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تاریخ: ۱۴۰۰/۰۷/۲۹
پیوست: ندارد



مدیر عامل محترم شرکت تامین فارمد تهران

موضوع: جمع آوری داروی وارداتی فلیوگاما از بازار مصرف به سری ساخت شماره 82N515

با سلام و احترام؛

با توجه به عدم تائید نتایج حاصل از آزمایش فرآورده ویال ۱۰۰ میلی لیتری (50 mg/ml) حاوی محلول تزریقی ایمونو گلوبولین نرمال وریدی با نام تجاری *FLEMOGAMMA* (با ارسال نمونه به شماره لیمس ۷۹۹۷۷۲، موضوع نامه ثبت شده به شماره ۹۱۳/۴۸۴۳۶ مورخ ۱۴۰۰/۶/۳۰ شرکت تامین فارمد تهران) مقرر فرمایید بلافاصله نسبت به جمع آوری فرآورده فوق از سری ساخت شماره 82N515، اقدام نموده و نتیجه ابتدایی و نهایی ریکال به ترتیب ظرف مدت ۳ و ۱۰ روز از تاریخ این نامه به این اداره کل اعلام گردد.

دکتر سید حیدر محمدی
مدیر کل امور دارو و مواد تحت کنترل

رونوشت :

جناب آقای مهندس امیری مدیر کل محترم دفتر فناوری اطلاعات و سامانه های سلامت: جهت اعمال ریکال IRC
معاون محترم غذا و داروی دانشگاه های علوم پزشکی و خدمات بهداشتی درمانی سراسر کشور
جناب آقای دکتر شمالی سرپرست محترم معاونت برنامه ریزی اداره کل امور دارو و مواد تحت کنترل

تهران روبروی درب اصلی دانشگاه تهران، خیابان فخر رازی، نبش خیابان شهید وحید نظری، سازمان غذا و دارو

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بسمه تعالی
«سال تولید، پشتیبانی ها، مانع زدائی ها»



شماره: ۱۶۳۴/ص/۰۰۱

تاریخ: ۱۴۰۰/۰۶/۳۰

پیوست: دارد

جناب آقای دکتر محمدی
مدیر کل امور دارو و مواد تحت کنترل سازمان غذا و دارو

موضوع: درخواست نمونه برداری از داروی فوریتی بیولوژیک

با سلام و احترام،

به استحضار می رساند شرکت تامین فارمد تهران یک محموله از داروی IVIG را وارد نموده است که اطلاعات آن ارسال می گردد.

نام ژنریک فرآورده:	نام تجاری فرآورده:
IMMUNE GLOBULIN INJECTION INTRAVENOUS	FLEBOGAMMA DIF INJECTION INTRAVENOUS
دوز: 50mg/1 mL	حجم: 100 mL
نام سازنده: Instituto Grifols S.a.	کشور سازنده: اسپانیا
تاریخ تولید: 04-2020	تاریخ انقضاء: 05-2022
شماره سری ساخت: 82N515	تعداد محموله: 1104
تعداد نمونه لازم: ۳ عدد	برگه آنالیز و روش آنالیز: پیوست شده است.

خواهشمند است جهت ارسال نمونه به آزمایشگاه کنترل غذا و دارو، دستورات لازم را مبذول فرمایید.

پیشاپیش از همکاری شما سپاسگزاریم.

