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Guidelines for Production and Quality Control of Antisera

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Abbreviations and Symbols

LD50:

The statistically determined quantity of a substance that, when administered by the specific route, may be expected to cause the death of 50 per cent of the test animals within a given period.

BRP:

Biological Reference Preparation.

GCC:

Gulf Cooperation Council

General Information

The manufacture of antisera is a very complex operation that usually consists of many steps. All steps and stages of the manufacturing must be controlled and validated to produce products of the highest quality (also flow chart of this manufacturing process should be included). This is necessary to protect every patient who will use the products and to ensure that the drug product will be both safe and effective [10].

Background

Immunosera are obtained from healthy animals immunized by injection with the appropriate toxins or toxoids, venoms, suspension of micro-organisms or other antigens. The globulins containing the immunizing substances may be obtained from the serum by enzyme treatment and fractional precipitation or by other chemical or physical methods.

The product (in a liquid or a freeze-dried form) must be sterile, pyrogen-free and preserved by any approved means of preservation [2].

The antisera produced from human origin should comply with all GCC requirements.

Definition

Immunosera for human use are preparations containing specific immunoglobulins obtained from serum of immunized animals after injection of specific antigens. These immunoglobulins have the power of specifically neutralizing venoms or the toxins formed by bacteria or of specifically combining with the bacteria, viruses or other antigens [6].

Production of Antisera:

I. Immunization of Animals:

a. Animals used for the production of sera

Only healthy animals shall be used for the production of sera. Animal immunoglobulins/immunosera are obtained from the sera of different species. Currently, these sera are collected from rabbits, horses, cattle, goats and sheep. Other species, like hens, could also be used. In general, it is desirable to have alternative products available from sera of different species for use in patients in the event of intolerance against heterologous protein. However, horses are mainly used for antisera preparation. Horses testing positive for glanders should be excluded [5] [19] & [20].

Cattle should be tuberculin tested and the reactors rejected. The risk of transmitting animal spongiform encephalopathy via human and veterinary medicinal products should be considered.

Animals used for production of serum shall be held under observation in quarantine for at least 7 days before immunization is started. Horses must be vaccinated against tetanus before immunization is started [18] & [19].

The marketing authorization holder of the immunoglobulin/immunosera has the responsibility for ensuring that the starting material comes from documented and recorded sources, and should perform regular audits of the farms supplying animals. The animals used should be from species approved by the competent authority, healthy and exclusively reserved for production of immunosera. The supplier of animals should be subjected to routine legal supervision by the competent veterinary authority [5].

The donor animals should be held in a closed breeding and production colony whenever possible. The strain, origin and number of the animals should be specified. Transport and introduction of the animals into production should follow specified procedures, including definition of quarantine measures. If different requirements apply to breeding and production animals this should be specified in the Marketing Authorization Dossier. For large animals, the differentiation between breeding and production animals may not be applicable. Source, identity and control animals taken to complete the herd should be

recorded. The feed should originate from a controlled source and no animal proteins should be added [5] .

If the animals are treated with antibiotics there should be a suitable withdrawal period before collection of blood or plasma. The animals must not be treated with penicillin group antibiotics. If a live vaccine is administered to the animals, a suitable waiting period should be imposed between the vaccination and collection of plasma for immunoserum/immunoglobulin production [5] & [19].

A regular health monitoring system should be in place which ensures that the animals are subject to continuous and systematic veterinary and laboratory monitoring for freedom from specified infectious agents. This should include constant monitoring of the animal colonies by the veterinarian, routine pathological examination of randomly selected animals, serological analysis for a range of viruses, bacteria and parasites and the examination of the health status of all animals by the responsible veterinarian, or a person under the responsibility of this veterinarian, prior to bleeding [5]& [18].

The results of the health monitoring of the animal colonies should be well documented and newly emerging serious veterinary diseases should immediately be reported to the competent authorities [5].

b. Antigens for immunization

Animals should be checked and found negative for potential virus contaminants (at least bovine viral diarrhoea, infectious bovine rhinotracheitis and para-influenza 3 virus). Ideally, inactivated bovine serum should be used. In addition, bovine sera and other bovine-derived biologicals used as supplements during the manufacturing procedure should comply with the requirements in the GCC Blood Products Guidelines.

A number of different antigens are used, including:

- human antigens like thymocytes or permanent lymphocyte cell lines to produce antilymphocyte-cell sera,
- venoms from snakes, scorpions and spiders to produce anti-venoms,
- microbial toxins to produce anti-toxins,

- viral and bacterial antigens to produce the corresponding antisera,
- other toxins (such as digitalis).

The antigens should be appropriately characterized. Information on the source and method of preparation should be provided. If appropriate, identification, sanitary status and age of the animal from which the antigen originates should be known. If the antigen is derived from a human donor, information concerning the health of the donor should be provided. Antigens derived from human tissues should be shown to be free of infectious agents [5].

c. Immunization protocol

The animals are immunized with antigens according to a defined scheme with booster injections at regular intervals. The use of adjuvant agents is permitted [5]. The schedules used for immunization of animals depend on many factors related to the particular antigen and animal. No single schedule for all antigens can be established. However, the schedule must lead to the highest titre of antibodies with the lowest protein content.

II. Blood or Plasma Collection

The blood or plasma collection must be conducted at a site separated from the area where the animals are kept or bred and the area where the immunoserum is purified [5] & [20]. Collection of blood or plasma from animals should be made by venipuncture or intracardiac puncture. The area surrounding the point at which insertion is made into the vein should be cleaned and disinfected. If the blood/plasma is held for any period before further processing, it should be treated and stored in such a way as to exclude microbial contamination. Further storage before processing must be validated to ensure that the quality of the product is not affected [5].

The serum from any animal shall not be further processed if the animal is subsequently found to be suffering from an infection or shows signs of persistent illness that is not attributed to the process of immunization, unless the cause of the infection or illness be established to the satisfaction of the regulatory authority by suitable diagnostic methods, which may

involve post-mortem examination, and such cause be judged to be of no significance. If the cause casts suspicion on the health of any other animals used for serum production and there is the possibility of danger to human health, the sera from those animals must not be used [5].

Mixing of serum or plasma from immunized animals of different species is not allowed.

Pool-Testing

Tests for Contaminating Viruses The earliest step at which the serum obtained from all animals is assembled should be defined as a serum pool. The pool should be tested for the absence of specific and adventitious viruses using appropriate in vitro and, if appropriate, in vivo tests. The program to be established to test for the absence of specific viruses depends on the individual manufacturing process. Thus, when human blood is used for absorption of unwanted antibodies and/or for immunization, the absence of human viruses, at least HCV, HIV 1/2 and HBV, must be demonstrated [5].

Potency test: A validated potency test for produced antisera has to be conducted and its result must be documented.

Characterization of the Animal Immunoglobulin/Immunoserum during Development

The active ingredient of any immunoglobulin/immunoserum should be characterized by chemical and biological methods. Particular attention should be paid to using a wide range of analytical techniques for exploring different physicochemical properties of the immunoglobulin. A clear distinction should be made between the analytical tests performed during development in order to fully characterize the immunoglobulin, and tests performed routinely on each batch of the finished product [5].

It should be demonstrated that the product has a characteristic pattern of antigen-binding. It should be shown that the product consists of a defined immunoglobulin G concentration. The content of other immunoglobulin classes should be investigated [5].

The product should not contain antibodies that cross-react with human tissues to a degree that would impair clinical safety. In the event that erythrocytes were used for absorption, the hemoglobin content should be demonstrated to be low [5].

III. Processing and Purification of Plasma

Plasma intended for further processing should be clearly defined. Methods used to purify the product and their in-process controls including their specification limits should be described in detail, justified and validated. It is important to ensure that purification procedures do not impair relevant immunobiological features of the immunoglobulin/ immunoserum [5].

Any optional variations of the manufacturing procedure should be validated. Criteria for reprocessing of any intermediate or of the final bulk should be carefully defined, and the procedure of reprocessing should be validated and justified [5].

