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Veterinary Immunosera

General Notices

(Immunosera for Veterinary Use, Ph. Eur. monograph 0030)

Veterinary Immunosera comply with the requirements of the European Pharmacopoeia monograph for Immunosera for Veterinary Use. These requirements are reproduced below.

The provisions of this monograph apply to the following immunosera.

Clostridium Tetani Antitoxin*

* Monograph of the European Pharmacopoeia

Ph Eur

DEFINITION

Immunosera for veterinary use are preparations containing immunoglobulins, purified immunoglobulins or immunoglobulin fragments obtained from serum or plasma of immunised animals. They may be preparations of crude polyclonal antisera or purified preparations.

The immunoglobulins or immunoglobulin fragments have the power of specifically neutralising the antigen used for immunisation. The antigens include microbial or other toxins, bacterial and viral antigens, venoms of snakes and hormones. The preparation is intended for parenteral administration to provide passive immunity.

PRODUCTION

GENERAL PROVISIONS

Immunosera are obtained from the serum or plasma of healthy animals immunised by administration of one or more suitable antigens.

The production method shall have been shown to yield consistently batches of immunosera of acceptable safety (*5.2.6*) and efficacy (*5.2.7*).

DONOR ANIMALS

The animals used are exclusively reserved for production of immunoserum. They are maintained under conditions protecting them from the introduction of disease, as far as possible. The donor animals, and any animals in contact with them, are tested and shown to be free from a defined list of infectious agents and re-tested at suitable intervals. The list of agents for testing includes not only those agents that are relevant to the donor animal, but also those that are relevant to the recipient target species for the product. Where the donor animals have not been demonstrated to be free from a relevant pathogen, a justification must be provided and a validated inactivation or purification procedure must be included in the manufacturing procedure. The feed originates from a controlled source. Where the donor animals are chickens, use chickens from a flock free from specified pathogens (*5.2.2*). Where applicable for the species used, measures are taken to avoid contamination with agents of transmissible spongiform encephalopathies.

As far as possible, animals being introduced into the herd are from a known source and have a known breeding and rearing history. The introduction of animals into the herd follows specified procedures, including defined quarantine measures. During the quarantine period the animals are observed and tested to establish that they are free from the list of agents relevant for the donor animals. It may be necessary to test the animals in quarantine for freedom from additional agents, depending on their known breeding and rearing history or any lack of information on their source.

Any routine or therapeutic medicinal treatment administered to the animals in quarantine or thereafter must be recorded.

IMMUNISING ANTIGEN

The principles described in the Production section of the general monograph *Vaccines for veterinary use (0062)* are applied to the production of the immunogen. The antigen used is identified and characterised. The starting materials used for antigen preparation must be controlled to minimise the risk of contamination with extraneous agents as described in general chapter *5.2.5*. The antigen may be blended with a suitable adjuvant. The immunogen is produced on a batch basis. The batches must be prepared and tested in such a manner that assures that each batch will be equally safe and free from extraneous agents and will produce a satisfactory, consistent immune response.

IMMUNISATION

The donor animals are immunised according to a defined schedule. For each animal, the details of the dose of immunising antigen, route of administration and dates of administration are recorded. Animals are kept under general health surveillance and the development of specific antibodies are monitored at appropriate stages of the immunisation process.

COLLECTION OF BLOOD OR PLASMA

Animals are thoroughly examined before each collection. Only healthy animals may be used as a donor animal. Collection of blood is made by venepuncture or plasmapheresis. The puncture area is shaved, cleaned and disinfected. The method of collection and the volume to be collected on each occasion are specified. The blood or plasma is collected in such a manner as to maintain sterility of the product. If the serum or plasma is stored before further processing, precautions are taken to avoid microbial contamination.

The blood or plasma collection is conducted at a site separate from the area where the animals are kept or bred and the area where the immunoserum is further processed.

Clear criteria are established for determining the time between immunisation and first collection of blood or plasma as well as the time between subsequent collections and the length of time over which collections are made. The criteria applied must take into account the effect of the collections on the health and welfare of the animal as well as the effect on the consistency of production of batches of the finished product over time.