مسئول فنی - **دکتر مریم لطیفی پور**



با تجدید احترام
دکتر مریم حبیبی
مدیر عامل تامین فارمد تهران



شماره های: ۰۹۱۲۲۴۳۹۲۵۸

CERTIFICATE OF ANALYSIS

FLEBOGAMMA DIF 50 mg/ml 100 ml

LOT NUMBER : 82N515

MANUFACTURING DATE : 27/APR/2020

FILE NUMBER : 40000917590

EXPIRY DATE : 27/MAY/2022

TESTS	SPECIFICATIONS	RESULTS
VOLUME	≥ 100.26 ml	100.83 ml
PRODUCT IDENTITY (EID)	Abnormal precipitation lines are not observed	Abnormal precipitation lines are not observed
Ig PURITY	≥ 97.0 %	99.8 %
PKA (3% w/V Ig)	≤ 10 IU/ml	< 2 IU/ml
ANTICOMPLEMENTARY ACTIVITY	≤ 1.00 CH50/mg Ig	0.62 CH50/mg Ig
HBsAg ANTIBODIES	≥ 2.5 IU/g Ig	47.3 IU/g Ig
ANTI-A HAEMAGGLUTININS (5% w/V Ig solution)	$\leq 1/64$ agglutination	1/16 agglutination
ANTI-B HAEMAGGLUTININS (5% w/V Ig solution)	$\leq 1/64$ agglutination	1/8 agglutination
IgA	< 0.05 mg/ml	< 0.0029 mg/ml
ANTI D ANTIBODIES (5% w/V Ig solution)	\leq Reference Preparation Ph. Eur. titre	$<$ Ph. Eur. Ref. preparat. titre
APPEARANCE	Clear or slightly opalescent, colourless or pale yellow solution	Clear or slightly opalescent, colourless or pale yellow solution
OSMOLALITY	240 .. 370 mOsm/Kg	328 mOsm/Kg
pH	5.0 .. 6.0	5.6
TOTAL PROTEIN	45 .. 55 mg/ml	49 mg/ml
MOLECULAR DISTRIBUTION (Monomer + Dimer)	≥ 95.0 %	99.9 %
MOLECULAR DISTRIBUTION (Fractions)	≤ 3.5 %	ND
MOLECULAR DISTRIBUTION (Polymers + Aggregates)	≤ 2.0 %	0.1 %
POLYETHYLENE GLYCOL	≤ 1.00 mg/ml	0.07 mg/ml
TNBP	≤ 2.0 μ g/ml	< 0.4 μ g/ml
POLYSORBATE 80	≤ 100.0 μ g/ml	20.2 μ g/ml
ACETATE	≤ 1.00 mg/ml	0.33 mg/ml
SORBITOL	45 .. 55 mg/ml	52 mg/ml
PYROGENS (20 ml/kg)	Pass test	Pass test
STERILITY	No microbiological growth	No evidence of microbial growth

Parrets del Vallès, May

By Order



GRIFOLS
Instituto Grifols, S.A.



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	Prepared by	Reviewed by		Approved by
Name	Olga Petrova	Taylor Bush	Margaret McCombes	Patrick Dogget
Designation	Asst. Manager-QP	Asst. Manager-QP	Executive-QA	Asst. Manager-QA
Sign and Date	<i>O. Petrova</i>	<i>Taylor B.</i>	<i>Maggie McCombes</i>	<i>Patrick</i>

1.0 OBJECTIVE

The objective of this STP is to provide instructions to perform the test for Human Normal Immunoglobulin for Intravenous use drug product.

2.0 SCOPE

The scope of this STP is applicable whenever tests are performed for Human Normal Immunoglobulin for Intravenous use Drug product manufactured from imported plasma at Instituto Grifols, S.A. Barcelona-Spain.

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3.0 RESPONSIBILITY

3.1 Executive

- 3.1.1 It is the responsibility of the Executive-QP to test the sample Human Normal Immunoglobulin for Intravenous Use Drug product and document as per this STP.

3.2 HOD/Designee

- 3.2.1 It is the responsibility of HOD/Designee-QP to provide training to all the concerned personnel and ensure overall compliance at the STP.
- 3.2.2 To coordinate or lead investigations.

3.3 QA Designee

- 3.3.1 To ensure the overall compliance of the STP.

3.4 Analytical QA

- 3.4.1 To review the document.
- 3.4.2 Participation of investigation like Deviation, Incident, OOS & OOT etc.

4.0 ABBREVIATIONS

- BP - British Pharmacopoeia
- BRP - Biological Reference Preparation
- HCl - Hydrochloric Acid
- HOD - Head of Department
- IgG - Immunoglobulin G
- mL - Milli Litre
- mm - Milli meter
- NA - Not Applicable
- NaCl - Sodium Chloride
- NLT - Not Less Than
- OOS - Out of Specification
- OOT - Out of Trend
- PBS - Phosphate Buffer Solution
- Ph. Eur - European Pharmacopoeia

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- PKA - Prekallikrein Activity
- QA - Quality Assurance
- QP - Quality Control Plasma
- RS - Reference Standard
- RST - Record Sheet
- STP - Standard Testing Procedure

5.0 MATERIAL REQUIRED

- NA

6.0 SAFETY REQUIREMENTS AND PRECAUTIONS

- NA

7.0 DEFINITIONS

- NA

8.0 FLOWCHART

- NA

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9.0 PROCEDURE

9.1 Description

9.1.1 Appearance (As per PH. Eur/BP)

Take the vial and observe visually as per Document no.: STP-U1-QP-011.

9.1.2 pH (As per Ph. Eur/BP)

Take 5mL of the sample and perform the test of PH as per Document No.: STP-U1-QP-011.

9.2 Identification

9.2.1 By Precipitation Test

9.2.1.1 Materials

- Agarose
- PBS buffer
- Petri plates

9.2.1.2 20mM Phosphate buffer saline: Weigh 0.54gm Potassium di hydrogen phosphate dissolve in 200mL water, weigh 2.272gm of disodium hydrogen phosphate in 800mL water, mix both solution to this weigh and add 16gm of sodium chloride.

9.2.1.3 Test Procedure

- Precipitation test is performed by Double Immuno diffusion (Ouchterlony method)
- This method is carried out in a semi solid medium such as agarose.
- Prepare 25mL of 0.8% agarose (0.20g/25mL) in 20mM PBS buffer by boiling to dissolve the agarose completely.
- Cool the solution to 55-60°C and pour into 2 petri-plates placed on a horizontal surface. Allow the gel to set for 10 min.
- Punch 5 wells in the semisolid agarose medium in both the plates.
- Prepare different dilutions of Ab (Which gives positive results for the presence of human protein and negative for the non-human protein) like 1:2, 1:4 and 1:8.
- To 40µl of 5% IVIG sample add 960µl of diluent (0.9% saline).

