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Application for a Product Licence

MEDICINES ACT 1968 AND 1971

APPLICATION FOR A PRODUCT LICENCE

HIGH POTENCY FACTORATE®

VOLUME I - CHEMISTRY AND PHARMACY

AP000982

Armour Pharmaceutical Company Limited, Eastbourne, Sussex

Armour Pharmaceutical Company Ltd

ARMOUR000354

ARMO0000023_0001

CONFIDENTIAL

COPY NO. 7

MEDICINES ACT 1968 AND 1971

APPLICATION FOR A PRODUCT LICENCE

HIGH POTENCY FACTORATE®

VOLUME I - CHEMISTRY AND PHARMACY

INDEX

SGB/JH
November 1978
Armour Pharmaceutical Company Ltd.,
Hampden Park,
EASTBOURNE,
East Sussex.

1

MEDICINES ACT 1968 AND 1971

APPLICATION FOR PRODUCT LICENCES

1 Name of Product: HIGH POTENCY FACTORATE

2 Full name and address of proposed licence holder:
Armour Pharmaceutical Company Ltd.,
Hampden Park,
EASTBOURNE,
East Sussex,
U.K.

3 Trading style to be shown on licence if different from above: N/A

4 Role of proposed licence holder:
~~(i) as person responsible for composition of product manufactured in UK.~~
[X] (ii) as person who imports or procures its importation.
[X] (iii) as person who first sells or supplies it as a medicinal product.

5 Activities for which licence is required:
[X] (i) selling or supplying product in the UK.
[X] (ii) procuring the manufacture or assembly of the product for sale or supply in the UK.
[X] (iii) importing or procuring the importation of the product.
(iv)

6 Applicants own reference no: SGB/JH

7 Details of earlier applications: FACTORATE (Dried Human Antihaemophilic Fraction)
Licence granted 25th March, 1976

8 To cover sale and supply of the product manufactured before the grant of the licence: YES/NO

9 Scientific Evidence:
(i) Chemistry and Pharmacy Pages 1 - 93
(ii) Experimental and Biological Studies Pages
(iii) Clinical Trials Pages 1 - 94

10 Number of pages of supplementary information: NONE

11 I/~~We~~ apply for the grant of a product licence to the proposed holder named above in respect of the product(s) to which the Product Particulars on Page 2 refer and in accordance with the other particulars annexed; the said licence to be for a period of five years and subject to the following provisions -

11.1. All the Standard Provisions applicable to product licences under regulations for the time being in force under Section 47 of The Medicines Act 1968.

11.2. The product shall not be recommended to be used for any purpose other than those specified in the Product Particulars as Uses, and shall be sold or supplied in accordance with the said Product Particulars except in so far as may from time to time be approved by the licensing authority.

11.3. The specification of the constituents and of the finished product shall be in accordance with the information contained in or furnished in connection with the application.

11.4. The product is to be manufactured only in accordance with the methods set out in this application or furnished in connection with it.

11.5. No material information has been omitted (within the knowledge of the signatory).

Date 20th November '78

GRO-C: Brooks

Signature ...
State capacity in which signed.
Regulatory Affairs Manager

AP000984

2

PRODUCT PARTICULARS

1	Name of Product:	HIGH POTENCY FACTORATE.
2	Pharmaceutical form:	A white to pale yellow lyophilised cake in a vial with vacuum for intravenous administration to human beings after reconstitution.
3	Active constituents	Antihaemophilic Factor (Human). Each vial contains a minimum of 800 AHF units to be reconstituted with 30 ml of Water for Injections B.P.
4	Uses:	In therapy of classical haemophilia (Haemophilia A)
5	Recommended dose and dosage schedule:	Dosage must be individualised according to the needs of the patients. Full recommended general dosages are given in the package insert leaflet.
6	Contra-indications, Precautions and Warnings:	There are no known contraindications.
7	Method of retail sale or supply:	Supplied in single dose vials with the stated antihaemophilic factor activity on the label. Also supplied if required - a vial of diluent and sterile needles for reconstitution, withdrawal and injection. For hospital supply only.
8	Manufacturer of dosage form:	Armour Pharmaceutical Company, P.O. Box 511, Kankakee, Illinois 60901, U.S.A. (Active constituent and dosage form)

Applicants reference number (as on page 1) SGB/JH

Applicants signature .. GRO-C: Brooks .....

AP000985

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MEDICINES ACT 1968 AND 1971

APPLICATION FOR A PRODUCT LICENCE
HIGH POTENCY FACTORATE

PART 1B SUPPLEMENTARY DETAILS

SECTION 1 Product Literature

1.1. Labelling and Package Insert

Details of the proposed labelling and package insert are as attached.

1.2. Data Sheets

It is intended to defer final drafting until the licence is granted.

DRAFT PACKAGE INSERT

Dried Human Antihaemophilic Fraction Sterile

HIGH POTENCY FACTORATE

Armour Pharmaceutical
Company Limited
Eastbourne, England

DRIED HUMAN ANTIHAEMOPHILIC FRACTION STERILE FACTORATE

FOR INTRAVENOUS USE

Dried Human Antihaemophilic Fraction HIGH POTENCY FACTORATE is a stable lyophilised concentrate of Factor VIII (AHF, AHG) prepared from pooled human plasma intended for use in therapy of classical haemophilia (Haemophilia A).

A hereditary disorder of blood coagulation occurring almost exclusively in males, Haemophilia A results in profuse bleeding in joints, muscles or internal organs as a result of minor trauma. The disease appears to be due to a deficiency of a specific plasma protein, antihaemophilic factor. Factor VIII provides temporary replacement of the missing clotting factor.

Affected individuals frequently require therapy following minor trauma. Surgery, when required in such individuals must be preceded by temporary corrections of the clotting abnormality with fresh plasma transfusions, cryoprecipitate or by injections of Factor VIII concentrates. Obvious advantages of the use of concentrates of Factor VIII are the avoidance of hyperproteinaemia, overloading the circulatory system and possible kidney dysfunction resulting from large volume transfusions.

Several different concentrations of Factor VIII have been used successfully. These range from Fraction I of Cohn to highly purified, potent preparations. HIGH POTENCY FACTORATE is a purified cryoglobulin with much of the fibrinogen, as well as other plasma proteins, removed. Upon reconstitution, as directed, HIGH POTENCY FACTORATE contains 20 to 40 times as much Factor VIII as does an equal volume of plasma. Thus it may be used to correct deficiencies in Factor VIII levels without overloading the circulatory system.

COMPOSITION AND STANDARDISATION

Each vial contains the labelled amount of antihaemophilic activity in International Units (One International Unit is the activity equivalent to the average Factor VIII content of 1 ml aliquots of 167 samples of fresh normal plasma, as determined in an international collaborative study).

Each vial also contains sodium chloride (approximately 20 to 40 mg per 100 International Units). When prepared for administration as directed under reconstitution, the resulting solution will have approximately twice the tonicity of isotonic saline.

RECOMMENDED RECONSTITUTION

Reconstitute HIGH POTENCY FACTORATE using 30 ml sterile Water for Injections B.P. using standard aseptic precautions.

Warm both diluent and HIGH POTENCY FACTORATE at from 20°C to 25°C. Direct diluent down the side of the vial and gently rotate the vial until contents are dissolved. DO NOT SHAKE VIAL. Vigorous shaking will cause frothing and prolong the reconstitution time. Complete solution usually takes 10 to 15 minutes. The solution is now ready for administration. If a gel forms on reconstitution, the preparation should not be used.

ADMINISTRATION

Standard asceptic techniques should be used at all times.

Intravenous Injection

Plastic disposable syringes are recommended with Factor VIII solution. The ground glass surfaces of all-glass syringes tend to stick with solutions of this type.

1. Attach a filter needle to a sterile disposable syringe. Insert filter needle into stopper of Factor VIII vial; inject air and withdraw the reconstituted solution from the vial.

2. Discard the filter needle and attach suitable intravenous needle.

3. Administer solution by slow intravenous injection, at a rate comfortable to the patient, and not exceeding 2 ml per minute.

Intravenous Infusion

The infusion equipment used should comply with that described in section 3 or 4 of British Standard 2463: 1962, Transfusion Equipment for Medical Use.

1. Prepare solution of HIGH POTENCY FACTORATE as recommended under Reconstitution.

2. Attach suitable infusion set.

3. If more than one vial is to be administered to the same patient the infusion set may be transferred to a second vial.

4. When infusion of HIGH POTENCY FACTORATE is complete, the infusion set may be flushed with sterile isotonic saline to avoid loss of any of the reconstituted solution.

5. After use, discard infusion set, needles and vials together with any unused solution.

DOSAGE

HIGH POTENCY FACTORATE is for Intravenous administration only. As a general rule one unit of Factor VIII activity per kg will increase by 2% the circulating Factor VIII level, and although dosage must be adjusted according to the needs of the patient (weight, severity of haemorrhage, presence of inhibitors) the following general dosages are suggested.

1. **Overt Bleeding** - initially 20 units per kg of body weight followed by 10 units per kg every eight hours for the first 24 hours and the same dose every 12 hours for 3 or 4 days. For massive wounds, give until bleeding stops and maintain with 20 units per kg 8-hourly to achieve a minimum Factor VIII level of 40%.

2. **Muscle Haemorrhages**

   (a) Minor haemorrhages in extremities or non-vital areas: 10 units per kg once a day for 2 or 3 days.

   (b) Massive haemorrhages in non-vital areas: 10 units per kg by infusion at 12 hour intervals for 2 days and then once a day for 2 more days.

   (c) Haemorrhages near vital organs (neck, throat, subperitoneal): 20 units per kg initially; then 10 units per kg every 8 hours. After 2 days the dose may be reduced by one half.

3. **Joint Haemorrhages** - 10 units per kg every 8 hours for a day; then twice daily for 1 or 2 days. If aspiration is carried out, 10 units per kg just prior to aspiration with additional infusions of 10 units per kg 8 hours later and again on the following day.

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HIGH POTENCY FACTORATE®

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EASTBOURNE,
East Sussex.

4. **Surgery** - Dosages of 30 to 40 units per kg bodyweight prior to surgery are recommended. After surgery 20 units per kg every 8 hours should be administered. Close laboratory control to maintain the blood level of Factor VIII above 40% of normal for at least 10 days post-operatively is suggested.

5. **Dental Extractions** - For simple extractions a preoperative dose of 20 - 25 units per kg sufficient to raise the Factor VIII level to 50% should be given, followed by intravenous administration of epsilon aminocaproic acid. For multiple extractions further doses of Factor VIII may be advisable 24 or 36 hours after the operation (Dormandy 1977).

WARNING

Factor VIII is prepared from human plasma, each donation of which has been found negative for hepatitis B surface antigen (HBsAg) by the radioimmunoassay (RIA) method. In addition, each batch, after reconstitution as recommended on page 3, has been tested and found negative by the RIA method. However, since no completely reliable laboratory test is yet available to detect all potentially infectious plasma donations, the risk of transmitting viral hepatitis is still present.

SIDE EFFECTS

Products of this type are known to cause mild chills, nausea or stinging at the infusion site.

PRECAUTIONS

Factor VIII contains low levels of group A and B isohaemagglutinins. When large volumes are given to patients of blood groups A, B or AB, the possibility of intravascular haemolysis should be considered. Such patients should be monitored by means of haematocrit and direct Coombs test for signs of progressive anaemia.

CONTRAINDICATIONS

There are no known contraindications to antihaemophilic fraction.

STORAGE

HIGH POTENCY FACTORATE is to be stored below $6^{o}C$.

When stored as directed, it will maintain its labelled potency for the dating period indicated on the label.

HOW SUPPLIED

HIGH POTENCY FACTORATE is supplied in single dose vials.

Potency is stated on each label.

REFERENCES

1. Abildgaard. "Current Concepts in the Management of Hemophilia". From "Current Problems in Pediatric Hematology" (Ed. Oski, Jaffe and Miescher), Grune and Stratton, 1975.

2. Abildgaard et al. "Treatment of Hemophilia with Glycine-Precipitated Factor VIII" *N. Engl. J. Med.*, 1966, **275**, 471.

3. Bangham, Biggs et al. "A Biological Standard for Measurement of Blood Coagulation Factor VIII Activity". *Bull. Wld. Hlth. Org.*, 1971, **45**, 337.

4. Biggs et al. "Factor VIII Concentrates Made in the United Kingdom and the Treatment of Haemophilia based on Studies made During 1969 - 1972". *Brit. J. Haemotol.*, 1974, **27**, 391.

5. Brinkhous et al. "A New High-Potency Glycine-Precipitated Antihemophilic Factor (AHF) Concentrate". *J. Amer. Med. Ass.*, 1968, **205**, 613.

6. Britton, Harrison and Abildgaard. "Early Treatment of Hemophilic Hemarthroses with Minimal Dose of New Factor VIII Concentrate". *J. Pediat.*, 1974, **85**, 245.

7. Dormandy. "Haemophilia A and B". *Prescribers J.*, 1977, **17**, 8.

8. George and Breckenridge. "The use of Factor VIII and Factor IX Concentrates During Surgery". *J. Amer. Med. Ass.*, 1970, **214**, 1673.

9. Mazza et al. "Antihemophilic Factor VIII in Hemophilia: Use of Concentrates to Permit Major Surgery". *J. Amer. Med. Ass.*, 1970, **211**, 1818.

10. Walsh et al. "The Therapeutic Role of Epsilon-Aminocaproic Acid (EACA) for Dental Extraction in Hemophiliacs". *Annals N.Y. Acad. Sci.*, 1975, **240**, 267.

Manufactured by Armour Pharmaceutical Company , USA

Distributed by

Armour Pharmaceutical Company Limited,
Eastbourne, England.

PL 0231/

LOT NO .....

EXPIRES .....

PLO231/.....

DRIED HUMAN ANTIHAEMOPHILIC FRACTION STERILE
HIGH POTENCY FACTORATE®

POM

For intravenous administration

INTERNATIONAL UNITS PER VIAL

This vial on reconstitution with 30ml of Sterile Water for Injections B.P. contains g/litre Total Protein, g/litre Fibrinogen

STORE BELOW 6°C

SEE LEAFLET FOR COMPLETE INFORMATION

Manufactured by Armour Pharmaceutical Company U.S.A.
Distributed by
ARMOUR PHARMACEUTICAL COMPANY LTD.
EASTBOURNE ENGLAND

KEEP OUT OF REACH OF CHILDREN

When reconstituted with 30 ml. of Sterile Water for Injections B.P. the contents of this vial are approximately two times isotonic.

Contains not more than 30 units of Heparin per vial.
Contains Glycine USP.
Contains no preservative.

Reconstitution:

The preparation must be warmed to 20°–25° C before reconstitution with 30ml of Sterile Water for Injections BP. Only gentle mixing should be employed to avoid frothing. If a gel forms on reconstitution the preparation should not be used. Use the reconstituted solution as soon as possible and in any case within three hours of reconstitution.

Caution:

The product is prepared from Pooled Human Plasma. Despite careful selection of donors and non-reactivity of the reconstituted solution for hepatitis B antigen by the radio-immuno assay procedure, freedom from causal agents of viral hepatitis cannot be assumed.

AP000993

SECTION 2 Background

2.1. Application in Other Countries

The application has been under consideration with the FDA (Bureau of Biologics) since 10th April, 1978, as a variant to the existing product licence for Factorate^{R} Antihaemophilic Factor (Human). Clinical investigational work completed under IND requirements (BB-IND 1229) is included as a separate volume to this present submission.

2.2. Background

Management of classical haemophilia has been transformed by the availability of concentrates of the substance which these patients lack, Factor VIII. Since major surgery is now often performed on haemophiliacs, concentrates of higher potency are needed, allowing administration of massive doses of Factor VIII. Very large doses are also used to treat haemophiliacs who have developed inhibitors to Factor VIII. High Potency Factorate is a product designed to meet the needs of haemophiliacs requiring these massive doses. It is a concentrate prepared by a modification of the Cohn process and is a product with a specific activity of approximately 1.0 AHF unit/mg protein.

High Potency Factorate is manufactured from human plasma donations collected at licensed establishments which are subject to U.S.A. Federal Law. Each donation and each product lot has been found negative for Hepatitis B Surface Antigen by radio-immunoassay. Vials from the final dosage form are examined for quality and safety aspects agreed for the Armour product Dried Antihaemophilic Fraction Sterile B.P. (PL 0231/0038) which was licensed for supply to hospitals on 25th March, 1976.

SECTION 3 Persons Involved in the Manufacture of the Finished Product and Distribution

3.1. Manufacturers and Assemblers

All plasma is collected in establishments licensed by the FDA, Bureau of Biologics, and transferred to Armour under conditions defined in Title 21 of the Code of Federal Regulations.

