CONFIDENTIAL
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MEDICINES ACT 1968

APPLICATION FOR A PRODUCT LICENCE

FACTORATE (FACTOR VIII)

VOLUME I - CHEMISTRY AND PHARMACY

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B. FINISHED PRODUCTS (Cont.)

SGB/FK
March 1975
Armour Pharmaceuticals Co. Ltd.,
Hampden Park,
Eastbourne,
Sussex.

MIA 201
PAGE 1

MEDICINES ACTS 1968 AND 1971 - APPLICATION FOR PRODUCT LICENCE

Full name and address of proposed licence holder:

Armour Pharmaceutical Co. Ltd.,
Hampden Park,
Eastbourne, Sussex

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

Role of proposed licence holder: (i) ~~as person who imports or procures importation of product manufactured outside UK~~
(ii) as person who imports or procures its importation
(iii) ~~as person who sells or supplies product manufactured outside UK~~

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

Applicants own reference no: SGB/FK

Name of Product: FACTORATE (FACTOR VIII)

Fees Information:

(i)	Does this product attract the major initial fee?	No
(ii)	If so, do you wish to pay this fee by instalments?	
(iii)	Is this product exempt from the initial fee?	Yes
(iv)	Do you hold any other product licence for which you are paying a duration fee?	/No
(v)	Are you a retail pharmacist?	/No
(vi)	Do you hold a manufacturer's licence?	Yes/~~No~~
(v)	Are you a retail pharmacist?	/No
(vi)	Do you hold a manufacturer's licence?	Yes/~~No~~
(vii)	Will this product be manufactured by a licensed manufacturer?	~~Yes~~/No
(viii)	Do you wish any surcharge to be adjusted by reference to sales?	~~Yes~~/No

X/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:-

1. All the provisions of Part I of Schedule 1 of the Medicines (Standard Provisions for Licences and Certificates) Regulations 1971 (S.I. 1971 No 1972) shall apply.
2. The product shall not be recommended to be used for any purpose other than those specified in the Product Particulars as Uses.
3. The specifications of the constituents and of the finished product shall be in accordance with information contained in or furnished in connection with the application.
4. The product is to be manufactured only in accordance with the methods set out in this application or furnished in connection with it.
5. The number of the Licence shall appear on all containers or packages in which the product(s) is/are packed and on any package inserts or accompanying literature.

Date 25th March 1975 Signature GRO-C

State capacity in which signed (Head of Regulatory Affairs)

Name and address for communications:-
Mr.S.G.Brooks
Armour Pharmaceutical Co. Ltd.,
Eastbourne, Sussex

Scientific Evidence:

(i)	Chemistry and Pharmacy	104 pages
(ii)	Experimental and Biological Studies	pages
(iii)	Clinical Trials	52 pages

Number of pages of supplementary information:-

AP000796

For licensing authority use

Product Particulars

2.1 Name of Product: FACTORATE (FACTOR VIII)

2.2 Pharmaceutical form: Injection. The preparation is in a form for administration to human beings.

2.3 Active constituents: Antihaemophilic Factor (Human)

2.4 Uses: In therapy of classical haemophilia (Haemophilia A).

Route of administration - intravenous infusion and intravenous injection

2.5 Recommended dose and dosage schedules: Dosage must be individualised according to needs of the patient. Full recommended general dosages are given in the package insert leaflet.

2.6 Contra-indications: There are no known contraindications to anti-haemophilic factor.

2.7 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.

2.8 Manufacturer of dosage forms: Armour Pharmaceutical Company, Phoenix, Arizona 85077 U.S.A.

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

Applicants signature ... GRO-C .....

AP000797

SUPPLEMENTARY PARTICULARS

3.1. Physical Characteristics

A white to pale yellow lyophilised cake in a vial with vacuum.

3.2. Manufacture

(a) Factorate is manufactured from fresh frozen human plasma which when tested is found to be negative for hepatitis B antigen activity. Cryoprecipitate as collected from thawed human plasma is dissolved in a buffer containing glycine, sodium chloride, and heparin. Impurities such as prothrombin are adsorbed with aluminium hydroxide after which the preparation is stablized with sodium citrate and heparin in an isotonic medium. Following the addition of stabilizers the solution is sterile filtered, filled into vials, and lyophilised.

(b) The Quali-quantitative formula:-

Factor VIII (Lyophilised)	NLT 125 U/Vial
Glycine	0.02 Molar
Sodium Chloride	0.04 Molar
Sodium Citrate	0.04 Molar
Heparin	NMT $2\frac{1}{2}$ U/ml

(c) The Name & Address of Place of Manufacture Assembly

Armour Pharmaceutical Company,
P.O. Box 511,
Kankakee,
Illinois, 60901
U.S.A.

(d) The Names & Addresses of Manufacturers of the Active Constituents

As in (c) above.

(e) The Arrangements for Storage and place of such Storage

The product is stored at -20°C during quarantine prior to labelling. It is stored at 2 - 8°C after labelling and held at Armour Pharmaceutical Co. P.O. Box 511, Kankakee, Illinois 60901.

3.3 Quality Control

Quality Control will be exercised on the raw materials, the ingredients used in the process during pharmaceutical manufacture and on the finished product. The vial appearance and fill weight is checked, tests for sterility and safety are carried out in accordance with Public Health Service Regulations, Title 42, Part 73 paragraphs 73.720 and 73.730 respectively.

3.4 Containers

The vials are 50 ml USP Type I glass with 20 mm finish closed with grey butyl lyophilisation style stoppers and having on aluminium flip-off seal.

3.5 Labelling

As provided in Volume II.

APPENDIX TO SUPPLEMENTARY INFORMATION
TO VOLUME I & II

3.3 Quality Control (Cont.)

Whether the proposed licensee will be responsible for deciding if any batch of the product is of acceptable quality for marketing.

In accordance with the "Guide to Good Pharmaceutical Manufacturing Practice" the Quality Controller at Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, will be responsible for the control of quality of products made by Armour Pharmaceutical Co., Kankakee, Illinois.

Safety Tests Employed

The test is performed as described in Public Health Service Regulations, Title 42, Part 273, Paragraph 73.720.*

Dosage into mice : 0.5 ml
Dosage into Guinea Pigs : 5.0 ml

Bulk and Final Container Tests for Sterility

The inoculum for final containers after reconstruction :-

1 ml in fluid Thioglycollate Medium
1 ml in Soyabean Casein digest

The above regulations apply*

* Food and Drug Regulations are as attached.

of all processes of manufacture which may affect compliance with the standard to which the test applies. The results of all tests performed shall be considered in determining whether or not the test results meet the test objective, except that a test result may be disregarded when it is established that the test is invalid due to causes unrelated to the product.

§ 73.710 Potency.

Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in § 73.101(t).

§ 73.720 General safety.

In addition to specified safety tests prescribed in this part for individual products, a general safety test shall be performed in final container material, from each filling of each lot of all products intended for administration to man, either after the labels have been affixed to the final container, or affixed, both outside and inside, to the multiple container storage receptacle just prior to its sealing for storage purposes. Exceptions to this procedure may be authorized by the Director, Division of Biologics Standards, when more than one lot is processed each day. The general safety test shall consist of the parenteral injection of the maximum volume tolerated into each of two mice weighing approximately 20 gms. each and into each of two guinea pigs weighing approximately 350 gms. each but no more than 0.5 ml. need be inoculated into each mouse and no more than 5.0 ml. need be inoculated into each guinea pig. After injection the animals shall be observed for a period of no less than seven days and if neither significant symptoms nor death results during the observation period, the product meets the requirements for general safety. Variations of this test, either in the volume injected or in the species of test animal used shall be made whenever required because of the human dose level demanded of the product or because of any individual demands of the product itself.

§ 73.730 Sterility.

Except as provided in paragraph (f), the sterility of each lot of each product shall be demonstrated by the performance of the tests prescribed in paragraphs (a) and (b) of this section for both bulk and final container material. Bulk material shall be tested separately from final container material and material from each final container shall be tested in individual test vessels.

(a) *The test*—(1) *Using Fluid Thioglycollate Medium.* The volume of product, as required by paragraph (d) of this section (hereinafter referred to also as the "inoculum"), from samples of both bulk and final container material, shall be inoculated into test vessels of Fluid Thioglycollate Medium. The inoculum and medium shall be mixed thoroughly and incubated at a temperature of 30° to 32° C. for a test period of no less than seven days and examined visually for evidence of growth on the third or fourth or fifth day and on the seventh or eighth day. If incubation is continued beyond eight days, an additional examination shall be made on the last day of the test period. If the inoculum renders the medium turbid so that the absence of growth cannot be determined reliably by visual examination, portions of this turbid medium in amounts of no less than 1.0 ml. shall be transferred on the third or fourth or fifth day of incubation, from each of the test vessels and inoculated into additional vessels of medium. The material in the additional vessels shall be incubated at a temperature of 30° to 32° C. for no less than seven days. Notwithstanding such transfer of material, examination of the original vessels shall be continued as prescribed above. The additional test vessels shall be examined visually for evidence of growth on the third or fourth or fifth day of incubation and on the seventh or eighth day and if incubation is continued beyond a period of eight days, an additional examination shall be made on the last day of the incubation period. If growth appears, repeat tests may be performed as prescribed in paragraph (b) of this section and interpreted as specified in paragraph (c) of this section.

(2) *Using Fluid Sabouraud Medium.* Except

for dried products, a test for fungi and yeast shall be made on final container material, following the procedures prescribed in subparagraph (1) of this paragraph except that (i) the medium shall be Fluid Sabouraud Medium; (ii) the incubation shall be at a temperature of 20° to 25° C.; (iii) the period of incubation shall be no less than ten days and an examination shall be made on the tenth or eleventh or twelfth day in lieu of an examination on the seventh or eighth day.

(b) *Repeat tests*—(1) *Repeat bulk test.* If growth appears in the test of the bulk material, the test may be repeated to rule out faulty test procedures by testing at least the same volume of material.

(2) *First repeat final container test.* If growth appears in any test (thioglycollate or Sabouraud) of final container material, that test may be repeated to rule out faulty test procedures by testing material from a sample of at least the same number of final containers.

(3) *Second repeat final container test.* If growth appears in any first repeat final container test (thioglycollate or Sabouraud), that test may be repeated provided there was no evidence of growth in any test of the bulk material and material from a sample of twice the number of final containers used in the first test is tested by the same method used in the first test.

(c) *Interpretation of test results.* The results of all tests performed on a lot shall be considered in determining whether or not the lot meets the requirements for sterility, except that tests may be excluded when demonstrated by adequate controls to be invalid. The lot meets the test requirements if no growth appears in the tests prescribed in paragraph (a) of this section. If repeat tests are performed, the lot meets the test requirements if no growth appears in the tests prescribed in paragraph (b)(2) or (b)(3) of this section, whichever is applicable.

(d) *Test samples and volumes*—(1) *Bulk.* Each sample for the bulk sterility test shall be representative of the bulk material and the volume tested shall be no less than 10 ml. (Note exceptions in paragraph (f) of this section.)

(2) *Final containers.* The sample for the final container and first repeat final container tests shall be no less than 20 final containers from each filling of each lot, selected to represent all stages of filling from the bulk vessel. If the amount of material in the final container is 1.0 ml. or less, the entire contents shall be tested. If the amount of material in the final container is more than 1.0 ml., the volume tested shall be the largest single dose recommended by the manufacturer or 1.0 ml., whichever is larger, but no more than 10 ml. of material or the entire contents from a single final container need be tested. (Note exceptions in paragraph (f) of this section.)

(e) *Culture medium*—(1) *Formulae.* (i) The formula for Fluid Thioglycollate Medium is as follows:

Fluid Thioglycollate Medium

1-cystine	0.5 gm.
Sodium chloride	2.5 gm.
($C_6H_{12}O_6 \cdot H_2O$) Dextrose	5.5 gm.
Granular agar (less than 15% moisture by weight)	0.75 gm.
Yeast extract (water-soluble)	5.0 gm.
Pancreatic digest of casein	15.0 gm.
Purified water	1,000 ml.
Sodium thioglycollate (or thioglycollic acid—0.3 ml.)	0.5 gm.
Resazurin (0.10% solution, freshly prepared)	1.0 ml.

Final pH 7.1±0.1.

(ii) The formula for Fluid Sabouraud Medium is as follows:

Fluid Sabouraud Medium

Dextrose	20 gm.
Pancreatic digest of casein	5 gm.
Peptic digest of animal tissue	5 gm.
Purified water	1,000 ml.

Final pH 5.7±0.1.

(2) *Culture medium requirements*—(i) *Quality and condition of medium and design of test vessel.* The growth promoting qualities and conditions of the culture medium, and the design of the test vessel, shall be such as are shown to provide conditions favorable to aerobic and anaerobic growth of microorganisms throughout the test period.

(ii) *Ratio of inoculum to culture medium.* The ratio of the volume of the inoculum to the volume of culture medium shall be such as will dilute the preservative in the inoculum to a

level that does not inhibit growth of contaminating microorganisms. Inhibitors or neutralizers of preservative may be considered in determining the proper ratio.

(f) *Exceptions.* Bulk and final container material shall be tested for sterility as described above in this section except as follows:

(1) *Different sterility tests prescribed.* When different sterility tests are prescribed for a product in this part.

(2) *Alternate incubation temperatures.* Two tests may be performed, in all respects as prescribed in paragraph (a)(1) of this section, one test using an incubation temperature of 18° to 22° C., the other test using an incubation temperature of 35° to 37° C., in lieu of performing one test using an incubation temperature of 30° to 32° C.

(3) *Different tests equal or superior.* A different test may be performed provided that prior to the performance of such a test a manufacturer submits data which the Director, National Institutes of Health, finds adequate to establish that the different test is equal or superior to the tests described in paragraphs (a) and (b) of this section in detecting contamination and makes the finding a matter of official record.

(4) *Test precluded or not required.* The tests prescribed in this section need not be performed for Whole Blood (Human), Cryoprecipitated Antihemophilic Factor (Human), Red Blood Cells (Human), Single Donor Plasma (Human), Smallpox Vaccine and other similar products concerning which the Director, National Institutes of Health, finds that the mode of administration, the method of preparation or the special nature of the product precludes or does not require a sterility test.

(5) *Viscous biological products.* Thioglycollate Broth Medium may be used in lieu of Fluid Thioglycollate Medium to test viscous biological products. The formula for Thioglycollate Broth Medium is as follows:

Thioglycollate Broth Medium. Certain biological products are turbid or otherwise do not lend themselves readily to culturing in Fluid Thioglycollate Medium because of its viscosity. In such instances, the following broth is acceptable in place of the Fluid Thioglycollate Medium, provided it is used in Smith fermentation tubes which have been heated within four hours in a boiling water bath or in free-flowing steam so as to drive the dissolved oxygen out of the medium in the closed arm:

l-cystine	0.5 gm.
Sodium chloride	2.5 gm.
($C_6H_{12}O_6 \cdot H_2O$) Dextrose	5.5 gm.
Yeast extract (water-soluble)	5.0 gm.
Pancreatic digest casein	15.0 gm.
Purified water	1,000 ml.
Sodium thioglycollate (or thioglycollic acid—0.3 ml.)	0.5 gm.

Final pH 7.1±0.1.

(6) *Number of final containers more than 20, less than 200.* If the number of final containers in the filling is more than 20 or less than 200, the sample shall be no less than 10 percent of the containers.

(7) *Number of final containers—20 or less.* If the number of final containers in a filling is 20 or less, the sample shall be two final containers, or the sample need be no more than one final container, provided (i) the bulk material met the sterility test requirements and (ii) after filling, it is demonstrated by testing a simulated sample that all surfaces to which the product was exposed were free of contaminating microorganisms. The simulated sample shall be prepared by rinsing the filling equipment with sterile 1.0 percent peptone solution, pH 7.1±0.1, which shall be discharged into a final container by the same method used for filling the final containers with the product.

(8) *Samples—large volume of product in final containers.* For Normal Serum Albumin (Human), Normal Human Plasma, Antihemophilic Plasma (Human), Plasma Protein Solution (Human) and Fibrinogen (Human), when the volume of product in the final container is 50 ml. or more, the final containers selected as the test sample may contain less than the full volume of product in the final containers of the filling from which the sample is taken: *Provided,* That the containers and closures of the sample are identical with those used for the filling to which the test applies and the sample represents all stages of that filling.

(9) *Diagnostic products not intended for injection.* For diagnostic products not intended

for injection, (i) only the Thioglycollate Medium test is required, (ii) the volume of material for the bulk test shall be no less than 2.0 ml., and (iii) the sample for the final container test shall be no less than three final containers if the total number filled is 100 or less, and, if greater, one additional container for each additional 50 containers or fraction thereof, but the sample need be no more than 10 containers.

(10) *Human immune globulin preparations.* For human immune globulin preparations, the test samples from the bulk material and from each final container need be no more than 2.0 ml.