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- To 40µl of 10% IVIG sample add 1960µl of diluent (0.9% saline).
- Neat concentration of antibody and sample concentration must be same.
- In plate-1 take 12µL of sample in the center well and in plate-2 (reference) take 12µL of Non-human protein in the center well.
- Fill the side wells with 12µL each of the antiserum (neat) and its dilutions.

S. No.	Well	Plate 1	Plate 2
1	Centre	Sample	Non-human protein
2	Side 1	Antibody (Neat)	Antibody (Neat)
3	Side 2	1:2	1:2
4	Side 3	1:4	1:4
5	Side 4	1:8	1:8

- Keep the plates overnight at room temperature.
- After incubation, observe for opaque precipitation lines between the antigen and antisera wells.

9.3 Immuno-Electrophoresis (As per Ph. Eur./BP)

9.3.1. Material

- 9.3.1.1 Agarose
- 9.3.1.2 Alcohol
- 9.3.1.3 Antiserum: Anti human IgG antibody produced in rabbit.
- 9.3.1.4 Conical flask
- 9.3.1.5 Distilled water
- 9.3.1.6 Filter paper
- 9.3.1.7 Glass Plate
- 9.3.1.8 Horizontal electrophoresis unit
- 9.3.1.9 Measuring cylinder
- 9.3.1.10 Micro pipette

9.3.2 Reagents Preparation

- 9.3.2.1 **Saline (0.9%) Solution:** Take 0.9gm of Sodium chloride and dissolve in small amount of purified water. Make up the volume to 100mL with the same solvent.

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9.3.2.2 **0.8% Agarose:** Take 0.08 gm of agarose and dissolve in 10 mL of 1 x TBE

Buffer.

9.3.2.3 **TBE (5 X) Buffer:** Take Boric acid 6.9 gm, Tris Base 13.5 gm and 5 mL of 0.5 M EDTA and make up the volume to 250 mL with water. From this dilute to 1 x by taking 50 mL of 5X buffer and diluting it to 250 mL.

9.3.2.4 **0.5M EDTA:** Take 930 mg of EDTA and dissolve in 5 mL of water.

9.3.2.5 **0.2% of test sample:** To 40µl of 5% IVIG sample add 960µl of diluent (0.9% saline). To 40µl of 10% IVIG sample add 1960µl of diluent (0.9% saline).

9.3.3 Principle

9.3.3.1 Test sample and normal human serum control are electrophoresed on the agarose plate, separating the Proteins according to their electrophoretic mobility. Antisera are then applied to trough in the plate and allowed to diffuse into agarose support medium. When a favorable antigen-to-antibody ratio exists, a precipitin arc will form on the plate.

9.3.3.2 Proteins are thus differentiated not only by their electrophoretic mobility, but also by their diffusion coefficient and antibody specificity.

9.3.3.3 Diffusion is halted by rinsing the plate in 0.9% saline. Unbound protein is washed from the plate by the saline and the antigen/antibody precipitin arcs are stained with a protein sensitive stain. The precipitin arcs formed by the test sample and the control are compared for a semi-quantitative protein analysis.

9.3.4 Procedure

9.3.4.1 Prepare 10mL of 0.8% agarose (0.08 g/10 mL) in 1X TBE buffer by heating slowly till agarose dissolves completely. Take care not to scorch or front the solution.

9.3.4.2 Pour the agarose mix onto a glass plate placed on a horizontal surface and allow it to solidify.

9.3.4.3 After solidification, punch the wells with gel puncher towards one edge of the plate. And also create trough in the gel between the two wells.

9.3.4.4 Place the glass plate in the electrophoresis tank; ensure that the wells are towards the cathode.

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- 9.3.4.5 Fill the tank with 1 X TBE buffer in to each outer section of chamber.
- 9.3.4.6 Place the filter paper in each buffer filled compartment, touching to both sides of gel. Allow the filter paper to become saturated with buffer.
- 9.3.4.7 Load 4 μ L of 0.2% of test sample and control (normal human serum) along with 1 μ L of 4 X Bromophenol blue dye in a separate wells. Take care not to damage the wells during sample application.
- 9.3.4.8 Make sure that the agarose makes good contact with buffer by filter paper.
- 9.3.4.9 Put the cover on the electrophoresis chamber and wait 30-60 seconds before applying current. This allows the plate to equilibrate with the buffer.
- 9.3.4.10 Connect the power cord and electrophorese the plate at 100 volts for the appropriate migration distance. Migration distance can be verified visually by observing the position of the dye.
- 9.3.4.11 After completion of electrophoresis, remove the plate from the chamber and put it on a flat surface.
- 9.3.4.12 Apply appropriate antiserum (200 μ L of 1:3 diluted antiserum) into a trough in the plate. Fill the trough by placing the tip of a pipette in the end of the trough farthest from the sample well. Holding the pipette in place, slowly depress the plunger and dispense the antiserum into the trough. The antiserum will flow down the trough by capillary section.
- 9.3.4.13 Before moving the plates allow the antiserum to absorb for approximately 3-5 minutes.
- 9.3.4.14 Place the plate in a moist chamber and incubate it for 18 hrs at room temperature.
- 9.3.4.15 After incubation place the place in 0.9% saline wash to stop the precipitin reaction.
- 9.3.4.16 Interpretation, observe the precipitin arc on the light box or dark back ground. Compare the test sample with control (normal human serum) in order to determine the presence or absence of an abnormal immunoglobulin.