Plasma and plasma records are inspected upon receipt at Armour Pharmaceutical Co., Kankakee, Illinois (U.S.A.), and, if satisfactory, the plasma is approved for fractionation.

Processing, sterile filling and quality control before release is carried out at Armour Pharmaceutical Co., Kankakee, Illinois (U.S.A.)

3.2. Arrangement for Storage

The finished vials are quarantined, relabelled and inspected by Quality Control Department at Armour Pharmaceutical Company Ltd., Hampden Park, Eastbourne, Sussex. Storage facilities are described in the manufacturing licence application ML 0231/01 (updated December, 1977).

3.3. Importer

The importer of the finished vials of lyophilised High Potency Factorate will be the licence holder.

3.4. Responsibility for Quality Control

(a) The licence holder will be responsible for deciding if any batch of the product is acceptable for release for marketing with prior reference to the NIBS & C.

(b) The quality control will be carried out initially at Armour Pharmaceutical Co., Kankakee, Illinois, U.S.A.

PART II PHARMACEUTICAL DATA ON THE DOSAGE FORM

SECTION 1 Finished Product

1.1. Description

A white to pale yellow lyophilised cake in a vial with a vacuum. The vial is fitted with a rubber closure and a one piece aluminium-plastic covered brown seal.

1.2. Complete Formula

1.2.1. Active Constituent

Factor VIII (lyophilised) .. .. NLT 800 units/vial

1.2.2. Other Constituents

Glycine U.S.P.	0.31 Molar
Sodium Chloride U.S.P.	0.15 Molar
Sodium Citrate U.S.P.	0.01 Molar
Heparin Sodium U.S.P.	NMT 30 units/vial

1.3. Containers

The lyophilised cake is contained in a 50 ml Type I Glass Vial with 20 mm finish. The closure is a butyl rubber grey stopper with an aluminium seal. A vial of sterile Water for Injection B.P. may be provided.

1.4. Formulation Used in Clinical Trials

The product as described was evaluated by Protocol 101 using Batches, K 852031 (1164 AHF units/vial), K 852032 (1218 AHF units/vial), K 852030 (1314 AHF units/vial). The specification for these batches was met by the Finished Product Specification given in Part II, Section 3, of this volume.

SECTION 2 Manufacture of the Dosage Form

2.1. Manufacturing Formulae

The attached flow diagram indicates the eleven phases of concentration and purification to which fresh frozen storage human plasma is subjected.

The following raw materials are involved in the preparation of the sterile bulk solution of Factorate Generation II:-

Sodium Hydroxide U.S.P.
Sodium Citrate U.S.P.
Sodium Chloride U.S.P.
Glycine (Aminoacetic Acid) U.S.P.
Glacial Acetic Acid U.S.P.
Sodium Heparin Injection U.S.P.
Rehsorptar (Aluminium Hydroxide Suspension)
Ethyl Alcohol 95%

2.2. Manufacturing Process

High Potency material is manufactured from fresh frozen human plasma which when tested complies with the Raw Material Specification and is negative for hepatitis B surface antigen activity. A cryoprecipitate is isolated from thawed human plasma and is dissolved at $25^{O} \pm 5^{O}C$ in glycine-saline buffer containing NMT 3 units/ml Sodium Heparin U.S.P. The pH is adjusted with 0.1N Acetic Acid and / or 0.5N NaOH and filtered. Impurities are adsorbed on to aluminium hydroxide sterile suspension, centrifuged at approximately $15^{O}C$ and the preparation stabilised with Sodium Citrate U.S.P. and Sodium Chloride U.S.P. (both pyrogen free). The solution is cooled to approximately $0^{O}C$ and cold Ethyl Alcohol (95%) added to a concentration of approximately 7%. The precipitate is isolated at low temperature and suspended in citrate-saline-glycine buffer. The pH is adjusted, the solution membrane filtered and is finally sterile filtered through bacterial retentive membrane filters (0.8µ down to 0.22µ), before filling into sterile Type I glass containers*. The filled vials are frozen, dried from the frozen state under vacuum, stoppered under vacuum and sealed.

*Manufacturing Protocol of Sterilising Procedures as attached.

SECTION 2 Manufacture of the Dosage Form (Cont.)

2.3. Assembling Process

The finished vials are transferred to an inspection area and inspected for particulate matter, vial stopper and seal defects and character of the cake. Acceptable vials are randomly sampled by Quality Control and checked for compliance to Specification.

The finished vials are stored in quarantine at below 6°C and when passed by Control are labelled as appropriate.

FLOW DIAGRAM

Manufacturing Process for Antihaemophilic Factor (Human), (High Potency)

PHASE A - Collection and Storage of Human Plasma

↓

PHASE B - Isolation of Cryoprecipitate

(a) Thawing at 0°C ± 2°C
(b) Centrifugation at 1°C ± 2°C ⟶ Cryo-Poor Plasma Supernatant

↓

PHASE C - Dissolution of Cryoprecipitate in Glycine-Saline Buffer Containing Heparin

(a) pH adjustment
(b) Centrifugation/Filtration ⟶ Precipitate discarded
(c) pH adjustment

↓

PHASE D - Aluminium Hydroxide Adsorption (Sterile 2% Suspension Added)

(a) Storing at 15°C ± 5°C
(b) Centrifugation and Filtration ⟶ Precipitate discarded

↓

PHASE E - Stabilisation and Alcohol Precipitation

(a) Sodium Citrate, Sodium Chloride Additions
(b) Addition of Ethanol at 0°C ± 2°C

↓

PHASE F - Isolation of Precipitate by Centrifugation at 0°C ± 3°C ⟶ Discarded Supernatant

↓

PHASE G - Resuspension of Precipitate in Citrate-Saline-Glycine Buffer

(a) pH adjusted

↓

PHASE H - Clarification through a Membrane Filtration Assembly

↓

PHASE I - Sterile Filtration into Sterile Holding Tanks.
(Sample Submitted for Bulk Sterility Testing)

↓ (see next page)

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FLOW DIAGRAM (CONT)

Manufacturing Process for Antihaemophilic Factor (Human), (High Potency)

PHASE J - Filling (Under Constant Positive Pressure)

↓

PHASE K - Lyophilisation (Under Vacuum) and Sealing of Vials for Inspection and Storage at 2 - 8°C or colder.

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ARMOUR PHARMACEUTICAL COMPANY
MANUFACTURING PROCEDURE
2ND MARCH, 1978

MANUFACTURING PROTOCOL FOR STERILISING PROCEDURES

LOT NO .....

YIELD .....

MP No 11007A
(FOR S-3665)

ANTIHAEMOPHILIC FACTOR (HUMAN)
STERILE BULK SOLUTION

INGREDIENTS AND SUPPLIES	SPEC NO	LOT OR R/R NO	THEORETICAL QUANTITY	ACTUAL QUANTITY
A.H.F. (Human) Non-Sterile Bulk Solution	3664	_____	5-40 L	_____
	ITEM NO			
Millipore AAWP29325 MF Filter AA 0.8 Micron, 293 mm	93170	_____	1	
or				
AA 0.8 Micron, 142 mm	92927	_____	1	
Millipore DAWP29325 MF Filter DA 0.65 Micron, 293 mm	93171	_____	1	
or				
DA 0.65 Micron, 142 mm	92928	_____	1	
Millipore HAWP29325 MF Filter HA 0.45 Micron, 293 mm	93172	_____	1	
or				
HA 0.45 Micron, 142 mm	92929	_____	1	
Millipore PHWP29325 MF Filter PH 0.3 Micron, 293 mm	93173	_____	1	
or				
PH 0.3 Micron, 142 mm	92930	_____	1	
Millipore GSWP29325 MF Filter GS 0.22 Micron, 293 mm	93174	_____	2	
or				
GS 0.22 Micron, 142 mm	92931	_____	2	
Millipore AP3225725 Dacron Separator, 257 mm	93785	_____	5	
or				
Separator, AP32, 124 mm	92922	_____	5	

BATCH SIZE AND EQUIPMENT: (Approximate Figures Only)

	Plasma Volume	Filtration Volume	Secondary Clarification Filters	Sterile Filters
☐	880 l	6.0 l	1	1
☐	1060 l	7.2 l	1	1
☐	1320 l	9.0 l	1	1
☐	1500 l	10.2 l	1	1
☐	2100 l	14.4 l	2	2
☐	3150 l	21.6 l	2	2
☐	4200 l	28.8 l	2	2
☐	5250 l	36.0 l	3	3

Date and Operator

PROCESS

A. PREPARATION OF SECONDARY CLARIFICATION FILTRATION ASSEMBLY

1. Assemble the number of 293 mm or 142 mm Millipore filters required by batch size per S.O.P. 11-11 for clarification using the following sequence of filters from outlet to inlet: _____No. Filters

   1 Dacron Separator
   1 MF Filter GS 0.22 Micron
   1 Dacron Separator
   1 MF Filter PH 0.3 Micron
   1 Dacron Separator
   1 MF Filter HA 0.45 Micron
   1 Dacron Separator
   1 MF Filter DA 0.65 Micron
   1 Dacron Separator
   1 MF Filter AA 0.8 Micron _____

2. Provide for connection via tubing directly to sterile filtration assembly as per drawing PD-48 _____

_____

_____

LOT NO. _____

Date and Operator

B. STERILE FILTRATION ASSEMBLY

1. Assemble a 2 - 20 l Millipore tank as receiver and one 293 mm or 142 mm Millipore filter per S.O.P. No. 11-11 for sterile filtration using the following sequence of filters from outlet to inlet:

   1 Dacron Separator
   1 MF Filter GS 0.22 Micron
   1 Dacron Separator
   1 MF Filter PM 0.3 Micron

   l-_____ Tank No.
   _____ Kg Tare Tank
   _____ mm Size of Holder

2. Sterilize receiver as per S.O.P. No. 11-6.

   l-_____ Autoclave No.
   _____ °C Temperature
   _____ Minutes Time

3. Sterilize Millipore filter holders as per S.O.P. No. 11-6.

   l-_____ Autoclave No.
   _____ °C Temperature
   _____ Minutes Time

4. Connect sterile filters and receiver within a sterile laminar air flow.

C. CLARIFICATION AND STERILIZATION FILTRATION

1. Weigh receiver to determine tare weight.

   _____ Kg Tare
   l-_____ Scale No.

2. Filter solution through clarification filters in series with sterilization filter per S.O.P. No. 11-11 for sterile filtration at 0.5 to 1 psi increasing gradually to 5 psi nitrogen to maintain a steady flow.

   Non-Sterile Bulk:
   _____ Kg Solution
   (from MP 11006A, C-2)

   NOTE: The solution is received in a 2 - 20 l Millipore tank and hence is not exposed to air-borne contamination during filtration.

Filtration started: _____ Time _____ PSI Pressure
Filtration finished: _____ Time _____ PSI Pressure

LOT NO. _____

AP001003

Date and Operator

2. Weigh sterile bulk solution.

Sterile Bulk:
G_____ Kg
T_____ Kg
N_____ Kg
1-_____ Scale No.

3. Label receiver, "Antihaemophilic Factor (Human), Sterile Bulk Solution, (High Potency), S-3665", Lot number, quantity and date.

NOTE: Upon completion of filtration, proceed immediately to filling.

D. CLEANUP

1. Wash filters per S.O.P. No. 11-9.

2. Wash bulk tank per S.O.P. No. 11-7.

NOTE: If equipment is not to be cleaned immediately after use, affix a tag reading, "To be Cleaned". The tag is to include the date and a verification signature by the checker or supervisor.

COMMENTS: _____

_____

APPROVED BY

PRODUCTION _____

TECHNICAL _____

CONTROL _____

REGULATORY _____

"THE ABOVE MANUFACTURING INSTRUCTIONS AS STATED, HAVE BEEN STRICTLY ADHERED TO EXCEPT AS NOTED."

Date Completed _____

Supervisor's Signature _____

LOT NO. _____

CONFIDENTIAL
ARMOUR PHARMACEUTICAL COMPANY
MANUFACTURING PROCEDURE
2ND MARCH, 1978

MANUFACTURING PROTOCOL FOR STERILISING PROCEDURES

LOT NO .....

YIELD .....

MP No 11008A
(FOR S-3666)

ANTIHAEMOPHILIC FACTOR (HUMAN), 50 ML VIAL

INGREDIENTS AND SUPPLIES		SPEC NO	LOT NO R/R NO	THEORETICAL QUANTITY	ACTUAL QUANTITY
A.H.F. (Human) Sterile Bulk Solution		3665	_____	5-40 l	_____
		ITEM NO			
Vials:	50 ml, 20 mm	64900	_____		
Stoppers:	Tompkins PT-24-B0854 Mod., Grey Butyl, 20 mm	65810	_____		
Seals:	One Piece, 20 mm Aluminium Plastic Flip-off Brown (Code 3770)	67263	_____		

BATCH SIZE: (Approximate Figures Only)

	Plasma Volume	Filtration Volume	Vials 30 ml Fill*
☐	880 l	6.0 l	150
☐	1060 l	7.2 l	180
☐	1320 l	9.0 l	225
☐	1500 l	10.2 l	255
☐	2100 l	14.4 l	360
☐	3150 l	21.6 l	540
☐	4200 l	28.8 l	720
☐	5250 l	36.0 l	900

CALCULATIONS

*Fill Weight: 30.2 ml x _____ S.G. = _____ g ± 0.2 g.

AP001005

SECTION 3 Quality Control

3.1. Specification of Constituents

	Specifications (attached)
Specification of the Source Plasma (Human), Flash Frozen for Antihaemophilic Factor (Human)	3029
Rehsorptar (F-5000 Aluminium Hydroxide Gel, sterile)	3232

3.1.1. Constituents Complying with Monographs

The following materials are used in manufacture and comply with the U.S.P. monographs and the "in-house" specification indicated:-

	Specifications*
Sodium Citrate U.S.P.	267
Sodium Chloride U.S.P. - Pyrogen free	271
Glycine (Aminoacetic Acid) U.S.P.	753
Glacial Acetic Acid U.S.P.	897
Sodium Heparin Injection U.S.P.	2951
Sodium Hydroxide U.S.P.	U.S.P.

*These Specifications were provided on pages 8 - 14 Volume I our Submission for Factorate.

3.1.2. Constituents Not Complying with Monographs

As attached.

SPECIFICATIONS OF NON-MONOGRAPHED CONSTITUENTS

1. Source Plasma (Human) Flash Frozen - Specification 3029

2. Rehsorptar (F-5000 Aluminium Hydroxide Gel) - Specification 3232

ARMOUR PHARMACEUTICAL COMPANY QUALITY STANDARDS Kankakee, Illinois	SOURCE PLASMA (HUMAN), FLASH FROZEN FOR ANTIHAEMOPHILIC FACTOR (HUMAN)	23

S P E C I F I C A T I O N 3029

Date:	Supersedes:	Prepared By:
1/6/77	10/10/73	M. S. Johnson

Description

Source Plasma (Human), Flash Frozen, for Antihaemophilic Factor (Human), S-3029, is the fluid portion of human blood which has been stabilised against clotting, collected by plasmapheresis from adult humans who have not been hyperimmunized to produce specific antibodies, and intended as source material for manufacture of Antihaemophilic Factor (Human), Immune Serum Globulin (Human), Plasma Protein Fraction (Human), and Normal Serum Albumin (Human). It is manufactured according to and conforms to all sections of Title 21 of the Code of Federal Regulations, Subchapter F, Parts 600, 601, 606, 607, 610, and Subpart G (640.60 through 640.76) of Subchapter F and is flash frozen as individual units at -70 °C or colder within one hour after separation from red blood cells and within two hours of withdrawal from the donor. The type of anti-coagulant contained in the plasma shall be separately agreed upon by authorised representatives of Armour Pharmaceutical Company and the supplier.

Plasma Properties (Specification S-3029)

1. Substantially free from red blood cells.

2. Maximum of 50 mg Haemoglobin per 100 ml.

3. Total Protein content of not less than 5.5% after processing to remove red blood cells.

4. Free of bacterial or pyrogenic contamination.

5. Free of Hepatitis B Surface Antigen as tested on individual units of plasma by Radioimmune Assay or other assay meeting requirements of Title 21 CFR 610.40.

6. Serologically non-reactive for Syphillis.

Labels and Shipping

1. The label affixed to each immediate container of plasma shall contain all information required in 21 CFR 640.70.

2. The following information shall appear on the label affixed to each carton of plasma.

   (a) Addressee - Armour Pharmaceutical Company.

   (b) Name and full address of blood establishment.

   (c) Proper name of and specification number for material in shipment. "Source Plasma (Human), Flash Frozen, For Antihaemophilic Factor (Human) S-3029".

   (d) Number and size of containers in carton.