§ 73.740 Purity.

Products shall be free from extraneous material. In addition, products shall be tested as provided in paragraphs (a) and (b) of this section.

(a) *Test for residual moisture.* Each lot of dried product shall be tested for residual moisture and other volatile substances.

(1) *Procedure.* The test for dried products shall consist of measuring the maximum loss of weight in a weighed sample equilibrated over anhydrous P_2O_5 at a pressure of not more than one mm. of mercury, and at a temperature of 20° to 30° C. for as long as it has been established is sufficient to result in a constant weight.

(2) *Test results; standard to be met.* The residual moisture and other volatile substances shall not exceed 1 percent except that for BCG Vaccine they shall not exceed 1½ percent, for Measles Virus Vaccine, Live, Attenuated, Measles-Smallpox Vaccine, Live, Rubella Virus Vaccine, Live and Antihemophilic Factor (Human), they shall not exceed 2 percent, and for Modified Plasma (Bovine); Thrombin; Fibrinogen; Streptokinase; Streptokinase-Streptodornase; and Anti-Influenza Virus Serum for the Hemagglutination Inhibition Test, they shall not exceed 3 percent.

(b) *Test for pyrogenic substances.* Each lot of any product intended for use by injection shall be tested for pyrogenic substances by intravenous injection into rabbits as provided in subparagraphs (1) and (2) of this paragraph: *Provided,* That notwithstanding any other provision of this part, the test for pyrogenic substances is not required for the following products: Products containing formed blood elements; Cryoprecipitated Antihemophilic Factor (Human); Single Donor Plasma (Human); Normal Horse Serum; Normal Rabbit Serum; bacterial, viral and rickettsial vaccines and antigens; toxoids; toxins; allergenic extracts; venoms; diagnostic substances and trivalent organic arsenicals.

(1) *Test dose.* The test dose for each rabbit shall be at least 3 milliliters per kilogram of body weight of the rabbit and also shall be at least equivalent proportionately, on a body weight basis, to the maximum single human dose recommended, but need not exceed 10 ml. per kilogram of body weight of the rabbit, except that: (i) Regardless of the human dose recommended, the test dose per kilogram of body weight of each rabbit shall be, at least 1 milliliter for immune globulins derived from human blood, at least 3 milliliters for Normal Human Plasma, and at least 30 milligrams for Fibrinogen (Human); (ii) for Streptokinase, Streptokinase - Streptodornase, Aggregrated Radio-Iodinated (I^{131}) Albumin (Human), Radio-Chromated (Cr^{51}) Serum Albumin (Human), Radio-Iodinated (I^{125}) Serum Albumin (Human) and Radio-Iodinated (I^{131}) Serum Albumin (Human), the test dose shall be at least equivalent proportionately on a body weight basis to the maximum single human dose recommended.

(2) *Procedure.* Products shall be tested for freedom from pyrogenic substances by intravenous injection of the test dose into three or more rabbits in overt good health and by recording for each rabbit a control temperature taken within one hour prior to injection, and three additional temperatures taken one, two, and three hours after injection. For purposes of subparagraph (3) of this paragraph, if there is no temperature increase over the control temperature (i.e. where the temperature remains unchanged or falls), the temperature rise shall be considered as zero. If there is an increase in temperature over the control temperature, the temperature rise shall be the difference between the highest of the three hourly

readings and the control temperature reading.

(3) *Test results; standards to be met.* The results recorded for all rabbits used in all tests of a lot of a product shall be included in determining whether the standard for purity is met. The product fails to meet test requirements if one half or more of all rabbits show a temperature rise of 0.6° C. or more or if the average temperature rise of all rabbits is 0.5° C. or more.

(c) *Different tests equal or superior.* A different test for residual moisture may be performed provided that prior to its performance the manufacturer submits data which the Director, National Institutes of Health, finds adequate to establish that the different test is equal or superior to the test described in paragraph (a) of this section and makes the finding a matter of official record.

§ 73.750 Test for *Mycoplasma*.

Except as provided otherwise in this part, prior to clarification or filtration in the case of live virus vaccines produced from in-vitro living cell cultures, and prior to inactivation in the case of inactivated virus vaccines produced from such living cell cultures, each virus harvest pool and control fluid pool shall be tested for the presence of *Mycoplasma*, as follows:

Samples of the virus for this test shall be stored either (1) between 2° and 8° C. for no longer than 24 hours, or (2) at −20° C. or lower if stored for longer than 24 hours. The test shall be performed on samples of the viral harvest pool and on control fluid pool obtained at the time of viral harvest, as follows: No less than 2.0 ml. of each sample shall be inoculated in evenly distributed amounts over the surface of no less than 10 plates of at least two agar media. No less than 1.0 ml. of sample shall be inoculated into each of four tubes containing 10 ml. of a semisolid broth medium. The media shall be such as have been shown to be capable of detecting known *Mycoplasma* and each test shall include control cultures of at least two known strains of *Mycoplasma*, one of which must be *M. pneumoniae*. One half of the plates and two tubes of broth shall be incubated aerobically at 36° C. ±1° C. and the remaining plates and tubes shall be incubated anaerobically at 36° C. ±1° C. in an environment of 5–10 percent CO_2 in N_2. Aerobic incubation shall be for a period of no less than 14 days and the broth in the two tubes shall be tested after 3 days and 14 days, at which times 0.5 ml. of broth from each of the two tubes shall be combined and subinoculated on to no less than 4 additional plates and incubated aerobically. Anaerobic incubation shall be for no less than 14 days and the broth in the two tubes shall be tested after 3 days and 14 days, at which times 0.5 ml. of broth from each of the two tubes shall be combined and subinoculated on to no less than four additional plates and incubated anaerobically. All inoculated plates shall be incubated for no less than 14 days, at which time observation for growth of *Mycoplasma* shall be made at a magnification of no less than 300×. If the Dienes Methylene Blue-Azure dye or an equivalent staining procedure is used, no less than a one square cm. plug of the agar shall be excised from the inoculated area and examined for the presence of *Mycoplasma*. The presence of the *Mycoplasma* shall be determined by comparison of the growth obtained from the test samples with that of the control cultures, with respect to typical colonial and microscopic morphology. The virus pool is satisfactory for vaccine manufacture if none of the tests on the samples show evidence of the presence of *Mycoplasma*.

§ 73.760 Identity.

The contents of a final container of each filling of each lot shall be tested for identity after all labeling operations shall have been completed. The identity test shall be specific for each product in a manner that will adequately identify it as the product designated on final container and package labels and circulars, and distinguish it from any other product being processed in the same laboratory. Identity may be established either through the physical or chemical characteristics of the product, inspection by macroscopic or microscopic methods, specific cultural tests, or in vitro or in vivo immunological tests.

§ 73.770 Requests for samples and protocols; official release.

Samples of any lot of any licensed product, together with the protocols showing results of applicable tests, may at any time be required to be sent to the Director, Division of Biologics Standards. Upon notification by the Director, Division of Biologics Standards, a manufacturer shall not distribute a lot of a product until the lot is released by the Director, Division of Biologics Standards: *Provided*, That the Director shall not issue such notification except when deemed necessary for the safety, purity or potency of the product.

§ 73.780 Cultures.

(a) *Storage and maintenance.* Cultures used

MEDICINES ACT 1968

APPLICATION FOR A PRODUCT LICENCE

FACTORATE

(A) ACTIVE CONSTITUENT

SECTION 1 NAMES

1.1. Approved Name

None

1.2. Monograph Name

None

1.3. U.S. Adopted Name

None

1.4. International Non-Proprietary Name

None

1.5. Laboratory Codes

Factor VIII (Lyophilised), AL - 1067

1.6. Chemical Name

None

1.7. Proprietary Name

FACTORATE

1.8. Other Names

None

SECTION 2 DESCRIPTION

A white to very pale yellow lyophilised cake in a vial with vacuum. The vial is fitted with a blue seal.

SECTION 3 METHOD OF MANUFACTURE

3.1 Description of the Manufacturing Process

Stage 1 - Frozen plasma is removed from the collection bags by thawing. The frozen plasma is crushed and pooled and allowed to reach 3°C. Samples are taken for assay to insure freedom from hepatitis associated antigen.

Stage 2 The cryoprecipitate is removed from the pool and dissolved in buffer solution containing glycine, sodium chloride and sodium heparin.

Stage 3 The buffer cryoprecipitate is treated with sterilised non-reactive aluminium hydroxide and centrifuged. The centrifugate is buffered with sodium citrate and sodium heparin, filtered, sterile filtered, vialed and lyophilised.

Stage 4 The final product is re-checked for hepatitis associated antigen.

SECTION 3 METHOD OF MANUFACTURE

3.2 Specifications of Starting Materials and Reagents Which make a Significant Contribution in the Method of Manufacture

	Specification
Plasma for Fractionation, normal (human) for Antihaemophilic Factor (human)	S-3029
Sodium Citrate USP	267
Sodium Bicarbonate USP	270
Sodium Chloride USP - Pyrogen Free	271
Glycine (Aminoacetic Acid) NF.	753
Glacial Acetic Acid USP	897
Sodium Heparin Injection USP	2951
Rehsorptar (F-5000 Aluminium Hydroxide Gel)	3232

The above specifications are attached in numerical order.

Armour Pharmaceutical Company
Quality Control
Kankakee, Illinois

Spec. No. 267

SODIUM CITRATE USP OR REAGENT

9

SPECIFICATION

Date:	Supersedes:	Prepared by:	Exp. Date:
11/1/73	5/23/56	G. A. Pontinga	1 year

DESCRIPTION:

Colorless crystals or a white, crystalline powder. It has a cooling, saline taste.

SAMPLING:

Submit one 100 cc. RWM bottle and one shell vial for reserve sample.
Group I: RWM bottle.

TEST	SPECIFICATION	METHOD
Solubility	O. K.	USP
*Identification	O. K.	USP
Alkalinity	O. K.	USP
*Loss on Drying	10 - 13%	USP
Tartrate	Nil	USP
Heavy Metals	NMt 10 ppm	USP
*Assay	NLT 99.0%; NMT 100.5%	USP

*Test run at Kankakee

Reason for Revision:

THIS SPECIFICATION WAS TRANSFERRED FROM AN APPROVED HALF SHEET. NO FURTHER APPROVALS ARE NECESSARY.

Approved by:

AP000810

Armour Pharmaceutical Company
Quality Control
Kankakee, Illinois

Spec. No. 270

SPECIFICATION

SODIUM BICARBONATE, USP OR REAGENT
(BLOOD INGREDIENT)

10

Date:	Supersedes:	Prepared by:	Exp. Date:
4/10/72	9/23/59	G. A. Portinga	1 year

DESCRIPTION:

Sodium Bicarbonate is a white, crystalline powder.

SAMPLING:

Submit one 100 cc. RWM bottle, one sterile, dry test tube, plus one shell vial for reserve sample. Group I: RWM bottle; Group X: test tube.

TEST	SPECIFICATION	METHOD
Solubility	Passes USP	USP
*Identification	Positive	"
Insoluble Substance	Complete and clear Soln.	"
Carbonate	No immediate red tint	"
Ammonia	No odor on heating	"
Arsenic	NMT 3 ppm	"
Heavy Metals	NMT 5 ppm	"
*Assay	NLT 99.0% – NMT 100.5%	"
Loss on Drying	NMT 0.25%	"
*Pyrogen	Satisfactory	203

*Test run at Kankakee

GRO-C 5/1/72

Reason for Revision:

Addition of Loss on Drying

Approved by:

GRO-C 4-18-72 9-20-72 4-28-72

AP000811

Armour Pharmaceutical Company
Quality Control
Kankakee, Illinois

Spec. No. 271 11

SPECIFICATION

SODIUM CHLORIDE USP - PYROGEN FREE

Date:	Supersedes: 3/2/71	Prepared by: G. A. Portinga	Exp. Date: 1 year

DESCRIPTION:

Colorless, cubic crystals or white crystalline powder with a saline taste.

SAMPLING:

Group I - 20 cc RWM bottle; Group X - 20 gms. in sterile bottle; Reserve - shell vial

TEST	SPECIFICATION	METHOD
Solubility	Satisfactory	USP
*Identification	Satisfactory	"
Acidity or Alkalinity	NMT 1.0 ml. of 0.02N NaOH NMT 2.0 ml. of 0.02N HCl	"
Loss on Drying	NMT 0.5%	"
Iodide or Bromide	No violet, orange or yellow color	"
Calcium & Magnesium	NMT 0.05%	"
Sulfate	NMT 150 ppm	"
*Sodium Ferrocyanide	Nil	"
*Assay	99.5 - 100.5% (DB)	"
*Pyrogen	Satisfactory	"

Reason for Revision:

THIS SPECIFICATION WAS TRANSFERRED FROM AN APPROVED HALF SHEET. NO FURTHER APPROVALS ARE NECESSARY.

Approved by:

AP000812

Armour Pharmaceutical Company
Quality Control
Kankakee, Illinois

Spec. No. 753.

12

SPECIFICATION

AMINOACETIC ACID NF (GLYCINE)

Date:	Supersedes:	Prepared by:	Exp. Date:
	3/9/56	G. A. Portinga	1 year

DESCRIPTION:

A white, odorless, crystalline powder having a sweetish taste.

SAMPLING:

Submit 5 grams to Group I: submit 5 grams sampled aseptically to Group X.

TEST	SPECIFICATION	METHOD
Solubility	1 gram in 4 cc H_2O	NF
Identity	O. K.	"
Moisture	NMT 0.2%	"
Ash	NMT 0.1%	"
Readily Carbonizable Substances	Colorless solution	"
Chloride	NMT 70 ppm.	"
Sulfate	NMT 65 ppm.	"
Heavy Metals	NMT 20 ppm.	"
Hydrolyzable Substances	O. K. - clear solution	"
Assay	NLT 98.5%; NMT 101.5%	"
Pyrogen	O. K.	208

kaf

Reason for Revision:

THIS SPECIFICATION WAS TRANSFERRED FROM AN APPROVED HALF SHEET. NO FURTHER APPROVALS ARE NECESSARY.

Approved by:

AP000813

Armour Pharmaceutical Company
Quality Control
Kankakee, Illinois

SPECIFICATION

Spec. No. 897
13

GLACIAL ACETIC ACID USP REAGENT (ACS)

Date:	Supersedes: 3/4/71	Prepared by: G. A. Portinga	Exp. Date:

DESCRIPTION:

A clear colorless liquid having a characteristic pungent odor

SAMPLING:

200 cc in a clean, dry, glass-stoppered Erlenmeyer flask.

TEST	SPECIFICATION	METHOD
*Assay (Freezing Point)	NLT 16.0° C	USP
Color (Apha)	NMT 10	ACS
Dilution Test	To pass test	"
*Residue After Evaporation	NMT 0.0010%	"
Chloride	NMT 0.0001%	"
Sulfate	NMT 0.0001%	"
*Heavy Metals (As Pb)	NMT 0.00005%	"
Iron (Fe)	NMT 0.00002%	"
*Substances Reducing Dichromate	To pass test	"
Sensitivity	To pass test	USP (Reagent)

half

Reason for Revision:

THIS SPECIFICATION WAS TRANSFERRED FROM AN APPROVED HALF SHEET. NO FURTHER APPROVALS ARE NECESSARY.

Approved by:

APC00814

Armour Pharmaceutical Company
Quality Control
Kankakee, Illinois

Spec. No. 2951

14

SPECIFICATION

SODIUM HEPARIN INJECTION, USP

Date:	Supersedes:	Prepared by:	Exp. Date:
10/12/71	New	G. A. Portinga	6 Months**

Description:

A clear sterile solution of sodium heparin in water for injection. The sodium heparin is derived from porcine stomach tissue. It exhibits a potency not less than 90.0 percent and not more than 110.0 percent of the potency stated on the label as expressed in U.S.P. Heparin Units. It meets USP requirements for injection.

Sampling:

To be determined

TEST	SPECIFICATION	METHOD
*Pyrogen	per USP	USP
*pH	5.0 - 7.5	USP
*Assay	1000 u/ml. ± 10%	1073

*Test run at Kankakee

Material must be used within six months after release by the Quality Control Department.

GRO-C

10-15-73

Reason for Revision:

New Specification

Approved by:

GRO-C

10-12-73

10-15-73

10-15-73

AP000815

ARMOUR PHARMACEUTICAL COMPANY
KANKAKEE, ILLINOIS

15

RAW MATERIAL SPECIFICATIONS

DATE: October 10, 1973 NUMBER: S-3029

PLASMA FOR FRACTIONATION, NORMAL (HUMAN)
FOR ANTIHEMOPHILIC FACTOR (HUMAN)

DESCRIPTION:

Plasma for Fractionation, Normal, (Human), is the liquid portion of whole blood containing no additives other than citrate, acid citrate dextrose, or citrate phosphate dextrose anticoagulant solution, drawn from adult humans by plasmapheresis who have not been hyperimmunized to produce specific antibodies (unless otherwise specified and mutually agreed upon by authorized representatives of Armour Pharmaceutical Company and the supplier), and who, at the time the blood is drawn, are in condition physically to give blood, insofar as can be determined by personal history, by physical examination and by appropriate tests on the day the blood is collected. Donors with a history of viral hepatitis, or an event of exposure to hepatitis within the normal incubation period, shall be excluded. The type of anticoagulant contained in the plasma shall be separately agreed upon by authorized representatives of Armour Pharmaceutical Company and the supplier.