The rate of clearance of any residues that may arise from the immunising antigen or medication given needs to be taken into account. In the case of the risk of residues from chemical substances, consideration could be given to the inclusion of a withdrawal period for the finished product. If the immunising agent consists of a live organism, the time between immunisation and collection may need to take into account the time required for the donor to eliminate the immunogen, particularly if any residual live organisms might be harmful to the recipient.

PREPARATION OF THE FINISHED PRODUCT

Several single plasma or serum collections from one or more animals may be pooled to form a bulk for preparation of a batch. The number of collections that may be used to produce a bulk and the size of the bulk are defined. Where pooling is not undertaken, the production procedure must be very carefully controlled to ensure that the consistency of the product is satisfactory.

The active substance is subjected to a purification and/or inactivation procedure unless omission of such a step has been justified and agreed with the competent authority. The procedure applied must have been validated and be shown not to adversely impair the biological activity of the product. The validation studies must address the ability of the procedure to inactivate or remove any potential contaminants such as pathogens that could be transmitted from the donor to the recipient target species and infectious agents such as those that cause ubiquitous infections in the donor animals and cannot be readily eliminated from these donor animals.

For purified immunosera, the globulins containing the immune substances may be obtained from the crude immunoserum by enzyme treatment and fractional precipitation or by other suitable chemical or physical methods.

Antimicrobial preservatives

Antimicrobial preservatives are used to prevent spoilage or adverse effects caused by microbial contamination occurring during use of a product. Antimicrobial preservatives are not included in freeze-dried products but, if justified, taking into account the maximum recommended shelf life after reconstitution, they may be included in the diluent for multidose freeze-dried products. For single-dose liquid preparations, inclusion of antimicrobial preservatives is not normally acceptable, but may be acceptable, for example where the same product is filled in single-dose and multidose containers and is for use in non-food producing species. For multidose liquid preparations, the need for effective antimicrobial preservation is evaluated taking into account likely contamination during use and the maximum recommended shelf life after broaching of the container.

During development studies the effectiveness of the antimicrobial preservative throughout the shelf life shall be demonstrated to the satisfaction of the competent authority.

The efficacy of the antimicrobial preservative is evaluated as described in general chapter *5.1.3. Efficacy of antimicrobial preservation*; for a multidose preparation, additional samples are taken to monitor the effect of the antimicrobial preservative over the proposed in-use shelf life. If neither the A criteria nor the B criteria can be met, then in justified cases the following criteria are applied to antisera for veterinary use: bacteria, no increase at 24 h and 7 days, 3 $\log_{10}$ reduction at 14 days, no increase at 28 days; fungi, no increase at 14 days and 28 days.

Addition of antibiotics as antimicrobial preservatives is not acceptable.

Unless otherwise prescribed in the monograph, the final bulk is distributed aseptically into sterile, tamper-evident containers which are then closed so as to exclude contamination.

The preparation may be freeze-dried.

In-process tests Suitable tests are carried out in-process, such as on samples from collections before pooling to form a bulk.

BATCH TESTS

The tests that are necessary to demonstrate the suitability of a batch of a product will vary and are influenced by a number of factors, including the detailed method of production. If a product is treated by a validated procedure for inactivation of extraneous agents, the test for extraneous agents can be omitted on that product with the agreement of the competent authority. Specific tests for extraneous agents may be required depending on the nature of the product and its use, in particular where the donor and recipient species are the same. A risk assessment should be conducted (see general chapter *5.2.5*), taking into account the nature of the preparation, its risk of contamination and the use of the product. If a product is treated by a validated procedure for inactivation of mycoplasmas, the test for mycoplasmas can be omitted on that product with the agreement of the competent authority.

Only a batch that complies with each of the relevant requirements given below under Identification, Tests and Potency and/or in the relevant specific monograph may be released for use. With the agreement of the competent authority, certain tests may be omitted where in-process tests give an equal or better guarantee that the batch would comply or where alternative tests validated with respect to the Pharmacopoeia method have been carried out.