   (e) Number of cartons in shipment and the individual number of each carton, e.g. Box No. 5 of 23 boxes.

   (f) The statement, "Store at -20°C or colder".

3. The following information will be included with each shipment of plasma to Armour Pharmaceutical Company. Records should be contained in an envelope or otherwise adequately bound and placed in one carton of the shipment; that carton should be adequately marked to facilitate its identification when received at Armour Pharmaceutical Company.

   (a) The name and full address of the licensed plasmapheresis center at which the plasma was collected.

   (b) Identification by number of each plasma unit included in the shipment.

   (c) Bleeding dates of all units.

   (d) Type of plasma in the shipment.

   (e) A statement defining the type of anticoagulant contained in all units in the shipment.

   (f) A statement confirming that all units have been tested and found non-reactive for Hepatitis B Surface Antigen as defined in Title 21, Code of Federal Regulations, S640.67.

   (g) A statement confirming that all units have been flash frozen at -70°C or colder within one hour after separation from red blood cells and within two hours of withdrawal from the donor.

NOTE: Forms similar to Attachments A and B may be used.

Inspection

The processing establishment, and all its equipment and records will be available for inspection during regular business hours by designated representatives of Armour Pharmaceutical Company.

Records

Records of all operations shall be maintained according to the requirements of the Source Plasma (Human) Regulations, but not less than 12 years after manufacture.

General

1. Failure to comply with any of the foregoing specifications shall be sufficient cause to reject any Plasma delivered to Armour Pharmaceutical Company.

2. Armour Pharmaceutical Company reserves the right to exclude any and all plasma that may, in its opinion, contribute to processing problems or unsatisfactory final products.

ATTACHMENT A

DONOR CENTER ______________________________

____

____

Carton Number	Plasma Unit Identity	Date of Bleeding	Plasma Volume	Carton Number	Plasma Unit Identity	Date of Bleeding	Plasma Volume

HUMAN PLASMA DATA SHEET

(To be submitted with each shipment of plasma sent to Armour Pharmaceutical Company, Kankakee, Illinois)

DONOR CENTER: Name _____

Address _____

TYPE OF PLASMA:

_____ Source Plasma (Human) Flash Frozen for Antihaemophilic Factor (Human)

_____ Source Plasma (Human) Flash Frozen for Tetanus Immune Globulin (Human) and Anti-haemophilic Factor (Human)

_____ Source Plasma (Human)

_____ Source Plasma (Human) for Tetanus Immune Globulin (Human)

_____ Source Plasma (Human) Salvaged

_____ Source Plasma (Human) Salvaged for Tetanus Immune Globulin (Human)

Shipment No. _____

Quantity _____ Liter

Date of Shipment _____

This shipment includes plasma collected from _____

through _____ inclusively, and sequentially numbered

from _____ through _____

ANTICOAGULANT:

_____ Anticoagulant Sodium Citrate

_____ Anticoagulant Citrate Dextrose

_____ Anticoagulant Citrate Phosphate Dextrose

HEPATITIS B SURFACE ANTIGEN

Test Used _____

All units in this shipment have been tested for and found non-reactive for Hepatitis B Surface Antigen (HbsAg).

Yes _____ No _____

Bleed numbers of plasma tested and found reactive for HbsAg from _____ through _____ (use same dates listed above:

_____

_____

Record or disposition of these units are attached.

PROCESSING INFORMATION:

All units in this shipment were flash frozen at -70°C or colder within one hour after separation from red blood cells and within two hours of withdrawal from the donor.

Yes _____ No _____

Explain Exceptions _____

_____

STORAGE CONDITIONS:

Room Temperature _____ Freezer (Below 0°C) _____

Refrigeration _____ Freezer (-20°C or lower) _____

Freezer (-40°C or lower) _____

SIGNATURE: _____ TITLE: _____

DATE: _____

OPERATIONS STANDARDS Kankakee, Illinois	REHSORPTAR, 1 LITER (F-5000 Aluminium Hydroxide Gel)	29
SPECIFICATION 3232		

Date:	Supersedes:	Prepared By:	Expiry Date:
6/5/77	New	M.S. Johnson	-

REHSORPTAR, 1 LITER
(ALUMINIUM HYDROXIDE STERILE SUSPENSION)

Description: Rehsorptar, 1 Liter is a sterile, opaque, white viscous, thixotropic gel.

Sampling: Group I - 1 x 1 liter bottle (partial fill if available);
Group III - 5 x 1 liter bottles; remainder returned to stock

TEST	SPECIFICATION	METHOD
*Aluminium Oxide	1.8 - 2.2%	286
*Protein Binding Capacity	Not less than 1 mg protein per mg Aluminium Oxide	344
*Specific Gravity	0.900 - 1.100	13
*Sterility	Satisfactory	303

*Test run at Kankakee using methods of analysis detailed and approved for Factorate^{R}.

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SECTION 3 Quality Control (Cont.)

3.1.3. Suppliers of Active Ingredient

The source is human plasma collected at approved centres in U.S.A.

3.2. In-Process Control During Pharmaceutical Manufacture

In process controls are exercised as follows:-

(a) On receipt of frozen plasma - plasma records are inspected before the plasma is approved for fractionation. All plasma donations must have been tested for hepatitis associated antigen.

(b) Checks are made on all temperature and pH recording apparatus.

(c) After sterile filtration samples are taken and submitted for bulk sterility testing and for protein content and specific gravity.

(d) During vial filling, the volume of fill is monitored throughout gravimetrically.

(e) A microbial profile of the filling area is regularly ascertained and monitored.

(f) All vials are checked for integrity.

(g) Filled vials are individually checked for particulate matter, vial stopper or seal defects and the character of the cake.

3.2.1. Analytical Control

As in (a) to (g) above.

3.2.2. Sampling for Quality Control

Sampling in respect of (c) above is made at the end of a production run.

Sampling in respect of (e) above is made on a daily basis.

SECTION 3 Quality Control (Cont.)

3.3. Final Product Specification

The Product Specification No. S-3666 is based on assays of 3 batches used in clinical evaluation and stability studies. The Specification includes all tests detailed in our Licence Application (Volume I, page 94, March 1975) for Dried Antihaemophilic Fraction B.P. (Factorate), and is a tentative check specification to which any sample should comply during shelf life.

The potency of the product is determined in International Units at the Oxford Haemophilic Centre, Headington, Oxford using a standard calibrated against the Second International Standard.

3.3.1. Tests and Limits Applied

The following test methods are included on subsequent pages of this Section and relate to the Finished Product Specification. All other tests, not provided, comply with the current B.P.

	METHODS (on subsequent pages)
Determination of Moisture (loss on drying)	43-D
Pyrogens Test	208
Sterility Test	303
Identity Test	351
Hepatitis Bs Antigen Test	379
Isoagglutinin Titre	386
Safety Test for Normal Serum Albumin	963
Determination of Total Protein ..	993
Determination of Fibrinogen	994
Determination of Aluminium	995
Heparin Content	1073
Solution Time	1257

The limits applied to the above methods are given on the Finished Product Specification.

SECTION 3 Quality Control (Cont.)

3.3.2. Pharmacopoeial Tests

The following tests are carried out to the current B.P. requirements:-

1. Determination of pH (when reconstituted)

2. Determination of potency - the biological assay for Human Antihaemophilic Fraction B.P.

3. Determination of sodium ions by flamephotometry

FINISHED PRODUCT SPECIFICATION

HIGH POTENCY FACTORATE

ARMOUR PHARMACEUTICAL COMPANY LTD.
EASTBOURNE ENGLAND

QUALITY CONTROL DEPARTMENT

TENTATIVE SPECIFICATION
S-3666

Description: A white to pale yellow lyophilised cake in a 50 ml vial with vacuum having a brown seal.

TEST	SPECIFICATION	METHOD
Potency	A minimum of 800 i.u./vial (26.5 i.u./ml reconstituted)*	B.P.
Heparin Content	A maximum of 30 units/vial	1073
Total Protein	Not more than 1600 mg/vial (NMT 5.3% reconstituted)*	993
Fibrinogen	Not more than 4.5% when reconstituted*	994
Aluminium	Maximum of 180 µg/vial	995
Moisture	Not more than 2%	43-D
Identity	Human - Positive, Bovine, Ovine and Porcine - Negative	351
Safety	Passes	963
Sterility	Passes	302-D
Pyrogens (10 i.u./kg)	Passes B.P. Test	208
Solution Time	Maximum of 30 minutes	1257
Isoagglutinins	Not more than 1:64 on any test	386
pH (reconstituted)	7.2 ± 0.4	B.P.
Sodium Ions	Not more than 200 mM/1 when reconstituted*	B.P.
Citrate Ions	Not more than 55 mM/1 when reconstituted*	B.P.
Hepatitis Bs Antigen	Non-reactive.	379

*Reconstituted in 30 ml Water for Injection B.P.

AP001019

RELATING ANALYTICAL METHODS

ARMOUR PHARMACEUTICAL COMPANY, KANKAKEE
QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

METHOD NO. 43D

METHOD TO DETERMINE LOSS OF WEIGHT ON DRYING

Preparation of Covered Moisture Pans or Weighing Bottles

Prepare the moisture pans or weighing bottles together with their matched covers or stoppers by placing them, with the cover removed, for 30 minutes under the same conditions to be employed in the determination. After 30 minutes, immediately replace the cover or stopper and accurately weigh the container. This is the tare or empty weight.

Procedure

Reduce the sample to a fine powder. Uniformly distribute 0.5 - 1.0 g of the sample material in the moisture container and accurately weigh to determine the gross weight or container plus undried sample weight. If possible, the exposed surface area of the powdered sample should not be less than 18 square centimeters.

Place the uncovered container, together with its matching cover, in a desiccator containing fresh phosphorus pentoxide or concentrated sulphuric acid for a period of 12 - 24 hours, under a pressure of not more than 500 microns* measured with a McLeod vacuum gauge, or equivalent. Maintain the temperature at 20 - 25°C (Room Temperature). After drying, remove the container and weigh immediately. This weight is the weight of container, cover, and dried sample.

Calculation

$$\frac{\text{Container + undried sample weight} - \text{tare weight}}{\text{Weight of undried sample}} = \frac{\text{Container + undried sample weight} - \text{Container + dried sample weight}}{\text{Loss of weight on drying}}$$

Then, $\frac{\text{Loss of weight on drying}}{\text{Weight of undried sample}} \times 100 = \%$ of sample weight lost of drying

*To obtain this pressure, a dry ice-acetone trap is necessary between the desiccator and the pump.

ARMOUR PHARMACEUTICAL COMPANY, KANKAKEE
QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

METHOD 208

PYROGEN TEST

The pyrogen test is designed to limit to an acceptable level the risks of febrile reaction in the patient, to the administration by injection, of the product concerned. The dose specified for the test is related to that generally given to the patient, but for practical reasons, it does not exceed 10 ml per kg of body weight of the test animal, injected in a brief period of time.

Apparatus

Render the syringes, needles, and glassware free from pyrogens by heating at 121°C for not less than 30 minutes or by any other suitable method. Just prior to injecting it, warm the product to be tested to approximately 37°C.

Test Animals

Use overtly healthy, mature rabbits. For a normal strain that is commonly used (New Zealand Whites) each should not weigh less than 1500 g. House the animals individually in an area of uniform temperature (±3°C(=5°F)) and free from disturbances likely to excite them. Before using an animal for the first time in a pyrogen test, condition it by a sham test that included all of the steps as directed under "Procedure" except the injection of the test dose. Do not use animals for pyrogen tests more frequently than once every 48 hours, nor prior to 2 weeks following their having been given a test sample that was adjudged pyrogenic.

Note: Perform the test under environmental conditions similar to those under which the animals are housed. During the test, withhold all food from the animals being used. Access to water may be allowed. If rectal temperature measuring probes are to remain inserted through-out the testing period, restrain the rabbits with light-fitting neck stocks that allow the rabbits to assume a natural resting posture.

Temperature Recording

Use an accurate clinical thermometer for which the time necessary to reach the maximum reading is known, or any other temperature-recording device of equal sensitivity. Insert the thermometer or probe into the rectum of the test animal to a depth of not less than 7.5 cm and after a period of time no less than that previously determined as sufficient, record the animal's body temperature.

Procedure

Not more than 40 minutes prior to the injection of the test dose, determine the "control temperature" of each animal; this is the base for the determination of any temperature increase resulting from the injection of a test solution. In any one test use only those animals the control temperatures of which do not vary by more than 1°C from each other, and do not use any animal with a temperature exceeding 39.8°C.

Unless otherwise specified, inject into an ear vein of each of three rabbits 10 ml of the product per kg of body weight, completing the injection within 10 minutes after start of administration. Record the temperature at 1, 2 and 3 hours subsequent to the injection.

Interpretation and Retest

Record observed temperature decreases as zero. If no rabbit shows an individual rise in temperature of 0.6°C or more above its respective control temperature, and if the sum of the three individual maximum temperature rises does not exceed 1.4°C the product meets the requirements for the absence of pyrogens. If any rabbit shows an individual temperature rise of 0.6°C or more, or if the sum of the three individual maximum temperature rise exceeds 1.4°C repeat the test using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.6°C or more, and if the sum of the eight individual maximum temperature rises does not exceed 3.7°C the material under examination meets the requirements for the absence of pyrogens.

Reference: U.S.P.; Code of Federal Regulations, 21, Section 610.13, 1976

ARMOUR PHARMACEUTICAL COMPANY, KANKAKEE
QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

METHOD 208
Supplement No. 33

ANTIHAEMOPHILIC FACTOR - HIGH POTENCY FACTORATE (A.H.F.)

Preparation of Sample

Reconstitute vial of A.H.F. with 30 ml of sterile water for injection using the solution procedure as stated in Method 1257. Dilute the reconstituted sample 1:4 with sterile water.

Dose

Assuming a potency of 1,200 units/vial, inject intravenously, 4ml/kg body weight, or 40 units/kg body weight.

ARMOUR PHARMACEUTICAL COMPANY, KANKAKEE
QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

METHOD 303

STERILITY TESTING - FINAL PRODUCT

Rigid aseptic techniques must be employed at all times. Testing should NOT be conducted in areas under aerosol treatment. Environmental control tests, such as exposure plates, must be performed daily in the aseptic sterility testing area.

Test each lot of medium for sterility and for its growth-promoting qualities. Inoculate two sets of the test medium with not more than 100 spores of Bacillus subtilis (ATCC No. 6633). Likewise, inoculate two sets of test medium with the same number of organisms of Candida albicans (ATCC No. 10231). For Fluid Thioglycollate Medium only, test also two additional sets of medium with no more than 100 organisms of Bacteriodes vulgatus (ATCC No. 8482). Additional organisms may be used. Incubate all inoculated sets of Soybean-Casein Digest medium at 20°C to 25°C and those of Fluid Thioglycollate Medium at 30°C to 32°C. The test media are satisfactory if evidence of substantial growth appears within 7 days. These tests may be conducted simultaneously with the use of the test media for sterility test purposes, provided, however, that the sterility test is considered unsatisfactory if the test medium shows poor or no growth response.

Confirm the sterility of each lot of medium used by incubation of samples at the temperature and for the length of time specified for it, where its use is called for in the tests.

If freshly prepared media are not used within 2 days, store them in the dark, preferably at 2° to 25°. Finished media may be stored in unsealed containers for more than 10 days, provided that they are tested weekly for growth promotion. If stored in sealed containers, the media may be used for one year, provided that they are tested for growth promotion every 3 months.

In order to avoid false negative results, establish the bacteriostatic and fungistatic activity for each product. If the product is bacteriostatic or fungistatic, a suitable sterile inactivating agent must be used or in the absence of such an agent, the established product inoculum-media ratios must be adhered to.

ALL STERILITY TESTING MUST BE CONDUCTED IN THE ASEPTIC AREA BY TRAINED PERSONNEL.

Product Sampling

For products which are sterilized with steam under pressure in the final sealed containers, select 20 or more units for each sterilizer load. These samples must be representative of all layers of the load. For all other products, select for the test a total of 20 or more units representative of each batch, taken at regular intervals throughout each filling operation.

In the process of testing a lyophilized product, it should be reconstituted according to the directions supplied with the product.

Testing Techniques

1. Liquids and Suspension

   (a) Sterilize the exterior surfaces of vials and ampoules with a suitable bactericidal agent.

   (b) Open ampoules by breaking off neck with sterile gloved fingers.

   (c) Remove liquids or suspensions for culturing with a sterile pipette or with a sterile syringe fitted with a sterile hypodermic needle.

   (d) Plant portions of the material from each container being tested into Fluid Thioglycollate Medium. In addition, plant portions from each container being tested into Soybean-Casein Digest Medium.