The parallel purification of several intermediate serum pools is possible. The maximum number of these intermediate pools and their volume should be defined [5].

All possible steps should be taken to prevent aggregation. It is important that the techniques used to demonstrate purity should be assessed using as wide a range of methods as possible, including physico-chemical and immunological techniques. Assays for endotoxin levels should be carried out [5].

- *Control of Infectious Risks*

The effectiveness of the manufacturing process to inactivate or remove potential viral contaminants is important for product safety. Unless otherwise justified, effective step(s) which inactivate or eliminate potential viral contaminants should be incorporated. Examples are solvent-detergent treatment, pasteurization or appropriate filtration methods. Any inactivation process should not compromise the biological activity of the product [4], [11],[13]& [22].

Procedures which make use of chromatography should be accompanied by appropriate measures to ensure that column substances or any additional potential contaminants arising from their use do not compromise the quality and safety of the final product. Characterization of column material or material used for the precipitation of the protein including data on the purification, cleaning, storage and repeated use of these materials should be provided. The stability of intermediates should be demonstrated [5].

The immunoglobulins are concentrated and purified by fractional precipitation, chromatography, immunoadsorption or by other approved chemical or physical methods. They may be processed further by enzyme treatment. The methods are selected and validated to avoid contamination at all steps of processing and to avoid formation of protein aggregates that affect immunobiological characteristics of the product. For products intended to consist of immunoglobulin fragments, the methods are validated to guarantee total fragmentation. The methods of purification used are such that they do not generate additional components that compromise the quality and the safety of the product.

Unless otherwise justified and authorized, validated procedures are applied for removal and/or inactivation of viruses. The procedures are also selected to avoid the formation of polymers or aggregates and to minimize the splitting of $F(ab')_2$ into Fab' fragments unless the product is intended to consist of Fab' fragments.

If an antimicrobial preservative has been added to the blood or plasma, the same substance must be used as antimicrobial preservative in the final bulk.

IV. In-Process Control

a. Minimum required tests to be done at each stage

1. Plasma

- Potency.
- Antimicrobial preservative concentration.

2. Bulk form

- Potency.
- Antimicrobial preservative concentration.
- Bioburden.

3. Final Bulk

- Potency.
- Bioburden,
- Pyrogen.
- Antimicrobial preservative concentration.
- Abnormal toxicity.
- Foreign matter.

4. Finished Product

- Potency.
- Sterility.
- Immunogenicity.
- Abnormal toxicity.
- Antimicrobial preservative concentration.
- Pyrogen.
- Protein content.
- Identity.
- Inspection of filled container.
- Physical characteristics [2], [6]. & [17].

b. Specifications of antisera according to the pharmacopoeial requirements

Characters. Immunoserum are clear to opalescent and almost colourless or very faint yellow liquids free from turbidity. Freeze-dried immunoserum consist of white or pale-yellow crusts or powders, freely soluble in water to form colourless or pale-yellow solutions having the same characters as the corresponding liquid preparations.

pH. The pH is 6.0 to 7.0

Foreign proteins. When examined by precipitation tests with specific antisera, only protein from the declared animal species is shown to be present.

Total protein content. 90 percent to 110 percent of the amount stated on the label, and not more than 100 g/l.

Albumins. When examined electrophoretically, they show not more than traces of albumins.

Preservatives. They must be preserved against microbial growth.

Sterility. They comply with the test for sterility.

Abnormal toxicity. They comply with the test for abnormal toxicity.

Pyrogen or Endotoxins. They comply with the test for pyrogen or endotoxins.

Potency. A validated potency test has to be conducted and its result must comply with the pharmacopial standards.

Storage. Store at a temperature of $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$. Liquid immunoserum should not be allowed to freeze.

Expiry date. The expiry date has to be determined based on a validated stability study that covers the period of expiration [1], [2], [6] & [17].

Antivenoms must effectively neutralize the venoms of snakes, scorpions or spiders inhabiting the GCC region [21].

Labeling

The label on the container must state the followings:

- Both generic and brand names,
- The number of International Units per millilitre where applicable,
- The batch number or other reference,
- The route of administration,
- The storage conditions,
- The expiry date,
- The animal species of origin.

In addition to the above, the followings must be stated on the outer package:

- The name and amount or concentration of any other additives,
- A declaration of any substance likely to cause any adverse reaction and any contra-indications to the use of the product,
- The name and address of the manufacturer.
- State if the product is sensitive to light and that freezing must be avoided for liquid forms.

For freeze-dried immunosera, the followings are also required:

- The name or composition and the volume of the reconstituting liquid to be added,
- The time required for complete dissolution,
- That the immunoserum should be used immediately after reconstitution.

In addition to the above mentioned under labeling, the pamphlet accompanying the package must include the followings:

- Indications and Usage,
- Dosage and Administration,
- Dosage Forms and Strengths,
- Contraindications,
- Warnings and Precautions,
- Adverse Reactions,

- Drug Interactions,
- Use in Specific Populations,
 - Pregnancy
 - Labor and delivery
 - Nursing mothers
 - Pediatric use
 - Geriatric use
- Description,
- Clinical Pharmacology,
 - Mechanism of action
 - Pharmacodynamics
 - Pharmacokinetics
- How Supplied/Storage and Handling [2], [6], [7]& [17].

V. Production Requirements

a. Processing Requirements

- Production should be carried out in a clean area class C. Walls and floors should be cleaned periodically with disinfectant according to an authorized protocol for cleaning which should be validated [9]&[16].
- Personnel shall be trained, educated and experienced for the job they are required to do. Training shall be in the particular operations that the employee performs and current good manufacturing practice as they relate to the employee's function [9] & [16].
- Contact parts of containers should be of stainless steel of pharmaceutical grade (316L) or higher, easily cleaned in place (CIP) and validated to be used for plasma processing [9]&[16].
- Water used should be of water for injection grade (WFI). Water supply should be controlled to decrease bioburden. WFI used in processing should be maintained at a high temperature (70-80°C) at all times to prevent contamination and decrease

of bioburden. Heat exchangers for cooling at the points of use or alternatively depyrogenating and bacterial filters should be fixed at the point of use [9] & [16].

- Equipment in the processing area must be maintained, cleaned, calibrated or validated periodically as needed [9]&[16].

b. Filling Requirements

- Sterile areas class A & B must be designed according to the assigned procedure. Differential pressure between areas of different classes must be maintained by positive pressure between high-pressure areas to low- pressure areas.
- Environmental control must be carried out periodically according to an authorized and validated schedule to prevent contamination.

- Environmental control includes:

Y control of viable matters (microbiology tests),

Y control of non viable matters (particle count control).

- Sterilization of equipment (including filling pumps, containers, stainless steel tanks and glass containers) & depyrogenating of final containers if glass, according to authorized and validated procedures.
- Validation of sterilizing equipment (autoclave) and depyrogenating equipment (ovens or tunnel sterilizer) which must be validated periodically according to an authorized protocol.
- Filling process validation by media fill must be carried out periodically to ensure maintenance of sterility conditions [9] & [5].

VI. Batch Record

There shall be written procedures for production and process control designed to ensure that the drug products have the identity, strength, quality, and purity they have or are presented to process. Such procedures shall include all requirements. Records shall be permanent and clearly indicate all steps in processing, testing, filling and distribution. Written records shall be kept of all tests irrespective of their results. The records shall be of a type approved by the regulatory authority. They shall be retained throughout the dating period of a lot or batch of a biological product and be available at all times for inspection by the regulatory authority. Records must make it possible to trace all steps in the manufacture and testing of a batch, and should include records of sterilization of all apparatus and materials used in its manufacture. Distribution records must be kept in a manner that permits rapid recall of any particular batch if necessary [9] & [16].