The specifications outlined herein are applicable to human plasma used in the production of "Licensed Products in Short Supply" as defined by the Director of the Food and Drug Administration in his directive dated January 25, 1972, and further defined in Title 21, Part 600, §601.22 of the Code of Federal Regulations.

COLLECTION AND PROCESSING OF BLOOD:

Blood collection clinics supplying human plasma under these specifications and licensed by the U.S. Department of Health, Education and Welfare, Food and Drug Administration, must comply with applicable requirements defined in the Code of Federal Regulations, Title 21, Part 600. Nonlicensed clinics supplying human plasma under these specifications must also comply with these regulations and specifically with §600.10, 600.11, 600.12, 600.3, and 600.4 thereof.

AP000816

ARMOUR PHARMACEUTICAL COMPANY
KANKAKEE, ILLINOIS

RAW MATERIAL SPECIFICATIONS

DATE: October 10, 1973 NUMBER: S-3029

COLLECTION AND PROCESSING OF BLOOD: (CONT'D.)

Blood shall be drawn from acceptable donors by licensed physicians or by specially trained assistants under their direct supervision. Determination of the suitability of the donor is the responsibility of the licensed physicians and must be made by them or under their supervision. Techniques and conditions normally associated with good medical practices must be maintained and exercised within these operations.

The Plasma is separated from the red cells by centrifugation and maintained as individual units for shipment to Armour Pharmaceutical Company as mutually agreed upon by authorized representatives of Armour and the supplier. The plasma is flash frozen as individual units at -70°C or lower within one hour of separation and within two hours of withdrawal from the donor, and maintained in the frozen state, at -20°C or lower, until delivered to Armour Pharmaceutical Company. All equipment and techniques used in handling the plasma must be scrupulously clean and designed to prevent pyrogenic or bacterial contamination.

Plasma obtained using plasmapheresis techniques must conform, in all respects, to requirements for Source Plasma (Human), defined in Paragraphs 640.60 through 640.70 of Title 21 Part 600 of the Code of Federal Regulations.

RECORDS:

Adequate records detailing the medical history of the donor, all physical examinations given him, and appropriate release statements he signs must be maintained. And, adequate systems identifying the blood, plasma, and serum, and correlating them with records supplied to Armour Pharmaceutical Company and records at the donor center, must also be maintained.

Records of all donors represented in the plasma supplied to Armour Pharmaceutical Company must be maintained for a period of at least six (6) months after the latest expiration date of the products prepared from the plasma. A period of twelve (12) years is recommended.

AP000817

RECORDS: (CONT'D.)

All shipments of plasma to Armour must be accompanied by records that include adequate donor identification by name and/or number, bleeding identification by number, bleeding dates, and cross-references to plasma pools, when applicable. The name and full address of the donor center must be included on each sheet of these records; forms similar to those prototyped as Attachments A and B may be used.

The records of each shipment of plasma must include a statement defining the type of anticoagulant used in all units in the shipment; when more than one type of anticoagulant is used, the number of units containing each type of anticoagulant must be given. And, the records of each shipment must include a statement confirming that all units have been tested and found non-reactive for Hepatitis B Antigen; the type of test used must be indicated. Finally, a statement certifying that the plasma had been flash frozen must be included. A form similar to that prototyped as Attachment C may be used. (The records accompanying the shipment should be contained in an envelope or otherwise adequately bound and placed in one carton of the shipment; that carton must be adequately marked to facilitate its identification when received at Armour Pharmaceutical Company.)

Each carton of plasma must be labeled with the following:

1. The addressee, Armour Pharmaceutical Company.
2. The name and address of the donor center.
3. The proper name and specification number of the material in the shipment, e.g. Plasma for Fractionation, Normal, (Human), S-3029.
4. The number and size of containers in the carton.
5. The recommended storage conditions.
6. The number of cartons in the shipment and the individual number of each carton (e.g. Box #5 of 23 Boxes).

AP000818

RAW MATERIAL SPECIFICATIONS

DATE: October 10, 1973 NUMBER: S-3029

RECORDS: (CONT'D.)

Each container of plasma must be labeled with the proper name of the product, the name and address of the donor center, the date of bleeding or pooling, and the unit donor number or plasma pool number.

FACILITIES INSPECTION:

Bleeding and processing establishments, and all processing equipment and records will be available for inspection, during regular business hours, by designated representatives of Armour Pharmaceutical Company. And, all facilities, equipment and records will also be available for inspection, during regular business hours, by designated representatives of the U.S. Department of Health, Education and Welfare, Food and Drug Administration.

Inspections are conducted to confirm total conformance to requirements of these specifications and regulations defined in 21CFR600.

PLASMA PROPERTIES:

Plasma for Fractionation (Human):

1. Shall be substantially free from red blood cells.
2. Shall contain no more than 50 mg. Hemoglobin per 100 ml.
3. Shall have a total protein content of not less than 5.5%.
4. Shall be free of bacterial or pyrogenic contamination, or, each container shall be marked otherwise.
5. Shall be packaged in plastic or glass, in volumes as as separately agreed upon by representatives of Armour Pharmaceutical Company and the supplier.

AP000819

ARMOUR PHARMACEUTICAL COMPANY
KANKAKEE, ILLINOIS

RAW MATERIAL SPECIFICATIONS

DATE: October 10, 1973 NUMBER: S-3029

PLASMA PROPERTIES: (CONT'D.)

6. Shall be stored and shipped frozen, below -20°C., unless otherwise specified and mutually agreed upon by authorized representatives of Armour Pharmaceutical Company and the supplier.

7. Shall be free of Hepatitis B Antigen as tested on individual units by Radio-Immune Assay.

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

GENERAL:

Failure to comply with any of the foregoing specifications shall be sufficient cause to reject any Plasma for Fractionation, Normal, (Human), delivered to Armour Pharmaceutical Company.

OPERATIONS STANDARDS
Kankakee, Illinois

20

REHSORPTAR, 1 LITER

SPECIFICATION 3232

(F-5000 Aluminium Hydroxide Gel).

DATE:	SUPERSEDES:	PREPARED BY:	EXP. DATE:
2/20/74	New	G. A. Portinga	- - - - - - - - - - - -

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

*Test run at Kankakee

GRO-C 3-11-74

REASON FOR REVISION:

New specification. Initial writing of specification for Rehsorptar, 1 Liter.

APPROVED BY:

GRO-C 3-1-74

3/13/74

ADI-6013 SG

SECTION 3 METHOD OF MANUFACTURE (Cont.)

3.3 Purpose of Each Stage in the Manufacturing Process

Stage 1 Samples are taken from the pooled liquid for radio-immuno assay to ensure no contamination has occurred in the individual plasma collection.

Stage 2 A buffered cryo precipitate is isolated.

Stage 3 An adsorption step is carried out for removal of non-active protein, and the aluminium hydroxide adsorbate removed by centrifugation.
The final product is filtered and lyophilised in vials.

Stage 4 Capped vials are submitted to Quality Control where they are tested in accordance with the finished product specification (sterility test being in accordance with current licensing requirements).

SECTION 3 METHODS OF MANUFACTURE (Cont.)

3.4. Quality Control Checks made at Each Stage in the Process.

Stage 1 The raw material must conform to Specification S-3029 and each shipment of plasma must include the type of anticoagulant, a statement of non-reactivity for Hepatitis B antigen, and a statement certifying that the plasma has been flash frozen.

Stage 2 The preparation of buffer solution is subject to Quality Control checks. Each kilogram of cryo-precipitate is dissolved in 2.8 litres of buffer solution containing fixed quantities of ingredients.

Stage 3 The adsorbate is examined for aluminium oxide content, protein binding capacity, specific gravity and sterility.

Stage 4 The lyophilised material is examined according to the Finished Product Specification provided in Section 11. It is also checked for Hepatitis B associated antigen.

SECTION 3 METHOD OF MANUFACTURE (Cont.)

The following analytical methods are summarised in the appropriate specifications referred to previously and are provided in full on the following pages.

	Method
Specific gravity of liquids	13
Method to determine loss of weight on drying	43D
Pyrogen test	208
Determination of aluminium oxide	286
Sterility test	303
Test for non-specific agglutinins	306
Quantitative determination of airborne bacterial and mould contamination	308
Determination of bacteriostatic and fungistatic properties of products to be tested for sterility	309
Microscopic examination of bacteria	310
Determination of protein binding capacity	344
Mammalian protein specie identification	351
Thromboplastin generation test	365
Safety test for normal serum albumin	963
Biuret assay for total protein content of cryoprecipitated antihaemophilic globulin	993
Determination of clottable protein	994
Atomic absorption analysis of aluminium in antihaemophilic factor	995
Determination of heparin content	1073
Solution time for antihaemophilic factor (human) lyophilised	1079

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD No. 13.

S.G. OF LIQUIDS

Pour a thin stream of the liquid from a clean 100 ml beaker or use the syringe provided to fill a 25 ml S.G. bottle. Maintain stoppered S.G. bottle at 20°C in a beaker of water for 1 hour.

Dry and weigh.

CALCULATION

Weight of S.G. bottle empty	=	x
Weight of S.G. bottle + water @ 20°C	=	y
Weight of S.G. bottle + liquid	=	z

Therefore, S.G. of liquid = $\frac{z - x}{y - x}$

By Hydrometer See Kankakee Method No. 83A

Of Pastes etc. see Kankakee Method No. 83B.

QUALITY CONTROL DEPARTMENT METHOD 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 by placing them, with the cover removed, in a hot air oven set at 100-105°C for at least one hour. Remove and immediately replace the cover and place the covered container in a suitable desiccator. Allow to cool to room temperature and accurately weigh the container and cover. Repeat the drying and weighing sequence until constant weight is attained. This is the tare or empty weight.

Procedure

Reduce the sample to a fine powder. Uniformly distribute 0.5-1.0 gm of the sample material in the moisture container and accurately weigh to determine the gross weight or container plus undried sample weight.

Place the uncovered container together with its matching cover in a vacuum desiccator containing phosphorus pentoxide or concentrated sulphuric acid for a period of 12-24 hours. Maintain the temperature at 20-25°C (Room Temperature). After drying remove the container and weigh immediately. This weight is the weight of the container, cover and dried sample.

Calculation

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

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

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD 208

PYROGEN

PURPOSE

To test a material to demonstrate the presence or absence of a pyrogenic reaction. The test is performed by intravenously injecting the material into rabbits in an amount commensurate with each rabbit's body weight.

1. Test Dose

The test dose for each rabbit shall be at least 3 millilitres per kilogram of body weight of the rabbit and also be at least equivalent proportionately, on a body weight basis, to the maximum single human dose recommended, but need not exceed 10 ml per kilogram of body weight of the rabbit.

2. Procedure

Products shall be tested for freedom from pyrogenic substances by intravenous injection of the test dose into three rabbits in overt good health and by recording for each rabbit a control temperature taken within one hour prior to injection, and three additional temperatures taken one, two, and three hours after injection. For purposes of paragraph (3) of this method, if there is no temperature increase over the control temperature (i.e. where the temperature remains unchanged or decreases), the temperature rise shall be considered as zero. If there is an increase in temperature over the control temperature, the temperature rise shall be the difference between the highest of the three hourly readings and the control temperature reading.

3. Test Results; Retests; Standards to be Met

If no rabbit shows an individual temperature rise of 0.6°C or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 1.1°C, the product meets the requirements for the absence of pyrogens. If one or two rabbits show a temperature rise of 0.6°C or more, or if the sum of the temperature rises exceeds 1.1°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 temperature rises does not exceed 3.0°C; the material meets the requirements for the absence of pyrogenic substances.

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD No. 286

DETERMINATION OF ALUMINIUM OXIDE IN ALUMINIUM CHLORHYDROL MICRO-DRY POWDER

REAGENTS

0.05M EDTA Solution

(Prepared as below or 0.1M EDTA as supplied by B.D.H. and diluted by half.

Dissolve 18.613 g of disodium ethylenediamine tetracetate dihydrate in distilled water, adjust to 1 litre exactly with distilled water. Mix.

Standardise EDTA solution as follows:
Take approximately 200 mg of calcium carbonate previously dried at 105° C for 4 hours. Transfer to a 250 ml conical flask with the aid of 50 ml of distilled water. Add sufficient dilute hydrochloric acid (approx. 2 ml 10% HCl) to dissolve the carbonate. Dilute with distilled water to 150 ml.

Add 15 ml of a 4% solution of sodium hydroxide and 300 mg hydroxy naphthol blue indicator.

Titrate with the 0.05M EDTA solution to a deep blue end point. Calculate the molarity of the solution using the formula w/(100 x v).

Where w = weight in mg of calcium carbonate taken
v = volume of 0.05M EDTA consumed

0.05M Zinc Sulphate Solution

Dissolve 14.377 g of zinc sulphate ($ZnSO_4.7H_2O$) in distilled water, adjust to 1 litre exactly with distilled water, Mix. Standardise zinc sulphate solution as follows:

Transfer 10 ml of 0.05M EDTA solution by pipette to a 250 ml conical flask. Add in the following order 10 ml of ammonia - ammonium acetate buffer, 50 ml of ethanol and 2 ml of Dithizone indicator solution.

Titrate with the 0.05M zinc sulphate solution to a clear rose pink colour.

Calculate the molarity of the zinc sulphate solution using the following formula:

$$\frac{\text{ml 0.05M EDTA x molarity of EDTA}}{\text{ml 0.05M Zinc Sulphate Solution}} = \text{Molarity Zinc Sulphate Solution.}$$

REAGENTS (Cont.) Method 286

Dilute Hydrochloric Acid B.P. Reagent

Ammonia-Ammonium Acetate Buffer Solution

Dissolve 7.71 g ammonium acetate in 20 ml of distilled water, transfer to a 100 ml volumetric flask, add 5.7 ml of glacial acetic acid. Dilute to volume with distilled water and mix well.

Dithizone Indicator Solution

Dissolve 25 mg of Dithizone in 100 ml of ethanol. This solution should not be used for more than 3 to 4 days after preparation.

PROCEDURE

Take approx. 2 g of aluminium chlorhydrol Micro-dry powder, or 2.5 g adsorbent aluminium hydroxide gel F5000 (or 10 g of Anti-diar preparation) accurately weighed into a 150 ml round flat bottomed flask, add 30 ml dilute hydrochloric acid and boil under a reflux condenser until dissolved. A few drops of concentrated hydrochloric acid may be required to effect solution. Cool, wash down the condenser with water and transfer to a 100 ml volumetric flask. Make up to volume with water. In F5000 preparation retain 5 ml of this solution for sulphate determination.

Transfer 40 ml of this solution to a 250 ml beaker. Add 20 ml of 0.05M EDTA by pipette, 15 ml of ammonium acetate buffer. Heat to just below boiling point for 5 minutes. Cool and wash down the sides of the beaker. From this point in the assay a pH meter and magnetic stirrer are used.

Add 60 ml ethanol. Adjust the pH to 4.5 - 4.7 with glacial acetic acid.

Add approximately 1 ml of Dithizone indicator and titrate with 0.05M zinc sulphate solution from a 25 ml burette. The tip of the burette should be immersed below the surface of the liquid in the beaker. Titrate until the first permanent pink-red colour appears. Do not over titrate to the orange-red end point.

Each ml of 0.05M EDTA = 0.002549 g Al_2O_3

Calculate the percentage Al_2O_3 in the weight of powder taken.

CALCULATIONS

Method 286

$$\frac{20 \times M\ EDTA}{0.05} - \frac{Titre \times ZnSO_4}{0.05} = Y \text{ (corrected volume)}$$

$$\frac{Y \times 0.002549 \times 100 \times 100}{\text{Weight taken (g)} \times 4} = \%$$

For F 5000 x FMA 11S:

$$\frac{Y \times 0.002549 \times 250 \times 100}{\text{Weight taken} \times 100} = \%$$

For Anti-diar

$$Y \times 0.002549 \times \frac{100}{10} \times \frac{100}{\text{Weight taken (g)}} \times S.G. = mg/ml$$

For Gastralar

$$Y \times 0.002549 \times \frac{250}{10} \times \frac{1000}{\text{Weight taken (g)}} \times \text{tablet weight (g)} = mg/tablet.$$

AP000830

ARMOUR000038

ARMO0000001_0038

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

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, including platelet counts (Method 308) must be performed daily in the aseptic sterility testing area.