   If the samples do not contain sufficient volume for seeding, of both the Fluid Thioglycollate and Soybean-Casein Digest Medium, use duplicate samples.

   If the product contains mercurial preservative, replace the Soybean-Casein Digest Medium with another tube of Fluid Thioglycollate Medium and incubate 14 days at 20 - 25°C.

2. Crystalline and Powdered Solids

   If the product is soluble or dispersible, the suitable amount of sterile diluent is added aseptically to the final container. After mixing, withdraw a quantity of the product corresponding to 300 mg from each container being tested, or the entire contents if less than 300 mg, and transfer to Fluid Thioglycollate Medium and Soybean-Casein Digest Medium, respectively, and mix.

Inoculum Size

Vary the minimum volume of medium used according to the content of the final container as follows:-

CONTAINER CONTENT	MINIMUM VOLUME OF PRODUCT	MINIMUM VOLUME OF MEDIUM IF PRESERVATIVE	MINIMUM VOLUME OF MEDIUM IF NO PRESERVATIVE
10 ml or less	1 ml of total content if less than 1 ml	80 ml	40 ml
From 10 to 50 ml	5 ml	80 - 250 ml	80 ml
More than 50 ml	10 ml	250 ml	80 - 250 ml

Incubation

Mix the liquid thoroughly with the medium. If the test material is an oil, shake the mixture at the time of planting and at frequent intervals thereafter during the incubation period. Incubate the tubes of Fluid Thioglycollate at 30°C - 32°C and the Soybean-Casein Digest Medium at 20°C to 25°C for not less than 14 days.

When the material to be tested renders the medium turbid so that the presence or absence of growth cannot be determined readily by visual examination, transfer on the third day, suitable portions of this turbid medium to additional tubes of medium.

Incubate both the original and sub-culture tubes for 7 or 14 days as indicated. Examine all tubes at the end of the incubation period. Examine all tubes daily for the presence of growth.

Check all tubes showing growth by a microscopic examination of stained smears.

Interpretation of Test Results

If on the first test no growth is found, the material under examination meets the requirements for STERILITY. If growth is found, the test may be repeated on an identical number of different containers to rule out laboratory contamination which may be introduced during the test (FIRST RESAMPLE).

If contamination is again evident, twice the original number of samples are tested (SECOND RESAMPLE). Observe special precautions to insure that the units for the resample are representative of the sterilizer load or filling operation.

If repeat tests confirm the presence of contamination, the material under examination fails to meet the requirements for sterility. The nature of the product determines the selection of the specific sterility test to be used.

References

United States Pharmacopoeia XIX

Code of Federal Regulations, Title 21, Part 610, Section 610.12

ARMOUR PHARMACEUTICAL COMPANY, KANKAKEE
QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

METHOD NO. 351

MAMMALIAN PROTEIN SPECIES IDENTIFICATION (AGAR DIFFUSION)

Test Summary

The agar diffusion test is used to demonstrate the presence or absence of human, bovine, ovine or porcine proteinaceous material in human blood fractions. In the agar diffusion method, an antiserum containing specific antibodies is diffused with a material which is suspected of containing the corresponding antigen.

Comments

A. Safety Precautions

1. Personal hygiene cannot be overemphasized. Special awareness of this preactice should be noted when handling human blood derivatives since there exists an inherent risk of hepatitis infection.

2. Practice general laboratory safety regulations.

B. General Precautions

1. If two different antiserums are placed in opposing wells, the antiserums will usually react with one another due to the presence of antigens used to absorb each antiserum.

2. Room temperature is the most suitable for this test procedure. Reactions appear too quickly and are fainter at 37°C. Refrigerator temperatures (2 to 8°C) cause a slower reaction rate. In fact, in some cases, the expected reaction of a known positive never appears.

Materials for Testing

A. Antiserums to be Used

1. Rabbit Anti-Ovine Serum
2. Rabbit Anti-Bovine Serum
3. Rabbit Anti-Porcine Serum
4. Rabbit Anti-Human Serum

All antiserums should be checked out for cross-reactions with other animal proteins. If any cross-reactions are noted, an antiserum may be specifically absorbed with appropriate protein (antigen). Antiserums are supplied through commercial distributors.

B. Controls

1. Positive Ovine Control - 25% human albumin prepared to contain approximately 70 ppm ovine albumin.
2. Positive Bovine Control - 25% human albumin prepared to contain approximately 19 ppm bovine albumin.
3. Positive Porcine Control - 25% human albumin prepared to contain approximately 100 ppm porcine albumin.
4. Negative Control - 25% human albumin previously tested and known to be negative against ovine, bovine and porcine antisera.

C. Equipment Necessary

1. Hyland Immuno-Plate® Immunodiffusion Plates (Ouchterlony), Pattern D.
2. Capillary pipettes
3. Saline Solution - 0.9% sodium chloride. Dissolve 9 g sodium chloride in 1000 ml distilled water.
4. Moist Chamber - Chamber with moist piece of sponge or filter paper.

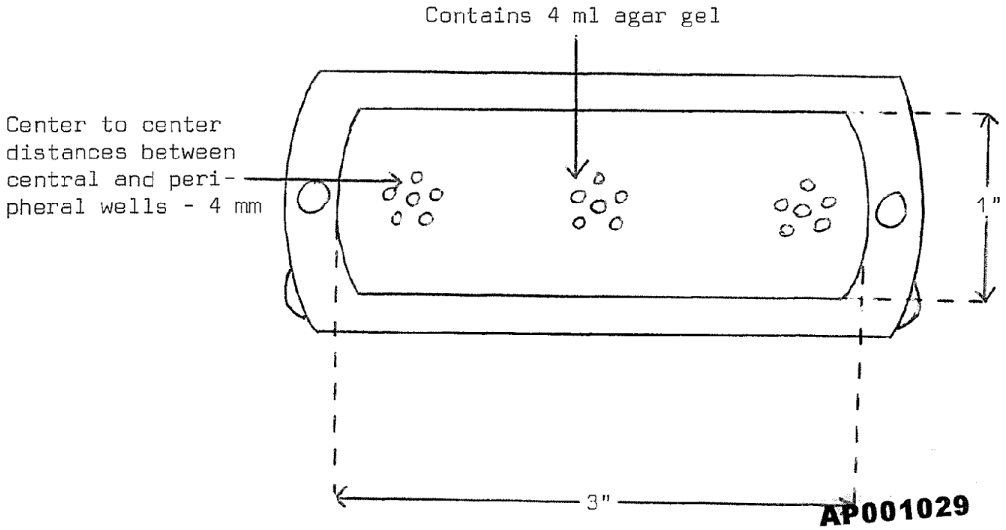
Test Procedure

A. Preparation of Samples for Testing

1. Albumins are ordinarily tested as a 25% solution, and, if necessary, are diluted to this percentage with saline solution. (1.25 g powder are dissolved in 5 ml saline solution.)
2. Gamma Globulins are ordinarily tested as a 16% solution (0.8 g powder are dissolved in 5 ml saline solution.)
3. Other blood products to be prepared as described on the package.

B. Double Diffusion Agar Gel Plates

Use Hyland Immuno-Plate® Immunodiffusion plates (Ouchterlony), Pattern D. The agar gel consists of Difco special 2% Noble agar, 7.5% glycine, 1% sodium chloride, 0.1% sodium azide with a pH of 7.0 to 7.2. The following diagram shows dimensions:



This pattern plate was chosen because of the speed with which the reactions occur. A final reading can usually be made within 6 hours. However, plates are held 24 hours before a test is recorded as negative. Caution must be exercised because of the speed of the reactions. Plates should be observed every half hour for the first 6 hours, and occasionally thereafter until 24 hours has elapsed. With familiar products a check at every half hour is unnecessary, however for new products a check is essential.

C. Set-Up and Assay

1. Using capillary pipettes, fill wells of agar plates in predetermined manner with samples to be tested and also controls.

2. Antigen is usually placed in outer wells; antiserum in center well. This conserves antiserum.

3. Place filled plates in moist chamber and incubate at room temperature.

4. Examine plates as necessary for 6 hours for precipitin reactions, i.e., an opaque zone or "line" between the antiserum well and each antigen well. (Indirect lighting may be used for this examination.)

5. Record positive results if they appear within 6 hours.

6. Examine the plate occasionelly (for the next 18 hours).

Results

If at the end of 24 hours no reaction has appeared the test is negative.

If excess antigen or antibody exists, the reaction will appear very quickly ($\frac{1}{2}$ to $1\frac{1}{2}$ hours), but may completely disappear by 6 hours or more. Positive reactions should be recorded as they appear and are considered a positive test even if the reaction disappears.

For the test to be valid, positive controls must be functioning properly; that is a positive reaction with the specific antiserum and no cross-reactions with other antiserums except Rabbit Anti-Human Albumin.

References

Armour Method No. 351

ARMOUR PHARMACEUTICAL COMPANY, KANKAKEE
QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

METHOD NO. 379

HEPATITIS ASSOCIATED ANTIBODY (ANTI-AUSTRALIA ANTIGEN) 125 I (AUSRIA II-125-ABBOTT)

Biological Principles of the Procedure

The Ausria II-125 system uses a "sandwich principle", a solid phase radioimmunoassay technique, to measure Hepatitis B Surface Antigen, HB_sAg, levels in serum and plasma. Plastic beads coated with guinea pig antibody are supplied in the kit. Patient serum or plasma is added and, during incubation, Hepatitis B Antigen, if present, is fixed to the antibody. When antibody tagged with iodine-125 is added, it binds to any Hepatitis B Antigen on the bead creating an antibody-antigen-antibody "sandwich".

Within limits, the greater the amount of antigen in the serum specimen, the higher the final count rate.

Reagents - (Store at 2° to 8°C - Ausria II-125-Abbott Kit No. 6119)

1. Negative Human Control (Recalcified human plasma non-reactive for HB_sAg and Anti-HB_s) Preservative: 0.1% sodium azide. Handle as though capable of transmitting hepatitis.

2. Positive Human Control (Human Plasma reactive for HB_sAg) 0.01 M TRIS Buffer containing 4% Bovine Serum Albumin is used as the diluent to adjust potency. Preservative: 0.1% sodium azide. Handle as though capable of transmitting hepatitis. Studies have indicated that the HB_sAg which is present in the Positive Human Control may possibly be a hepatitis agent or be carried in close association with such an agent. Because of this possibility, the Positive Human Control has been heated for 10 hours at 60°C. It is generally accepted that exposure of normal serum albumin to this time and temperature will inactivate the hepatitis agent. Nevertheless, complete inactivation should not be assumed.

3. Antibody to Hepatitis B Surface Antigen 125 I (Human). 0.005 M TRIS (hydroxymethyl) Aminomethane Buffer containing 50% Calf Serum, 2% Normal Human Serum and 0.5% Bovine Serum Albumin is used as the diluent to adjust potency. Activity: 0.74 microcurie or less/ml. Preservative: 0.1% sodium azide. Handle as though capable of transmitting hepatitis.

4. Antibody to Hepatitis B Surface Antigen (Guinea Pig) - Coated beads (polystyrene beads coated with guinea pig Anti-HB_s). Handle as though capable of transmitting hepatitis.

Handling

The following precautions should be observed in handling Ausria materials:

1. Handling should preclude any pipetting by mouth.

2. There should be no smoking or eating while radioactive or antigen-containing materials are being handled.

3. Hands should be covered with rubber gloves during, and thoroughly washed after, handling of radioactive materials.

4. Spills should be wiped up quickly and thoroughly and contaminated materials added to radioactive waste matter.

5. Certain small quantities of I-125 liquid waste may be disposed of through a selected sink drain. Details are available from the Diagnostic Division of Abbott Laboratories, Abbott Park AP-8, North Chicago, Illinois 60064. Reference can be made to Title 10, Code of Federal Regulations, Part 20.

   USAEC Form 483 in vitro registrants may dispose of solid waste by conventional means.

6. The specimens found to be reactive by the Ausria II-125 test and all materials used to perform the test should be disposed of as if they contained the infectious agent of viral hepatitis. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Rubber gloves worn throughout the entire procedure should also be deconta minated before discarding. Disposable materials may be incinerated. Liquid wastes may be mixed with sodium hypochlorite in volumes such that the final mixture contains 2.5% sodium hypochlorite. Allow 30 minutes for sterilization to be completed.

7. To avoid microbial contamination of reagents, aseptic techniques should be used in removal of aliquots from the primary vials. If the reagents are to be used within 48 hours, seals and stoppers of the primary vials may be removed and the contents may be utilized providing aseptic technique is employed.

General

Do not mix materials from different master lots.

Do not use kit components beyond the expiration date.

All materials should be brought to room temperature before use.

Materials should not be exposed to strong light during storage or incubation.

Although the association of infectivity and a positive result for HB_sAg is strong, it is recognized that presently available methods for HB_sAg detection are not sensitive enough to detect all infectious units of blood or all possible cases of hepatitis.

False positive results may be obtained with any diagnostic test. Two types of false positive results may occur with Ausria II-125:

1. Non-Repeatable Positives:

   Some of the Ausria II-125 positives may test non-reactive on repeat. This phenomenon is highly dependent on technique used in running the test. The most common sources of such non-repeatable positives are: (a) inadequate rinsing of bead, (b) contamination of sample holders in the gamma counter, and (c) cross-contamination of non-reactive samples caused by transfer of residual droplets of high titer, antigen containing sera on the pipetting device.

2. Non-Specific False Positives:

   The non-specific false positives resulting from cross reactions in the sandwich technique (antibody-antigen-antibody) appear to have been virtually eliminated by using a heterologous antibody system (guinea pig Anti-HB_s coated beads and 125 I labelled human Anti-HB_s). All highly sensitive immune systems have a potential for false positive reactions, but it is highly unusual to find Ausria II-125 repeatable reactive specimens which cannot be confirmed by the licensed Ausria Confirmatory Neutralization Test Kit, No. 8310.

Equipment

1. Precision pipettes or similar equipment to deliver 0.2 ml.

2. Device for delivery of rinse solution such as Cornwall syringe, Filamatic or equivalent.

3. An aspiration device for washing coated beads such as a cannula, aspirator tip, Uniwash™ II with a vacuum source and a trap for retaining the aspirate.

4. A well-type gamma scintillation detector capable of efficiently counting I 125.

5. Gently circulating water bath capable of maintaining temperature at $45^{o} \pm 1^{o}C$.

6. Abbott No. 8310, Antibody to Hepatitis B Surface Antigen (Human) AUSRIA Confirmatory Neutralization Test Kit.

   This radioimmuno assay must be performed on all Ausria II-125 reactive specimens unless they can be confirmed as positive by other licensed HB_sAg test systems.

Procedure A: (Incubation: 2 hours at $45^{o}C$; 1 hour at $45^{o}C$)

Seven negative and three positive controls should be assayed with each run of unknowns. Insure that reaction trays containing controls and reaction trays of unknowns are subjected to the same process and incubation times.

CAUTION: Use a clean pipette or disposable tip for each transfer to avoid cross-contamination.

1. Adjust temperature of water bath to 45°C.

2. Remove cap from clear plastic tube that contains antibody coated beads and attach dispensing tip to the open end. Remove dispensing tip cover and hold bead dispenser directly over top of reaction tray incubation well. Push down on dispensing tip with index finger to release one bead into a well for each sample to be tested.

3. Using precision pipettes, add 0.2 ml of serum (or recalcified plasma) and positive and negative controls to the bottom of their respective wells. Make sure that the antibody coated bead is completely surrounded by specimen. Tap the reaction tray to release any air bubbles that may be trapped in specimen.

4. Apply a cover sealer to each tray and incubate the trays in the 45°C water bath for two hours.

5. At the end of two hours remove the trays from the water bath. Remove the cover sealer and discard. Using a semi-automated aspiration and rinsing system, i.e. Uniwash II or Pentawash II and an automatic delivery system and vacuum source, follow the directions supplied with the semi-automated system and aspirate the specimen, rinse each well and bead with 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

   A manual system of washing the wells and beads may also be used. Using disposable pipettes or cannulas attached to a vacuum source and a Cornwall syringe delivery system, or equivalent, rinse each well and bead using extreme care not to overflow the reaction well but assure that the bead is totally immersed throughout the wash procedure. Place the pipette or cannula, attached to the vacuum source, into the bottom of the well next to the bead and simultaneously slowly add with the Cornwall syringe 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

6. With precision pipettes, add 0.2 ml of 125 I-Anti-HB_S (Human) to the bottom of each reaction well. Make sure that the antibody coated bead is completely surrounded by the labelled antibody solution. Tap to release any air bubbles that may be trapped in the solution.