- *Sampling Procedure*

There should be written procedures for sampling, which include the person(s) authorized to take samples, the methods and equipment to be used, the amount to be taken and any precautions to be observed to avoid contamination of the material or any deterioration in its quality. It is not sufficient to sample and test only the end product. In-process sampling and testing must be conducted. Samples should be withdrawn and tested at several steps along the way. The samples must be of sufficient number, size and selection to be statistically representative. Criteria or specifications for allowable variation must be sufficiently strict to assure that desirable quality is assured. Sampling and evaluation should be thoroughly documented at every stage. Protocols are commonly used to assure that this is controlled [9].

- *Stability*

The stability studies must be done according to recent ICH guidelines for the biological products.

It is extremely important that the manufacturer conducts the stability tests of:

- Y raw materials,
- Y intermediate stages if stored for long time,
- Y finished products.

Extreme conditions of storage should be taken into consideration.

Major factors which affect stability are:

- temperature and relative humidity,
- light.

A protocol for stability study has to be implemented to provide evidence that the antisera are stable, i.e. remain within specification during the re-test period when stored under the recommended environmental conditions.

The stability program should extend not only to the end of the shelf life period but also in addition to accelerated study and should include, but not be limited to, the following parameters:

- Y number of batches,
- Y relevant physical, chemical, microbial and biological test method,
- Y acceptance criteria,
- Y reference to test method,
- Y description of the container closure system(s),
- Y testing interval,
- Y description of the conditions of storage.

A summary of all data generated, including any interim conclusions on the program, should be written and maintained.

Findings outside specifications or significant atypical trends should be investigated. Any confirmed findings outside specifications, or significant negative trends, should be reported to the regulatory authority.

An on-going stability program after marketing should be monitored according to a continuous appropriate program that will permit the detection of any stability issues [8].

- *Validation*

Validation studies should reinforce Good Manufacturing Practice and be conducted in accordance with defined procedures. Results and conclusions should be recorded.

When any new manufacturing formula or method of preparation is adopted, steps should be taken to demonstrate its suitability for routine processing. The defined process, using the materials and equipment specified, should be shown to yield a product consistently of the required quality.

Significant amendments to the manufacturing process, including any change in equipment or materials which may affect product quality and/or the reproducibility of the process, should be validated.

Processes and procedures should undergo periodic critical revalidation to ensure that they remain capable of achieving the intended results.

Each material, each item of equipment and each step of manufacturing is validated before the product is produced for distribution:

- Y e.g. for processing validation: media fill has to be done to simulate all stages of processing and filling.
- Y e.g. for equipment: temperature mapping for autoclave, and depyrogenation for ovens or tunnel sterilizer.
- Y e.g. validation methods used to ensure freedom from viruses [9].

- *Complaints and Product Recall*

All complaints and other information concerning potentially defective products must be carefully reviewed according to written procedures. In order to provide for all contingencies, a system should be designed to promptly and effectively recall, if necessary, products known or suspected to be defective from the market.

Complaints

A person should be designated responsible for handling complaints and deciding the measures to be taken together with sufficient supporting staff to assist him. If this person is not the authorized person, the latter should be made aware of any complaint, investigation or recall.

There should be written procedures describing the action to be taken, including the need to consider a recall, in the case of a complaint concerning a possible product defect.

Any complaint concerning a product defect should be recorded with all the original details and thoroughly investigated.

If a product defect is discovered or suspected in a batch, consideration should be given to checking other batches in order to determine whether they are also affected. In particular, other batches which may contain reworks of the defective batch should be investigated.

All the decisions and measures taken as a result of a complaint should be recorded and referenced to the corresponding batch records.

Complaints records should be reviewed regularly for any indication of specific or recurring problems requiring attention and possibly the recall of marketed products.

Special attention should be given to establishing whether a complaint was caused because of counterfeiting.

The regulatory authority should be informed if a manufacturer is considering action following possibly faulty manufacture, product deterioration, and detection of counterfeiting or any other serious quality problems with a product.

Recalls

A person should be designated as responsible for execution and coordination of recalls and should be supported by sufficient staff to handle all the aspects of the recalls with the appropriate degree of urgency. This responsible person should normally be independent of the sales and marketing organization. If this person is not the authorized person, the latter should be made aware of any recall operation.

There should be established written procedures, regularly checked and updated when necessary, in order to organize any recall activity.

Recall operations should be capable of being initiated promptly and at anytime.

All competent authorities of all countries to which products may have been distributed should be informed promptly if products are intended to be recalled because they are, or are suspected of being, defective.

The distribution records should be readily available to the person(s) responsible for recalls, and should contain sufficient information on wholesalers and directly supplied customers (with addresses, phone and/or fax numbers during and after working hours, batches and amounts delivered), including those for exported products and medical samples.

Recalled products should be identified and stored separately in a secure area while awaiting a decision.

The progress of the recall process should be recorded and a final report issued, including reconciliation between the delivered and recovered quantities of the products.

The effectiveness of the arrangements for recalls should be evaluated regularly [9].

- *Clinical Assessment of Antisera*

Preclinical, clinical and post-marketing surveillance of all antisera, should be carried out according to WHO's most current guidelines and recommendations.

Sera From Animal Origin

I. Antivenoms

1. Polyvalent snake antivenom

Definition:

A preparation containing antitoxic equine globulins [F (ab')₂] that have the power of neutralizing the venoms of snakes inhabiting the GCC region.

Each venom of one species of snake inhabiting the Arabian peninsula is usually injected in a group of horses to get monovalent antivenom, and then these monovalents are pooled to prepare potent polyvalent antivenom.

It should comply with the requirements stated under the Specification of antisera according to the pharmacopial requirements in addition to the followings:

Identification: It must neutralize the venoms of the following snakes:

- *Walterinnesia aegyptica*
- *Naja haje arabicus*
- *Cerastes cerastes*
- *Echis carinatus*
- *Echis coloratus*
- *Bitis arietans*

rendering them harmless to susceptible animals. The antivenom must not only neutralize lethality, but also neutralize the other pharmacological properties of the venoms, such as:

- *Haemorrhagic effect,*
- *Necrotizing effect,*
- *Haemolytic effect,*
- *Fibrinolytic effect,*

- *Neuromuscular paralytic activity [14] & [15].*

Assay

Each millilitre of the preparation to be examined contains sufficient antibodies to neutralize not less than 25 mouse LD50 of each of the 6 venoms mentioned above.

The potency of antivenom depends first on determining the LD50 of each venom.

Selection of Venoms:

Due to venom variation within the same species from region to region, the venom used for evaluating the antivenom has to be a standard national venom reference of a pooled collection from different regions.

Venom should be pooled from more than 50 different snakes of the same species and of various ages and sizes [20] & [21].

These should be collected from different geographical regions, representing the distribution of that species of snake in the GCC region.

The median lethal dose (LD50) of each venom should be determined in addition to the most relevant pathophysiological activities of each venom (e.g. haemorrhagic, necrotizing, fibrinolytic...) according to the methods adopted by WHO and printed on the label of the venom's containers [14] & [21].

Determination of the potency of antivenom:

Should be carried out according to current international pharmacopoeia.

Labeling:

The label must state the venom or venoms against which the antivenom is effective and the nominal volume; it must also state that it was prepared from horse serum [2], [6] & [20].

2. Polyvalent scorpion antivenom

Definition:

A preparation containing antitoxic equine globulins [F (ab')₂] that have the power of neutralizing the venoms of medically important scorpions inhabiting the GCC region.

Each venom of one species of scorpion inhabiting the Arabian peninsula is usually injected in a group of horses to get monovalent antivenom, and then these monovalents are pooled to prepare potent polyvalent antivenom.

It should comply with the requirements stated under the Specification of antisera according to the pharmacopial requirements in addition to the followings:

Identification: it must neutralize the venoms of the following clinically important scorpions:

- *Leiurus quinquestriatus*
- *Androctonus crassicauda*

rendering them harmless to susceptible tested animals.

Assay

Each millilitre of the preparation to be examined contains sufficient antibodies to neutralize not less than 50 mouse LD₅₀ of each of the 2 venoms mentioned above.

The potency of antivenom depends first on determining the LD₅₀ of each venom.

Selection of Venoms:

Due to venom variation within the same species from region to region, the venom used for evaluating the antivenom has to be a standard national venom reference of a pooled collection from different regions.

