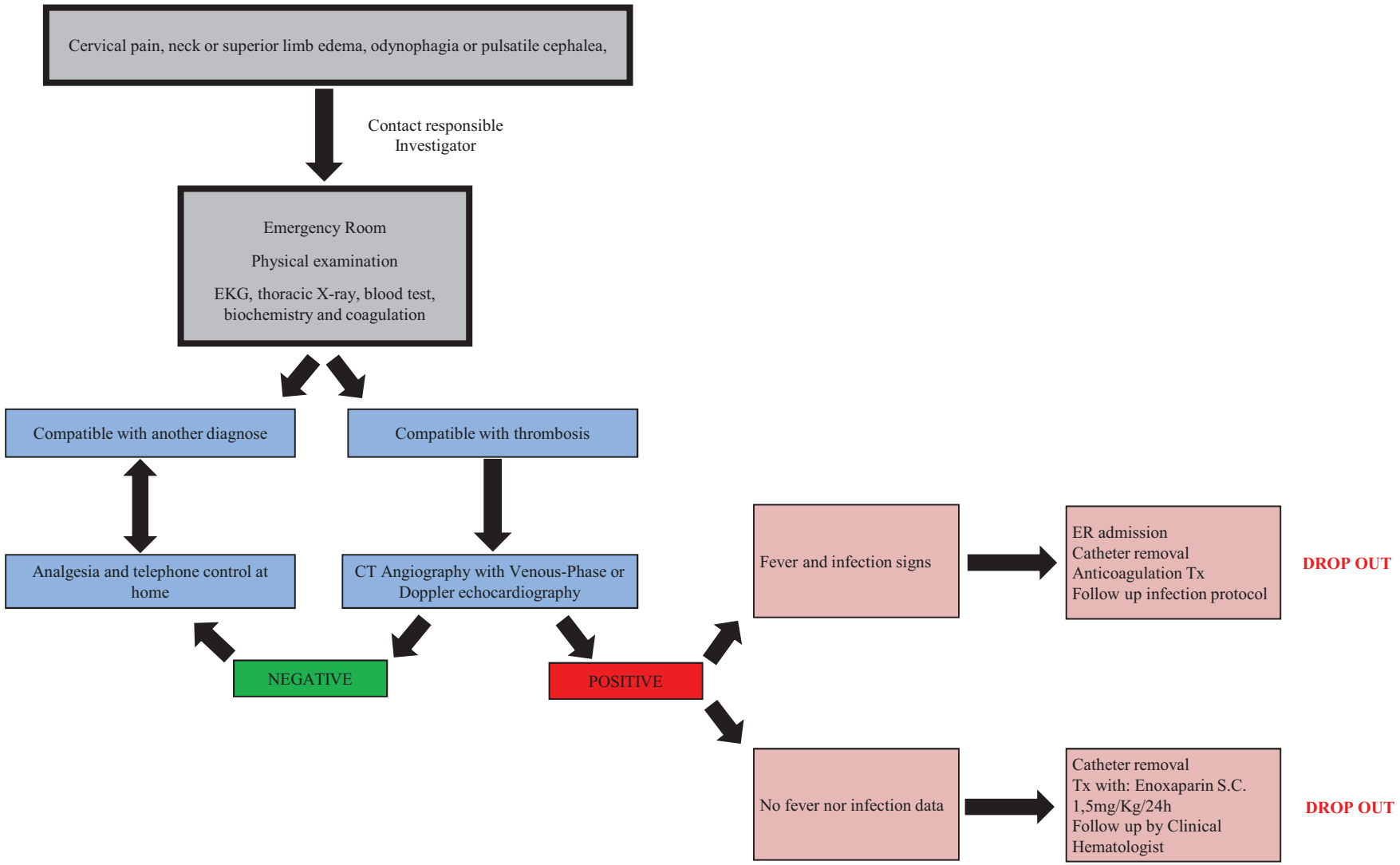
Debourdeau, P. et al., 2013. International clinical practice guidelines for the treatment and prophylaxis of thrombosis associated with central venous catheters in patients with cancer. *Journal of thrombosis and haemostasis* : JTH, 11(1), pp.71–80.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/23217208 [Accessed February 7, 2013].