Certain tests, e.g. for antimicrobial preservatives, for foreign proteins and for albumin, may be carried out by the manufacturer on the final bulk rather than on the batch, batches or sub-batches of finished product prepared from it. In some circumstances, e.g. when collections are made into plasmapheresis bags and each one is, essentially, a batch, pools of samples may be tested, with the agreement of the competent authority.

It is recognised that, in accordance with General Notices (section 1.1. General statements), for an established antiserum the routine application of the safety test will be waived by the competent authority in the interests of animal welfare when a sufficient number of consecutive batches have been produced and found to comply with this test, thus demonstrating consistency of the manufacturing process. Significant changes to the manufacturing process may require resumption of routine testing to re-establish consistency. The number of consecutive batches to be tested depends on a number of factors such as the type of antiserum, the frequency of production of batches, and experience with the immunoserum during developmental safety testing and during application of the batch safety test. Without prejudice to the decision of the competent authority in the light of information available for a given antiserum, testing of 10 consecutive batches is likely to be sufficient for the majority of products. For products with an inherent safety risk, it may be necessary to continue to conduct the safety test on each batch.

Animal tests In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. The criteria for judging tests in monographs must be applied in the light of this. For example, if it is indicated that an animal is considered to show positive, infected etc. when typical clinical signs occur then as soon as sufficient indication of a positive result is obtained the animal in question shall be either euthanised or given suitable treatment to prevent unnecessary suffering. In accordance with the General Notices, alternative test methods may be used to demonstrate compliance with the monograph and the use of such tests is particularly encouraged when this leads to replacement or reduction of animal use or reduction of suffering.

pH (*2.2.3*)

The pH of crude and purified immunosera is shown to be within the limits set for the products.

Formaldehyde

If formaldehyde is used for production of immunoserum, a test for free formaldehyde is carried out as prescribed under Tests.

Other inactivating agents

When other inactivation methods are used, appropriate tests are carried out to demonstrate that the inactivating agent has been removed or reduced to an acceptable residual level.

Batch potency test

If a specific monograph exists for the product, the test described under Potency is not necessarily carried out for routine testing of batches of antiserum. The type of batch potency test to be carried out will depend on the claims being made for the product. Wherever possible, *in vitro* tests must be used. The type of test required may include measurement of antibodies against specific infectious organisms, determination of the type of antibody (e.g. neutralising or opsonising). All tests must be validated. The criteria for acceptance must be set with reference to a batch that has been shown to comply with the requirements specified under Potency if a specific monograph exists for the product, and which has been shown to have satisfactory efficacy, in accordance with the claims being made for the product.

Total immunoglobulins

A test for the quantities of total immunoglobulins and/or total gammaglobulins and/or specific immunoglobulin classes is carried out. The results obtained must be within the limits set for the product and agreed with the competent authority. The batch contains not more than the level shown to be safe in the safety studies and, unless the batch potency test specifically covers all appropriate immunoglobulins, the level in the batch is not less than that in the batch or batches shown to be effective in the efficacy studies.

Total protein

For products where claims are being made which relate to the protein content, as well as demonstrating that the batch contains not more than the stated upper limit, the batch shall be shown to contain not less than that in the batch or batches shown to be effective in the efficacy studies.

Extraneous agents (*5.2.5*)

Immunosera for veterinary use are free from extraneous agents.

Water

Where applicable, the freeze-drying process is checked by a determination of water and shown to be within the limits set for the product.

IDENTIFICATION

The identity of the product is established by immunological tests and, where necessary, by determination of biological activity. The potency test may also serve for identification.

TESTS

The following requirements refer to liquid immunosera and reconstituted freeze-dried immunosera.

Foreign proteins

When examined by precipitation tests with specific antisera against plasma proteins of a suitable range of species, only protein from the declared animal species is shown to be present.