Test each lot of medium for sterility and for its growth-promoting qualities, using two or more strains of microorganisms that are exacting in their nutritive requirements. In order to avoid false negative results, establish the bacteriostatic and fungistatic activity as outlined in Method 309. If the product is baceriostatic 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(see Method 309).

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

Product Sampling:

For products which are sterilised by steam under pressure in the final sealed containers, select 10 or more units from from each steriliser load. These samples must be representative of all layers of the load.

For diagnostic reagents not for human use, licensed by the Division of Biologics Standards, sample and test as specified under Title 42, Part 73, Section 73.730 of the Public Health Service Regulations. These samples should be selected at regular intervals throughout the filling operation. In the process of testing a lyophilised product, it should be reconstituted according to the directions supplied with the product

For all other products, select for the test a total of 20 or more units representative of each batch. In the case of aseptically-filled products, select the units at regular intervals throughout each filling operation.

Testing Techniques

1. Liquids and Suspensions

   a. Sterilise the exterior surfaces of vials and ampules by submerging them for two hours under a suitable bactericidal agent.

   b. Open ampules by breaking off neck with fingers after first wrapping with sterile filter paper.

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 when the final container has not been heated to at least 100°C for as long as 15 minutes.

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

If product contains mercurial preservative, replace the Soybean-Casein Digest Medium with another tube of Fluid Thioglycollate Medium and incubate 14 days at 22 - 25°C.

2. Crystalline and Powdered Solids

If the product is soluble or dispersible, a suitable amount of sterile diluent is added aseptically to the final container and after mixing is withdrawn aseptically and inoculated into the testing mediums as directed under Liquids and Suspensions.

Inoculum Size

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

Container	Min. Vol. of Product	Min. Vol. of Medium	
		a. if preserv.	b. if no pres.
10 ml or less	1 ml or total content if less than 1 ml.	70 ml	40 ml
From 10 to 50 ml.	5 ml.	70-250 ml	70 ml
More than 50 ml.	10 ml	250 ml	70-250 ml.

Incubation Method 303

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 to 32°C and the Soybean-Casein Digest Medium at 22°C to 25°C for not less than 14 days. In cases where the product is sterilised with steam under pressure in the final sealed containers, incubate the Fluid Thioglycollate at 30°C to 32°C for at least 7 days. Examine all tubes daily for the presence of growth.

When the material to be tested renders the medium turbid so that the presence or absence of growth cannot be readily determined 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 (Method 310)

Interpretation of Test Results

If on the first day no growth is found, the material under examination meets the requirements for Sterility. If growth is found, the test may be repeated to rule out laboratory contamination which may be introduced during the test. Retest those containers showing contamination.

If contamination is again evident, twice the original number of samples are tested (Resample). Observe special precautions to insure that the units for the resample are representative of the steriliser 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

U.S. Public Health Service
Regulations, Title 42, Section 73.730

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD 306

TEST FOR NON-SPECIFIC AGGLUTININS

Ref.: Michael Reese Research Foundation

REAGENTS

1. Positive Control: A batch of bovine albumin known to contain agglutinins.

   Negative Control: A batch of albumin known to be free of agglutinins.

2. Red Blood Cells:
   Type A Rh positive
   Type A Rh positive
   Type A Rh positive
   Type A Rh negative
   Type B Rh positive
   Type O Rh positive
   Type O Rh negative

TECHNIQUE

1. Prepare a 2% Red Blood Cell suspension of each of the above cells using a 20% solution of the test albumin as the diluent.
2. Introduce 0.1 ml of each of the above cell suspensions into correspondingly marked 10 x 70 mm test tubes.
3. Add 0.1 ml of the undiluted test albumin to each of the tubes and shake them thoroughly.
4. Repeat the above procedures for each albumin sample to be tested.
5. Incubate all tubes for one hour in a 37^{o}C water bath
6. Centrifuge tubes for 2 minutes at 2000 r.p.m.
7. Very gently resuspend cells and observe for agglutination.
8. Both positive and negative controls are run.

ARMOUR PHARMACEUTICAL COMPANY METHOD 308
QUALITY CONTROL DEPARTMENT

QUANTITATIVE DETERMINATION OF AIRBORNE BACTERIAL AND MOULD CONTAMINATION

MATERIALS

Petri Dishes (Sterile)
Petri Dish Cans
Trypticase Glucose Extract Agar (Sterile)
Potato Dextrose Agar (Sterile)

PROCEDURE

In an aseptic area, prepare two sets of agar plates using respectively Trypticase Glucose Extract Agar (G.T.) and Potato Dextrose Agar (P.T.) which has been melted and then cooled to 50°C.

Allow the media to solidify, place in Petri Dish Cans in an inverted position, and incubate the G.T. plates for 48 hours at 32°C and the P.T. plates for 24 hours at 32°C followed by 24 hours at room temperature (approximately 25°C). At the end of the incubation period, examine each individual plate for signs of contamination. DISCARD ALL PLATES SHOWING GROWTH.

These plates are supplied for use in the aseptic filling areas and a prescribed number of plates are exposed daily in designated locations throughout the aseptic area.

In rooms that are currently in use, the plates, both G.T. (for bacteria) and P.T. (for mould) are exposed for the duration of the operation or a minimum of four hours. The plates in rooms that are not currently in use are exposed for not less than four hours.

All exposed G.T. plates are incubated for 48 hours at 32°C, while all exposed P.T. plates are incubated 48 hours at room temperature (approximately 25°C). At the end of the incubation period, the colonies on each plate are counted and recorded. Bacteria and mould counts are reported to the proper interested parties as quickly as possible.

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD 309

DETERMINATION OF BACTERIOSTATIC AND FUNGISTATIC PROPERTIES OF PRODUCTS TO BE TESTED FOR STERILITY

A TO DETERMINE BACTERIOSTASIS:

1. Prepare 18-24 hour cultures of each of the following organisms:

   E. Coli
   M. Pyrogenes var. aureus
   B. Subtilis
   Cl. Novyi
   Cl. Sporogens

2. To a series of tubes containing 70 ml of Fluid Thioglycollate medium add the specified amount of product being tested.

Container Content	Minimum Volume of Product
10 ml or less	1 ml or total content
10 ml to 50 ml	5 ml
More than 50 ml	10 ml

3. Inoculate tubes of product-medium mixtures, and several tubes containing medium only (positive controls), with 1.0 ml of a 1:100,000 dilution of each of the cultures.

4. Prepare several negative control tubes, i.e. tubes containing product and media only which will not be inoculated with test organisms.

5. Incubate all tubes at 32-35^{o}C for seven days.

6. Compare degree of growth of product-media tubes with the control tubes. Record degree of growth as +, ++, +++, ++++.

7. If bacteriostasis is observed, divide inoculum into 2 x 70 ml tubes of Fluid Thioglycollate medium.

8. If bacteriostasis is still displayed, repeat the test using 4 x 70 ml tubes of Fluid Thioglycollate medium

B. TO DETERMINE FUNGISTASIS

1. Prepare a 24-48 hour culture of candida albicans and a 7 day culture of penicillum notatum.

2. To a series of tubes containing 70 ml Sabouraud Liquid Medium, add the specified amount of product being tested.

3. Inoculate these tubes of product-medium mixtures, and several containing medium only (positive controls), with 1.0 ml of a 1:1000 dilution of the two cultures.

4. Incubate 10 days at 23-25°C.

5. Compare degree of growth in each tube to that obtained in the culture control (no product) tubes.

6. Growth essentially equal to the culture control indicates the correct inoculum.

7. As with the test for bacteriostasis, it may be necessary to repeat the procedure using 2 x 70 ml and 4 x 70 ml volumes of Sabouraud Liquid Media.

Reference: United States Pharmacopoeia.

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD 310

MICROSCOPIC EXAMINATION OF BACTERIA

Ref. : Tanner; Frobisher
Staining Procedures
Conn & Darrow 1946

I. SIMPLE STAIN - FIXED PREPARATION

A. With a sterile needle or loop, mix a small part of a culture in a drop of sterile water and spread over the surface of a clean glass microscope slide making a thin film. Cultures in liquid media are applied directly.

B. Dry the film by slowly waving the slide in the air.

C. Fix the film by passing the slide through a Bunsen flame three times with the film side up.

D. Methylene blue (or gentian violet, crystal violet or methyl violet) is put on the surface and allowed to act for one-half to one and one-half minutes, depending on the stain employed.

E. Wash off excess stain with water.

F. Air dry or blot preparation with absorbent paper.

G. Examine slide under microscope using the oil immersion objective.

II. GRAM STAIN

A. Prepare film, dry, and fix in the same manner as outlined above under Simple Stain.

B. Stain for one to two minutes with Carbol Gentian Violet.

C. Rinse with deionised water quickly until no more stain comes off.

D. Cover with Gram's Iodine Solution for 30 seconds to 1 minute.

E. Rinse in water and remove excess.

F. Decolorise with 95% alcohol-acetone mixture until the colour ceases to run from the preparation.

G. Counterstain with 1% Aqueous Safranin for two to three minutes.

H. Rinse in deionised water, blot or air dry, and examine with the oil immersion objective.

BACTERIA RETAINING THE ORIGINAL STAIN (VIOLET) ARE GRAM-POSITIVE, WHILE THOSE WHICH ARE DECOLORISED BY THE ALCOHOL AND TAKE THE COUNTERSTAIN (RED) ARE GRAM-NEGATIVE.

III PREPARATION OF STAIN SOLUTIONS

A. Carbol Gentian Violet

1. Carbol Gentian Violet made from Gentian Violet Crystals.

Gentian Violet - 1 gram
Carbolic acid crystals - 2 grams
Absolute alcohol - 10 ml
Distilled water to make 100 ml

Rub up the Gentian Violet and the alcohol in a mortar. Add the carbolic acid crystals and mix. Add 2/3 of the water, a small amount at a time, stirring constantly. Pour the mixture into a bottle, and rinse the mortar with the remaining water, add it to the mixture in the bottle. Filter through filter paper after 24 hours.

2. Alternate Method

a. Stock saturated alcoholic solution of Gentian Violet

Gentian Violet - 5 grams
95% Ethyl Alcohol - 100 ml

Grind the Gentian Violet in a mortar, adding the alcohol slowly, until all the dye is in solution. Place in a glass stoppered bottle and filter through filter paper after 24 hours.

b. Carbol Gentian Violet made from stock saturated alcoholic solution.

Add 10 ml stock saturated alcoholic solution of Gentian Violet to 50 ml 1% carbolic acid solution in a 100 ml graduated cylinder. Dilute to 100 ml with 1% carbolic acid solution. Filter with filter paper after 24 hours.

B. Gram's Iodine

1. Gram's Iodine made from Iodine crystals.

Iodine, resublimed - 1 gram
Potassium iodide c.p. - 2 grams
Distilled water - 300 ml.

Rub up the iodine and the potassium iodide in a mortar until they are thoroughly mixed. Add the water a small amount at a time and grind it into the mixture until all the water has been added. Allow to stand in a glass stoppered bottle and filter with filter paper after 24 hours.

2. Gram's Iodine made from stock Lugol's solution.

a. Stock Lugol's Solution

This solution is 15 times the strength of Gram's Iodine solution and may be used as stock from which Gram's Iodine is made.

Iodine, resublimed - 5 grams
Potassium iodide c.p. - 10 grams
Water (distilled) - 100 ml

Rub up the iodine and potassium iodide until they are thoroughly mixed. Add the water, a small amount at a time, and grind it into the mixture until all the water has been added. Allow to stand in a glass stoppered bottle and filter with filter paper after 24 hours.

b. Gram's Iodine

Add 10 ml stock Lugol's solution to about 100 ml distilled water in a graduated cylinder. Dilute to 150 ml with distilled water and mix. Filter with filter paper after 24 hours.

C. One Percent Aqueous Solution of Safranin

Dissolve 1 gram of safranin in 100 ml distilled water by grinding it in a mortar and adding the water a small amount at a time until all the dye has been dissolved. Filter with filter paper after 24 hours.

IV. HANGING DROP - LIVING ORGANISM STUDY

A. Using a toothpick, put a little vaseline around the concavity on a special hanging drop slide.

B. If the culture medium is solid, a small amount of the colony growth is emulsified with a small amount of sterile water. A drop of the emulsion is then placed in the centre of the cover slip with an inoculating loop.

C. Lower the prepared slide over the cover-slip so that the drop is centred in the concavity of the slide.

D. Invert the slide and adjust the cover-slip gently over the depression in the slide so that the drop hangs suspended in the concavity, sealed with vaseline.

E. Examine with the low-power objective to locate the hanging drop.

F. Centre it and then lower the high-power objective gradually, focussing until the organisms come into view.

Bacteria are studied in hanging drop preparations to observe motility and other characteristics of the living organism which may be destroyed upon staining.

V. SPORE STAIN (DORNER'S METHOD)

A. Make a heavy suspension of the organism in 2 to 3 drops of distilled water in a small test tube.

B. Add an equal quantity of freshly filtered Ziehl's carbol fuchsin, C.C.).

C. Allow the mixture to stand in a boiling water bath 10 minutes or longer.

D. On a cover-slip or slide, mix one loopful of the stained preparation with a loopful of a 5-10% aqueous solution of nigrosin(C.C.). (This solution must be filtered before use and may be kept indefinitely if preserved with a few drops of formalin.)

E. Smear as thinly as possible and do not dry too slowly.

NOTE: If even background for exhibiting or photographing are required, especially in the case of slime-producing bacteria, the following procedure is recommended:

A. Make the suspension in a 0.5 ml bacterial nutrient broth or water.

B. Add 1 ml of 10% gelatin solution.

C. Add 1 ml of carbol fuchsin and stain as above.

D. Wash out the colloids with warm tap water with the help of centrifuge or sedimentation.

E. Mix with nigrosin and proceed as above.

Results: Spores - red
Vegative cells - unstained
Background - dark grey

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD SHEET 344
Amended July 1974

DETERMINATION OF PROTEIN BINDING CAPACITY

REAGENTS

1. Bovine Albumin Solution:
Dissolve 2 g Bovine Albumin Fraction V in water and dilute to 100 ml in a volumetric flask.

2. Protein Standard Solution, Armour:
This contains approximately 10 mg protein nitrogen per ml. To obtain the concentration (mg/ml) of protein in this solution, calculate as pure bovine albumin, multiply the stated protein nitrogen concentration by 6.25.

3. Dilute Protein Solution:
Pipette 4 ml Armour Protein Standard solution into a 25 ml volumetric flask and dilute to volume with water. This solution contains approximately 10 mg protein per ml.

4. Biuret Reagent:
Dissolve 3.75 g copper sulphate $CuSO_4.5H_2O$ in 250 ml water. Dissolve 15.0 g potassium sodium tartrate in 250 ml water. Mix the two solutions thoroughly. Dissolve 75 g sodium hydroxide in 750 ml water and cool. Add this to the copper sulphate/tartrate solution, dilute to 2.50 litres with water, store under nitrogen and protect from light. After use the nitrogen atmosphere over the solution should be replenished.

ASSAY PROCEDURE

1. Preparation of a Standard Curve:
Pipette 5 ml aliquots of Dilute Protein Solution into 25, 50 and 100 ml volumetric flasks labelled A, B and C. Add 5 ml water to flask B and 15 ml to flask C. Adjust the volume of all the flasks to fill volume with Biuret Reagent, mix and allow to react at 20° to 25°C for between 30 and 45 minutes. Read the absorbance of each solution at 540 nm against a blank prepared by diluting 5 ml water to 25 ml Biuret Reagent.

   Plot concentration of protein in the final solutions against extinction at 540 nm.

2. Preparations of Suspensions for Test

   (a) Antidiar Preparations:
   Weigh about 10 g accurately into a 50 ml volumetric flask. Pipette 40 ml Bovine Albumin Solution into the flask and adjust to volume with water. Mix thoroughly.

(b) F5000 Gel:
Weigh about 2.5 g accurately into a dry 50 ml volumetric flask. Add 5 ml of water and shake hard until an even dispersion is obtained. Pipette 40 ml Bovine Albumin Solution into the flask and adjust to volume with water. Mix thoroughly.

3. Procedure:
From the time suspensions for test are prepared, allow them to stand for between one and three hours at 20° to 25°C, inverting the flasks at 15 minute intervals. Centrifuge 10 ml of the suspension at about 3,000 rpm for 10 minutes and filter the supernatant through a 0.45 micron membrane filter with glass fibre pre-filter.

Transfer 5 ml of the filtrate to a 50 ml volumetric flask, add 5 ml water and adjust to volume with Biuret Reagent. At the same time prepare a solution containing 5 ml Bovine Albumin Solution and 5 ml water made up to 50 ml with Biuret Reagent. Incubate at 20° to 25°C for 30 - 45 minutes, then record the absorbance of the solutions at 540 nm against a blank prepared by diluting 5 ml water to 25 ml with Biuret Reagent.