Stavroulopoulos, A., Aresti, V. & Zounis, C., 2012. Right atrial thrombi complicating haemodialysis catheters. A meta-analysis of reported cases and a proposal of a management algorithm. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - *European Renal Association,* 27(7), pp.2936–44.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/22187317 [Accessed January 29, 2013].

alzheimer management by albumin replacement

Appendix 12 – Guideline for thromboembolic events

A) Suspicion of venous thrombosis related to the catheter

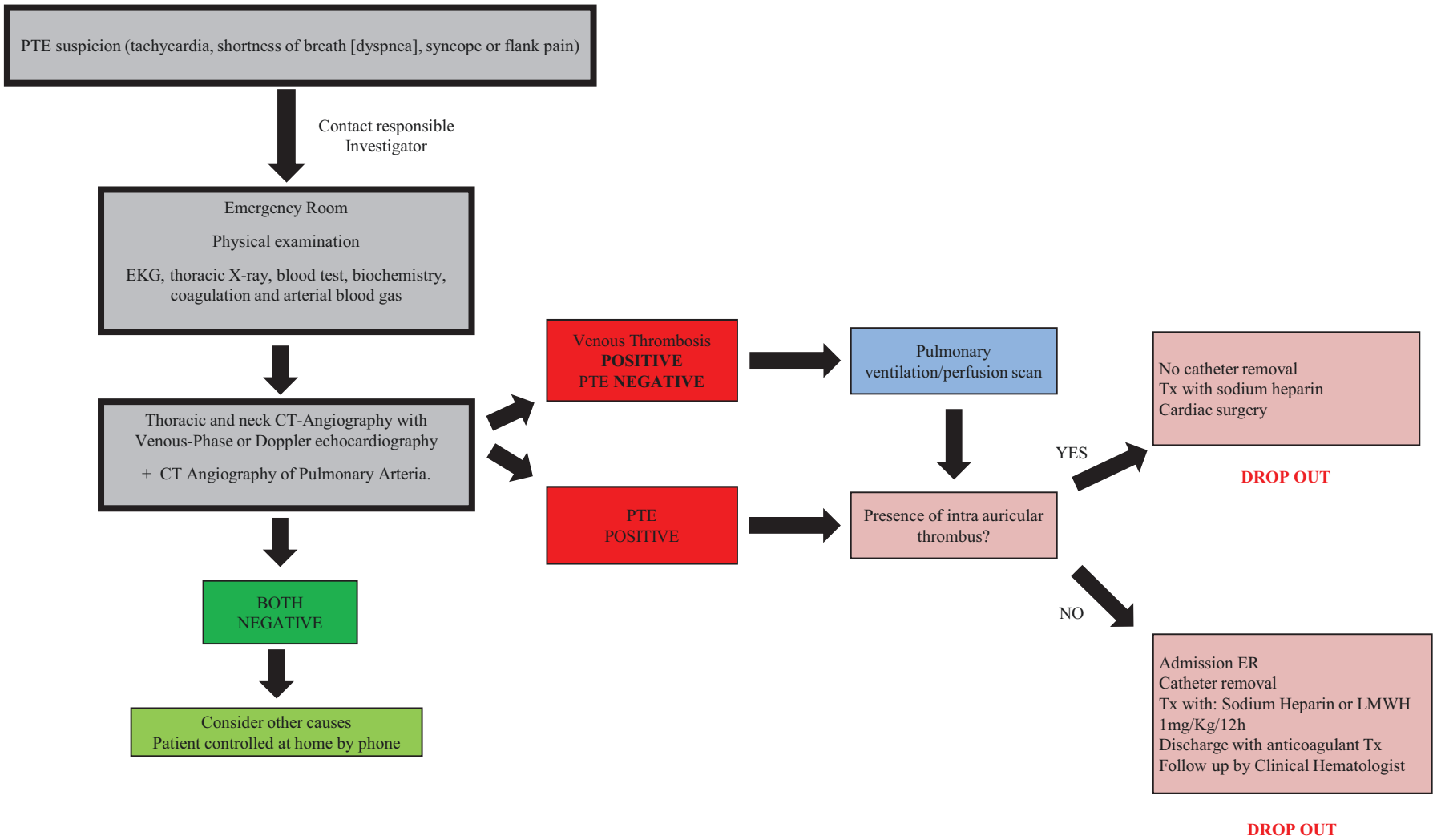


GRIFOLS

alzheimer management by albumin replacement

Appendix 12 – Guideline for Thromboembolic Events

B) Pulmonary thromboembolism (PTE) suspicion



GRIFOLS