7. Apply new cover sealer to each tray and incubate the trays in the 45°C water bath for one hour.

8. At the end of one hour remove the trays from the water bath. Remove cover sealer, aspirate the antibody solution from each well and rinse the well and antibody coated bead it contains with a total of two 5 ml portions of distilled or deionized water as in Step 5.

9. Transfer beads from reaction wells to properly identified counting tubes; align inverted rack of oriented counting tubes over reaction tray, press tubes tightly over wells, then invert tray and tubes together so that beads fall into properly labelled tubes.

10. Place the counting tubes in a suitable well type gamma scintillation counter and determine the count rate. The position of the bead at the bottom of the counting tube is not important. Although it is not critical that the counting be done immediately, preferably, it should be done within 24 hours after the final wash. All control samples and unknowns must be counted together.

Procedure B: (Incubation: Overnight at Room Temperature; 1 hour at 45°C)

Seven negative and three positive controls should be assayed with each run of unknowns. Insure that the reaction trays containing controls and reaction trays of unknowns are subjected to the same process and incubation times.

CAUTION: Use a clean pipette or disposable tip for each transfer to avoid cross-contamination.

1. Remove cap from clear plastic tube that contains antibody coated beads. Hold bead dispenser directly over top of reaction tray incubation well and push down with index finger to release one bead into a well for each sample to be tested.

2. Using precision pipettes, add 0.2 ml of serum, plasma, or recalcified plasma and positive and negative controls to the bottom of their respective wells. Make sure that the antibody coated bead is completely surrounded by specimen. Tap the reaction tray to release any air bubbles that may be trapped in specimen.

3. Apply a cover sealer to each tray and incubate on a level surface at room temperature for 16 hours (12 to 20 hours).

4. At the end of the incubation period remove the cover sealer and discard. Using a semi-automated aspiration and rinsing system, i.e. Uniwash II or Pentawash II and an automatic delivery system and vacuum source, follow the directions supplied with the semi-automated system and aspirate the specimen; rinse each well and bead with 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

   A manual system of washing the wells and beads may also be used. Using disposable pipettes or cannulas attached to a vacuum source and a Cornwall syringe delivery system, or equivalent, rinse each well and bead using extreme care not to overflow the reaction well but assure that the bead is totally immersed throughout the wash procedure. Place the pipette or cannula, attached to the vacuum source, into the bottom of the well next to the bead and simultaneously slowly add with the Cornwall syringe 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

5. Adjust the temperature of water bath to 45°C.

6. With precision pipettes, add 0.2 ml of 125 I-Anti-HB_s (Human) to the bottom of each reaction well. Make sure that the antibody coated bead is completely surrounded by the labelled antibody solution. Tap to release any air bubbles that may be trapped in the solution.

7. Apply new cover sealer to each tray and incubate the trays in the 45°C water bath for one hour.

8. At the end of one hour remove the trays from the water bath. Remove cover sealer, aspirate the antibody solution from each well and rinse the well and antibody coated bead it contains with a total of two 5 ml portions of distilled or deionized water as in Step 4.

9. Transfer beads from reaction wells to properly identified counting tubes: align inverted rack of oriented counting tubes over reaction tray, press tubes tightly over wells, then invert tray and tubes together so that beads fall into properly labelled tubes.

10. Place the counting tubes in a suitable well type gamma scintillation counter and determine the count rate. The position of the bead at the bottom of the counting tube is not important. Although it is not critical that the counting be done immediately, preferably, it should be done within 24 hours after the final wash. All control samples and unknowns must be counted together.

Results

The presence or absence of HB_sAg is determined by relating net counts per minute of the unknown sample to net counts per minute of the negative control mean times the factor 2.1.

Unknown samples whose net count rate is higher than the mean cutoff value established with the negative control are to be considered positive with respect to HB_sAg.

The mean value for the positive control samples should be at least 5 times the negative control mean. If not, technique may be suspect and the run should be repeated.

Calculation for Determining Cutoff Value

1. Calculation of the negative control mean

   a. Example:

Negative Control Sample No.	Net Count Rate Per Minute
1	380
2	400
3	410
4	375
5	350
6	390
7	400
Total	2705

$$\frac{\text{Total Net cpm}}{7} = \frac{2705}{7} = 386 \text{ net cpm (mean)}$$

b. Elimination of aberrant values

Method

Discard those individual values in the negative control samples which fall outside the range 0.5 to 1.5 times the mean.

Example*

0.5 x 386 = 193 and 1.5 x 386 = 579
Range = 193 cpm to 597 cpm

*In the example, no negative control sample is rejected as aberrant.

c. The negative control mean, therefore need not be revised. Typically, all negative control values should fall within the range 0.5 to 1.5 times the control mean. If more than one value is consistently found outside this range, technique problems should be investigated.

2. Calculation of the cutoff value (see NOTE)

a. Multiply the net negative control mean, 386 cpm, by the factor 2.1.

b. The calculated cutoff value is then 811 cpm.

c. Unknowns whose net count rate is higher than the cutoff value should be considered positive with respect to HB_sAg.

NOTE: Many gamma counters have no capacity for automatically subtracting background. In this case, as an alternative to subtracting instrument background manually from each sample, uncorrected sample counts per minute can be compared with a cutoff modified as follows:-

(Negative control mean - Background) x 2.1 + Background = Cutoff

Example

Gross negative control mean = 436 cpm

Instrument background = 50 cpm

Cutoff = (436 - 50) x 2.1 + 50 = 861 cpm

Samples with gross count rates greater than 861 cpm are to be considered reactive with respect to HB_sAg.

3. Calculation of positive control: negative control ratio

a. Divide the positive control mean value by the negative control mean value after correcting for background:

$$\frac{\text{Net Positive Control Mean}}{\text{Net Negative Control Mean}} = \text{P/N Ratio}$$

b. This ratio should be at least 5 or technique may be suspect and the run should be repeated.

Example

Net positive control mean value = 5906 cpm

Net negative control mean value = 386 cpm

P/N Ratio = 5906 ÷ 386 = 15.3

Technique is acceptable and data should be considered valid.

Interpretation of Results

Further testing of the sample in question will verify whether it is repeatedly positive. In making an evaluation of data, consideration should be given to the actual test values obtained. 2.1 times the negative control mean is used as the cutoff for single determinations. This value has been selected in order to decrease the total number of non-repeatable positives.

If repeat testing shows the sample to be less than 2.1 times the negative control mean, the original result may be classified as nonrepeatably reactive. If repeats are above the cutoff value, the sample should be presumed positive for HB_sAg. Such results are contingent on determination of the specificity of the repeatably reactive specimens.

Specificity analysis must be performed prior to informing a donor that he is a HB_sAg carrier.

Antibody to Hepatitis B Surface Antigen (Human), Kit No. 8310, provides a method for confirmation of screening procedure reactive specimens. This radioimmuno assay must be performed on all reactive specimens unless they can be confirmed as positive by other licensed HB_sAg test systems.

Repeatably Ausria II reactive specimen, confirmed by neutralization with human antiserum or other licensed HB_sAg tests must be considered HB_sAg positive.

Reference: Abbott Laboratories Brochure to Hepatitis - Associated Antibody - Ausria II - 125 Kit, Rev. Feb., 1976.

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METHOD NO. 379
Supplement No. 1

RECONSTITUTION OF ANTIHAEMOPHILIC FACTOR (A.H.F.) FOR HEPATITIS TESTING

Materials

1. Sterile Water for Injection, U.S.P. (30 ml/vial)
2. Double-ended Reconstitution Needle
3. Stopwatch or Timer
4. Water Bath at 37°C

Test Procedure

1. Warm both the 30 ml diluent of sterile water for injection and vial of A.H.F. (unopened vials) to 37°C in a water bath (not less than 15 minutes).

2. Remove caps from both vials to expose the central portion of rubber stoppers.

3. Insert one end of the double-ended needle into the rubber stopper of the diluent vial. Invert the diluent vial and insert the other end of the double-ended needle into the rubber stopper of the A.H.F. vial. Allow the diluent to be drawn into the A.H.F. vial by vacuum and direct the stream over the surface of the cake.

4. Release the vacuum by removing the diluent vial from the double-ended needle allowing the incoming airstream to agitate the vial contents. Then remove the double-ended needle from the A.H.F. vial.

5. Permit the A.H.F. vial to sit at room temperature for 1 minute without any agitation. This permits thorough wetting of the A.H.F. cake and improves the rate of reconstitution.

6. Manually swirl the A.H.F. vial very gently (avoid foaming) to promote breakup of the cake. As more cake goes into solution, more rapid swirling is permissible to get the last few particles into solution (avoid foaming).

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METHOD NO. 386

DETERMINATION OF ISOAGGLUTININ TITERS IN ANTIHAEMOPHILIC FACTOR (HUMAN)

Apparatus

12 x 75 mm test tubes
Test tube racks
Physiological saline (9 g/litre)
Adams Sero-fuge
Agglutination viewer with light and mirror
Coombs Serum
Automatic pipette to deliver 0.1 ml
Disposable Fibro-tip Pipettes
37°C water bath
Fresh anticoagulated A and B red blood cells
Control serum -- O serum or plasma with a known A and B titer
AHF sample to be tested - Reconstituted according to label.

Preparation of Cell Suspensions

1. A and B test cells are freshly prepared with sufficient volume of the suspension for the complete titration.

2. Cells are washed with saline a minimum of three times in an Adams Sero-fuge spinning one minute at each wash.

3. Prepare 2% cell suspensions in saline by pipetting 0.2ml of each blood sample into tubes containing 9.8 ml saline. Label either A or B respectively, mix well.

Technique for Anti-A and Anti-B Saline-Antiglobulin Titration

1. Label 4 rows of 12 x 75 mm test tubes according to the serum dilution -- usually 1:1 through 1:512.

2. Label the first tube in row 1 "Sample-A"; the first tube in row 2 "Control-A"; the first tube in row 3 "Sample-B"; and the first tube in row 4 "Control-B".

3. Use an automatic pipette to deliver 0.1 ml of saline into the bottom of all tubes except the first tube in each row.

4. Pipette 0.1 ml of sample being tested into tubes 1 and 2 of the first and third sample rows. Pipette 0.1 ml of the serum control into tubes 1 and 2 of the second and fourth control rows.

5. With a clean pipette, mix the contents of tube 2 (1:2) in row one several times. Transfer 0.1 ml to tube labelled 4 (1:4 dilution) in the same row.

6. Continue same procedure through dilutions 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512. A separate pipette must be used for each dilution if "carryover" of antibody from one tube to the next is to be avoided.

7. Repeat procedures 5 and 6 on the next three rows.

8. Pipette 0.1 ml of the saline suspension of A red blood cells into all tubes in rows 1 and 2; pipette 0.1 ml of the saline suspension of B red blood cells into all tubes in rows 3 and 4. Shake tubes well.

9. Incubate all tubes 15 minutes at room temperature.

10. Centrifuge all tubes in Adams Sero-fuge for 45 seconds.

11. Gently dislodge red cell button and observe macroscopically for agglutination starting with the 1:512 tubes. Record results. Express endpoints at Anti-A and Anti-B room temperature saline titer.

12. Incubate all unagglutinated or weakly agglutinated specimens at 37°C for one hour.

13. Centrifuge all tubes as before and again observe macroscopically for agglutination. Express endpoints as Anti-A and Anti-B 37°C saline titer.

14. Wash all tubes 3 times with saline centrifuging 45 seconds at each wash. Perform an antiglobulin (Coombs) test by adding 2 drops commercial Coombs serum to each tube and centrifuging 45 seconds.

15. Again observe macroscopically for agglutination starting with the 1:512 tubes. Express endpoint as Anti-A and Anti-B antiglobulin (immune) titer.

16. Titers on the control serum should be in the expected range to assure assay results on the product being tested are correct.

17. Titers on the product tested should not exceed 1:256 on any of the three tests - room temperature, 37°C, or Coombs.

References

Technical Methods and Procedures of the AABB, Fifth Edition 1970, Reprinted, 1973.

Bray's Clinical Methods and Procedures, Bauer, Ackermann, Toro. Seventh Edition, Copyright 1968, C.V. Mosley Company.

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METHOD NO. 386
Supplement No. 1

PREDILUTION OF ANTIHAEMOPHILIC FACTOR (HUMAN) FOR DETERMINATION OF ISOAGGLUTININ TITERS

After vial of sample has been reconstitued as described in Method No. 1073, Supplement No. 1, dilute solution to be tested to approximately 10 units/ml based on potency assigned to the lot (Method No. 365).

Proceed as described in Method No. 386.

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METHOD NO. 963

GENERAL SAFETY TEST

Product to be Tested

The general safety test shall be conducted upon a representative sample of the product in the final container from every final filling of each lot of the product. If any product is processed further after filling, such as by freeze-drying, sterilization, or heat treatment, the test shall be conducted upon a sample from each filling of each drying chamber run, sterilization chamber, or heat treatment bath.

Test Animals

Only overtly healthy guinea pigs weighing less than 400 grams each and mice weighing less than 22 grams each shall be used. The animals shall not have been used previously for any test purpose.

Procedure

The duration of the general safety test shall be 7 days for both species, except that a longer period may be established for specific products in accordance with the following paragraph entitled Test Variations. After a specific duration of the test period for a specific product has been established, it cannot be varied subsequently, except in accordance with the paragraph entitled Test Variations. Each test animal shall be weighed and the individual weights recorded immediately prior to injection and on the last day of the test. Each animal shall be observed every working day. Any animal response including any which is not specific for or expected from the product and which may indicate a difference in its quality shall be recorded on the day such response is observed. The test product shall be administered as follows:-

1. Liquid product or freeze-dried product which has been reconstituted as directed on the label. Inject intraperitoneally 0.5 ml of the liquid product or the reconstituted product into each of at least two mice; and 5.0 ml of the liquid product or the reconstituted product into each of at least two guinea pigs.

2. Freeze-dried product for which the volume of reconstitution is not indicated on the label. The route of administration, test dose, and diluent shall be as approved by the Director, Bureau of Biologics, in accordance with the paragraph entitled Test Variations. Administer the test product as approved on at least two mice and at least two guinea pigs.

3. Non-liquid products other than freeze-dried product. The route of administration, test dose, and diluent shall be as approved by the Director, Bureau of Biologics, in accordance with the paragraph entitled Test Variations. Dissolve or grind and suspend the product in the approved diluent. Administer the test product as approved on at least two mice and at least two guinea pigs.

Test Requirements

A safety test is satisfactory if all animals meet all of the following requirements:-

1. They survive the test period.

2. They do not exhibit any response which is not specific for or expected from the product and which may indicate a difference in its quality.

3. They weigh no less at the end of the test period than at the time of injection.

Repeat Tests

1. First repeat test. If a filling fails to meet the requirements of the Test Requirements in the initial test, a repeat test may be conducted on the species which failed the initial test, as prescribed in Procedure. The filling is satisfactory only if each retest animal meets the requirements prescribed in Test Requirements.

2. Second repeat test. If a filling fails to meet the requirements of the first repeat test, a second repeat test may be conducted on the species which failed the test; provided that 50% of the total number of animals in that species has survived the initial and first repeat tests. The second repeat test shall be conducted as prescribed in the Procedure, except that the number of animals shall be twice that used in the first repeat test. The filling is satisfactory only if each second repeat test animal meets the requirements prescribed in paragraph Test Requirements.

Test Variations

Variations in the general safety test, such as test dose, route of administration, or duration of the test period may be offered as an amendment to the product licence and must receive written approval by the Director, Bureau of Biologics, Food and Drug Administration. Approval will be given only if the license amendment provides substantial evidence demonstrating that the proposed test variation will assure sensitivity equal to or greater than the test described in this method.

Reference: Code of Federal Regulations, Title 21, Paragraph 610.11.

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METHOD NO. 993

BIURET ASSAY FOR TOTAL PROTEIN CONTENT OF CRYOPRECIPITATED ANTIHAEMOPHILIC GLOBULIN (AHF)

I REAGENTS

1. Standard Protein Solution (3 ml/ml)

   Dissolve 300 mg of crystallised human albumin in 75 ml of distilled water and dilute to 100 ml with distilled water. The solution should be stored at 2 - 8°C and is stable for 2 months.

2. 3 Normal Sodium Hydroxide (NaOH)

   Dissolve 120 g of sodium hydroxide pellets in 750 ml of distilled water and dilute to 1 litre with distilled water.

3. 6 Normal Sodium Hydroxide (NaOH)

   Dissolve 240 g of sodium hydroxide pellets in 750 ml of distilled water and dilute to 1 litre with distilled water.

4. Biuret Reagents

   a. Dissolve 17.3 g of copper sulphate (anhydrous) in 75 ml of distilled water and dilute to 100 ml with distilled water.

   b. Dissolve 173 g of sodium citrate dihydrate and 100 g of sodium carbonate (anhydrous) in 700 ml of distilled water. Stir the solution to facilitate solubilisation of the reagents.

   c. Cool the mixture (2) and pour the copper sulphate into the sodium citrate - sodium carbonate mixture.

   d. Stir and dilute to 1 litre with distilled water.

   e. The Biuret reagent is stable indefinitely at room temperature.