CALCULATION

Example:

Concentration protein in Bovine Albumin Solution calculated from standard curve		18.8 mg/ml
Concentration after dilution 4 to 5		15.0 mg/ml
Concentration of protein in filtrate		10.0 mg/ml
Total protein absorbed	= (15-10) x 50 =	250 mg
Weight of Antidiar		9.8 g
Specific Gravity		1.09
Volume Antidiar		9.0 ml

Therefore weight of protein calculated as Bovine Albumin absorbed by Antidiar =

$$\frac{250}{9} = 27.8 \text{ mg/ml}$$

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Equipment Necessary (Cont.) Method 351

3. Physiological Saline Solution - 0.85 M NaCl.

4. Test Solution.

5. Moist Chamber.

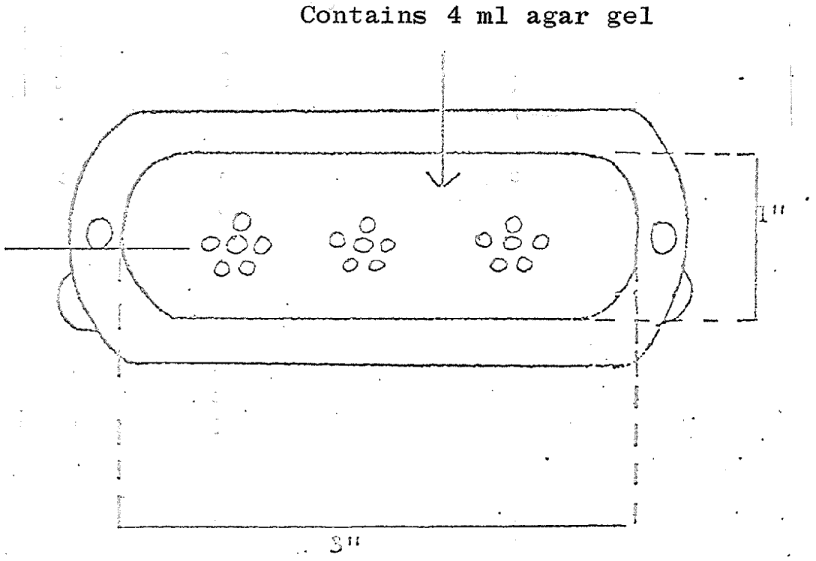
Preparation of Samples for Testing

1. Albumins are ordinarily tested as a 25% solution, and, if necessary, are diluted to this percentage with physiological saline solution. (1.25 g powder to 5 ml of saline solution).

2. Gamma Globulins are ordinarily tested as a 16% solution. (0.8 g powder to 5 ml of saline solution).

Double Diffusion Agar Gel Plates

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



Centre to Centre Distances Between Central and Peripheral Wells - 4 mm.

MAMMALIAN PROTEIN SPECIE IDENTIFICATION (AGAR DIFFUSION)

Purpose of Test

The agar diffusion test is used to demonstrate the presence or absence of human, bovine or porcine proteinaceous material in Normal Serum Albumin, Polio Immune Globulin, Immune Serum Globulin and Fibrinogen Powder. In the agar diffusion method, an antiserum containing specific antibodies is diffused with a material which is suspected of containing the corresponding antigen.

Antiserums to be used

1. Rabbit Anti-Bovine Albumin.
2. Rabbit Anti-Bovine Serum - this antiserum has been absorbed with Normal Human Serum to prevent cross-reactions.
3. Rabbit Anti-Porcine Albumin.
4. Rabbit Anti-Human Albumin.

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).

Controls

1. Positive Bovine Control - 25% Albumin containing approximately 19 ppm bovine albumin.
2. Positive Porcine Control - 30% Albumin containing approximately 100 ppm porcine albumin.
3. Negative Control - 25% Albumin known to be negative.

Equipment Necessary

1. Hyland Immuno-Plate® Immunodiffusion Plates (Ouchterlony), Pattern D.
2. Capillary pipettes.

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. If an antigen or antibody excess exists, the reaction will appear very quickly (half to one and a half 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 reaction later disappears.

Test Procedure

1. Prepare sample dilutions to be tested.
2. Using capillary pipettes, fill wells of agar plates in predetermined manner with samples to be tested and also controls.
3. Antigen is usually placed in outer wells; antiserum in centre well. This conserves antiserum.
4. Place filled plates in moist chamber (i.e., plastic box with wet piece of sponge or filter paper) and incubate at room temperature.
5. Examine plates every 30 minutes for precipitin reactions for 6 hours. (Indirect lighting may be used for this examination.)
6. Record positive results as they appear.
7. After the first 6 hours, examine plate occasionally. If no reaction has appeared at the end of 24 hours, record results as negative.
8. For tests 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 other than Rabbit Anti-Human Albumin.

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 temperature (2 to 8°C) cause a slowing down of the reaction. In fact, in some cases, the expected reaction of a known positive is not obtained.

Reference

Letter to R.C. Johnson dated December 20 1968, from E.H. Mealey, Director of Quality Assurance, Hyland Laboratories, Los Angeles, California.

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD 365

THROMBOPLASTIN GENERATION TEST - AHF POTENCY DETERMINATION

I Equipment

A. 37°C water bath

B. Test tubes - disposable 12 x 75 mm.

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

C. Stopwatch - 1 hour calibrated in seconds

D. Ice bath

E. 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 FibroTube cups and FibroTip plastic tips

II Reagents

A. Imidazole - NaCl buffer (IBS)

Imidazole 3.4 gm (0.05 moles)
NaCl 3.85 gm (0.066 moles)
0.1 N HCl 183.0 ml (0.0183 moles)
qs 1 litre with distilled water

The pH should be 7.3 ± 0.2
The ionic strength is 0.08
The osmolarity is 0.2006

Sterile filter the solution and store in the refrigerator (6°C). Discard if cloudy.

II Reagents (Cont.) Method 365

B. $CaCl_2$: 0.25 M

$CaCl_2.2H_2O$ 1.838 gm
qs 500 ml with distilled water

Store in the refrigerator (6^oC)

C. Inositihin: 25 mg % in 0.15 M NaCl

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

Suspend 25 mg of inositihin in 100 ml normal saline, allow to stand overnight at room temperature, shake to produce as fine an emulsion as possible. Dispense into 2 ml aliquots, and store in a freezer at -20^oC.

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

D. Stock Bovine Serum

ACD Bovine Plasma is recalcified by the addition of 1 M $CaCl_2$ solution to a final concentration of 1/40 M. It is allowed to stand overnight at room temperature and then centrifuged (2,000 g's) for 10 minutes to remove the clot. It is then frozen in aliquots of 2.5 ml at -20^oC.

E. Stock Human Serum

ACD Bank Plasma is recalcified by the addition of 1 M $CaCl_2$ solution to a final concentration of 1/40 M. It is allowed to stand overnight at room temperature and then centrifuged (2,000 g's) for 10 minutes to remove the clot. It is then frozen in aliquots of 2.0 ml at -20^oC.

F. Substrate Plasma

Fresh ACD Human Plasma (pool of at least 3 units) is rendered platelet poor by centrifugation in a refrigerated International Centrifuge for 20 minutes at 4,000 g's at 4^oC. It is frozen (within 3 hours of collection) in aliquots of 5.0 ml at -20^oC.

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-NaCl 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 batch. 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-NaCl Buffer and placed in the ice bath.

D. Inositihin

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 $CaCl_2$ solution into a sufficient number of FibroTube cups.

B. Pre-warm 5 ml of 0.025M $CaCl_2$ solution to 37°C for use in the reaction mixture.

IV. Pretest Procedure (Cont.)

C. Reconstitute all samples and standards. (Do not put in ice bath). Any sample or standard which is a lyophilised plasma instead of a concentrate must be absorbed with a $BaCl_2$ solution. 0.1 ml 15% $BaCl_2$ 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 min., remove the supernate for testing.

D. Make up the following dilutions (with Imidazole-NaCl Buffer, IBS) of each sample and standard for each test run just prior to the test run.

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)

E. Place 0.6 ml of substrate plasma on thermal block to preheat.

F. Place a set (5) of FibroTubes, containing the 0.25M $CaCl_2$ 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 inosithin.

*These dilutions are not tested

V. Test Procedure (Cont.)

A. Reaction Mixture (Cont.)

Then add 0.1 ml of the prewarmed 0.025M $CaCl_2$ solution to the first tube in the series. Immediately following the $CaCl_2$ solution, 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 $CaCl_2$ 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 dilutions in the series).

B. Clotting mixture

Place one of the prefilled FibroTubes beneath the probe of the fibrometer.

When the watch in the procedure above registers 5 min., using the automatic pipette in the "off" position, pipette 0.1 ml of the first reaction mixture into the FibroTube. Using a new pipette tip, withdraw 0.1 ml of the pre-warmed substrate plasma, switch the pipette to the "on" position and pipette the substrate plasma into the FibroTube. This will automatically activate the Fibrometer timer, which will automatically stop when the probe detects the formation of the clot.

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

VI. Results

A. Results are expressed in terms of relative potency of the house standard. The results of standard curve are plotted on millimetre rectangular graph paper; the clotting time is plotted vs the percent concentration of the standard (1/100 dilution is 100%, 1/200 is 50%, 1/400 dilution is 25% etc.) The relative activity of the sample tested is compared to the standard curve.

N.B. All testing should be in duplicate or triplicate and these results averaged. Each result should agree within 10% of the average.

VI. Results (Cont.)

B. Preparation of the standing curve

1. Calculate the average clotting time for each dilution.
2. Plot the average clotting time (in seconds) against the percent concentration of the standard.
3. Construct a curve that best fits the five points.

C. Calculation of percent AHF activity in the sample

1. By interpolation, determine the percent AHF for each test 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.

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD No. 963

SAFETY TEST FOR NORMAL ALBUMIN SERUM

Guinea Pig: Inject, intraperitoneally, 5 ml of the product into each of two guinea pigs. Animals must be unused healthy guinea pigs. (250-300 g)

Mice: Inject, intraperitoneally, 0.5 ml of the product into each of two mice. Animals must be unused healthy mice (18-20 g)

Interpretation of Results :

If all animals appear healthy and do not show a weight loss during the test period, the material is acceptable.

A questionable test should be repeated using twice the original number of animals.

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD No. 993

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

I Reagents

1. Standard Protein Solution (3ml/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 Reagent
   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
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

II Assay Procedure (Cont.) Method 993

3. To all test tubes add equal amounts of 6 Normal Sodium Hydroxide.
4. To a, b, c & d, add the required amounts of 3 Normal Sodium Hydoxide 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.

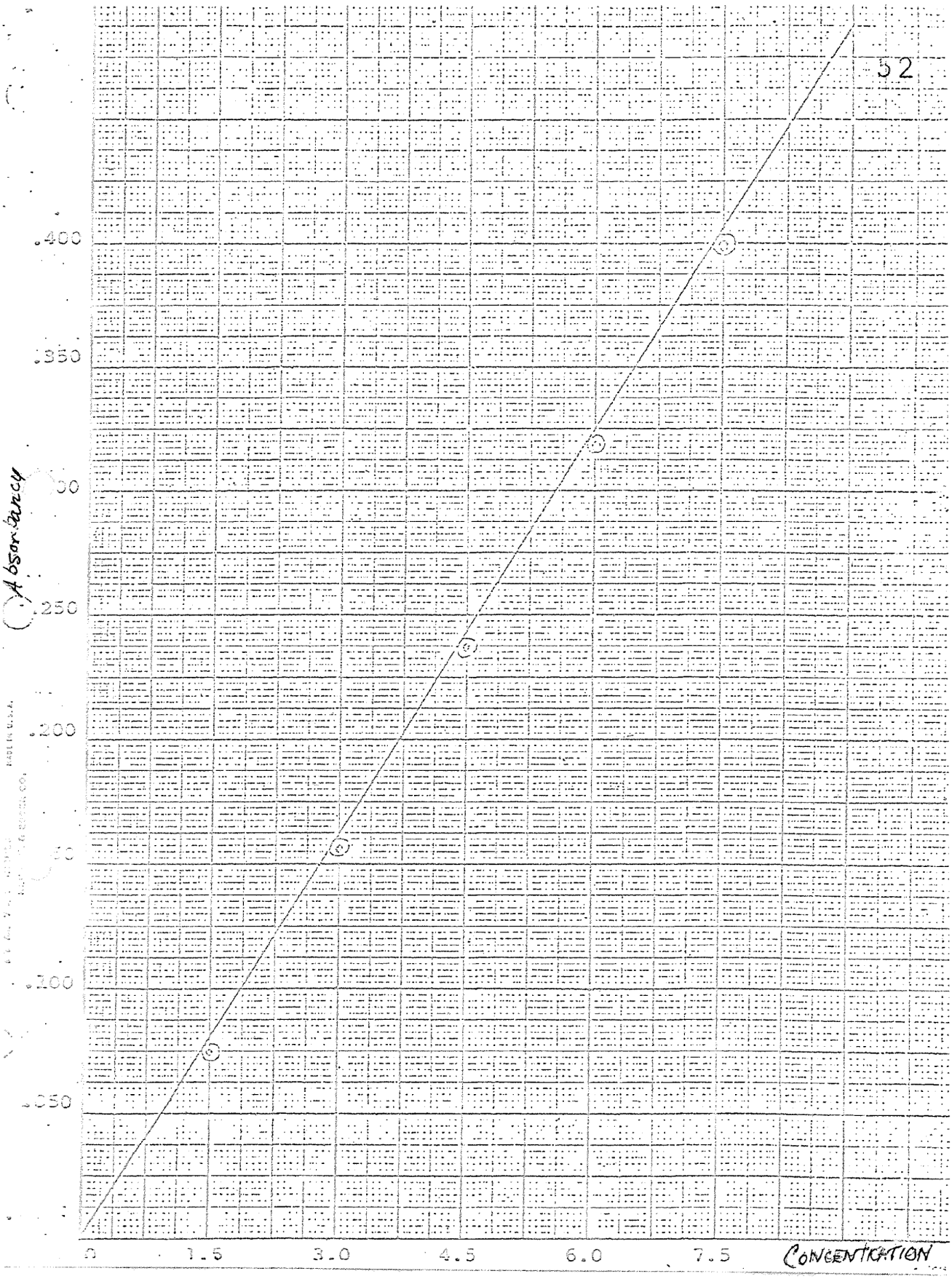
III Total Protein Control of Test Sample

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 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 25 ml.



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ARMO0000001_0067

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

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. 1/10x2.

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.

Sample Preparation (Cont.) Method 994

4. Heat in a 37°C water bath for 15 minutes.

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 $(\frac{mg}{ml})$.

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD No. 995

ATOMIC ABSORPTION ANALYSIS OF ALUMINUM IN ANTIHAEMOPHILIC FACTOR

Reagents

Aluminum Standard Solution - 1000 ppm from Harleco, Item No. 7689 or equivalent.

Sodium Chloride - Analytical Reagent Grade

Equipment

Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer equipped with an aluminium lamp and a nitrous oxide burner head.

Procedure

Preparation of Standard Aluminum Solutions - Prepare a solution of aluminum and sodium chloride so that the final concentration is 10 γ/ml aluminum and 1000 γ/ml sodium chloride.

Preparation of Blank Solution - Prepare a solution of sodium chloride so that the final solution is 1000 γ/ml.

Preparation of Sample Solution - Prepare a solution using sample and sodium chloride so that the final concentration is 1.0% weight/volume sample and 1000 γ/ml sodium chloride.