Albumin

Purified immunosera comply with a test for albumin. Unless otherwise prescribed in the monograph, when examined electrophoretically, purified immunosera show not more than a trace of albumin, and the content of albumin is in any case not greater than 30 g/L of the reconstituted preparation, where applicable.

Total protein

Dilute the preparation to be examined with a 9 g/L solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 mL. To 2 mL of this solution in a round-bottomed centrifuge tube add 2 mL of a 75 g/L solution of *sodium molybdate R* and 2 mL of a mixture of 1 volume of *nitrogen-free sulfuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, discard the supernatant and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulfuric acid digestion (*2.5.9*) and calculate the content of protein by multiplying by 6.25. The results obtained are not greater than the upper limit stated on the label.

Antimicrobial preservative

Determine the amount of antimicrobial preservative by a suitable physico-chemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Formaldehyde (*2.4.18*)

Where formaldehyde has been used in the preparation, the concentration of free formaldehyde is not greater than 0.5 g/L, unless a higher amount has been shown to be safe.

Sterility (*2.6.1*)

Immunosera for veterinary use comply with the test for sterility. When the volume of liquid in a container is greater than 100 mL, the method of membrane filtration is used wherever possible. If this method is used, incubate the media for not less than 14 days. Where the method of membrane filtration cannot be employed, the method of direct inoculation may be used. Where the volume of liquid in each container is at least 20 mL, the minimum volume to be used for each culture medium is 10 per cent of the contents of the container or 5 mL, whichever is the least. The appropriate number of items to be tested (*2.6.1*) is 1 per cent of the batch with a minimum of 4 and a maximum of 10.

Mycoplasmas (*2.6.7*)

Immunosera for veterinary use comply with the test for mycoplasmas.

Safety

A test is conducted in one of the species for which the product is recommended. Unless an overdose is specifically contraindicated on the label, twice the maximum recommended dose for the species used is administered by a recommended route. If there is a warning against administration of an overdose, a single dose is administered. For products to be used in mammals, use 2 animals of the minimum age for which the product is recommended. For avian products, use not fewer than 10 birds of the minimum age recommended. The birds are observed for 21 days. The other species are observed for 14 days. No abnormal local or systemic reaction occurs.

Extraneous agents (*5.2.5*)

Immunosera for veterinary use are free from extraneous agents. A test for extraneous agents is conducted using a suitable method (e.g. polymerase chain reaction (PCR)) or by inoculation of cell cultures (*2.6.37*) susceptible to pathogens of the species of the donor animal and cell cultures susceptible to pathogens of each of the recipient target species stated on the label. Specific tests for extraneous agents may be required depending on the nature of the preparation, its risk of contamination and the use of the product. In particular, specific tests for important potential pathogens may be required when the donor and recipient species are the same.

For immunosera of avian origin, if other suitable methods are insufficient to detect potential extraneous agentsthen inoculation of embryonated eggs from flocks free from specified pathogens (*5.2.2*) can be used.

POTENCY

Carry out a suitable test for potency.

Where a specific monograph exists, carry out the biological assay prescribed in the monograph and express the result in International Units per millilitre when such exist.

STORAGE

Protected from light, at a temperature of 5 ± 3 °C. Liquid immunosera must not be allowed to freeze.

LABELLING

The label states:

— that the preparation is for veterinary use;

— whether or not the preparation is purified;

— the minimum number of International Units per millilitre, where such exist;

— the volume of the preparation in the container;

— the indications for the product;

— the instructions for use including the interval between any repeat administrations and the maximum number of administrations that is recommended;

— the recipient target species for the immunoserum;

— the dose recommended for different species;

— the route(s) of administration;

— the name of the species of the donor animal;

— the maximum quantity of total protein;

— the name and amount of any antimicrobial preservative or any other excipient;

— any contra-indications to the use of the product including any required warning on the dangers of administration of an overdose;

— for freeze-dried immunosera:

  — the name or composition and the volume of the reconstituting liquid to be added;

  — the period within which the immunoserum is to be used after reconstitution.