Venom must be obtained from 500 to 1000 scorpions of the same species by electrical stimulation, not by extraction of the dried telsons.

These should be collected from different geographical regions, representing the distribution of that species of scorpion in the GCC region [21].

Determination of the potency of antivenom:

Should be carried out according to current international pharmacopoeia.

Labeling:

The label must state the venom or venoms against which the antivenom is effective and the nominal volume; it must also state that it was prepared from horse serum [1].

3.Spider antivenom**Definition:**

A preparation containing antitoxic equine globulins [F (ab')₂] that have the power of neutralizing the venoms of spiders inhabiting the GCC region.

It should comply with the requirements stated under the Specification of antisera according to the pharmacopoeial requirements in addition to the followings:

Identification: it must neutralize the venoms of the following spiders:

- *Lactodectus mactans*
- *Loxosceles* [3]

rendering them harmless to the susceptible animals. The antivenom must not only neutralize lethality but also neutralize the other pharmacological properties of the venom, such as: Haemorrhagic effects and necrotizing effects.

Assay

Each millilitre of the preparation to be examined contains sufficient antibodies to neutralize 2400 mouse LD₅₀ of each venom of spiders.

The potency of antivenom depends first on determining the LD₅₀ of each venom.

Determination of the potency of antivenom to be examined:

Should be carried out according to current international pharmacopoeia.

Labeling:

The label must state the venom or venoms against which the antivenom is effective and the nominal volume; it must also state that it was prepared from horse serum [17].

** For more details, refer to the General Information.*

II. Bacterial antitoxins

1. Botulinum antitoxin

Definition:

Botulinum antitoxin is a preparation containing antitoxic globulins that have the power of specifically neutralizing the toxins formed by *Clostridium botulinum* type A, B or E, or any mixture of these types.

When mixed botulinum antitoxin or botulinum antitoxin is prescribed or demanded and the types to be present are not stated, botulinum antitoxin prepared from type A, B and E shall be dispensed or supplied.

It is obtained by fractionation from the serum of horses, or other mammals that have been immunized against *Clostridium botulinum* type A, B and E toxins.

It should comply with the requirements stated under the Specification of antisera according to the pharmacopoeial requirements in addition to the followings:

Identification: It specifically neutralizes the type of *Clostridium botulinum* toxins stated on the label, rendering them harmless to susceptible tested animals.

Assay

Not less than 500 IU of antitoxin per ml for each of type A and B and not less than 50 IU of antitoxin per ml of type E.

Determination of the potency of botulinum antitoxin:

Should be carried out according to current international pharmacopoeia.

Labeling

The label states the types of *Clostridium botulinum* toxin neutralized by the preparation. The label may states “Bot/Ser” followed by a letter or letters indicating the type or types present [2], [6] & [17].

2. Diphtheria antitoxin

Definition:

Diphtheria antitoxin is a preparation containing antitoxic globulins that have the power of specifically neutralizing the toxins formed by *Corynebacterium diphtheriae*.

It is obtained by fractionation from the serum of horse, or other mammals, that have been immunized against diphtheria toxin.

It should comply with the requirements stated under the Specification of antisera according to the pharmacopial requirements in addition to the followings:

Identification: It specifically neutralizes the toxin formed by *Corynebacterium diphtheriae*, rendering it harmless to susceptible tested animals.

Assay

Not less than 1000 IU of antitoxin per ml for antitoxin obtained from horse serum. Not less than 500 IU of antitoxin per ml for antitoxin obtained from the serum of other mammals.

Determination of the potency of diphtheria antitoxin:

Should be carried out according to current international pharmacopoeia.

Labeling:

The label states the types of *Corynebacterium diphtheriae* toxin neutralized by the preparation. The label may state “Dip/Ser” [2], [6] & [17].

3. Gas-gangrene antitoxin

A) Gas-gangrene antitoxin (novyi)

Definition:

Gas-gangrene antitoxin (novyi) is a preparation containing antitoxic globulins that have the power of specifically neutralizing the alpha toxins formed by *Clostridium novyi* (former nomenclature: *Clostridium oedematiens*).

It is obtained by fractionation from the serum of horse, or other mammals, that have been immunized against *Clostridium novyi* toxin.

It should comply with the requirements stated under the Specification of antisera according to the pharmacopoeial requirements in addition to the followings:

Identification: It specifically neutralizes the alpha toxin formed by *Clostridium novyi*; rendering it harmless to susceptible tested animals.

Assay

Not less than 3750 IU of antitoxin per ml.

Determination of the potency of gas-gangrene (novyi) antitoxin:

Should be carried out according to current international pharmacopoeia.

Labeling:

The label states the types of *Clostridium* Gas-gangrene (*novyi*) toxin neutralized by the preparation. The label may state “Nov/Ser” [2], [6] & [17].

b) Gas-gangrene antitoxin (*perfringens*)

Definition:

Gas-gangrene antitoxin (*perfringens*) is a preparation containing antitoxic globulins that have the power of specifically neutralizing the alpha toxins formed by *Clostridium perfringens*.

It is obtained by fractionation from the serum of horses, or other mammals, that have been immunized against *Clostridium perfringens* toxin.

It should comply with the requirements stated under the Specification of antisera according to the pharmacopoeial requirements in addition to the following:

Identification: It specifically neutralizes the alpha toxin formed by *Clostridium perfringens*, rendering it harmless to susceptible tested animals.

Assay

Not less than 1500 IU of antitoxin per ml.

Determination of the potency of gas-gangrene (*perfringens*) antitoxin:

Should be carried out according to current international pharmacopoeia.

Labeling:

The label states the types of *Clostridium* Gas-gangrene (*perfringens*) toxin neutralized by the preparation. The label may state “Perf/Ser” [2], [6] & [17].

c) Gas-gangrene antitoxin (*Septicum*)

Definition:

Gas-gangrene antitoxin (*Septicum*) is a preparation containing antitoxic globulins that have the power of specifically neutralizing the alpha toxins formed by *Clostridium septicum*.

It is obtained by fractionation from the serum of horses, or other mammals, that have been immunized against *Clostridium septicum* toxin.

It should comply with the requirements stated under the Specification of antisera according to the pharmacopoeial requirements in addition to the following:

Identification: It specifically neutralizes the alpha toxin formed by *Clostridium septicum*, rendering it harmless to susceptible tested animals.

Assay

Not less than 1500 IU of antitoxin per ml.

Determination of the potency of gas-gangrene (*septicum*) antitoxin:

Should be carried out according to current international pharmacopeia.

Labeling:

The label states the types of *Clostridium* Gas-gangrene (*septicum*) toxin neutralized by the preparation. The label may state “Sep/Ser” [2], [6] & [17]

D) Mixed gas-gangrene antitoxin

Definition:

Mixed gas-gangrene antitoxin is prepared by mixing gas-gangrene antitoxin (*novyi*), gas-gangrene antitoxin (*perfringens*) and gas-gangrene (*septicum*) in appropriate quantities.

It is obtained by fractionation from the serum of horses, or other mammals, that have been immunized against gas-gangrene (*novyi*), gas-gangrene (*perfringens*) and gas-gangrene (*septicum*).

It should comply with the requirements stated under the Specification of antisera according to the pharmacopoeial requirements in addition to the following:

Identification: It specifically neutralizes the alpha toxin formed by *Clostridium novyi*, *Clostridium perfringens* and *Clostridium septicum*, rendering them harmless to susceptible tested animals.

Assay

Gas-gangrene antitoxin (*novyi*), not less than 1000 IU of antitoxin per ml, gas- gangrene antitoxin (*perfringens*), not less than 1000 IU of antitoxin per ml, gas- gangrene antitoxin (*septicum*) not less than 500 IU of antitoxin per ml.

Determination of the potency of mixed gas-gangrene antitoxin:

Should be carried out according to current international pharmacopoeia.

Labeling:

The label states the types of the mixed *Clostridium* Gas-gangrene (*novyi*, *perfringens* and *septicum*) toxin neutralized by the preparation [2], [6] & [17].