Instrumental Conditions

Wavelength: 309.3 nanometers
Range: UV
Slit: 4
Source: As given on lamp
Scale: 10
Fuel: Acetylene Flow - 5.5 (metal ball)
Oxidizer: Nitrous Oxide Flow 6.0

Analysis

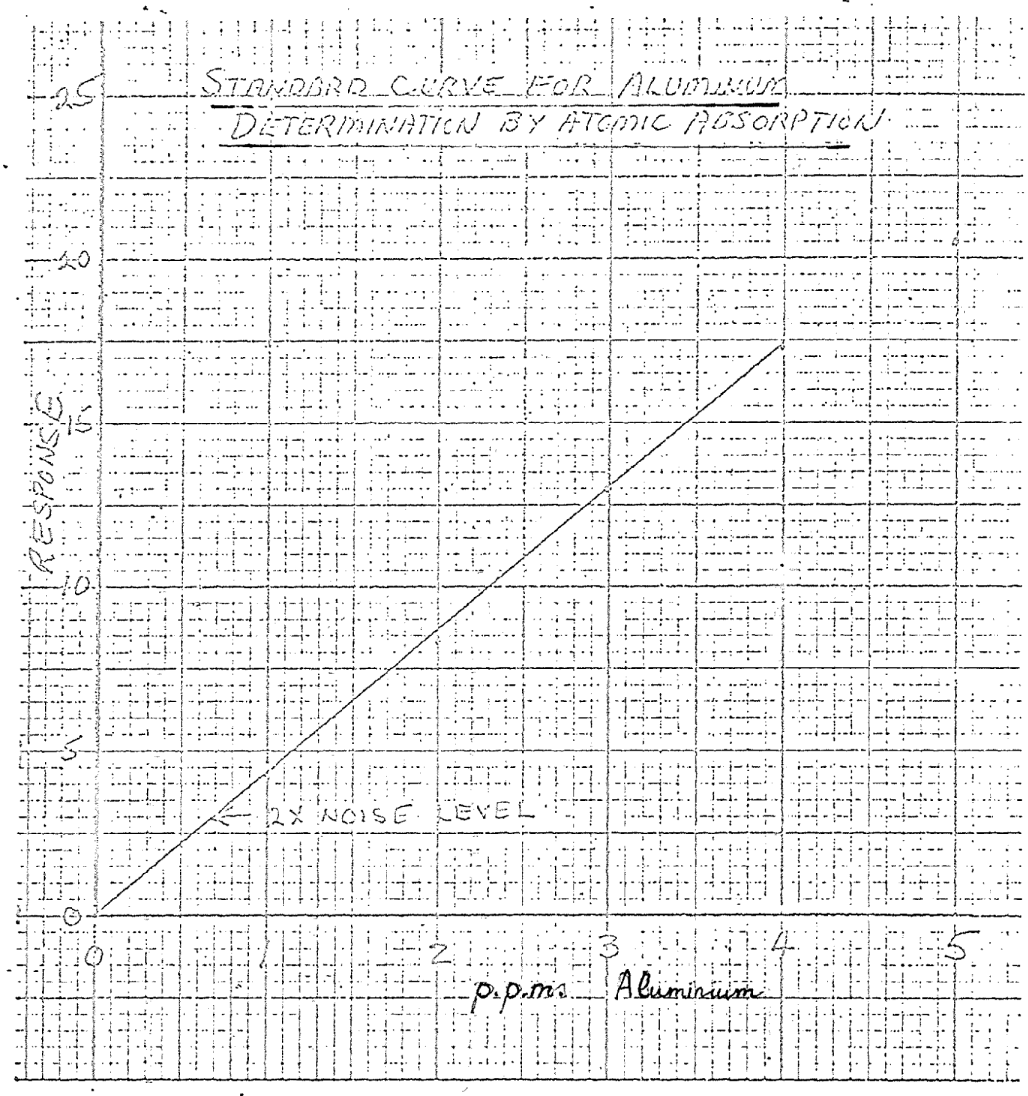
Record absorption values for sample and standard solutions versus blank values.

Calculations

Since the absorption values obtained in this analysis are below 10% there is no need to convert them to absorbance for calculation purposes.

ppm Al = (Sample Absorption x 1000)/Standard Absorption

NOTE: To avoid the possibility of an explosion when lighting flame following procedure found in the September 1968 edition of Perkin-Elmer's Analytical Methods for Atomic Absorption Spectrophotometer - page 11.


STANDARD CURVE FOR ALUMINUM
DETERMINATION BY ATOMIC ABSORPTION
RESPONSE
25
20
15
10
5
0
2X NOISE LEVEL
0
1
2
3
4
5
p.p.m. Aluminum

ARMOUR PHARMACEUTICAL COMPANY
ANALYTICAL CONTROL DEPARTMENT

METHOD No. 1073

MANUFACTURING PROCEDURE FOR THE 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.

I Reagents and Equipment

1. Heparin (Abbott 1000 units/ml.)

2. Activated Partial Thromboplastin

3. Cryoprecipitated AHF (Lyophilised)
   (a) Heparin Containing
   (b) Non-Heparin Containing

4. Normal Control Plasma

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

6. 0.025 molar calcium chloride.

7. A Thermal Incubation Block (37^{o}C)

II Procedure:

A. Reconstitution of Reagents

   1. Reconstitute the normal coagulation control plasma (lyophilised) with 1 ml of 3(a) or (b).

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

   3. Reconstitute the AHF sample (lyophilised) with 20 ml of distilled water.

      (a) Heparin Containing

      (b) Non-Heparin Containing

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

   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.

II Procedure (Cont.) Method 1073

C. Determination of 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 5 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
QUALITY CONTROL DEPARTMENT

METHOD No. 1079

SOLUTION TIME FOR ANTIHAEMOPHILIC FACTOR (HUMAN) LYOPHILISED

Obtain a finished vial of Antihaemophilic Factor (Human) Lyophilised. Remove the stopper and accurately pipette 25 ml of distilled water into the vial and start a timer. Replace the stopper and swirl slowly until solution is complete. The elapsed time, from the addition of the water until the solution is complete, is recorded as the solution time. The solution time must be less than 30 minutes to be acceptable.

SECTION 4 IMPURITIES

4.1. Impurities likely to be Present from the Method of Manufacture.

Aluminium and non-active protein.

4.2. Methods used for the Detection and Evaluation of Impurities in Batches of Final Product Examined.

(a) As described in Analytical Methods 995, 993 and 994 for aluminium, total protein and clottable protein. These methods are provided in Section 3.4.

(b) The levels of aluminium and non-active protein remaining in batches of final product examined are provided in Section 7.

4.3. Identification of Impurities

As given in 4.2 above.

SECTION 5 DEVELOPMENT CHEMISTRY

The cryoprecipitate was previously known as an anti-haemophilic factor and it was considered important to standardise potency and present a product of reasonable stability by employing a simple fractionation. The techniques employed were common to blood fractionation manufacture and the buffering reagents were acceptable to the F.D.A. in our submission of 11th February 1972.

SECTION 6 SPECIFICATION

6.1. Specification Tests Applied

Assays on the crude cryoprecipitate are as follows:

Test	Specification	Reference
Total Protein	Not more than 5 mg per AHF unit	Method 993
Heparin	Not more than 2 units per ml of reconstituted product.	Method 1073
Solution Time	Readily soluble within 30 minutes in full volume of diluent.	Method 1079

The above methods are provided in Section 3.4.

SECTION 7 BATCH ANALYSIS

Batch analyses for batches used in initial evaluation, pre-clinical trial studies and stability testing are provided in the following data for lot numbers M46307, J20201, J21604, J24008 and L30706. The specification limits to which these batches conform are provided in Section 11.

ARMOUR PHARMACEUTICAL COMPANY
KANKAKEE, ILLINOIS

PROTOCOL OF PRODUCTION AND ASSAY

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ANTIHEMOPHILIC FACTOR (HUMAN)

Lot No. M46307 Date August 16, 1974

1. Starting Material Plasma for Fractionation, Normal (Human) for Antihemophilic Factor (Human) S-3029 Lot No. RR1-103245X RR1-102607X RR1-102031X

2. Manufacturing Process Used As Described in License Application

3. Number of Final Containers Filled 889

4. Chemical Tests

Total Protein	38.7	mg./ml. After Reconstitution
Clottable Protein	26.8	mg./ml. After Reconstitution
Heparin	1.6	units/ml. After Reconstitution
Moisture	0	%
Solution Time	Satisfactory	
pH	7.5	
Residual Aluminum	<0.001	mg./25 ml. reconstituted volume in final container determined by analysis. Aluminum is used in the manufacturing process in the form of aluminum hydroxide.

5. Sterility Test (Final Container)

Result Sterile Date 8/5/74

AP000872

Lot No. M46307 ANTIHEMOPHILIC FACTOR (HUMAN)

6. Pyrogen Tests - Temperature in °C

Dosage: 10 u/kg Date Injected: 7/23/74 Date Injected: 7/24/74

Rabbit	INITIAL Initial Temp.	1st Hour	2nd Hour	3rd Hour	Max. Rise	RECHECK Initial Temp.	1st Hour	2nd Hour	3rd Hour	Max. Rise
1	39.5	39.7	39.4	39.4	0.2	39.6	39.6	39.6	39.6	0.0
2	39.2	39.9	39.3	39.3	0.7	39.6	39.9	40.7	41.0	1.4
3	39.7	39.9	39.8	39.7	0.2	39.8	39.8	39.7	39.7	0.0
4						39.7	39.6	39.6	39.7	0.0
5						39.3	39.4	39.3	39.3	0.1

7. Safety Test

	Quantity on Test	Results	Date
Guinea Pigs	2	Satisfactory	7/30/74
Mice	2	Satisfactory	7/30/74

8. Identity Tests

Human Serum Protein Positive

Bovine Serum Protein Negative

Porcine Serum Protein Negative

9. Potency Test

AHF units/ml. After Reconstitution 10.6 Expiration Date: Aug. 12, 197

10. Isoagglutinins

Anti-A Titer: A+ 1:64 Anti-D Titer: O+ NIL

Anti-B Titer: B+ 1:32 O+ (Coombs) NIL

O- NIL

O- (Coombs) NIL

GRO-C

GRO-C

AP000873

ARMOUR000081

ARMO0000001_0081

SUBMITTED FOR TESTING PRIOR TO PRODUCT LICENSE APPROVAL

ARMOUR PHARMACEUTICAL COMPANY
Kankakee, Illinois

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PROTOCOL OF PRODUCTION AND ASSAY

ANTIHEMOPHILIC FACTOR (HUMAN)

LOT NO. J20201 DATE April 25, 1972

1. Starting Material Plasma for Fractionation, Normal, (Human), for Anti-hemophilic Factor, (Human), S-3029, Lot No. K576201

2. Manufacturing Process used As described in license application

3. Number of Final Containers Filled 319

4. Chemical Tests

Total Protein	31.85	mg./ml. after reconstitution
Clottable Protein	24.05	mg./ml. after reconstitution
Aluminum	<1	ppm after reconstitution
Heparin	1.19	units/ml. after reconstitution
Moisture	0.04	%
Solution Time	18	Minutes

5. Sterility Test (Final Container)

Result Sterile Date February 2, 1972

AP000874

ANTIHEMOPHILIC FACTOR (HUMAN)

LOT NO. J20201

6. Pyrogen Tests - Temperature in °C

Dosage: 10 u/kg. Date Injected: 2/11/72

	INITIAL					RETEST				
Rabbit	Initial Temp.	1st Hour	2nd Hour	3rd Hour	Max. Rise	Initial Temp.	1st Hour	2nd Hour	3rd Hour	Max. Rise
1	39.2	39.7	39.6	39.3	0.5					
2	39.0	39.0	38.9	38.9	0.0					
3	38.9	38.7	38.5	38.7	0.0					

7. Safety Test

	Quantity on Test	Results	Date
Guinea Pigs	2	Satisfactory	1/25/72
Mice	2	Satisfactory	1/25/72

8. Identity Tests

Human Serum Protein — Positive

Bovine Serum Protein — Negative

Porcine Serum Protein — Negative

9. Potency Test

AHF units/ml. after reconstitution 11.73 Date: 2/3/72

10. Isoagglutinins To be supplied at a later date

GRO-C

IN CHARGE OF TESTING

GRO-C

IN CHARGE OF PROCESSING

AP000875

ARMOUR PHARMACEUTICAL COMPANY
Kankakee, Illinois

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PROTOCOL OF PRODUCTION AND ASSAY

ANTIHEMOPHILIC FACTOR (HUMAN)

LOT NO. J21604 DATE July 25, 1972

1. Starting Material Plasma for Fractionation, Normal (Human) for Antihemophilic Factor (Human) S-3029, Lot No. 51642, 51917, and 52195

2. Manufacturing Process used As described in license application

3. Number of Final Containers Filled 240

4. Chemical Tests

Total Protein	36.0	mg./ml. after reconstitution
Clottable Protein	22.5	mg./ml. after reconstitution
Aluminum	< 1	ppm after reconstitution
Heparin	0.20	units/ml. after reconstitution
Moisture	Nil	%
Solution Time	Satisfactory	

5. Sterility Test (Final Container)

Result Sterile Date 5/15/72

AP000876

ANTIHEMOPHILIC FACTOR (HUMAN)

LOT NO. J21604

6. Pyrogen Tests - Temperature in °C

Rabbit	Dose: 10 u/kg. Date Injected: 5/3/72 INITIAL Initial Temp.	1st Hour	2nd Hour	3rd Hour	Max. Rise	Date Injected: REPEST Initial Temp.	1st Hour	2nd Hour	3rd Hour	Max. Rise
1	39.1	39.3	39.3	39.2	0.2					
2	39.2	39.3	39.3	39.3	0.1					
3	39.2	39.2	39.0	39.0	0.0					

7. Safety Test

	Quantity on Test	Results	Date
Guinea Pigs	2	Satisfactory	5/10/72
Mice	2	Satisfactory	5/10/72

8. Identity Tests

Human Serum Protein — Positive

Bovine Serum Protein — Negative

Porcine Serum Protein — Negative

9. Potency Test

AHF units/ml. after reconstitution 11.14 Date: 5/4/72

10. Isoagglutinins Satisfactory

GRO-C

IN CHARGE OF TESTING

GRO-C

IN CHARGE OF PROCESSING

AP000877

ARMOUR PHARMACEUTICAL COMPANY.
Kankakee, Illinois

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PROTOCOL OF PRODUCTION AND ASSAY

ANTIHEMOPHILIC FACTOR (HUMAN)

LOT NO. J24008 DATE 9/25/72

1. Starting Material Plasma for Fractionation, Normal (Human) for Antihemophilic Factor (Human) S-3029, Lot No's. 50992 & 74654

2. Manufacturing Process used As described in license application

3. Number of Final Containers Filled 292

4. Chemical Tests

Total Protein	33.50	mg./ml. after reconstitution
Clottable Protein	19.75	mg./ml. after reconstitution
Aluminum	<1	ppm after reconstitution
Heparin	0.82	units/ml. after reconstitution
Moisture	Nil	%
Solution Time	Satisfactory	

5. Sterility Test (Final Container)

Result Sterile Date September 6, 1972

AP000878

3. Pyrogen Tests - Temperature in °C

Rabbit	Dose: 10 u/kg. Date Injected: 9/7/72 INITIAL Initial Temp.	1st Hour	2nd Hour	3rd Hour	Max. Rise	Date Injected: RETEST Initial Temp.	1st Hour	2nd Hour	3rd Hour	Max. Rise
1	39.4	39.6	39.6	39.4	0.2					
2	39.2	39.0	39.3	39.2	0.1					
3	39.3	39.4	39.3	39.2	0.1					

4. Safety Test

	Quantity on Test	Results	Date
Guinea Pigs	2	Satisfactory	9/14/72
Mice	2	Satisfactory	9/14/72

5. Identity Tests

Human Serum Protein — Positive

Bovine Serum Protein — Negative

Porcine Serum Protein — Negative

6. Potency Test

AHF units/ml. after reconstitution 8.06 Date: 8/29/72

7. Isoagglutinins Satisfactory

GRO-C

By CHARGE OF TESTING

GRO-C

IN CHARGE OF PROCESSING

AP000879

ARMOUR PHARMACEUTICAL COMPANY
Kankakee, Illinois

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PROTOCOL OF PRODUCTION AND ASSAY

ANTIHEMOPHILIC FACTOR (HUMAN)

LOT NO. L30706 DATE July 9, 1973

1. Starting Material Plasma for Fractionation, Normal (Human) for Antihemophilic Factor (Human) S-3029, Lot No. 1-79129X

2. Manufacturing Process used As described in license application

3. Number of Final Containers Filled 607

4. Chemical Tests

Total Protein	39.81	mg./ml. after reconstitution
Clottable Protein	25.00	mg./ml. after reconstitution
Aluminum	<1	ppm after reconstitution
Heparin	1.9	units/ml. after reconstitution
Moisture	Nil	%
Solution Time	Satisfactory	

5. Sterility Test (Final Container)

Result Sterile. Date 7/6/73

AP000880

ANTIHEMOPHILIC FACTOR (HUMAN)

Lot No. I30706

6. Pyrogen Tests - Temperature in °C

Dosage: 10 u/kg.	Date Injected: 6/27/73					Date Injected:				
	INITIAL					RETEST				
Rabbit	Initial Temp.	1st Hour	2nd Hour	3rd Hour	Max. Rise	Initial Temp.	1st Hour	2nd Hour	3rd Hour	Max. Rise
1	39.2	39.2	39.4	39.3	0.2					
2	39.1	39.2	38.9	38.8	0.1					
3	39.3	39.0	39.1	38.9	0.0					

7. Safety Test

	Quantity on Test	Results	Date
Guinea Pigs	2	Satisfactory	6/29/73
Mice	2	Satisfactory	6/29/73

8. Identity Tests

Human Serum Protein — Positive

Bovine Serum Protein — Negative

Porcine Serum Protein — Negative

9. Potency Test

AHF units/ml. after reconstitution 12.2 Expiration Date: 6/27/74

10. Isoagglutinins Satisfactory

GRO-C

IN CHARGE OF TESTING

GRO-C

IN CHARGE OF PROCESSING

AP000881

SECTION 8 STABILITY REPORTS

Stability data is not available for the crude cryoprecipitate since the product is used immediately by being processed with activated aluminium hydroxide adsorbate to effect a purification.