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9.4 Osmolality (As per Ph.Eur./BP)

9.4.1 Materials

9.4.1.1 Standard Solution

9.4.2 Instrument/Apparatus

9.4.2.1 20 μ L pipette

9.4.2.2 Micro-Osmometer

9.4.3 Test procedure

9.4.3.1 Draw a 20 μ L sample with the 20 μ L pipette.

9.4.3.2 Insert the pipette tip fully into the bottom of a sample tube and smoothly eject the sample without any splash or spray.

9.4.3.3 Place the loaded sample tube into the sample well.

9.4.3.4 Push fully the operating head into the sample tube.

9.4.3.5 Initiate the test by pressing [TEST] button as indicated on the user interface.

9.4.3.6 When the test is finished, lift the sample probe.

9.4.3.7 Place a probe-cleaner in the sample tube and clean the probe by pushing the operating head down.

9.4.3.8 When all measurements are finished push the operating head down into an empty sample tube.

9.5 Assay: Estimation of Protein Concentration

9.5.1 Perform the Estimation of Protein content as per Document No.: STP-U1-QP-013.

9.5.2 Perform the Estimation of Protein Content as per Document No.: STP-U1-QP-018 (As per Ph.Eur/BP)

9.6 Determination of Protein Composition (As per Ph.Eur/BP)

9.6.1 Perform the Test for determination of Protein composition as per Document no.: STP-U1-QP-014.

9.7 Molecular size Distribution: By Liquid Chromatography (as per Ph. Eur/BP)

9.7.1 Materials

9.7.1.1 Disodium hydrogen phosphate dihydrate

9.7.1.2 Human Immunoglobulin Reference solution

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9.7.1.3 Sodium azide

9.7.1.4 Sodium chloride

9.7.1.5 Sodium dihydrogen phosphate monohydrate

9.7.2 Instrument/Apparatus

9.7.2.1 HPLC

9.7.2.2 Stainless steel column 60cm x 7.5mm or 30cm x 7.8mm packed with hydrophilic silica.

9.7.3 Reagents preparation

9.7.3.1 **Saline Solution:** Take 0.9 g of Sodium Chloride and dissolve it in a small amount of purified water and make up the volume to 100 mL STP with the same solvent.

9.7.3.2 **Test Solution:** Dilute the sample with the saline solution to obtain concentration in the range of 0.4 to 1.2% w/v.

9.7.3.3 Injection of 50 to 600 µg of protein is usually suitable.

9.7.3.4 Briefly, dilute the test sample to 5 mg/mL, from 5 mg/mL, inject 20 µL of sample of analysis.

9.7.3.5 **Reference solution (HIS):** Dilute the Human Immunoglobulin RS with the saline solution to the same protein concentration as the test solution.

9.7.3.6 **Chromatographic system:** A stainless steel column 60cm x 7.5mm or 130cm x 7.8mm packed with hydrophilic silica gel.

9.7.3.7 **Mobile Phase:** Dissolve 4.873 g of Disodium hydrogen phosphate dihydrate 1.741 g of Sodium dihydrogen phosphate monohydrate, 11.688 g of Sodium chloride and 50 mg of Sodium Azide in 1000 mL of water.

9.7.4 Instrument Parameters:

Flow Rate	0.5 mL/Minute
Run Time	40 Minutes
Injection Volume	20 µL
Wavelength	280 nm
Sample temperature	12°C
Elution mode	Isocratic

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Column	Stainless steel column 60cm x 7.5mm or 30cm x 7.8mm packed with hydrophilic silica or equivalent.
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9.7.5 Test Procedure

9.7.4.1 Maintain Flow rate at 0.5mL/min.

9.7.4.2 Spectrophotometer set at 280 nm.

9.7.4.3 System suitability will be performed before doing the actual sample analysis. System suitability criteria, HIS monomer is considering for Asymmetry factor, As (NMT 2) and Theoretical plates, N/m (NLT 1000). Record the system suitability results in STP-U1-QP-008/ANX-01.

9.7.4.4 In the chromatogram obtained, identify the peaks representing the monomer and the dimer. Compare the peaks of the chromatogram of sample with that of the reference.