II Assay Procedure

A. Preparation of Protein Standard

1. Label a series of test tubes in triplicate as follows:

   a. 1.5 mg/ml
   b. 3 mg/ml
   c. 4.5 mg/ml
   d. 6 mg/ml
   e. 7.5 mg/ml

II Assay Procedure (Cont.)

A. Preparation of Protein Standard (Cont.)

2. To a, add 0.5 ml of the stock protein solution
To b, add 1.0 ml of the stock protein solution
To c, add 1.5 ml of the stock protein solution
To d, add 2.0 ml of the stock protein solution
To e, add 2.5 ml of the stock protein solution

3. To all test tubes add equal amounts of 6 Normal Sodium Hydroxide.

4. To a, b, c and d add the required amounts of 3 Normal Sodium Hydroxide to bring the total volume to 5.0 ml*.

*NOTE: e will already be at the required volume of 5.0 ml.

5. To all 5 test tubes, add 1.0 ml of the Biuret reagent and mix well.

6. Prepare a blank by mixing 5 ml of the 3 Normal Sodium Hydroxide and 1.0 ml of the Biuret reagent and mix well.

7. Use the blank to standardise the spectrometer and determine the optical density of the test solution at 545 mn.

8. Plot the results on log-log graph paper, absorbancy vs concentration.

III Total Protein Control of Test Sample

1. The amount of sample to be used is estimated so that its value will fall on the standard curve. This is usually done by trial runs. It has been found that the following sample size can be used to obtain readings on the standard curve.

a. AHF cryoprecipitate samples - 1/10 ml of the sample - estimated concentration is 35 mg/ml (± 5 mg)*

2. To the amount of sample used, add equal amounts of 6 Normal Sodium Hydroxide. Prepare the test sample in triplicate.

3. Dilute to 5 ml with 3 Normal Sodium Hydroxide.

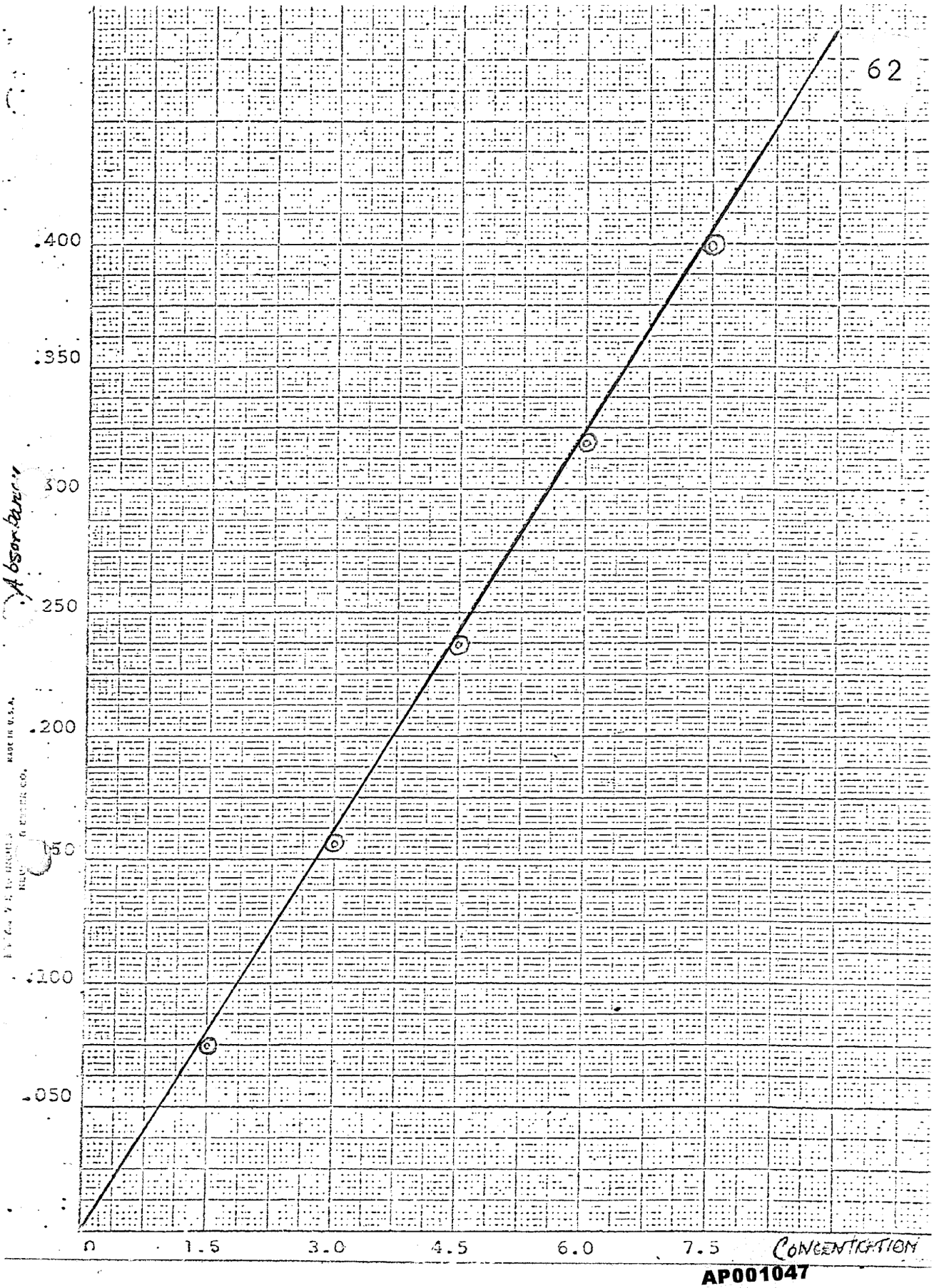
4. Add 1.0 ml of Biuret reagent and mix well.

5. Prepare blank in the same manner as for the standard curve.

6. Read at the same wave length as for the standard curve.

7. Calculate the amount of protein in the test sample from the standard curve. Correct for sample dilution and express the total protein content in mg/AHF unit.

*AHF sample reconstituted to 30 ml for High Potency Factorate.



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METHOD NO. 994

AHF BIURET ASSAY FOR FIBRINOGEN (DETERMINATION OF CLOTTABLE PROTEIN)

Sample Preparation

1. Use the same sample size as used for the protein assay - 1/10 x 2.

Sample	Estimated Range Value
CRV (Cryoprecipitate) conc.	35±5
CRV (Cryoprecipitate) lyophilised	35±5
AHF conc.	11±5
AHF lyophilised	11±5
AHF plasma fraction	60±5
AHF tris fraction	14±5
AHF $(OH)_3$ fraction	12±5
AHF 4.0% P.E.G.	4±2
AHF 5.8% P.E.G.	3±2
AHF 6.0% P.E.G.	3±2
AHF 9.0% P.E.G.	14±5
AHF 12.0% P.E.G.	5±2
Squibb - R.C., AHF	10±2
Hyland, AHF	40±5
Cortland, AHF	30±5

2. Add 0.2 ml of the 0.04M $CaCl_2$.
3. Add 0.2 ml of the thrombin reagent.
4. Heat in a 37°C water bath for 15 minutes.

Sample Preparation (Cont.)

5. (A) Small Clot - Take out the fibrin and discard it. Measure the volume of supernatant and add an equal amount of 6N NaOH, dilute to 5 ml with 3N NaOH, and add 1 ml of the Biuret reagent. Read as for protein assay.

Calculation

Protein Value $(\frac{mg}{mt})$ - Supernatant value $(\frac{mg}{mt})$ = Fibrinogen $(\frac{mg}{mt})$

(B) Large Clot - Wash the clot with distilled water - repeat two or more times, Suspend clot in 2.5 ml of 6N NaOH, heat in a 37°C bath for 15 minutes, mix well, and add 2.5 ml of distilled water. Add 1 ml of the Biuret reagent - mix well. Read as for protein assay.

Calculation

Read directly from Curve $(\frac{mg}{ml})$

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METHOD NO. 995

ATOMIC ABSORPTION ANALYSIS OF ALUMINUM IN ANTIHAEMOPHILIC FACTOR

Reagents and Instrumentation

1. Aluminum Standard Solution, 1000 ppm

   Purchased from Harleco, Item No. 7689 or equivalent.

2. Sodium Chloride Solution, 1000 ppm

   Dissolve 1 g of Analytical Reagent Grade Sodium Chloride in 1 liter of water.

3. Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer

   Equipped with an aluminum lamp and a nitrous oxide burner head. An equivalent unit may be used.

4. Perkin-Elmer Recorded Readout or Equivalent

5. Omni-Scribe Recorder (Houston Instruments) or equivalent

Preparation of Standard Aluminum Solutions

Transfer a 2 ml aliquot of the Aluminum Standard Solution, 1000 ppm, to a 200 ml volumetric flask, dilute to volume with water and mix well. Transfer a 10 and 20 ml aliquot to two 100 ml volumetric flasks to produce two final solutions containing 1 and 2 ppm aluminum respectively. Dilute each solution to volume with water and mix well.

Preparation of Sample Solution

a. Vials labelled to be reconstituted with 25 or 50 ml of diluent. Reconstitute the contents of a vial with 25 or 50 ml as required with Sodium Chloride Solution, 1000 ppm as the diluent.

b. Vials labelled to be reconstituted with 10 ml of diluent. Reconstitute the contents of a vial with 25 ml of Sodium Chloride Solution, 1000 ppm, as the diluent. In no case must the sample solution contain more than 4% solids.

c. Products requiring dilutions that are not covered by paragraphs a. or b.; reconstitute according to the labelling instructions.

Procedure

Using a Perkin Elmer Model 303 Atomic Absorption Spectrophotometer or equivalent equipped with an aluminum lamp and a nitrous oxide burner head, determine the absorption values for the sample and standard solution versus a blank solution consisting of a solution of 1000 ppm sodium chloride. Suggested instrument settings for the instruments being used are:

Perking-Elmer, Model 303 Atomic Absorption Spectrophotometer
Wavelength - 309.0 nanometers
Range - UV
Slit - 4
Source - As recorded on the lamp
Scale - 1
Oxidizer - Nitrous Oxide - Air - 6.0
Fuel Flow - Initially white ball at 8, light flame, increase flow until metal ball is 5.5, then switch from air to nitrous oxide.

Recorder Readout
Noise Suppression - 3
Scale - 10x

Recorder Settings
0.5 inch/min

Note: The following Flame Ignition Procedures are presented by Perkin-Elmer. The analyst is advised to read the instruction manual and the General Information section of the Perkin-Elmer Analytical Methods book before using the instrument.

Flame Ignition Procedures

With the exception of the nitrous oxide-acetylene flame, all flames may be ignited directly as described in the instruction manual for the appropriate instrument.

Always turn fuel on last and off first.

Experience has shown that with the nitrous oxide-acetylene flame, flash-back is most likely to occur when the flame is either ignited or turned off. These flashbacks can generally be avoided if the flame is turned on or off with air as the oxidant. This procedure requires a means of rapidly switching from air to nitrous oxide. Most instrument gas control systems now have this capability. For those which do not, a T-junction valve is available as an accessory (Perkin-Elmer part number 303-0225). The ignition sequence given below for the nitrous oxide-acetylene flame is usable on all Perkin-Elmer atomic absorption spectrophotometers except those equipped with gas control boxes providing automatic switchover to nitrous oxide (gas control boxes 040-0301, 057-0345 057-0134 and 057-0262). With gas control boxes providing automatic switchover, it is merely necessary to install the nitrous oxide burner head. All secondary acetylene adjustments take place automatically.

1. Install a nitrous oxide burner head.

2. Turn on the acetylene flow (without igniting the flame) and adjust the flow rate to the appropriate value specified for the nitrous oxide-acetylene flame. Turn the acetylene flow off.

3. With both air and nitrous oxide supplies turned on, set the switching valve to nitrous oxide and adjust the flow rate to the value specified.

4. Turn the switching valve to the air position.

5. Turn the acetylene on and ignite the flame (air-acetylene). Allow the burner head to warm up for several minutes.

6. Increase the acetylene flow to the value specified for nitrous oxide-acetylene operation in the appropriate table.

7. With a rapid motion, turn the switching valve from air to nitrous oxide.

The nitrous oxide flame is now operating normally. Fuel and oxidant flow can be adjusted as described under Gas Flow Optimization.

When extinguishing the flame, the reverse procedure is followed. The oxidant switching valve is switched rapidly from the nitrous oxide to the air position after which the acetylene flow is reduced, then turned off.

Calculations

Since the absorption values obtained in this analysis are below 10% there is no need to convert them to absorbance for calculation purposes. Use the absorption of the aluminum standard nearest the sample absorption and calculate the aluminum content of the sample by the following formula:

a. For samples labelled to be diluted to 25 or 50 ml.

$$\frac{\text{Sample Absorption}}{\text{Standard Absorption}} \times \text{Concentration Al Std (mcg/ml)} = \text{mcg Al/ml}$$

(When vial is reconstituted for use)

Note: If the sample absorption is less than the 1 ppm aluminum standard, report the aluminum content of the sample as less than 1 ppm.

b. For samples labelled to be diluted to 10 ml

$$\frac{\text{Sample Absorption}}{\text{Standard Absorption}} \times \text{Concentration Al Std (mcg/ml)} \times 2.5 = \text{mcg Al/ml}$$

(when vial is reconstituted for use)

Note: If the sample absorption is less than the 1 ppm aluminum standard, report the aluminum content of the sample as less than 2.5 ppm.

ARMOUR PHARMACEUTICAL COMPANY, KANKAKEE
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ANALYTICAL METHODS

METHOD NO. 1073

DETERMINATION OF THE HEPARIN CONTENT OF CRYOPRECIPITATED AHF

The following in vitro method is used in the determination of the Heparin content of cryoprecipitated AHF. The test is based on the in vitro inhibition by Heparin of coagulation time as measured by the activated partial thromboplastin time procedure.

1. Reagents and Equipment

   a. Heparin-potency 1000 units/ml obtained from a reputable source.

   b. Activated Partial Thromboplastin

   c. Cryoprecipitated AHF (Lyophilized)
      (i) Heparin Containing
      (ii) Non-Heparin Containing

   d. Normal Control Plasma

   e. A BBL Fibrometer for the determination of clotting times. This instrument should be equipped with an automatic pipette capable of dispensing 0.1 ml.

   f. 0.025 molar calcium chloride

   g. A thermal Incubation Block (37°C)

2. Procedure

   A. Reconstitution of Reagents

      1. Reconstitute the normal coagulation control plasma (lyophilized) with 1 ml of c (i) or c (ii).

      2. Reconstitute the activated partial thromboplastin (lyophilized) with 2 ml distilled water.

      3. Reconstitute the AHF sample (lyophilized) with 25 ml of distilled water or see appropriate supplement.

B. Dilution of Heparin for the Preparation of a Heparin Standard

1. Dilute the stock Heparin Solution of 1000 u/ml with distilled water to provide the following concentrations, in 1 ml of normal coagulation control plasma:

   0.25 u/ml, 0.30 u/ml, 0.35 u/ml, and 0.40 u/ml.

C. Determination of the Clotting Time of the Plasma with Heparin Added

1. Incubate the plasma, partial thromboplastin and 0.025 molar calcium chloride at 37°C for five minutes.

2. Using an automatic pipette, add 0.1 ml of the plasma to a fibrometer reaction cup.

3. Add 0.1 ml of partial thromboplastin to the plasma in the reaction cup and incubate exactly four minutes.

4. At the end of four minutes, add 0.1 ml of calcium chloride and start timing of the clotting reaction.

5. Determine the clotting times for these Heparin containing plasma in 5 replicate determinations and plot on standard graph paper. Plot clotting time on the vertical axis and the Heparin content on the horizontal axis.

D. Determination of the Heparin Concentration in the AHF Sample

1. Reconstitute 1.0 ml of normal coagulation control plasma with 1.0 ml of the reconstituted AHF sample. Mix and incubate at 37°C for five minutes in a thermal block.