Calibration Curve (Cont.)

The DBS-AHF reference plasma is absorbed with 0.1 ml of 15% $BaCl_2$ solution per ml of reconstituted volume and allowed to stand for 5 minutes. The material is centrifuged for 10 minutes, the supernate removed and tested using the following procedure:

(A) Test Dilutions:

1. 1/100 (0.1 ml of the reconstituted AHF standard in 9.9 ml of imidazole-saline buffer)
2. 1/200 (1.0 ml of buffer + 1.0 ml of 1/100 dilution)
3. 1/400 (1.0 ml of buffer + 1.0 ml of 1/200 dilution)
4. 1/800 (1.0 ml of buffer + 1.0 ml of 1/400 dilution)

3. Reaction Mixture

Add to each tube, in a series of 5 (12 x 75 mm) glass tubes in a 37°C water bath or thermal incubation block 0.1 ml of prewarmed phospholipid, 0.1 ml of prewarmed diluted bovine serum and 0.1 ml of diluted human serum. Mix and allow to equilibrate at 37°C for five minutes. After this mixture has been incubated, add 0.1 ml of the prewarmed 0.025M $CaCl_2$ solution to the first tube in the 5 tube assay series. Immediately following the addition of the calcium chloride, add 0.1 ml of the AHF test dilutions, mix, and start timing the reaction. At minute intervals, initiate the above addition to the remaining 4 tubes.

4. Clotting Mixture

Place a rack of fibrometer reaction cups which have been prefilled with 0.025M $CaCl_2$ solution on the thermal block for prewarming. Next, place one of the prefilled reaction cups beneath the probe of the fibrometer. When 5 minutes of the reaction time have elapsed, transfer 0.1 ml of the contents of the first reaction mixture to the reaction cup beneath the fibrometer probe containing $CaCl_2$. Next, using a clean pipette, transfer 0.1 ml of control plasma to the test cup and start timing the clotting time in seconds. The reaction is automatically terminated by fibrin formation in the test cup. This procedure, listed above, is followed for each dilution under test whether a standard or test solution.

5. Results

Results are expressed in terms of relative potency of the standard. The clotting time of the AHF standard are plotted on log-log graph paper; the clotting time is plotted vs AHF standard dilution. The relative activity of the sample tested is compared to the calibration curve.

N.B. All of the testing should be in triplicate. The triplicate results are averaged. These results should be in good agreement. When plotted, the test material should be parallel to the standard.

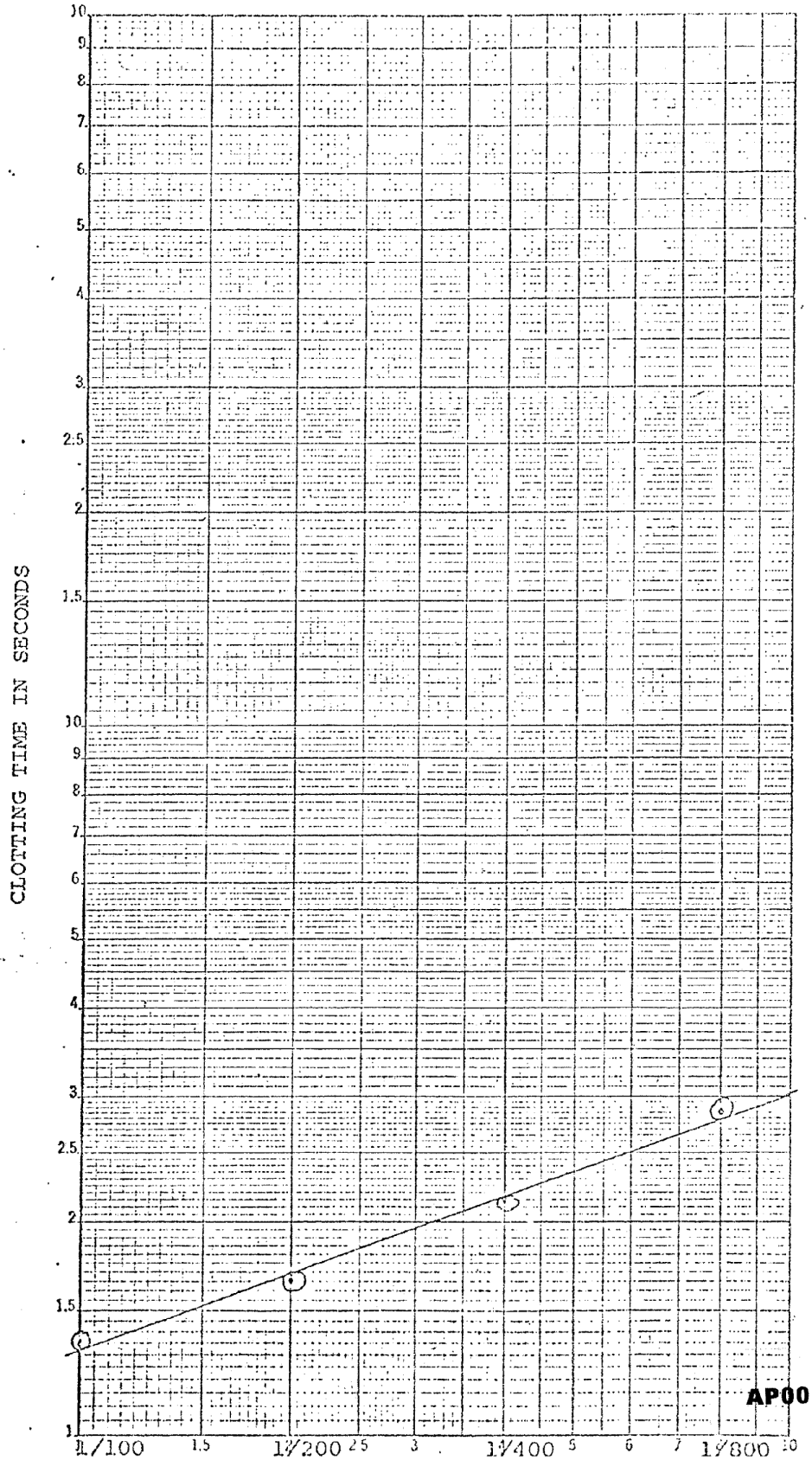
6. Calculations

(1) Preparation of the Calibration Curve

(a) Calculate the average clotting time of each dilution of the standard.
(b) Calculate the average clotting time of each dilution of the test material
(c) Plot on log-log graph paper the clotting times of the standard vs dilution of the standard. The plot should be linear
(d) Plot on log-log graph paper the clotting times of the test material vs dilution of the test material. The plot should be linear and parallel to the standard.

(2) Calculation of Units of AHF Activity in the Material Under Test.

(a) By interpolation, determine the clotting time and dilution concentration from the graph.
(b) By selection of a second difference determine the clotting time and dilution concentration from the graph.
(c) Divide (a) by (b) and multiply by 1.3 (AHF units/ml of AHF standard) times dilution of unknown. This is equal to the total units of AHF activity per ml of solution.
(d) Multiply AHF units per ml by the fill volume to give total units per vial.



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 Antigen (Hepatitis B Surface Antigen, HB_sAg) levels in serum. 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 #6119)

1. Negative Human Control (Recalcified human plasma non-reactive for Hepatitis B Antigen and Hepatitis Associated Antibody) Preservative: 0.1% sodium azide. Handle as though capable of transmitting hepatitis.
2. Positive Human Control (Human plasma reactive for Hepatitis B Antigen). 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 Hepatitis B Antigen 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. Hepatitis Associated Antibody (Anti-Australia Antigen) 125 I (Human). 0 005 M TRIS (hydroxymethyl) Aminomethane Buffer containing 50% Calf Serum, 2% Normal Human Serum

3. (Con't) and 0 5% Bovine Serum Albumin is used as the diluent to adjust potency. Preservative: 0.1% sodium azide. Handle as though capable of transmitting hepatitus.

4. Hepatitis Associated Antibody (Guinea Pig) - Coated beads (polystyrene beads coated with guinea pig Hepatitis Associated Antibody). 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 Diagnostics 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 decontaminated before discarding. Disposable materials may be incinerated. Liquid wastes may be mixed with sodium hypochlorite in volumes such that the final mixture

6. (Con't) 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 Hepatitis B Antigen is strong, it is recognized that presently available methods for Hepatitis B Antigen 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 nonreactive 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 Hepatitis Associated Antibody coated

2. (Con't) beads and 125I labeled human Hepatitis Associated Antibody). 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 ^{TM} II or Pentawash^{TM}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 $45^{\circ} \pm 1^{\circ}C$.

6. Hepatitis Associated Antibody (Anti-Australia Antigen) (Human) AUSRIA Confirmatory Neutralization Test Kit. Abbott Number 8310.

   This radioimmunoassay 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 - (Incubation: Overnight at Room Temperature; 1 hour at $45^{\circ}C$)

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.

(Procedure Con't)

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 speciment.

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-Hepatitis Associated Antibody (Human) to the bottom of each reaction well. Make sure that the antibody coated bead

(Procedure Con't)
is completely surrounded by the labeled 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 labeled 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 Hepatitis B Antigen 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 Hepatitis B Antigen.

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.

RESULTS: (Continued)

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 of 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 579 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.

RESULTS: (Continued)

C. Unknowns whose net count rate is higher than the cutoff value should be considered positive with respect to Hepatitis B Antigen.

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 Hepatitis B Antigen.

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 a non-repeatable positive. If repeats are above the cutoff value, the sample should be presumed reactive for HB_sAg. Such results are contingent on determination of the specificity of the repeatable positives.

Specificity analysis must be performed prior to informing a donor that he is a Hepatitis B Antigen carrier.

Hepatitis Associated Antibody (Anti-Australia Antigen) (Human), No. 8310, provides a method for confirmation of screening procedure reactive specimens. This radioimmunassay must be performed on all reactive specimens unless they can be confirmed as positive by other licensed HB_sAg test systems.

A repeatable Ausria II reactive specimen, confirmed by neutralization with human antiserum or other licensed HB_sAg tests must be considered Hepatitis B Antigen positive.

Reference: Abbott Laboratories brochure to Hepatitis - Associated Antibody - Ausria II-125 kit, Rev. Dec. 1974

11.4 FINISHED PRODUCT SPECIFICATIONS

The specifications are as listed below:-

Description: A white to very pale yellow lyophilised cake in a vial with vacuum having a medium blue seal.

TEST	SPECIFICATION	METHOD
Potency	A minimum of 6.25 i.u./reconstituted* ml (equivalent to a minimum of 125 i.u./vial)	B.P.
Heparin Content	A maximum of 3.125 units/reconstituted ml (equivalent to a maximum of 62.5 units/vial)	1073
Total Protein	NMT 3.0% when reconstituted*	993
Fibrinogen	NMT 2.5% when reconstituted*	994
Aluminium	Maximum of 5 ppm	995
Moisture	Maximum of 0.5% of its weight	43-D
Identity	Human - Positive Bovine - Negative Porcine - Negative	351
Safety	Passes	963
Sterility	Passes	303
Pyrogens (10 i.u./kg)	Passes D.B.S. and B.P. test	208
Solution Time	Maximum of 30 minutes	1079
Isoagglutinin Titre	Complies	378
pH	When reconstituted the pH of the resulting solution is 6.8 to 7.5	B.P.
Sodium ions	Not more than 200 mM/l*	B.P.
Citrate ions	Not more than 55 mM/l*	B.P.
Hepatitis B_S Antigen	Negative	379

*Reconstituted with 20 ml Water for Injections B.P.

SECTION 11 QUALITY CONTROL (Cont.)

11.5 Analytical Control Procedures Applied to the Finished Product

The following methods have been provided in Section 3 (page 23):-

	Analytical Method
The determination of moisture (loss on drying)	43-D
Pyrogens test	208
Sterility test	303
Mammalian protein specie identification ..	306
Safety test for normal serum albumin	963
The determination of total protein	993
The determination of fibrinogen (determination of clottable protein)	994
The determination of aluminium	995
Heparin Potency	1073
Solution time	1079

The following method has been provided in Section 11.3 (page 78):-

Hepatitis B_s Antigen	379

The following method is included in subsequent pages of this Section:-

Isoagglutinin Titre	378

(B) FINISHED PRODUCT

SECTION 9 FORMULATION

9.1. Complete Formula for Product Used in Clinical Trials and Reported in Support of the Marketing Application.

(a) Clinical supplies of Factorate consisted of unit packages each containing a vial of lyophilised AHF & vial of water for injection USP (25 ml). The relevant batch numbers and chemical data are provided on Table I (attached).

9.2. Complete Formula of Products Proposed for Marketing

(a) The composition is as in 9.1 above. Where appropriate, Water for Injection BP (25 ml) can be provided. Each vial contains sufficient sodium chloride USP to make the reconstituted solution approximately isotonic.

SECTION 10 METHODS OF MANUFACTURE OF THE DOSAGE FORM.

As detailed in Section 3.1.

TABLE I

SUMMARY OF CHEMICAL DATA

ON AL-1067 USED IN CLINICAL STUDIES

Preparation			AHF Activity	Protein						
				Total		Clottable				
Batch No.	Date	Size *	u/vial	mg/vial	mg/unit	mg/vial	mg/unit	AL+++ p.p.m.	Heparin u/vial	Evaluated by
K499096	30/4/71	100	132	360	2.7	315	2.4	$\leq$ 0.7	--	Dr. Lazerson
K499104	19/5/71	100	198	570	2.9	375	1.9	$\leq$ 0.8	--	Dr. Gehrke
K499105	7/5/71	100	278	495	1.8	337	1.2	$\leq$ 0.7	--	Drs. Gehrke, Lazerson
K499129	17/11/71	50	187	720	3.8	398	2.1	$\leq$ 0.7	17	Dr. Honig
K499131	16/11/71	100	184	780	4.2	520	2.8	$\leq$ 0.7	19	Dr. Lazerson
K499137	17/11/71	300	208	765	3.7	515	2.5	$\leq$ 0.7	13	Dr. Gehrke

*Litres of plasma in the starting material

SECTION 11 QUALITY CONTROL

11.1. Specifications of the Constituents of the Dosage Form.

As provided in Section 3.2.

11.2. Control Tests Applied to Individual Constituents of a Formulation before Manufacture of the Finished Product.

As detailed in Section 3.4 together with the following Method 992, a copy of which is attached:

Antihaemophilic Factor Potency Assay - 2 Stage.

11.3. In-Process Control Carried Out During the Course of Manufacture of the Finished Product.

The following control is employed:-

1. All plasma is collected in an approved anticoagulant and plasma is tested for hepatitis associated antigen. If it is found negative its use is permitted.

2. Protein content and specific gravity of the bulk product is determined. (Methods 993 and USP respectively) The fill quantity is then calculated in grams, to contain approximately 200 - 250 AHF units/vial and controlled gravimetrically throughout the filling process.

3. Standard operating procedures ensure that injection vials are approved by Quality Control before inspection and filling.

4. A re-check for absence of hepatitis associated antigen is carried out according to Method 379 provided on subsequent pages in this Section.

AHF POTENCY ASSAY — 2-STAGE

Thromboplastin Generation Assay

I Equipment

1. $37^{o}C \pm 0.5^{o}C$ Water Bath

2. Test Tubes; Disposable 12 x 75 mm Plastic Tubes

3. Serological and Measuring Pipettes. Acid washed, distilled water rinsed and dried.

   (1) 1.0 ml, gradaated in 0.01 ml
   (2) 25 ml, graduate in 0.01 ml
   (3) 0.1 ml, graduated in 0.01 ml
   (4) 0.1 ml measuring pipette graduated in 0.01 ml
   (5) 1.0 ml measuring pipette graduated in 0.01 ml
   (6) 2.0 ml measuring pipette graduated in 0.01 ml
   (7) 5.0 ml measuring pipette graduated in 0.01 ml
   (8) disposable pipettes capable of delivering 0.1 ml

4. Timers - (2) Automatic 1 hour timers calibrated in seconds
   (2) Automatic clotting timers calibrated in .01 of a second.

5. Ice Bath - Insulated

6. Clotting System - (2) Fibrometer Coagulation Timers
   (2) Thermal Blocks for dry type heating, Controlled to $37^{o}C \pm 0.05^{o}C$
   (2) Automatic Pipettes which deliver reagents (0.1 ml) into a reaction tube or cup and may be used to initiate the clot reaction.

7. Disposable test tubes and reaction cups.

II Reagents

1. Buffer: Imidazole - NaCl, pH 7.3 $\pm$ 0.02

(A) Components
   (1) Imidazole
   (2) Sodium Chloride (NaCl)
   (3) 0.1N Hydrochloric Acid (HCl)

(B) Method: (1) Dissolve 3.4 g (0.05M) Imidazole in 500 ml of distilled water. (2) Add 3.85 g of NaCl and mix until dissolved. (3) Adjust the pH of the solution, if necessary, with 0.1N HCl to 7.3 $\pm$ 0.02. (4) Dilute to volume with

II Reagents (Cont.)