9.7.4.5 Calculate the sum of peak areas of monomer and dimer in sample chromatogram.

9.7.4.6 For monomer and dimer, the relative retention to the corresponding peak to the chromatogram obtained with the reference solution is 1 ± 0.02 .

9.8 Anti-Complementary Activity Test (As per Ph. Eur/BP)

9.8.1 **Principle:** For the measurement of Anti complementary activity of immunoglobulin, a defined amount of test material (10 mg of immunoglobulin) is incubated with a defined amount of guinea-pig complement ($20CH_{50}$) and the remaining (unreated) complement is titrated; the anti-complementary activity is expressed as percentage consumption of complement relative to the Complement control considered as 100%.

9.8.2 **Hemolytic Unit:** The hemolytic unit of complement activity (CH_{50}) is the amount of complement that, in the given reaction conditions, will produce the lysis of 2.5×10^8 out of a total of 5×10^8 optimally sensitized red blood cells.

9.8.3 Materials

9.8.3.1 Barbital Sodium

9.8.3.2 Calcium Chloride

9.8.3.3 Gelatin

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9.8.3.4 Glucose

9.8.3.5 Guinea-pig complement

9.8.3.6 Haemolysin

9.8.3.7 Hydrochloric Acid

9.8.3.8 Immunoglobulin preparation (sample)

9.8.3.9 Magnesium chloride

9.8.3.10 Sheep red blood cells

9.8.3.11 Sodium Chloride

9.8.3.12 Sodium Citrate

9.8.4 Instrument/Apparatus

9.8.4.1 Centrifuge

9.8.4.2 pH meter

9.8.4.3 UV-Visible spectrophotometer

9.8.4.4 Water-bath

9.8.4.5 Cold room 2-8°C

9.8.5 Reagents preparation:

9.8.5.1 **Magnesium and calcium stock solution:** Dissolve 1.103 g of calcium chloride and 5.083 g of magnesium chloride in water and dilute to 25mL with the water.

9.8.5.2 **Buffer Stock:** Barbital buffer/Equivalent buffer

9.8.5.3 **Barbital buffer:** Dissolve 207.5 g of Sodium chloride and 25.48 g of Barbital sodium in 4000 mL of water and pH adjusted to 7.3 using 1M hydrochloric acid. Add 12.5 mL of Magnesium and calcium stock solution and dilute to 5000 mL with water.

9.8.5.4 **Gelatin solution:** Dissolve 12.5 g of gelatin in about 800 mL of water and heat to boiling in a water-bath. Cool to 20°C and dilute to 10 lit with water. Filter through a membrane filter (pore size: 0.22µm). Store at 4°C (Use clear solutions only).

9.8.5.5 **Citrate solution:** Dissolve 8.0g of Sodium citrate, 4.2 g of Sodium chloride and 20.5 g of glucose in 750 mL of water. Adjust pH to 6.1 using a 10.0% w/v solution of citric acid and dilute to 1000mL with water.

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9.8.5.6 **Buffer Solution:** Add 4 volumes of gelatin solution to 1 volume of above prepared Buffer stock and mix. Adjust pH to 7.3, if necessary, using 1M Sodium Hydroxide or 1M hydrochloric acid. Maintain at 4°C (Prepare fresh solutions daily).

9.8.5.7 **Stabilized sheep blood:** Collect 1 volume of sheep blood into 1 volume of citrate solution and mix. Store at 4°C for not less than 7 days and not more than 28 days.

9.8.5.8 **Haemolysin:** Antiserum against sheep red blood cells prepared in rabbits.

9.8.5.9 **Guinea-pig complement:** Prepare a pool of serum from the blood of not fewer than 10 guinea-pigs. Separate the serum from the clotted blood by centrifugation at about 4°C. Store the serum in small amounts below -70°C.

9.8.6 Test procedure

9.8.6.1 Preparation of standardized 5% sheep red blood cell suspension:

- Separate sheep red blood cells by centrifuging an appropriate volume of stabilized sheep blood and wash the cells at least three times with Buffer Solution and prepare as a 5% v/v suspension in the same solution.
- Measure the cell density of the suspension as follows: add 0.2mL to 2.8mL of water and centrifuge the lysed solution for 5 min at 1000 g. The cell density is suitable if the absorbance of the supernatant liquid at 541 nm is 0.62 ± 0.01 . Correct the cell density by adding solution A according to the formula:

$$V_f = \frac{V_i \times A}{0.62}$$

V_f = final adjusted volume;

V_i = initial volume; &

A = Absorbance of the original suspension of 541nm

- The adjusted suspension contains about 1×10^9 cells per mL.

9.8.6.2 **Haemolysin titration:** Prepare Haemolysin Dilutions: as shown in below table.