4. Tetanus antitoxin

Definition:

Tetanus antitoxin is a preparation containing antitoxin globulins that have the power of specifically neutralizing the toxin formed by *Clostridium tetani*.

It is obtained by fractionation from the serum of horses, or other mammals, that have been immunized against tetanus toxin.

It should comply with the requirements stated under the Specification of antisera according to the pharmacopoeial requirements in addition to the following:

Identification: It specifically neutralizes the toxin formed by *Clostridium tetani*, rendering it harmless to susceptible animals.

Assay

Not less than 1000 IU of antitoxin per ml when intended for prophylactic use. Not less than 3000 IU of antitoxin per ml when intended for therapeutic use.

Determination of the potency of tetanus antitoxin:

Should be carried out according to current international pharmacopoeia.

Labeling:

The label states the types of *Clostridium tetanis* antitoxin neutralized by the preparation. The label may state “Tet/Ser” [2] & [6].

** For more details, refer to the General Information.*

III. viral Antisera

Antirabies serum

Definition:

Antirabies is a preparation containing antiviral globulins that have the power of specifically neutralizing the rabies virus.

It is obtained by fractionation from the serum of horses that have been immunized against the rabies virus (the CVS strain).

It should comply with the requirements stated under the Specification of antisera according to the pharmacopial requirements in addition to the following:

Determination of the potency of antirabies serum:

Should be carried out according to current international pharmacopoeia.

Labeling:

The label states that it is prepared in horses as well as the number of IU/ml [17].

Sera From Human Origin

Normal Immunoglobulin

1. Normal immunoglobulin for injection

Definition:

Human normal immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G (IgG). Other proteins may be present. Human normal immunoglobulin contains the IgG antibodies of normal subjects. It is intended for intramuscular injection.

Human normal immunoglobulin is obtained from plasma that complies with the requirements of the monograph on *Human plasma for fractionation*. No antibiotic is added to the plasma used.

Production

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses, it shall have been shown that any residues present in the final product have no adverse effects on the patients treated with the immunoglobulin.

The product shall have been shown, by suitable tests in animals and evaluation during clinical trials, to be well tolerated when administered intramuscularly.

Human normal immunoglobulin is prepared from pooled material from at least 1000 donors by a method that has been shown to yield a product that:

- does not transmit infection;
- at a protein concentration of 160 g/l, contains antibodies for at least 2 of which (one viral and one bacterial) an International Standard or Reference Preparation is available, the concentration of such antibodies being at least 10 times that in the initial pooled material.

Human normal immunoglobulin is prepared as a stabilized solution, for example in a 9 g/l solution of sodium chloride, a 22.5 g/l solution of glycine or, if the preparation is to be

freeze-dried, a 60 g/l solution of glycine. Multi-dose preparations contain an antimicrobial preservative. Single-dose preparations do not contain an antimicrobial preservative. Any antimicrobial preservative or stabilizing agent used shall have been shown to have no deleterious effect on the final product in the amount present. The solution is passed through a bacteria retentive filter.

The stability of the preparation is demonstrated by suitable tests carried out during development studies.

Characteristics

The liquid preparation is clear and pale-yellow to light-brown; during storage it may show formation of slight turbidity or a small amount of particulate matter. The freeze-dried preparation is a white or slightly yellow powder or solid, friable mass.

For the freeze-dried preparation, reconstitute as stated on the label immediately before carrying out the identification and the tests, except those for solubility and water.

Identification

- A. Using a suitable range of species-specific antisera carry out precipitation tests on the preparation to be examined. It is recommended that the test be carried out using antisera specific to the plasma proteins of each species of domestic animal commonly used in the preparation of materials of biological origin in the country concerned. The preparation is shown to contain proteins of human origin and gives negative reactions with antisera specific to plasma proteins of other species.
- B. Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal human serum and the preparation to be examined, both diluted to contain 10 g/l of protein. The main component of the preparation to be examined corresponds to the IgG component of normal human serum. The solution may show the presence of small quantities of other plasma proteins.

Quality Control Tests:

Solubility: For the freeze-dried preparation, add the volume of the liquid stated on the label. The preparation dissolves completely within 20 min. at 20-25°C.

pH: Dilute the preparation to be examined with 9 g/l solution of *sodium chloride* to obtain a solution containing 10 g/l protein. The pH of the solution is 6.4 to 7.2.

Total protein: Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride* to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid* and 30 volumes of *water*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion and calculate the content of protein by multiplying by 6.25. The preparation contains not less than 100 g/l and not more than 180 g/l of protein and not less than 90 per cent and not more than 110 per cent of the quantity of protein stated on the label.

Protein composition: Examine by zone electrophoresis, using strips of suitable cellulose acetate gel as the supporting medium and *barbital buffer solution pH 8.6* as the electrolyte solution.

Test solution. Dilute the preparation to be examined with 9 g/l solution of *sodium chloride* to a protein concentration of 50 g/l.

Reference solution. Reconstitute *human Immunoglobulin for electrophoresis BRP* and dilute with a 9 g solution of *sodium chloride* to a protein concentration of 50 g/l.

To a strip apply 2.5 µl of the test solution as a 10 mm band or apply 0.25 µl per millimeter if a narrower strip is used. To another strip apply in the same manner the same volume of the reference solution. Apply a suitable electric field such that the albumin band of normal human serum applied on a control strip migrates at least 30 min. Stain the strip with a *amido black 10B solution R* for 5 min. Decolourise with a mixture of 10

volumes of *glacial acetic acid* and 90 volumes of *methanol* so that the background is just free of colour. Develop the transparency of the strips with a mixture of 19 volumes of *glacial acetic acid* and 81 volumes of *methanol*. Measure the absorbance of the bands at 600 nm in an instrument having a linear response over the range of measurement. Calculate the result as the mean of 3 measurements of each strip. In the electropherogram obtained with the test solution, not more than 10 per cent of the protein has a mobility different from that of the principal band. The test is not valid unless, in the electropherogram obtained with the reference preparation, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

Distribution of molecular size. Examine by liquid chromatography.

Test solution. Dilute the preparation to be examined with 9 g/l solution of *sodium chloride* to a concentration suitable for the chromatographic system used. A concentration in the range 4 g/l to 12 g/l and injection of 50 µg to 600 µg of protein are usually suitable.

Reference solution. Dilute *human immunoglobulin BRP* with a 9 g/l solution of *sodium chloride* to the same protein concentration as the test solution.

The chromatographic procedure may be carried out using:

- a column 0.6 m long and 7.5 mm in internal diameter packed with *hydrophilic silica gel for chromatography*.
- as mobile phase, at a flow rate of 0.5 ml/min a solution containing per litre: 4.873 g of *disodium hydrogen phosphate dehydrate*, 1.741 g of *sodium dihydrogen phosphate monohydrate*, 11.688 g of *sodium chloride* and 50 mg of *sodium azide*.
- as detector, a spectrophotometer set at 280 nm.

In the chromatogram obtained with the reference solution, the principal peak corresponds to IgG monomer and there is a peak corresponding to dimer with a retention time relative to monomer of about 0.85. Identify the peaks in the chromatogram obtained with

the test solution by comparison with the chromatogram obtained with the reference solution; any peak with a retention time shorter than that of dimer corresponds to polymers and aggregates. The preparation to be examined complies with the test if, in the chromatogram obtained with the test solution: for monomer and dimer, the retention time relative to the corresponding peak in the chromatogram obtained with the reference solution is 1 ± 0.02 , the sum of monomer and dimer represents not less than 85 per cent of the total area of the chromatogram and polymers and aggregates represent not more than 10 per cent of the total area of the chromatogram.

Water. Determined by a suitable method, such as the semi-micro determination of water, loss on drying or near infrared spectrophotometry, the water content is within the limits approved by the competent authority.

Sterility: It complies with the test for sterility.

Pyrogens: It complies with the test for pyrogens. Inject 1 ml per kilogram of the rabbit's mass.