2. Determine the activated partial thromboplastin clotting times in five replicate determinations.

3. Calculate the Heparin concentrations of the AHF sample from the Heparin Standard and correct for dilution.

4. Express the Heparin concentration in units/ml.

ARMOUR PHARMACEUTICAL COMPANY, KANKAKEE
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ANALYTICAL METHODS

Method No. 1073
Supplement No. 1

RECONSTITUTION OF ANTIHAEMOPHILIC FACTOR (A.H.F.) FOR DETERMINATION OF HEPARIN CONTENT

Materials

1. Sterile Water for Injection, U.S.P. (30 ml/vial)
2. Double-ended Reconstitution Needle
3. Stopwatch or Timer
4. Water Bath at 37°C

Test Procedure

1. Warm both the 30 ml diluent of sterile water for injection and vial of AL 1259 A.H.F. (unopened vials) to 37°C in a water bath (not less than 15 minutes)

2. Remove caps from both vials to expose the central portion of rubber stoppers.

3. Insert one end of the double-ended needle into the rubber stopper of the diluent vial. Invert the diluent vial and insert the other end of the double-ended needle into the rubber stopper of the A.H.F. vial. Allow the diluent to be drawn into the A.H.F. vial by vacuum and direct the stream over the surface of the cake.

4. Release the vacuum by removing the diluent vial from the double-ended needle; then remove the double-ended needle from the A.H.F. vial allowing the incoming airstream to agitate the vial contents.

5. Permit the A.H.F. vial to sit at room temperature for 1 minute without any agitation. This permits thorough wetting of the A.H.F. cake and improves the rate of reconstitution.

6. Manually swirl the A.H.F. vial very gently (avoid foaming) to promote breakup of the cake. As more cake goes into solution, more rapid swirling is permissable to get the last few particles into solution (avoid foaming).

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METHOD NO. 1257

SOLUTION TIME FOR HIGH POTENCY FACTORATE ANTIHAEMOPHILIC FACTOR (A.H.F.)

Test Summary

The purpose of this method is to determine the rate of reconstitution time of High Potency Factorate.

Comments

(a) Safety Precautions: None.

(b) General Precautions: Care should be exercised when swirling the vials so as to minimize the foaming.

Materials for Testing

1. Sterile Water for Injection, U.S.P. (30 ml/vial)
2. Double-ended Reconstitution Needle.
3. Stop Watch or Timer.

Test Procedure

1. Equilibrate the 30 ml diluent of Sterile Water for Injections and vial of Factorate A.H.F. (not less than 15 minutes).
2. Remove caps from both vials to expose the central portion of rubber stoppers.
3. Insert one end of the double-ended needle into the rubber stopper of the diluent vial. Invert the diluent vial and insert the other end of the double-ended needle into the rubber stopper of the A.H.F. vial. Allow the diluent to be drawn into the A.H.F. vial by vacuum and direct the stream over the surface of the cake.
4. Release the vacuum by removing the diluent vial from the double-ended needle; then remove the double-ended needle from the A.H.F. vial allowing the incoming airstream to agitate the vial contents. Start a stop-watch.

Method No. 1257

5. Permit the A.H.F. vial to sit at room temperature for 1 minute without any agitation. This permits thorough wetting of the A.H.F. cake and improves the rate of reconstitution.

6. Manually swirl the A.H.F. vial very gently (avoid foaming) to promote breakup of the cake. As more cake goes into solution, more rapid swirling is permissable to get the last few particles into solution (avoid foaming).

7. Discontinue assay when the A.H.F. is in solution. The A.H.F. must be in solution. The A.H.F. must be in solution after 30 minutes of continual swirling at room temperature 20 - 25°C. Record reconstitution time.

Interpretation of Results

Report the reconstitution time to the nearest minute.

SECTION 4 Development Pharmaceutical and Biological Availability

4.1. Formulation Studies

(a) Clinical Requirements

The clinical requirements were for a Factor VIII concentrate required to avoid over-loading of the circulatory system in such cases as preparation for elective surgery and control of haemorrhage in surgery or trauma. The product was required to have more units per volume and have lower amounts of protein/unit than the previous Generation I product. Factorate^{R} High Potency II (AL-1259) meets these criteria and is produced from human plasma that has been tested and found negative for Hb_sAg by radio-immunological techniques. The final product developed was similarly tested and found negative for Hb_sAg.

(b) Physical Characteristics

A lyophilised product with a low moisture content was needed to obtain a finished product with acceptable reconstitution times. Development work has concentrated on maintaining a moisture content below 2% for the final lyophilised cake, and an initial vial temperature during the first 30 hours of the drying cycle which does not exceed $-20^{o}C$.

4.2. Analytical Development

The analytical methods of assay procedures selected for routine control of the finished product were those established for Factorate^{R} Generation I and monographed in B.P. 1973. Minor variations in methods were made in order that determinations should in general be made on the reconstituted product (reconstitution volume now 30 ml Water for Injections B.P.)

Batch to batch uniformity of the product is demonstrated in the attached results for consecutive batches, K 852030, 852031 and 852032. The following data is also relevant:-

4.2. Analytical Development (Cont.)

BATCH	DATE OF MANUFACTURE	BATCH SIZE	POTENCY (BY METHOD 365)
K 825030	July 1977	133 vials	1314 units/vial
K 852031	July 1977	186 vials	1164 units/vial
K 852032	May 1977	174 vials	1218 units/vial

4.3. Analytical Results

The batches from production runs have been tested to the Finished Product Specification with potencies determined using the Thromboplastin Generation Assay as described in our previous submission (Factorate, Volume I, p. 79). This assay has been re-written to include the appropriate statistics necessary to determine confidence limits and their validity, and included at the end of this Section.

Minor differences exist between the U.S.A. assay Method 365 and that in the current B.P. These are as follows:-

(a) A different standard is currently employed.

(b) An automated fibrometer is used.

(c) The imidazole buffer is sterile filtered.

(d) Substrate plasma is from human sources only and flash frozen (-20^{o}).

Batch to batch consistency is demonstrated in the attached analytical results from 3 consecutive batches. These have been tested both to our Finished Product Specification and also to the requirements of the American IND Specification (which includes particulate matter and potency 3 hours post reconstitution at Room Temperature.

BATCH ANALYSIS RESULTS

LOT K852030

TEST	SPECIFICATION*	ASSAY DATA
AHF Potency a	NLT 30 AHF U/Recon. ml and NLT 900 AHF U/Vial	43.8 U/ml 1314 U/Vial
AHF Potency Recon. Stability 3 hrs at Cont. Rm. Temp.[a]	NLT 80% of 0 Hour	1278 U/vial 97.3% of 0 hr
Heparin Assay [a]	NMT 1U/Recon. ml NMT 30 U/Vial	0.4 U/ml 12 U/Vial
Total Protein[a]	For Calculation	45.4 mg/ml or 1362 mg/Vial
Clottable Protein[a]		39.5 mg/ml or 1185 mg/Vial
Specific Activity	NLT 0.5 AHF U/mg protein or NMT 2.0 mg protein/AHF U	0.965 U/mg 1.036 mg/Vial
Aluminium [a]	LT 0.0002 mg/Recon. ml and LT 0.006 mg/Vial	LT 0.001 mg/25 ml
Moisture	NMT 2% w/w	0.04%
pH[a]		7.07
Identity	Human - Positive Bovine - Negative Porcine - Negative Ovine - Negative	Passes
Safety [a]	Passes	Passes
Sterility	Passes U.S.P.	Passes
Pyrogens [a] (40 AHF U/kg)	Passes U.S.P.	Passes (.2/1.1/.6/.3/.5/.4/.4/.1)
Solution Time[a]	NMT 30'	14'
Isoagglutinins [a] (10 AHF U/ml)	NMT 1:256	Passes
Hepatitis B Surface Antigen (HBsAg) [a]	Negative	Negative
Appearance of Cake	White to Nearly White	White
Particulate Matter		Thres. 10µ 1106/ml Thres. 25µ 418/ml

NOTE: a - Reconstituted with 30 ml Sterile Water for Injection U.S.P.

LOT K852031

TEST	SPECIFICATION*	ASSAY DATA
AHF Potency [a]	NLT 30 AHF U/Recon. ml and NLT 900 AHF U/Vial	38.8 U/ml 1164 U/Vial
AHF Potency Recon. Stability 3 hrs at Cont. Rm. Temp.[a]	NLT 80% of 0 Hour	1203 U/Vial 103.4% of 0 hr
Heparin Assay [a]	NMT 1 U/Recon. ml NMT 30 U/Vial	0.4 U/ml 12 U/vial
Total Protein[a]	For Calculation	46.3 mg/ml or 1389 mg/Vial
Clottable Protein[a]		32 mg/ml or 960 mg/Vial
Specific Activity	NLT 0.5 AHF U/ml protein or NMT 2.0 mg protein/AHF U	0.838 U/mg 1.193 mg/U
Aluminium [a]	LT 0.0002 mg/Recon. ml and LT 0.006 mg/Vial	LT 0.001 mg/25 ml
Moisture	NMT 2% w/w	0.03%
pH[a]		7.15
Identity	Human - Positive Bovine - Negative Porcine - Negative Ovine - Negative	Passes
Safety [a]	Passes	Passes
Sterility	Passes U.S.P.	Passes
Pyrogens [a] (40 AHF U/kg)	Passes U.S.P.	Passes (.1/.5/.2)
Solution Time[a]	NMT 30'	14'
Isoagglutinins [a] (10 AHF U/ml)	NMT 1:256	Passes
Hepatitis B Surface Antigen (HBsAg) [a]	Negative	Negative
Appearance of Cake	White to Nearly White	White
Particulate Matter		Thres. 10µ 1010/ml Thres. 25µ 422/ml

NOTE: a - Reconstituted with 30 ml Sterile Water for Injection U.S.P.

LOT K852032

TEST	SPECIFICATION*	ASSAY DATA
AHF Potency [a]	NLT 30 AHF U/Recon. ml and NLT 900 AHF U/Vial	40.6 U/ml 1218 U/Vial
AHF Potency Recon. Stability 3 hrs at Cont. Rm. Temp.[a]	NLT 80% of 0 hour	1119 U/Vial 91.8% of 0 hr
Heparin Assay [a]	NMT 1 U/Recon. ml NMT 30 U/Vial	0.4 U/ml 12 U/Vial
Total Protein[a]	For Calculation	44.3 mg/ml or 1329 mg/Vial
Clottable Protein[a]		28.9 mg/ml or 867 mg/Vial
Specific Activity	NLT 0.5 AHF U/mg protein or NMT 2.0 mg protein/AHF U	0.916 U/mg 1.091 mg/U
Aluminium [a]	LT 0.0002 mg/Recon. ml and LT 0.006 mg/Vial	LT 0.001 mg/25 ml
Moisture	NMT 2% w/w	0.08%
pH[a]		7.23
Identity	Human - Positive Bovine - Negative Porcine - Negative Ovine - Negative	Passes
Safety [a]	Passes	Passes
Sterility	Passes U.S.P.	Passes
Pyrogens [a] (40 AHF U/kg)	Passes U.S.P.	Passes (.5/0/0)
Solution Time[a]	NMT 30'	26'
Isoagglutinins[a] (10 AHF U/ml)	NMT 1:256	Passes
Hepatitis B Surface Antigen (HBsAg) [a]	Negative	Negative
Appearance of Cake	White to Nearly White	White
Particulate Matter		Thres. 10 μ 1154/ml Thres. 25 μ 551/ml

NOTE: a - Reconstituted with 30 ml Sterile Water for Injection U.S.P.

LOT K852032

TEST	SPECIFICATION*	ASSAY DATA
AHF Potency [a]	NLT 30 AHF U/Recon. ml and NLT 900 AHF U/Vial	40.6 U/ml 1218 U/Vial
AHF Potency Recon. Stability 3 hrs at Cont. Rm. Temp.[a]	NLT 80% of 0 hour	1119 U/Vial 91.8% of 0 hr
Heparin Assay [a]	NMT 1 U/Recon. ml NMT 30 U/Vial	0.4 U/ml 12 U/Vial
Total Protein[a]	For Calculation	44.3 mg/ml or 1329 mg/Vial
Clottable Protein[a]		28.9 mg/ml or 867 mg/Vial
Specific Activity	NLT 0.5 AHF U/mg protein or NMT 2.0 mg protein/AHF U	0.916 U/mg 1.091 mg/U
Aluminium [a]	LT 0.0002 mg/Recon. ml and LT 0.006 mg/Vial	LT 0.001 mg/25 ml
Moisture	NMT 2% w/w	0.08%
pH[a]		7.23
Identity	Human - Positive Bovine - Negative Porcine - Negative Ovine - Negative	Passes
Safety [a]	Passes	Passes
Sterility	Passes U.S.P.	Passes
Pyrogens [a] (40 AHF U/kg)	Passes U.S.P.	Passes (.5/0/0)
Solution Time[a]	NMT 30'	26'
Isoagglutinins[a] (10 AHF U/ml)	NMT 1:256	Passes
Hepatitis B Surface Antigen (HBsAg) [a]	Negative	Negative
Appearance of Cake	White to Nearly White	White
Particulate Matter		Thres. 10 µ 1154/ml Thres. 25 µ 551/ml

NOTE: a - Reconstituted with 30 ml Sterile Water for Injection U.S.P.

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ARMOUR PHARMACEUTICAL COMPANY, KANKAKEE
QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

METHOD NO. 365

THROMBOPLASTIN GENERATION TEST
ANTI-HAEMOPHILIC FACTOR (AHF)

I. Equipment

(a) 37°C water bath

(b) Test tubes - disposable 12 x 75 mm

(c) Pipettes - acid washed, distilled water rinsed and dried
1. 0.1 ml serological pipette graduated in 0.01
2. 1.0 ml serological pipette graduated in 0.01
3. 2.0 ml measuring pipette graduated in 0.10
4. 10.0 ml measuring pipette graduated in 0.10

(d) Stopwatch - 1 hour calibrated in seconds

(e) Ice bath

(f) Fibrometer system
1. Fibrometer precision coagulation timer
2. Thermal preparation block, controlled to 37.2 ± 0.7°C.
3. Automatic pipette which delivers reagents into the test receptacle and simultaneously starts the fibrometer timer.
4. Disposable Fibro Tube cups and Fibro Tip plastic tips

II. Reagents

(a) Imidazole - Sodium Chloride Buffer

In a liter volumetric flask, add 500 ml distilled water, 3.4 g imidazole, 3.85 g sodium chloride, and 183.0 ml 0.1N hydrochloric acid. Swirl contents until dissolved and add sufficient distilled water to make 1 liter. The pH is 7.3 ± 0.02. If it is not, adjust the solution with hydrochloric acid or sodium hydroxide dropwise until the proper pH is reached.

Sterile filter the solution using a 0.3 micron Millipore filter and store in the refrigerator (6°C). Discard if cloudy.

(b) Calcium Chloride, 0.025 M

To a 500 ml volumetric flask add 1.838 gm calcium chloride ($CaCl_2.2H_2O$) and add sufficient distilled water to make 500 ml.

(c) Normal Saline, 0.85%

Add 1.7 g sodium chloride to a 200 ml volumetric flask and dilute to volume with deionized water.

(d) Inosithin, 25 mg % in 0.15 M Sodium Chloride

Inosithin is the trade name for a mixture of soy bean phospholipids produced by Associated Concentrates, Woodside, Long Island, New York.

Suspend 25 mg of inosithin in 100 ml normal saline, allow to stand overnight at room temperature, shake to produce as fine an emulsion as possible. Dispense into 5 ml aliquots, and store in a freezer at -20°C.

Do not use the inosithin - (1) before it has been frozen for 3 days, or (2) after it is 4 weeks old.

(e) Anticoagulant 4% Sodium Citrate Solution N.F.

Purchased from Fenwal Laboratories, Morton Grove, Illinois, 60053.

(f) Stock Bovine Serum

Bovine Plasma collected in anticoagulant citrate dextrose solution is recalcified by the addition of 1 M Calcium Chloride solution to a final concentration of 0.025 M. It is allowed to stand overnight at room temperature and then centrifuged (2,000g) for 10 minutes to remove the clot. It is then frozen in aliquots of 2.5 ml at -20°C.

(g) Stock Human Serum

Bank Plasma collected in anticoagulant citrate dextrose solution is recalcified by the addition of 1M calcium chloride solution to a final concentration of 0.025M. It is allowed to stand overnight at room temperature and then centrifuged (2,000 g) for 10 minutes to remove the clot. It is then frozen in aliquots of 2.0 ml at -20°C.

(h) Substrate Plasma

Fresh Human Plasma collected in anticoagulant citrate dextrose solution (pool of at least 3 donors) is rendered platelet poor by centrifugation in a refrigerated International Centrifuge for 20 minutes at 4,000 g at 4°C. It is frozen (within 3 hours of collection) in aliquots of 5.0 ml at -20°C.

(i) Standard - Normal Human Plasma Lyophilized

Potency (AHF units/ml) is confirmed by Bureau of Biologics.

III. Preparation of Reagents for Test

(a) Human Serum

The stock human serum is thawed rapidly at 37°C.