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Required dilution of Haemolysin	Prepared Using		
	Buffer Solution		Haemolysin Volume (in mL)
	Volume (in mL)	Dilution (1:)	
7.5	0.65	Undiluted	0.1
10	0.90	Undiluted	0.1
75	1.80	7.5	0.2
100	1.80	10	0.2
150	1.00	75	1.0
200	1.00	100	1.0
300	1.00	150	1.0
400	1.00	200	1.0
600	1.00	300	1.0
800	1.00	400	1.0
1200	1.00	600	1.0
1600	1.00	800	1.0
2400	1.00	1200	1.0
3200*	1.00	1600	1.0
4800*	1.00	2400	1.0

* Discard 1.0 mL of the mixture

- Add 1.0mL of 5% sheep red blood cell suspension to each tube of the haemolysin dilution series, starting at the 1:75 dilution, and mix. Incubate at 37°C for 30 min.
- Transfer 0.2mL of each of these incubated mixtures to new tubes and add 1.10 mL of Solution A and 0.2 mL of diluted guinea-pig complement (for example, 1: 150). Perform this in duplicate.
- As the unhaemolysed cell control, prepare three tubes with 1.4 mL of solution A and 0.1 mL of 5% sheep red blood cell suspension.
- As the fully haemolysed control, prepare three tubes with 1.4 mL of water and 0.1 mL of 5% sheep red cell suspension.
- Incubate all tubes at 37°C for 60 min. & centrifuge at 1000 g for 5min. Measure the absorbance of the supernatants at 541 nm and calculate the percentage degree of haemolysis in each tube using the expression:

Aa - A1

————— **X 100**

Ab -A1

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Aa=absorbance of tubes with haemolysin dilution

Ab=mean absorbance of the three tubes with full haemolysis

A1=mean absorbance of the three tubes with no haemolysis

- Plot the percentage degree of haemolysis as the ordinate against the corresponding reciprocal value of the haemolysin dilution as the abscissa on linear graph paper. Determine the optimal dilution of the haemolysin from the graph by inspection.
- Select a dilution such that further increase in the amount of haemolysin does not cause appreciable change in the degree of haemolysis. This dilution is defined as One Minimal Haemolytic Unit (1MHU) in 1.0 mL. The optimal haemolytic haemolysin dilution for the preparation of sensitized sheep red blood cells contained 2MHU/mL.
- The haemolysin titration is not valid unless the maximum degree of haemolysis is 50-70%. If the maximum degree of haemolysis is not in this range, repeat the titration with more or less diluted complement solution.

9.8.7 Preparation of Optimized Sensitized Sheep Red Blood Cells (Haemolytic System)

9.8.7.1 Prepare an appropriate volume of diluted haemolysin containing 2 MHU/mL and an equal volume of standardized 5% sheep red blood cell suspension.

9.8.7.2 Add the haemolysis dilution to the standardized cell suspension and mix. Incubate at 37°C for 15min., store at 2-8°C and use within 6hrs.

9.8.8 Titration of complement

9.8.8.1 Prepare an appropriate dilution of complement with Solution A and perform the titration in duplicate: as shown in below table.

Tube Number	Volume of diluted complement (in mL) (for example 1: 250)	Buffer Solution (in mL)
1	0.1	1.2
2	0.2	1.1
3	0.3	1.0
4	0.4	0.9
5	0.5	0.8

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6	0.6	0.7
7	0.7	0.6
8	0.8	0.5
9	0.9	0.4
10	1.0	0.3
11	1.1	0.2
12	1.2	0.1
3 tubes (as cell control) at 0% Haemolysis	-	1.3
3 tubes at 100% Haemolysis	-	1.3mL of water

9.8.8.2 Add 0.2 mL of sensitized sheep red blood cells to each tube, mix well and incubate at 37°C for 60 min. Cool the tubes in an ice bath and centrifuge at 1000g for 5 min.

9.8.8.3 Measure the absorbance of the supernatant liquid at 541 nm and calculate the degree of haemolysis (Y) using the expression.

$$Y = \frac{Ac - A1}{Ab - A1}$$

Ac=absorbance of tubes 1 to 12,

Ab=mean absorbance of tubes with 100% haemolysis

A1=mean absorbance of cell controls with 0% haemolysis.

9.8.8.4 Plot Y/(1-Y) as the abscissa against the amount of diluted complement in millilitres as the ordinate on log-log graph paper. Fit the best line to the points and determine the ordinate for the 50.0% haemolytic complement dose where Y/ (1-Y) =1.0.

9.8.8.5 Calculate the activity in haemolytic units (CH₅₀/mL) from the expression.

$$CH_{50} = \frac{Cd}{Ca \times 5}$$

Cd=reciprocal value of the complement dilution,

Ca=volume of diluted complement in millilitres resulting in 50% haemolysis;

5=scaling factor to take account of the number of red blood cells.

9.8.8.6 The test is not valid unless the plot is a straight line between 15% and 85% haemolysis and the slope is 0.15 to 0.40, and preferably 0.18 to 0.30.

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9.8.9 Test for Anticomplementary activity

9.8.9.1 Prepare complement dilution having 100 CH₅₀/mL by diluting titrated guinea-pig complement with Buffer solution.

9.8.9.2 Dilute IVIG 10% sample (100mg/mL) to 50mg/mL with 10% maltose solution.

9.8.9.3 If necessary, adjust the immunoglobulin to be examines to pH-7. Prepare incubation mixtures as follows for an immunoglobulin containing 50mg/mL.