Antibody to hepatitis B surface antigen: Not less than 0.5 IU/g of immunoglobulin, determined by a suitable immunochemical method.

Antibody to hepatitis C surface antigen and HIV 1 & 2: The final products shall be tested for viral marker as HCV, HBsAg, and HIV 1 & 2 by screening and confirmatory ELISA and PCR assay and final products shall be free from these pathogens.

Antibody to hepatitis A virus: If intended for use in the prophylaxis of hepatitis A, it complies with the following additional requirement. Determine the antibody content by comparison with a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity.

The International Unit is the activity contained in a stated amount of the International Standard for anti-hepatitis A immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Human hepatitis A immunoglobulin BRP is calibrated in International Units by comparison with the International Standard.

The stated potency is not less than 100 IU/ml. The estimated potency is not less than the stated potency. The fiducial limits of error ($P = 0.95$) of the estimated potency are not less than 80 per cent and not more than 125 per cent.

Storage

For the liquid preparation, store in a colourless glass container, protected from light. For the freeze-dried preparation, store in a colourless glass container, under vacuum or under an inert gas, protected from light.

Labeling

The label states:

- for liquid preparations, the volume of the preparation in the container and the protein content expressed in grams per liter,
- for freeze-dried preparations, the quantity of protein in the container,
- the route of administration,
- for freeze-dried preparations, the name or composition and the volume of the reconstituting liquid to be added,
- where applicable, that the preparation is suitable for use in the prophylaxis of hepatitis A infection,
- where applicable, the anti-hepatitis A virus activity in International Units per millilitre,
- where applicable, the name and amount of antimicrobial preservative in the preparation [2], [6], [12], [13].

** For more details, refer to the General Information.*

2. Normal immunoglobulin for intravenous use

Definition:

Human normal immunoglobulin for intravenous administration is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G (IgG). Other proteins may be present. Human normal immunoglobulin for intravenous administration contains the IgG antibodies of normal subjects.

Human normal immunoglobulin for intravenous administration is obtained from plasma that complies with the requirements of the monograph on *Human plasma for fractionation*. No antibiotic is added to the plasma used.

Production

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses, it shall have been shown that any residues present in the final product have no adverse effects on the patients treated with the immunoglobulin.

The product shall have been shown, by suitable tests in animals and evaluation during clinical trials, to be well tolerated when administered intravenously.

Human normal immunoglobulin is prepared from pooled material from not fewer than 1000 donors by a method that has been shown to yield a product that:

- does not transmit infection,
- at an immunoglobulin concentration of 50 g/l, contains antibodies for at least 2 of which (one viral and one bacterial) an International Standard or Reference Preparation is available, the concentration of such antibodies being at least 3 times that in the initial pooled material,
- has a defined distribution of immunoglobulin G subclasses,
- complies with the test for Fc function of immunoglobulin.

Human normal immunoglobulin for intravenous administration is prepared as a stabilized solution or as a freeze-dried preparation. A stabilizer may be added. In both cases the

preparation is passed through a bacteria-retentive filter. No antimicrobial preservative is added either during fractionation or at the stage of the final bulk solution.

The stability of the preparation is demonstrated by suitable tests carried out during development studies.

Characteristics

The liquid preparation is clear or slightly opalescent and colourless or pale yellow. The freeze-dried preparation is a white or slightly yellow powder or solid, friable mass.

For the freeze-dried preparation, reconstitute as stated on the label immediately before carrying out the identification and the tests, except those for solubility and water.

Identification

- A. Using a suitable range of species-specific antisera, carry out precipitation tests on the preparation to be examined. It is recommended that the test be carried out using antisera specific to the plasma proteins of each species of domestic animal commonly used in the preparation of materials of biological origin in the country concerned. The preparation is shown to contain proteins of human origin and gives negative reactions with antisera specific to plasma proteins of other species.
- B. Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal human serum and the preparation to be examined, both diluted to contain 10 g/l of protein. The main component of the preparation to be examined corresponds to the IgG component of normal human serum. The solution may show the presence of small quantities of other plasma proteins; if human albumin has been added as a stabilizer, it may be seen as a major component.

Quality Control Tests

Solubility: For the freeze-dried preparation, add the volume of the liquid stated on the label. The preparation dissolves completely within 30 min. at 20-25°C.

pH: Dilute the preparation to be examined with 9 g/l solution of *sodium chloride* to obtain a solution containing 10 g/l protein. The pH of the solution is 4.0 to 7.4.

Osmolality: Not less than 240 mosmol/kg.

Total protein: Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride* to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid* and 30 volumes of *water*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion and calculate the content of protein by multiplying by 6.25. The preparation contains not less than 30 g/l of protein and not less than 90 per cent and not more than 110 per cent of the quantity of protein stated on the label.

Protein composition: Examine by zone electrophoresis, using strips of suitable cellulose acetate gel as the supporting medium and *barbital buffer solution pH 8.6* as the electrolyte solution.

Test solution. Dilute the preparation to be examined with 9 g/l solution of *sodium chloride* to a protein concentration of 30 g/l.

Reference solution. Reconstitute *human immunoglobulin for electrophoresis BRP* and dilute with a 9 g/l solution of *sodium chloride* to a protein concentration of 30 g/l.

To a strip apply 4.0 µl of the test solution as a 10 mm band or apply 0.4 µl per millimeter if a narrower strip is used. To another strip apply in the same manner the same volume of the reference solution. Apply a suitable electric field such that the albumin band of normal human serum applied on a control strip migrates at least 30 min. Stain the

strip with an *amido black 10B solution* for 5 min. Decolourise with mixture of 10 volumes of *glacial acetic acid* and 90 volumes of *methanol* so that the background is just free of colour. Develop the transparency of the strips with a mixture of 19 volumes of *glacial acetic acid* and 81 volumes of *methanol*. Measure the absorbance of the bands at 600 nm in an instrument having a linear response over the range of measurement. Calculate the result as the mean of 3 measurements of each strip. In the electropherogram obtained with the test solution, not more than 5 per cent of the protein has a mobility different from that of the principal band. This limit is not applicable if albumin has been added to the preparation as a stabilizer; for such preparation, a test for protein composition is carried out during manufacture before addition of the stabilizer. The test is not valid unless, in the electropherogram obtained with the reference preparation, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

Distribution of molecular size: Examine by liquid chromatography.

Test solution. Dilute the preparation to be examined with 9 g/l solution of *sodium chloride* to a concentration suitable for the chromatographic system used. A concentration in the range 4 g/l to 12 g/l and injection of 50 µg to 600 µg of protein are usually suitable.

Reference solution. Dilute *human immunoglobulin BRP* with a 9 g/l solution of *sodium chloride* to the same protein concentration as the test solution.

The chromatographic procedure may be carried out using:

- a column 0.6 m long and 7.5 mm in internal diameter packed with *hydrophilic silica gel for chromatography*.
- as mobile phase, at a flow-rate of 0.5 ml/min a solution containing per litre: 4.873 g of *disodium hydrogen phosphate dehydrate*, 1.741 g of *sodium dihydrogen phosphate monohydrate*, 11.688 g of *sodium chloride* and 50 mg of *sodium azide*.
- as detector, a spectrophotometer set at 280 nm.

In the chromatogram obtained with the reference solution, the principal peak corresponds to IgG monomer and there is a peak corresponding to dimer with a retention time relative to monomer of about 0.85. Identify the peaks in the chromatogram obtained with the test solution by comparison with the chromatogram obtained with reference solution; any peak with a retention time shorter than that of dimer corresponds to polymers and aggregates. The preparation to be examined complies with the test if, in the chromatogram obtained with the test solution: for monomer and dimer, the retention time relative to the corresponding peak in the chromatogram obtained with reference solution is 1 ± 0.02 , the sum of monomer and dimer represents not less than 90 per cent of the total area of the chromatogram and polymers and aggregates represent not more than 3 per cent of the total area of the chromatogram. This requirement does not apply to products where albumin has been added as a stabilizer; for products stabilized with albumin, a test for distribution of molecular size is carried out during manufacture before addition of the stabilizer.