1.0 ml of thawed serum is diluted with 9.0 ml of Imidazole-Sodium Chloride Buffer and allowed to stand in the water bath for at least 2 hours, but not more than 2 hours and 15 minutes. At the end of the incubation period, it is transferred to the ice bath.

(b) Substrate Plasma

The plasma is thawed rapidly at 37°C and then stored in the ice bath. Remove to the thermal preparation block only the amount needed (approximately 0.6 ml) for each run. The remainder is kept in the ice bath. Do not return the plasma which has been warmed to the cold stock of plasma. Leftover, thawed plasma is discarded at the end of the test.

(c) Bovine Serum

The stock Bovine Serum is thawed rapidly at 37°C. 0.5 ml of thawed serum is diluted with 9.5 ml of Imidazole-Sodium Chloride Buffer and placed in the ice bath.

(d) Inosithin

Thaw and place in the 37°C water bath or thermal preparation block.

IV. Pretest Procedure

(a) Using the automatic pipette, pipette 0.1 ml of 0.025 M Calcium Chloride Solution into a sufficient number of Fibro Tube cups.

(b) Pre-Warm 5 ml of 0.025 M Calcium Chloride solution to 37°C for use in the reaction mixture.

(c) Reconstitute all sample and standards. (Do not put in ice bath.) Any sample or standard which is a lyophilized plasma instead of a concentrate must be absorbed with a Barium Chloride solution. 0.1 ml 15% Barium Chloride solution per ml of reconstituted volume is added to the reconstituted sample or standard. Let stand for at least 12 minutes, then centrifuge for approximately 10 minutes, remove the supernatant for testing.

Lyophilized Vial	:	Using distilled water, reconstitute with the volume directed for it's intended use.
Powder	:	Using distilled water, reconstitute 1 g of powder to a volume of 25 ml.
Standards	:	Using distilled water, reconstitute as directed on the standard vial.

(d) Make the following dilutions (with Imidazole Sodium Chloride Buffer, IBS) of each sample and standard for each test run just prior to the test run. Samples and standards are run in duplicate or triplicate.

Predilution: A variable dilution of a sample reducing its concentration to approximately that of the standard.

1/10* (0.1 ml of sample, or its predilution, + 0.9 ml IBS)

1/100 (0.1 ml of 1/10 dilution + 0.9 ml IBS)

1/200 (0.5 ml of 1/100 dilution + 0.5 ml IBS)

1/400 (0.5 ml of 1/200 dilution + 0.5 ml IBS)

1/800 (0.5 ml of 1/400 dilution + 0.5 ml IBS)

1/1600 (0.5 ml of 1/800 dilution + 0.5 ml IBS)

*These dilutions are not tested.

(e) Place 0.6 ml of substrate plasma on thermal block to preheat.

(f) Place a set (5) of Fibro Tubes, containing the 0.025 M Calcium Chloride Solution, on the thermal block to preheat.

V. Test Procedure**

(a) Reaction Mixture

Add to each test tube, in a series of 5, in the thermal prep block: (1) 0.1 ml diluted human serum, (2) 0.1 ml diluted bovine serum, (3) 0.1 ml inositihn.

Then add 0.1 ml of the prewarmed 0.025 M Calcium Chloride Solution to the first tube in the series. Immediately following this addition, add 0.1 ml of the first test dilution (1/100) and simultaneously start a stop watch.

When the watch registers 15 seconds before the minute, add the prewarmed Calcium Chloride solution to the second tube in the series and on the minute add 0.1 ml of the second test dilution to the second tube. Repeat for each of the remaining tubes (third, fourth and fifth tubes and respective dilution) in the series.

(b) Clotting Mixture

Place one of the prefilled Fibro Tubes beneath the probe of the fibrometer.

When the watch in the procedure above registers 5 minutes, using the automatic pipette in the "off" position, pipette 0.1 ml of the first reaction mixture into the Fibro Tube. Using a new pipette tip withdraw 0.1 ml of the prewarmed substrate plasma, switch the pipette to the "on" position and pipette the substrate plasma into the Fibro Tube. The timer automatically stops when the probe detects the formation clot.

Repeat the above procedure for each reaction tube in the series.

**Acceptable alternate method (wireloop) may be employed if desired.

VI. Results

(a) Preparation of the Standard Curve

(1) Calculate the average clotting time of each dilution.

(2) Plot the average clotting time (in seconds) against the percent concentration of the standard on millimeter rectangular graph paper.

(3) Construct a curve that best fits the five points.

(b) Calculation of AHF Activity in Sample

(1) By interpolation, determine the percent AHF activity for each dilution of the sample.

(2) Multiply the resulting percent AHF factor by the dilution factor (test dilution divided by the 100% dilution of the reference preparation).

(3) The average of these results represents the AHF activity of the test material in terms of percent of the reference preparation. This percentage multiplied by the potency of the standard will give an answer in AHF units/ml.

(c) Statistical Review

(1) Three 5 dilution sets are run per aliquot, and results averaged. Following the recommended procedure of the Bureau of Biologics, the results should agree within ± 10% of the mean of the replicate assays.

(2) Assays are repeated on the following days with results complying with the ± 10% agreement within an assay.

(3) At least two vials will be reconstituted and assayed.

(4) All acceptable values over aliquots, days and vials, are averaged.

(5) Any values which exceed ± 20% of the mean, are excluded. Only one of each three daily assays can be excluded, other-wise, the entire assay must be rejected. The remaining total of assays must not be exclusively the result of one analyst.

(6) Calculate the mean ($\bar{x}$) and standard deviation (S) of the remaining assay values.

(a) Mean = $\frac{\text{Sum the Values}}{\text{No. of Values}} = \frac{\Sigma x}{n} = \bar{x}$

(b) Standard Deviation

1. Square each value, sum the squares ($[\Sigma x^2]$)

2. Sum the individual values; square this sum and divide by n $(\frac{(\Sigma x)^2}{n})$

3. Subtract the quotient of (2) from (1)

$$(\Sigma x^2 - \frac{(\Sigma x)^2}{n})$$

4. Divide the difference obtained by n-1.

5. Extract the square root of this quotient; this is the standard deviation S.

$$S = \sqrt{\frac{\Sigma x^2 - (\Sigma x)^2/n}{n-1}}$$

(7) Calculate the 95% Confidence Limits for the mean of the assays as follows:-

(a) A value of $T/\sqrt{n}$, previously calculated and set up in a table for each n, where n is the number of values used in the calculation in Step 6, is obtained.

(b) This value is multiplied by the standard deviation as

$(t/\sqrt{n}) * S$

then divided by the mean ($\bar{x}$) and multiplied by 100. This is the expression of the apparent assay error in terms of percent of the mean.

(8) This value is compared to the precision of the assay of ± 7.5% (previously determined - June, 1973).

(9) If the value is 7.5% or less, the assay mean potency stands.

(10) If the value is greater than 7.5%, additional assays must be run and the new values combined with all the previous values. Return to Step 4 and again follow the described procedure.

(11) The same accept-reject criterion must hold.

P (Probability) / n (Degrees of Freedom)	0.05
	't'
1	12.71
2	4.303
3	3.182
4	2.776
5	2.571
6	2.447
7	2.365
8	2.306
9	2.262
10	2.228
11	2.201
12	2.179
13	2.160
14	2.145
15	2.131
16	2.120
17	2.110
18	2.101
19	2.093
20	2.086
21	2.080
22	2.074
23	2.069
24	2.064
25	2.060
26	2.056
27	2.052
28	2.048
29	2.045
30	2.042
40	2.021
50	2.009
60	2.000
80	1.990
100	1.984
120	1.980
∞	1.960

ARMOUR PHARMACEUTICAL COMPANY, KANKAKEE
QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

Method No. 365
Supplement No. 1

RECONSTITUTION OF ANTIHAEMOPHILIC FACTOR (A.H.F.) FOR THROMBOPLASTIN GENERATION TEST (POTENCY)

Materials

1. Sterile Water for Injection, U.S.P. (30 ml/vial)
2. Double-ended Reconstitution Needle
3. Stopwatch or Timer
4. Water Bath at 37°C.

Test Procedure

1. Warm both the 30 ml diluent of sterile water for injection and vial of A.H.F. (unopened vials) to 37°C in a water bath (not less than 15 minutes).

2. Remove caps from both vials to expose the central portion of rubber stoppers.

3. Insert one end of the double-ended needle into the rubber stopper of the diluent vial. Invert the diluent vial and insert the other end of the double-ended needle into the rubber stopper of the A.H.F. vial. Allow the diluent to be drawn into the A.H.F. vial by vacuum and direct the stream over the surface of the cake.

4. Release the vacuum by removing the diluent vial from the double-ended needle allowing the incoming airstream to agitate the vial contents. They remove the double-ended needle from the A.H.F. vial.

5. Permit the A.H.F. vial to sit at room temperature for one minute without any agitation. This permits thorough wetting of the A.H.F. cake and improves the rate of reconstitution.

6. Manually swirl the A.H.F. vial very gently (avoid foaming) to promote breakup of the cake. As more cake goes into solution, more rapid swirling is permissible to get the last few particles into solution (avoid foaming).

SECTION 4 Development Pharmaceutical and Biological Availability (Cont.)

4.4. Availability Studies

4.4.1. In-Vitro Tests

Development work has included *in-vitro* comparison studies in which Factorate, Factorate II and Haemofil (Hylands) have been compared. The following results were obtained for the particular product reconstituted in the appropriate volume of Water for Injection U.S.P.

PRODUCT	TOTAL PROTEIN (MG/ML)	FIBRINOGEN (MG/ML)	UNITS/VIAL	SALT CONCENTRATION
Factorate	15 - 20	7.5 - 10	300	Isotonic
Factorate II*	40	28 - 36	1000	2 x Isotonic
Haemofil	34	18	1000	3 x Isotonic

4.4.2. In-Vivo Tests

Clinical evaluation of bioavailability *in-vivo* has been made by clinicians at separate study centres in the U.S.A. Each investigator accepted the protocol as a guide for his study and completed a case report for each patient. Full details are provided in Volume II of this Submission.

The biological half life in the circulation was determined by Professor P. H. Levine to be 8 - 12 hours following injections of Batch 852030 (1314 units/vial).

*Factorate II is synonymous with High Potency Factorate

SECTION 5 Stability

5.1. Batches Examined

Four batches were studied K 852029, K 852030, K 852031 and K 852032.

5.2. Conditions of Storage

Three temperature conditions were used - room temperature (23 ± 2°C), 2 - 8°C and 40°C. Duration of studies were for up to 26 weeks for physical and chemical stability.

5.3. Containers

The product was stored in 50 ml Type I glass vials fitted with a rubber closure and aluminium seal.

5.4. Results

The results are shown in Tables I and II (Physical Stability Tests) and Table III (Potency Values on reconstitution and 3 hour post-reconstitution).

5.5. Analytical Methods

The methods used to determine stability were those listed below:-

	Method*
1. Method to Determine Loss of Weight on Drying	43-D*
2. Thromboplastin Generation Test - Anti-haemophilic Factor	365ø
3. Solution Time for Generation II Antihaemophilic Factor	1257*

*These methods have been provided in Section 3
øThis method has been provided in Section 4

TABLE I

DOSAGE FORM PHYSICAL/CHEMICAL STABILITY DATA

Product - Antihaemophilic Factor, High Potency Factorate

Test - Solution Time

Packaging - Vial: Type I Glass, 50 ml
Stopper: T ompkins Rubber Co., Compound No. 0845, 20 mm
Seal: 1 Piece Aluminium, 20 mm

Analytical Method - 1257

Specification - NMT 30 minutes

Storage Conditions	Lot K852029 Solution Time	Lot K852030 Solution Time	Lot K852031 Solution Time	Lot K852032 Solution Time
Initial Solution Time	Satisfactory	Satisfactory	Satisfactory	Satisfactory
13 Weeks				
2 - 8°C	Satisfactory	Satisfactory	Satisfactory	Satisfactory
23 ± 2°C	Satisfactory	Satisfactory	Satisfactory	Satisfactory
40°C	Satisfactory	Satisfactory	Satisfactory	Unsatisfactory
26 Weeks				
2 - 8°C	Satisfactory	Satisfactory	Satisfactory	Satisfactory
23 ± 2°C	Satisfactory	Satisfactory	Satisfactory	Satisfactory
40°C	Satisfactory	Satisfactory	Unsatisfactory	Unsatisfactory

TABLE II

DOSAGE FORM PHYSICAL/CHEMICAL STABILITY DATA

Product - Antihaemophilic Factor (Human) High Potency Factorate

Test - Moisture

Packaging - Vial: Type I Glass, 50 ml
Stopper: T ompkins Rubber Co., Compound No. 0854, 20 mm
Seal: 1 Piece Aluminium, 20 mm

Analytical Method - 43D

Specification - NMT 2%

Storage Conditions	Lot K852029 % Moisture	Lot K852030 % Moisture	Lot K852031 % Moisture	Lot K852032 % Moisture
Initial Moisture	0.2	<0.1	<0.1	0.1
26 Weeks				
2 - 8°C	0.1	0.4	<0.1	0.1
23 ± 2°C	0.1	0.6	<0.2	0.1
40°C	0.1	Not Tested	<0.1	0.1

TABLE III

DOSAGE FORM POTENCY STABILITY DATA 3 HOUR POST-RECONSTITUTION

PRODUCT - Antihaemophilic Factor (Human) High Potency Factorate

PACKAGING - Vial: Type I Glass, 50 ml
Stopper: Tompkins Rubber Co., Compound No. 0854, 20 mm
Seal: 1 Piece Aluminium, 20 mm

ANALYTICAL METHOD - 365

SPECIFICATION - Minimum 800 u/vial

Storage Conditions	Lot K852029			Lot K852030			Lot K852031			Lot K852032		
	u/vial*	3 hr**	% Initial	u/vial	3 hr	% Initial	u/vial	3 hr	% Initial	u/vial	3 hr	% Initial
Initial Potency	1377	1290	94	1314	1278	97	1164	1203	103	1218	1119	92
2 - 8°C												
13 Weeks	1431	1416	99	1281	1212	95	1020	1233	121	1188	1170	99
26 Weeks	1278	1260	99	1230	1314	107	1230	1095	89	1173	1209	103
52 Weeks												
23 ± 2°C												
13 Weeks	1359	1305	96	1146	1200	105	1194	1131	95	1350	1347	100
26 Weeks	1350	1284	95	1278	1296	101	1200	1164	97	1137	1185	104
52 Weeks												
40°C												
13 Weeks	1251	1200	96	1206	1305	108	1038	1083	104	1269	1014	80
26 Weeks	1140	1056	93	1068	1236	116	927	906	98	1089	1128	104
52 Weeks												

*Potency determined by two-stage Throboplastin Generation Test immediately after reconstitution with 30 ml Sterile Water for Injection, U.S.P.

**Potency of solution determined three hours post-reconstitution

AP001076

90

ARMO0000023_0095

ARMOUR000448

SECTION 5 Stability (Cont.)

5.6. Summary of Results

(a) Physical Characteristics

It was noticed that a slight yellowing of the lyophilised cake took place in the period studied particularly at Room Temperature and 40°C temperature conditions. This appears to be characteristic of lyophilised blood products generally and did not affect solution time on reconstitution. Moisture uptake was not significant at any check up to 26 weeks of storage at 2 - 8°C.

(b) Potency

This was studied immediately after reconstitution by American methodology (Method 365). The results in Table III show that potency is maintained for 26 weeks at Room Temperature and below.

5.7. Shelf Life

A shelf life of one year is requested for the product stored at below 6°C from the date of manufacture. This is defined as the date on which the last valid potency test is completed.

5.8. Storage Conditions to be included on the Label

Store at a temperature below 6°C.

5.9. On-Going Stability Trials

Stability is being studied on the potency of the dosage form, itsreconstitution time and assay post-reconstitution.

SECTION 6 Containers

These are, for the finished product - 50 ml Type I glass vials with a grey butyl stopper. The rubber closure is covered with a brown plastic and aluminium flip-off seal.

The vials are bulk packed for shipment to the U.K. using polythene shrink-wrapping for each tray of vials. All information pertaining to the vial is provided on the outer carton label.

PART II (ADDENDUM) CHEMISTRY OF THE DRUG SUBSTANCE

SECTION 7 Identity of Material

7.1. Nomenclature

British Approved Name: None
International Non-Proprietary Name: None
U.S. Adopted Name: None
Laboratory Code: AL-1259
Chemical Name: None
Other Names: High Potency Factorate

7.2. Description

Physical Form: White to pale yellow lyophilised cake

The drug substance is synonomous with the dosage form and no development chemistry or stability work is carried out on the crude cryoprecipitate. The crude product is always immediately processed with aluminium hydroxide sterile suspension to give the finished product as detailed in Section 2 Part II.