	Immunoglobulin to be examined	Complement control (in duplicate)
Immunoglobulin (50mg/mL)	0.2 mL	-
Solution A	0.6 mL	0.8 mL
Complement	0.2 mL	0.2 mL

9.8.9.4 Carryout the test on the immunoglobulin to be examined and prepare ACA negative and positive controls using human immunoglobulin BRP, as indicated in the leaflet accompanying the reference preparation.

9.8.9.5 Higher or lower volumes of sample and of Buffer Solution are added if the immunoglobulin concentration varies from 50mg/mL.

9.8.9.6 Close the tubes and incubate at 37°C for 60 min.

9.8.9.7 Add 0.2 mL of each incubation mixture to 9.8 mL of Buffer Solution to dilute the complement.

9.8.9.8 Perform complement titrations as described above on each tube to determine the remaining complement.

9.8.9.9 Calculate the Anti-complementary activity of the preparation to be examined relative to the complement control considered as 100%, from the expression:

$$\frac{a - b}{A} \times 100$$

a=mean complement activity (CH₅₀/mL) of complement control,

b=complement activity (CH₅₀/mL) of tested sample.

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9.8.10 The test is not valid unless

9.8.10.1 The Anti-complementary activities found for ACA negative control and ACA positive control are within the limits stated in the leaflet accompanying the reference preparation,

9.8.10.2 The complement activity of the complement control (a) is in the range of 80-120 CH₅₀/mL.

9.9 Prekallikerein test (As per Ph.Eur/BP)

9.9.1 Preparation of Reagents/Buffers

9.9.1.1 **Human Prekallikrein:** Reconstitute in 2.5mL sterile distilled water.

9.9.1.2 **Kallikrein Substrate:** H-D-Pro-Phe-Arg-pNA 3.68 mg/vial plus mannitol. Reconstitute in 1 mL sterile distilled water and then dilute 1 mL with 9 mL Buffer B (below) before use. Stability before dilution: 8 hours at room temperature, 48 hours at 4°C, or at -20°C for 6 months. Stability after dilution: 6 hours at room temperature or 24 hours at 4°C.

9.9.1.3 **PKA Standard 32 IU/mL:** Reconstitute in 1.0 mL of sample/standard diluent. This gives a PKA concentration of 32IU/mL. Store at 4°C before use or for up to 8 hours or at -20°C for 6 months.

9.9.1.4 **Buffer A Concentrate:** Tris-HCl buffer (100 mmol/L Tris) containing NaCl (24 mmol/L). Store at 4°C. The vial contained 6mL of concentrated buffer. Before use dilute the contents of each vial with sterile distilled water to give a final volume of 12mL for each vial (Buffer A).

9.9.1.5 **Buffer B:** Dilute 1mL of Buffer A with 9mL sterile distilled water.

9.9.1.6 **Sample/Standard Diluent:** Dissolve vial contents in 6mL sterile distilled water. Store at room temperature for 8 hours or for longer storage at -20°C up to 6 months.

9.9.1.7 **Immunoglobulin Pretreatment reagent:** All immunoglobulin fractions must be pretreated with this reagent before being tested. Add 100 µL of pretreatment reagent to 900 µL of immunoglobulin sample in a plastic tube. Mix well and perform assay as per the test schedule.

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9.9.1.8 **Blank activity reagent (BABR):** Reconstitute in 1.0 mL sterile distilled water. To this, add 11mL of diluted buffer A (1 part buffer A + 1 part sterile distilled water). Mix and use within 4 hrs at room temperature or store in 3.0 mL aliquots at -20°C.

9.9.2 Test Procedure

9.9.2.1 Test sample preparation:

- ◆ 3% w/v immunoglobulin sample preparation from 5% IVIG: to 600µl of sample 1.5 mL polypropylene Eppendorf tube add 400µl of 0.9% saline, mix well.
- ◆ 3% w/v immunoglobulin sample preparation from 10% IGIG: To 300µl of sample in 1.5mL polypropylene Eppendorf tube add 700µl of 0.9% saline, mix well.
- ◆ Add 100 µL of pretreatment reagent to 900 µL of 3% w/v immunoglobulin sample in a 1.5 mL poly propylene Eppendorf tube. Mix well and dilute 100 µL of test sample with 100 µL of standard/sample diluent.

9.9.2.2 **Standard preparation:** Dilute the PKA standard with standard/sample diluent to give PKA values of 0,1.0,2.0,4.0,8.0 and 16.0 OU/mL as given in below table.

PKA concentration IU/mL	PKA standard µL	Standard/sample Diluent µL
0	0	200
1.0	12.5	387.5
2.0	25	375
4.0	50	350
8.0	100	300
16.0	100	100

9.9.2.3 Preparation of Standards and test samples

- Take 25µL of each standard and sample solution, in 1.5 mL micro centrifuge tubes.
- Add 50µL of Human Prekallikrein each of the tubes as prepared in 9.9.1.1.