Anticomplementary activity: the consumption of complement is not greater than 50 per cent.

Prekallikrein activator: Not more than 35 IU/ml, calculated with reference to a solution containing 30 g/l of immunoglobulin.

Anti-A and anti B haemagglutinins: Carry out the tests for anti-A and anti-B haemagglutinins. If the preparation to be examined contains more than 30 g/l of immunoglobulin, dilute to this concentration before preparing the dilutions to be used in the test. The 1:64 dilutions do not show agglutination.

Water. Determined by a suitable method, such as the semi-micro determination of water, loss on drying or near infrared spectrophotometry, the water content is within the limits approved by the competent authority.

Sterility. It complies with the test for sterility.

Pyrogens. It complies with the test for pyrogens. Inject per kilogram of the rabbit's mass a volume equivalent to 0.5 g of immunoglobulin but not more than 10 ml per kilogram of body mass.

Antibody to hepatitis B surface antigen: Not less than 0.5 IU/g of immunoglobulin, determined by a suitable immunochemical method.

Storage

For the liquid preparation, store in a colourless glass container, protected from light, at the temperature stated on the label. For the freeze-dried preparation, store in a colourless glass container, under vacuum or under an inert gas, protected from light, at a temperature not exceeding 25°C.

Labeling

The label states:

- for liquid preparations, the volume of the preparation in the container and the protein content expressed in grams per litre,
- for freeze-dried preparations, the quantity of protein in the container,
- the amount of immunoglobulin in the container,
- the route of administration,
- for freeze-dried preparations, the name or composition and the volume of the reconstituting liquid to be added,
- the distribution of subclasses of immunoglobulin G present in the preparation,
- where applicable, the amount of albumin added as a stabilizer,
- the maximum content of immunoglobulin A [2], [6], [12], and [13] .

** For more details, refer to the General Information.*

Specific Immunoglobulin

1. Anti-D Immunoglobulin

Definition:

Human anti-D immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma from D-negative donors immunized against D-antigen. It contains specific antibodies against erythrocyte D-antigen and may also contain small quantities of other blood-group antibodies. Human normal immunoglobulin may be added.

It complies with the monograph on human normal immunoglobulin, except for the minimum number of donors and the minimum total protein content.

Stability:

For liquid preparations, an accelerated degradation test is carried out on each batch of the final product by heating it at 37°C for 4 weeks; the loss of anti-D activity after heating does not exceed 20 per cent of the initial value.

Potency:

Should be carried out according to current international pharmacopoeia.

Storage:

See *Human normal immunoglobulin*

Labeling:

See *Human normal immunoglobulin*. The label states the number of International Units per container [2], [6] & [17].

2. Anti-D immunoglobulin for Intravenous Use

Definition:

Human anti-D immunoglobulin for intravenous administration is a liquid or freeze- dried preparation containing immunoglobulins, mainly immunoglobulin G. It is obtained from plasma from D-negative donors immunized against D-antigen. It contains specific antibodies against erythrocyte D-antigen and may also contain small quantities of other blood-group antibodies. *Human normal immunoglobulin for intravenous administration* may be added.

It complies with the monograph on *Human normal immunoglobulin for intravenous administration*, except for the minimum number of donors, the minimum total protein content, the limit for osmolality and the limit for prekallikrein activator. For products prepared by a method that eliminates immunoglobulins with specificities other than anti-D where authorized, the test for antibodies to hepatitis B surface antigen is not required; a suitable test for Fc function is carried out.

Stability: for liquid preparations, an accelerated degradation test is carried out on each batch of the final product by heating it at 37°C for 4 weeks; the loss of anti-D activity after heating does not exceed 20 per cent of the initial value.

Potency:

Should be carried out according to current international pharmacopoeia.

Storage:

See *Human normal immunoglobulin for intravenous administration*.

Labeling:

The label states the number of International Units per container [2], [6] & [17].

3. Hepatitis A Immunoglobulin:

Definition:

Human hepatitis A immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma from selected donors having antibodies against hepatitis A virus. *Human normal immunoglobulin* may be added.

It complies with the monograph on *Human normal immunoglobulin*, except for the minimum number of donors and the minimum total protein content.

Storage:

See *Human normal immunoglobulin*.

Labeling:

See *Human normal immunoglobulin*. The label states the number of International Units per container [2] & [6].

4. Hepatitis B Immunoglobulin:

Definition:

Human hepatitis B immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from selected and/or immunized donors having antibodies against hepatitis B surface antigen. *Human normal immunoglobulin* may be added.

It complies with the monograph on *Human normal immunoglobulin*, except for the minimum number of donors and the minimum total protein content.

Storage:

See *Human normal immunoglobulin*.

Labeling:

See *Human normal immunoglobulin*. The label states the number of International Units per container [2] & [5].

5. Hepatitis B Immunoglobulin for Intravenous Use:

Definition:

Human hepatitis B immunoglobulin for intravenous administration is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. It is obtained from plasma from selected and/or immunized donors having antibodies against hepatitis B surface antigen. *Human normal immunoglobulin for intravenous administration* may be added.

It complies with the monograph on *Human normal immunoglobulin for intravenous administration*, except for the minimum number of donors and the minimum total protein content and the limit for osmolality.

Storage:

See *Human normal immunoglobulin for intravenous administration*.

Labeling:

See *Human normal immunoglobulin for intravenous administration*. The label states the minimum number of International Units of hepatitis B immunoglobulin per container [2] & [5].

6. Measles Immunoglobulin

Definition:

Human measles immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma containing specific antibodies against the measles virus. *Human normal immunoglobulin* may be added.

It complies with the monograph on *Human normal immunoglobulin*, except for the minimum number of donors and the minimum total protein content.

Storage:

See *Human normal immunoglobulin*.

Labeling:

See *Human normal immunoglobulin*. The label states the minimum number of International Units per container [2] & [6].

7. Rabies Immunoglobulin**Definition:**

Human rabies immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma from donors immunized against rabies. It contains specific antibodies neutralizing the rabies virus. *Human normal immunoglobulin* may be added.

It complies with the monograph on *Human normal immunoglobulin*, except for the minimum number of donors and the minimum total protein content.

Storage:

See *Human normal immunoglobulin*.

Labeling:

See *Human normal immunoglobulin*. The label states the minimum number of International Units per container [2] & [6].

8. Rubella Immunoglobulin**Definition:**

Human rubella immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma containing specific antibodies against rubella virus. *Human normal immunoglobulin* may be added.

It complies with the monograph on *Human normal immunoglobulin*, except for the minimum number of donors and the minimum total protein content.

Storage:

See *Human normal immunoglobulin*.

Labeling:

See *Human normal immunoglobulin*. The label states the minimum number of International Units per container [2] & [6].

9. Tetanus Immunoglobulin**Definition:**

Human tetanus immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma containing specific antibodies against the toxin of *clostridium tetani*. *Human normal immunoglobulin* may be added.

It complies with the monograph on *Human normal immunoglobulin*, except for the minimum number of donors and the minimum total protein content.

Storage:

See *Human normal immunoglobulin*

Labeling:

See *Human normal immunoglobulin*. The label states the minimum number of International Units per container [2], [6] & [17].

10. Varicella Immunoglobulin**Definition:**

Human varicella immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma from selected donors having antibodies against *herpes virus varicellae*. *Human normal immunoglobulin* may be added.

It complies with the monograph on *Human normal immunoglobulin*, except for the minimum number of donors and the minimum total protein content and, where authorized, the test for antibody to hepatitis B surface antigen.

Storage:

See *Human normal immunoglobulin*.

Labeling:

See *Human normal immunoglobulin*. The label states the minimum number of International Units per container [2] & [6].

11. Varicella Immunoglobulin for Intravenous Use

Definition:

Human varicella immunoglobulin for intravenous administration is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. It is obtained from plasma from selected donors having antibodies against *Human herpes virus* (varicella-zoster virus 1). *Human normal immunoglobulin for intravenous administration* may be added.