(B) Method (Cont.)

distilled water. The ionic strength is 0.08 and the osmolarity is 0.2006.

The buffer is stored at 2-8°C. Fresh buffer should be prepared if the solution becomes cloudy or is stored for more than one month.

2. Calcium Chloride ($CaCl_2$ - 0.025M)
(a) Method: Dissolve 1.84 g of $CaCl_2.2H_2O$) in distilled water and dilute to 500.0 ml. Store in a suitable container at 2-8°C.

3. Phospholipid
Prepare either:
(A) Inositihin - 50 g% - Dissolve 50 g of Inositihin in 50 ml of sodium chloride (0.9%) and mix gently overnight. Dilute to volume with 0.9% NaCl. The preparation should be a uniform emulsion of soybean phospholipid. Store in small aliquots at -80°C. Stable for one month only.

(B) Use of a Non-activated Partial Thromboplastin as a Source of Phospholipid
(1) Platelin - A phospholipid prepared by Warner Chillcott. Store at 2-8°C and reconstitute the lyophilised product with 2.5 ml of distilled water.

4. Stock Bovine Serum
Whole blood is collected in the absence of anticoagulants and allowed to clot at 37°C for 2 hours. The blood is then centrifuged at 3000 rpm for 20 minutes with the serum being drawn off and stored in small aliquots at -80°C.

5. Stock Human Serum
Whole blood is collected in the absence of any anticoagulants and allowed to clot at 37°C for 2 hours. The blood is then centrifuged at 3000 rpm for 20 minutes with the serum being drawn off and stored in small aliquots at -80°C.

6. Substrate Plasma - Human
Metrix lyophilised coagulation control plasma is used as the assay control substrate. The blood is freshly collected from human donors, pooled, centrifuged and the plasma buffered and lyophilised.

III Preparation of Test Reagents

1. Human Serum
The human serum is thawed rapidly at 37°C and placed in an ice bath for use during the assay period. One (1.0) ml of serum is diluted with 9.0 ml of Imidazole - NaCl buffer and and placed in the ice bath for use during the assay period

III Preparation of Test Reagents (Cont.)

All serums (diluted and non-diluted) not used in the assay period are discarded and fresh serums prepared daily.

2. Bovine Serums

The bovine serum is thawed rapidly at 37°C and placed in an ice bath for use during the assay period. One half (0.5) ml of the serum is diluted with nine and one half (9.5) ml of imidazole - NaCl buffer and placed in the ice bath for use during the assay period. All serums (diluted and non-diluted) not used in the assay period are discarded and fresh serums prepared daily.

3. Human Substrate Plasma

Metrix coagulation control plasma is reconstituted with 1.0 ml of distilled water and placed in an ice bath during the assay period. All plasma not used in the assay period is discarded. Do not return plasma which has not been warmed to 37°C to the ice bath for future use.

4. Phospholipid

Either
(a) Reconstitute the "platelin" preparation with 2.5 ml of distilled water and place in an ice bath for use during the assay period, or
(b) Inosithin: Thaw and place in an ice bath for use during the assay period.

IV Assay Procedure

1. Incubate for 5 minutes at 37°C sufficient (a) phospholipid, (b) dilute bovine serum, (c) dilute human serum, (d) 0.025M $CaCl_2$, (e) AHF-test or standard solution and (f) control plasma for the number of tests to be run in an assay cycle. Prepare fresh reagents for each assay sequence.

2. Calibration Curve -

Reconstitute the DBS-AHF Reference Plasma Number 2 or Armour AHF Standard with distilled water. Reconstitute using 2.0 ml for the AHF Reference Plasma and 1.0 ml for the Armour AHF Standard. The Armour Standard requires no absorption prior to use.

ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT

METHOD No. 378

DETERMINATION OF ISOAGGLUTININ TITRES IN HUMAN BLOOD PRODUCTS

A. EQUIPMENT NEEDED

1. 12 x 75 mm test tubes
2. Test tube racks
3. Physiological saline (9 gms/l)
4. Adams Sero-fuge
5. Agglutination viewer with light and mirror
6. Coombs serum
7. Automatic pipetter to deliver 0.1 ml
8. Disposable Fibro-Tip pipettes
9. Serologically acceptable 30% bovine albumin
10. 37°C water bath
11. Washed red blood cells (preferably citrated)
    (a) 1 x A negative
    (b) 1 x B negative
    (c) 1 x O positive
    (d) 1 x O negative
12. Vortex mixer

B. PREPARATION OF CELL SUSPENSIONS

1. Using 0.9% solution of sodium chloride, wash each red blood cell three times in Adams Sero-fuge, spinning 1 minute at each wash.

2. Prepare 2% cell suspensions in saline by pipetting 0.2 ml of each blood cell into tubes containing 9.8 ml of physiological saline. Mix well.

3. Prepare 2% cell suspensions in bovine using only the O positive and O negative cells by pipetting 0.2 ml of each cell into tubes containing 9.8 ml 15% bovine albumin. (Dilute 30% bovine albumin to 15% with physiological saline.) Mix well.

4. The above six suspensions should be kept refrigerated when not being used. Washed cells may be stored and used for 2 - 3 days; however, 2% suspensions must be prepared daily.

C. TITRATION PROCEDURE

1. Use one test tube rack for each sample to be tested. Place six rows of 12 x 75 mm test tubes in the rack with 12 tubes in each row.

2. Pipette 0.1 ml of normal saline into all tubes in the first four rows using the automatic pipetter and fibro-tip.

3. Into all tubes in the remaining two rows, pipette 0.1 ml of 20% bovine albumin. (Dilute 30% bovine albumin to 20% with normal saline.)

4. Into the first tube of each row, pipette 0.1 ml of sample to be tested. Label the first tube in the first row with lot number of sample to be tested and with A negative, saline. The second row is labelled B negative, saline; the third row is labelled O positive, saline; the fourth, O negative, saline; the fifth, O positive, bovine; and the last row is labelled O negative, bovine.

5. Prepare serial twofold dilutions over a range covering the expected titre. In this case, the first tube would be a 1:2 dilution and the final tube would represent a 1:4096 dilution. Carryover of a higher concentration of sample to the tube of the next greater dilution must be avoided by using a fresh fibro-tip pipette for each dilution. Also, each dilution must be mixed thoroughly. If desired, a Vortex mixer can be used.

6. Into the first row of saline dilutions, pipette 0.1 ml of the A negative, saline cell suspension into each tube, starting with the 1:4096 dilution and working toward the 1:2 dilution.

7. Repeat the above procedure using the B negative, saline cell suspension in the second row; the O positive, saline cell suspension in the third row; and the O negative, saline cell suspension in the fourth row. Always use a clean pipette for each cell suspension.

8. Into the fifth row, pipette 0.1 ml of the O positive, bovine albumin cell suspension into each tube and in the sixth row, 0.1 ml of the O negative, bovine albumin cell suspension - again starting with the greatest dilution working toward the first dilution.

9. Shake rack vigorously and incubate all tubes at room temperature for 15 minutes.

10. Centrifuge all tubes 45 seconds on an Adams Sero-fuge and observe for agglutination over a lighted mirror. The last tube in the series should be read first, followed by the other tubes in sequence toward the first tube, i.e., tube 4096, 2048, 1024, 512 . . . 2. The final tube showing 1 plus agglutination is the end point. Any doubtful reaction should be recorded as negative.

11. Shake rack vigorously and incubate all tubes for 1 hour in a 37°C water bath. Centrifuge 45 seconds and again observe over a lighted mirror. Record 37°C agglutination by reading as above.

12. Centrifuge all unagglutinated or weakly agglutinated specimens for 45 seconds and sling off protein. Wash tubes three times with physiological saline, spinning 45 seconds on each wash.

13. Add two drops commercial Coombs serum to each tube. Centrifuge 45 seconds and observe for agglutination as above.

D. ISOAGGLUTININ TITRE REQUIRED

An acceptable product shall have no more than the following titres:

ANTIBODY	CELL	INCUBATION	MAXIMUM TITRE OR VALUE
Anti-A	A negative	R.T. saline	1:128
Anti-A	A negative	37°C saline	1:256
Anti-A	A negative	Coombs saline	1:256
Anti-B	B negative	R.T. saline	1:128
Anti-B	B negative	37°C saline	1:256
Anti-B	B negative	Coombs saline	1:256
Anti-D	O positive	R.T. saline	1:32
Anti-D	O negative	R.T. saline	1:32
Anti-D	O positive	37°C saline	1:64
Anti-D	O negative	37°C saline	1:64
Anti-D	O positive	Coombs saline	1:64
Anti-D	O negative	Coombs saline	1:64
Anti-D	O positive	R.T. bovine albumin	1:32
Anti-D	O negative	R.T. bovine albumin	1:32
Anti-D	O positive	37°C bovine albumin	1:64
Anti-D	O negative	37°C bovine albumin	1:64
Anti-D	O positive	Coombs bovine albumin	1:64
Anti-D	O negative	Coombs bovine albumin	1:64

SECTION 12 DEVELOPMENT PHARMACEUTICS

Effectiveness in elevating Factor VIII levels is regarded as directly related to the total amount of antihaemophilic factor activity available and independent of the state of purification. Other factors such as infusion volume, protein concentration, stability, incidence of reactions, clarity of solution and convenience of administration had to be considered in establishing the extent of purification. Selection of the method of processing has been based on a balance between the degree of purification desired and the maximum yield of antihaemophilic factor activity.

SECTION 13 BIOAVAILABILITY

This is discussed in published papers provided in Volume 2 of the Submission.

SECTION 14 METABOLIC STUDIES

Not applicable to this product.

15.1 Batch Numbers and Dates of Manufacture of Batches Examined

Four batches of filled vials have been stored at temperatures of 2-8° C for up to 52 weeks and in the case of two batches for periods up to 24 months.

Potency has been determined in AHF units per ml of reconstituted solution by application of Method 992 provided in Section 11.

The dates of manufacture of the batches examined are as follows:-

Batch Examined	Date of Manufacture
K 499129	8 Sept 1971
K 499131	8 Sept 1971
K 499137	8 Sept 1971
J 20201	31 Jan 1972
J 21604	20 May 1972
J 24008	13 Sept 1973

15.2 Assay Methods Employed and Results Obtained for the Purpose of Determination of Potency

The Antihaemophilic Factor (AHF) Potency has been determined by Method 992 provided in Section 11.

15.3 Methods Employed for Presence of Degradation Products After Period of Storage

Not applicable to this product.

15.4 Physical Characteristics During Storage

The sterile white to very pale yellow lyophilised cake showed no change in physical appearance during the periods examined.

15.5 Nature of the Containers in which Products Stored

50 ml Type I glass vials fitted with grey butyl rubber stoppers (manufactured by Thompkins Rubber Co.) and having an aluminium seal.

KANKAKEE, ILLINOIS

STABILITY PROTOCOL

PRODUCT: Antihemophilic Factor (Human) LOT NO. K499129

DOSAGE FORM: 50 Ml. Vial

PACKAGING COMPONENTS: 50 Ml. Type I Glass Vial with grey butyl lyophilization style stopper, manufactured by Thompkins Rubber Co. of Plymouth Meeting, Pa., Catalog No. PT23, Compound No. 0854, and aluminum seal.

STORAGE CONDITIONS: Refrigeration (2-8°C.)

DATE BEGUN: September 8, 1971

		AHF Units per ml. of Reconstituted Solution*
INITIAL POTENCY:		7.5
	(Three hours after reconstitution)	7.8
POTENCY AFTER:	4 Weeks	6.1
	13 Weeks	6.3
	26 Weeks	6.8
	39 Weeks	6.6
	52 Weeks	8.1

* Reconstituted with 25 ml. sterile water for injection, USP.

AP000908

KANKAKEE, ILLINOIS

STABILITY PROTOCOL

PRODUCT: Antihemophilic Factor (Human) LOT NO. K406121

DOSAGE FORM: 50 Ml. Vial

PACKAGING COMPONENTS: 50 Ml. Type I Glass Vial with grey butyl lyophilization style stopper, manufactured by Thompkins Rubber Co. of Plymouth Meeting, Pa., Catalog No. PT23, Compound No. 0854, and aluminum seal.

STORAGE CONDITIONS: Refrigeration (2-8°C.)

DATE BEGUN: September 8, 1971

	AHF Units per ml. of Reconstituted Solution*
INITIAL POTENCY:	7.3
(Three hours after reconstitution)	6.1
POTENCY AFTER: 4 Weeks	6.0
13 Weeks	6.2
26 Weeks	6.9
39 Weeks	6.9
52 Weeks	6.9

*Reconstituted with 25 ml. sterile water for injection, USP.

AP000909

ARMOUR000117

ARMO0000001_0117

STABILITY PROTOCOL

PRODUCT: Antihemophilic Factor (Human) LOT NO. K499137

DOSAGE FORM: 50 Ml. Vial

PACKAGING COMPONENTS: 50 Ml. Type I Glass Vial with grey butyl lyophilization style stopper, manufactured by Thompkins Rubber Co. of Plymouth Meeting, Pa., Catalog No. PT23, Compound No. 0854, and aluminum seal.

STORAGE CONDITIONS: Refrigeration (2-8°C.)

DATE BEGUN: September 8, 1971

	AHF Units per ml. of Reconstituted Solution*
INITIAL POTENCY:	8.3
(Three hours after reconstitution)	8.0
TIME AFTER: 4 Weeks	-
13 Weeks	8.5
26 Weeks	7.3
39 Weeks	6.5
45 Weeks	8.0
52 Weeks	9.9

* Reconstituted with 25 ml. sterile water for injection, USP.

AP000910

KANKAKEE, ILLINOIS

101

STABILITY PROTOCOL

PRODUCT: Antihemophilic Factor (Human) LOT NO. 720201

DOSAGE FORM: 50 Ml. Vial

PACKAGING COMPONENTS: 50 Ml. Type I Glass Vial with grey butyl lyophilization style stopper, manufactured by Thompkins Rubber Co. of Plymouth Meeting, Pa., Catalog No. PT23, Compound No. 0854, and aluminum seal.

STORAGE CONDITIONS: Refrigeration (2-8°C.)

DATE BEGUN: January 31, 1972

	AHF Units per ml. of Reconstituted Solution*
INITIAL POTENCY:	11.7
POTENCY AFTER: 12 Months	12.3
24 Months	13.5

* Reconstituted with 25 ml. sterile water for injection, USP.

AP000911

KANKAKEE, ILLINOIS

102

STABILITY PROTOCOL

PRODUCT: Antihemophilic Factor (Human) LOT NO. J21604

DOSAGE FORM: 50 Ml. Vial

PACKAGING COMPONENTS: 50 Ml. Type I Glass Vial with grey butyl lyophilization style stopper, manufactured by Thompkins Rubber Co. of Plymouth Meeting, Pa., Catalog No. PT23, Compound No. 0854, and aluminum seal.

STORAGE CONDITIONS: Refrigeration (2-8°C.)

DATE BEGUN: May 20, 1972

		AHF Units per ml. of Reconstituted Solution*
INITIAL POTENCY:		12.5
POTENCY AFTER:	3 Months	12.6
	12 Months	11.6
	24 Months	10.2

*Reconstituted with 25 ml. sterile water for injection, USP.

AP000912

ARMOUR000120

ARMO0000001_0120

KANKAKEE, ILLINOIS

103

STABILITY PROTOCOL

PRODUCT: Antihemophilic Factor (Human) LOT NO. J24000

DOSAGE FORM: 50 Ml. Vial

CONTAINING COMPONENTS: 50 Ml. Type I Glass Vial with grey butyl lyophilization style stopper, manufactured by Thompkins Rubber Co. of Plymouth Meeting, Pa., Catalog No. PT24, Compound No. 0854, and aluminum seal.

STORAGE CONDITIONS: Refrigeration (2-8°C.)

DATE BEGUN: September 13, 1973

	AHF Units per ml. of Reconstituted Solution*
INITIAL POTENCY:	15.0
POTENCY AFTER: 12 Months	13.0

*Reconstituted with 25 ml. sterile water for injection, USP.

AP000913

ARMOUR000121

ARMO0000001_0121

SECTION 16 PROPOSED SHELF LIFE FOR THE PRODUCT

One year from the date of manufacture. The date of manufacture is defined as the date on which the last valid potency test is completed. The product is stored at between -15^{o} to -40^{o}C during quarantine prior to labelling. Subsequently it is stored at $2-8^{o}$C.

SECTION 17 CONTAINERS

The vials are 50 ml USP Type I glass, with 20 mm finish. The stoppers are grey butyl lyophilisation style 20 mm. The rubber closure is covered with a blue plastic and aluminium flip-off seal.