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9.9.2.4 Preparation of Standards and normal test sample blanks

- Take 25µL of each standard and sample solution, in 1.5 mL micro centrifuge tubes.
- Add 50µL of Buffer A in each of the tubes as prepared in 9.9.1.4.
- Incubate all the above tubes for exactly 45 minutes at 37°C.

9.9.2.5 Preparation of additional test sample blanks

- Take 25µL of each diluted test sample solution, in 1.5mL micro centrifuge tubes.
- Add 50µL of BABR in each of the tubes as prepared in 9.9.1.8.

9.9.2.6 Preparation of standard, test sample, test sample blanks and BABR blanks

- Pre-warm diluted kallikrein substrate at 37°C
- Transfer 25 µL volume from all of the above incubates to a 96 well microtitre plate using a multi pipette.
- Add 100 µL diluted kallikrein substrate.
- Incubate the plate at 37°C for exactly 5 minutes. Then add 50 µL of 50% acetic acid solution to each well to stop the reaction.
- Transfer the microtitre plate immediately to a plate reader set to read at an optical density of 405 nm.

9.9.3 Calculation

9.9.3.1 Plot a linear graph by taking concentration of Prekallikrein activator IU/mL on X-axis and Absorbance at 405 nm on Y-axis.

9.9.3.2 Record the values of standard curve equation $Y=mX+C$.

9.9.3.3 Calculate Prekallikrein units in sample from the recorded data.

9.10 Anti-D antibodies (As per Ph.Eu/BP)

9.10.1 This test is outsourced as per SOP-U1-QP-022.

9.11 Immunoglobulin A (As per Ph. Eur./BP)

9.11.1 This test shall be performed as per Document No.: QC-QP-STP-032.

9.12 Detection of Antibodies to Hepatitis B Surface Antigen (As per Ph. Eur/BP)

9.12.1 Perform the test as per STP-U1-QP-022

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9.13 Pyrogen (As per Ph. Eur/BP)

9.13.1 Perform the test as per STP-U1-QP-002.

9.14 Sterility test (As per Ph.Eur/BP)

9.14.1 Perform the test as per STP-U1-MB-023

9.15 Viral Safety Analysis

9.15.1 Perform the test as per STP-U1-QP-004

9.16 Anti-A and Anti-B Haemagglutinins (As per Ph.Eur/BP)

9.16.1 Perform the test as per STP-U1-QP-020.

9.17 Estimation of Maltose Content (in House)

9.17.1 Perform the test as per QC-QP-STP-019

10.0 REFERENCE

- Ph. Eur. 8.0
- BP 2013

11.0 DOCUMENTATION

- The details of Appearance and pH shall be recorded in STP-U1-QP-011/RST-01 (STP-U1-QP-011-F01-04).
- Record the data (Osmolality, Precipitation, Immunoelectrophoresis, Molecular size distribution, Anti complementary activity, Prekallikrein Activator, Anti D antibodies, Viral safety analysis in STP-U1-QP-008/RST-01 (STP-U1-QP-008-F02-14).
- The details of system suitability check shall be recorded in STP-U1-QP-008/ANX-01 (STP-U1-008-F01-14).
- The details Antibody to HBsAg shall be recorded in STP-U1-QP-022/RST-011 (STP-U1-QP-022-F01-01).
- The details of Anti-A and Anti B Haemagglutinins shall be recorded in STP-U1-QP-020/RST-01 (STP-U1-QP-020-F01-01).
- The details of Immunoglobulin-A Assay results shall be captured in QC-QP-STP-032/RST-01.
- The details of Protein composition shall be recorded in STP-U1-QP-014/RST-01 (STP-U1-QP-014-F01-03).
- The details of Estimation of protein content shall be recorded in STP-U1-QP-

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013/RST-01 (STP-U1-QP-F01-03).

- The details of Estimation of protein content (As per Ph.Eur/B.P.) shall be recorded in STP-U1-QP-018/RST-01 (STP-U1-QP-018-F01-02)
- The details of Estimation of Maltose content shall be recorded in QC-QP-STP-019/RST-01.
- The details of Test for Sterility shall be recorded in STP-U1-MB-023/RST-01 (STP-U1-MB-023/F01-12).
- The details of Test for pyrogens shall be recorded in STP-U1-QP-002/RST-01 (STP-U1-QP-002-F01-03).

12.0 REVISION SUMMARY

Revision No.	Effective Date	Change Control Ref. No.	Changes Made
12	17/03/2020	CCFU1QP004/20	Viral safety analysis details included in RST STP-U1-QP-005/RST-01
13	15/06/2020	CCFU1QP008/20	Antibody to HBsAg test removed
14	04/11/2020	CCFU1QP011/20	Included IVIG 10% sample details in Identification, Anticomplementary and Prekallikrein test.

END OF DOCUMENT