It complies with the monograph on *Human normal immunoglobulin for intravenous administration*, except for the minimum number of donors and the minimum total protein content and the limit for osmolality.

Storage:

See *Human normal immunoglobulin for intravenous administration*.

Labeling:

See *Human normal immunoglobulin for intravenous administration*. The label states the minimum number of International Units per container [2], [6] & [17].

12. Anti-T Lymphocyte Immunoglobulin for Human Use:

Refer to Anti-T Lymphocyte Immunoglobulin for human use, Animal (Ph Eur monograph 1928).

** For more details, refer to the General Information.*

Plasma for Fractionation

Refer to SFDA requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (www.sfda.gov.sa)

Annex 1. Immunochemical Methods

Immunochemical methods are based on the selective, reversible and non-covalent binding of antigens by antibodies. These methods are employed to detect or quantify either antigens or antibodies. The formation of an antigen-antibody complex may be measured using a variety of techniques. The provisions of this general method apply to immunochemical methods using labeled or unlabelled reagents, as appropriate.

The results of immunochemical methods depend on the experimental conditions and the nature and quality of the reagents used. It is essential to standardize the components of an immunoassay and to use, wherever available, international reference preparation for immunoassays.

The reagents necessary for many immunochemical methods are available as commercial assay kits, that is a set including reagents (particularly the antigen or the antibody) and materials intended for the in vitro estimation of a specified substance as well as instructions for their proper use. The kits are used in accordance with the manufacturers' instruction; it is important to ascertain that the kits are suitable for the analysis of the substance being examined, with particular reference to selectivity and sensitivity. Guidance concerning immunoassay kits is provided by the World Health Organization, *Technical Report Series* 658(1981).

Methods in which a labeled antigen or a labeled antibody is used

Methods using labeled substances may employ suitable labels such as enzymes, fluorophores, luminophores and radioisotopes. All work with radioactive materials must be carried out in conformity with national legislation and internationally accepted codes of practice for protection against radiation hazards.

Methods in which an unlabeled antigen or antibody is used

Immunoprecipitation methods Immunoprecipitation methods include flocculation and precipitation reactions. When a solution of an antigen is mixed with its corresponding antibody under suitable conditions, the reactants form flocculating or precipitating aggregates.

The ratio of the reactants that gives the shortest flocculation time or the most marked precipitation is called the optimal ratio, and is usually produced by equivalent amounts of antigen and antibody. Immunoprecipitation can be assessed visually or by light-scattering techniques (nephelometric or turbidimetric assay). An increase in sensitivity can be obtained by using antigen-or antibody coated particles (such as latex) as reactants.

In flocculation methods, stepwise dilutions of one of the reactants are usually used whereas in immunodiffusion (ID) methods the dilution is obtained by diffusion in a gel medium. Concentration gradients of one or both of the reactants are obtained, thus creating zones in the gel medium where the ratio of the reactants favours precipitation. Whereas flocculation methods are performed in tubes, immunodiffusion methods may be performed using different supports such as tubes, plates, slides, cells or chambers.

Where the immunoprecipitating system consists of one antigen combining with its corresponding antibody, the system is referred to as *simple*; when it involves related but not serologically identical reactants, the system is *complex* and where several serologically unrelated reactants are involved, the system is *multiple*.

In *simple diffusion methods*, a concentration gradient is established for only one of the reactants diffusing from an external source into the gel medium containing the corresponding reactant at a comparatively low concentration.

Single radial immunodiffusion (SRID) is a simple quantitative immunodiffusion technique. When the equilibrium between the external and the internal reactant has been established, the circular precipitation area, originating from the site of the external reactant, is directly proportional to the amount of the antigen applied and inversely proportional to the concentration of the antibody in the gel.

In *double diffusion methods*, concentration gradients are established in a neutral (inert) gel by allowing both reactants to diffuse into the gel from separate sites.

Comparative double diffusion methods are used for qualitatively comparing various antigens to versus a suitable antibody or *vice versa*. The comparison is based on the presence or absence of interaction between the precipitation patterns. Reactions of identity, non-identity or partial identity of antigens! antibodies can be distinguished.

Immunoelectrophoretic methods: Immunoelectrophoresis (IE) is a qualitative technique combining two methods: gel electrophoresis followed by immunodiffusion.

Crossed immunoelectrophoresis is a modification of the IE method. It is suitable for both qualitative and quantitative analysis. The first part of the procedure is an ordinary gel electrophoresis, after which a longitudinal gel strip, containing the separated fractions to be determined, is cut out and transferred to another plate. The electrophoresis in the second direction is carried out at an angle of 90° to the previous electrophoretic run in a gel containing a comparatively low concentration of antibodies corresponding to the antigens. For a given antibody concentration and gel thickness, the relationship between the area of the respective precipitation peaks and the amount of the corresponding antigen is linear.

Electroimmunoassay, often referred to as rocket immunoelectrophoresis, is a rapid quantitative method for determining antigens with a charge differing from that of the antibodies or vice versa. The electrophoresis of the antigen being determined is carried out in a gel containing a comparatively lower concentration of the corresponding antibody. The test material and dilutions of a standard antigen used for calibration are introduced into different wells in the gel. During electrophoresis, migrating peak-shaped precipitation zones originating from the wells are developed. The edge of the precipitate becomes stationary when the antigen is no longer in excess. For a given antibody concentration, the relationship between the distance traveled by the precipitate and the amount of antigen applied is linear.

Counter-immunoelectrophoresis is a rapid quantitative method allowing concentration gradients of external antigen and external antibody to be established in an electric field depending on the different charges. Dilutions of a standard for calibration and dilutions of the test material are introduced into a row of wells in a gel and a fixed amount of the corresponding reactant is introduced into an opposite row of wells. The titre of the test material may be determined as the highest dilution showing a precipitation line.

A number of modifications of crossed immunoelectrophoresis and electroimmunoassay methods exist. Other techniques combine separation of antigens by molecular size and serological properties.

Visualization and characterization of immunoprecipitation lines:

These may be performed by selective or non-selective stains, by fluorescence, by enzyme or isotope labeling or other relevant techniques. Selective staining methods are usually performed for characterization of non-protein substances in the precipitates. In translucent gels such as agar or agarose, the precipitation line becomes clearly visible in the gel, provided that the concentration of each of the reactants is appropriate.

Validation of the method

Validation criteria A qualitative immunochemical method is not valid:

1. if the antibody or antigen significantly discriminates between the test and standard, or if, for a labelled reactant, the corresponding reactant significantly discriminates between labeled and unlabelled compounds;
2. unless the method is unaffected by the assay matrix, that is, any component of the test sample or its excipients that can vary between samples. These may include high concentrations of other proteins, salts, preservatives or contaminating proteolytic activity;
3. unless the limit of quantitation is below the acceptance criteria stated in the individual monograph;
4. unless the precision of the assay is such that the variance of the results meets the requirements stated in the individual monographs;
5. if the order in which the assay is performed gives rise to systematic errors.

Validation methods: In order to verify the validation criteria, the validation design includes the followings:

1. the assay is performed at least in triplicate;
2. the assay includes at least three different dilutions of the standard preparation and three dilutions of sample preparation of presumed activity similar to that of the standard preparation;
3. the assay layout is randomized;

4. if the test sample is presented in serum formulated with other components, the standard is prepared in an identical manner;
5. the test includes measurements of non-specific binding of the labeled reactant;
6. for displacement immunoassay: (a) maximum binding (zero displacement) is determined; (b) dilutions cover the complete response range from values close to non-specific binding to maximum binding, preferably for both standard and test preparations.

Statistical calculation

To analyze the results, response curves for test and standard may be analyzed using a suitable method. Significant non-parallelism indicates that the antibody or antigen discriminates between test and standard and the results are not valid.

In displacement immunoassay, the values for non specific binding and maximum displacement at high test or standard concentration must not be significantly different. Differences may indicate effects due to the matrix, either inhibition of binding or degradation of tracer [2] & [6].

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