**TESTS**

**Solution S.** Dissolve 4.0 g in carbon dioxide-free water R prepared from distilled water R and dilute to 100 ml with the same solvent.

**Appearance of solution.** Solution S is clear (2.2.1) and colourless (2.2.2, Method II).

**pH (2.2.3):** 9.0 to 9.6 for solution S.

**Sulphates (2.4.13):** maximum 50 ppm, determined on solution S. Use in this test 1.0 ml of sulphate standard solution (10 ppm SO₄) R and 12 ml of distilled water R.

**Ammonium (2.4.1):** maximum 10 ppm. Dilute 6 ml of solution S to 14 ml with water R. Prepare the standard using a mixture of 2.5 ml of ammonium standard solution (1 ppm NH₄) R and 7.5 ml of water R.

**Arsenic (2.4.2, Method A):** maximum 5 ppm, determined on 5 ml of solution S.

**Calcium (2.4.3):** maximum 100 ppm, determined on solution S. Prepare the standard using a mixture of 6 ml of calcium standard solution (10 ppm Ca) R and 9 ml of distilled water R.

**Heavy metals (2.4.8):** maximum 25 ppm. 12 ml of solution S complies with test A. Prepare the reference solution using a mixture of 2.5 ml of lead standard solution (2 ppm Pb) R and 7.5 ml of water R.

**ASSAY**

Dissolve 20 g of mannitol R in 100 ml of water R, heating if necessary, cool and add 0.5 ml of phenolphthalein solution R and neutralise with 0.1 M sodium hydroxide until a pink colour is obtained. Add 3.00 g of the substance to be examined, heat until dissolution is complete, cool, and titrate with 1 M sodium hydroxide until the pink colour reappears. 1 ml of 1 M sodium hydroxide is equivalent to 0.1907 g of Na₂B₄O₇·10H₂O.

**B. Solution S (see Tests) is acid (2.2.4).**

**TESTS**

**Solution S.** Dissolve 3.3 g in 80 ml of boiling distilled water R, cool and dilute to 100 ml with carbon dioxide-free water R prepared from distilled water R.

**Appearance of solution.** Solution S is clear (2.2.1) and colourless (2.2.2, Method II).

**pH (2.2.3):** 3.8 to 4.8 for solution S.

**Solubility in ethanol (96 per cent).** The solution is not more opalescent than reference suspension II (2.2.1) and is colourless (2.2.2, Method II).

Dissolve 1.0 g in 10 ml of boiling ethanol (96 per cent) R.

**Organic matter.** It does not darken on progressive heating to dull redness.

**Sulphates (2.4.13):** maximum 450 ppm. Dilute 10 ml of solution S to 15 ml with distilled water R.

**Heavy metals (2.4.8):** maximum 15 ppm. 12 ml of solution S complies with test A. Prepare the reference solution using a mixture of 2.5 ml of lead standard solution (2 ppm Pb) R and 7.5 ml of water R.

**ASSAY**

Dissolve 1.000 g with heating in 100 ml of water R containing 15 g of mannitol R. Titrate with 1 M sodium hydroxide, using 0.5 ml of phenolphthalein solution R as indicator, until a pink colour is obtained.

1 ml of 1 M sodium hydroxide is equivalent to 61.8 mg of H₃BO₃.

**01/2008:2113**

**BOTULINUM TOXIN TYPE A FOR INJECTION**

Toxinum botulinicum typum A ad injectabile

**DEFINITION**

Botulinum toxin type A for injection is a dried preparation containing purified botulinum neurotoxin type A which may be present in the form of a complex with haemagglutinins and non-toxic proteins. Botulinum neurotoxin type A or its haemagglutinin complex is prepared by a suitable purification process of the liquid supernatant from a broth-culture of a suitable strain of Clostridium botulinum type A.

The purified complexes consist of several proteins and can be of various sizes. The largest complex (relative molecular mass about 900 000) consists of a 150 000 relative molecular mass neurotoxin, a 130 000 relative molecular mass non-toxic protein and various haemagglutinins ranging between relative molecular mass 14 000 and 43 000.

The purified toxin moiety is composed of only the same 150 000 relative molecular mass neurotoxin as is found in the 900 000 relative molecular mass neurotoxin complex, which is initially produced as a single chain and further cleaved (nicked) by endogenous proteases into a fully active, disulphide-linked, 54 000 relative molecular mass light chain and a 97 000 relative molecular mass heavy chain. The preparation is reconstituted before use, as stated on the label.
PRODUCTION

GENERAL PROVISIONS

Production of the toxin is based on seed cultures, managed in a defined seed-lot system in which the ability to produce toxin is conserved. The production method must be shown to yield consistently product of activity and profile comparable to that of lots shown in clinical studies to be of adequate safety and efficacy.

The production method is validated to demonstrate that the product, if tested, would comply with the general test of abnormal toxicity (2.6.9) using not less than the maximum human clinical dose, in the presence of a suitable amount of specific botulinum type A antitoxin used for neutralisation.

The production method and stability of the finished product and relevant intermediates are evaluated using the tests below. Such tests include the specific toxin activity per milligram of protein of purified toxin in an appropriate functional model of toxin activity and may be supported by tests confirming the presence of botulinum toxin type A and, if appropriate, associated non-toxic proteins.

BACTERIAL SEED LOTS

A highly toxicogenic strain of C. botulinum of known toxin type A and confirmed absence of genes encoding other botulinum toxins (particularly botulinum toxin type B), with known origin and history, is grown using suitable media. The bacterial strain, used for the master seed lot, shall be identified by historical records that include information on its origin and the tests used to characterise the strain. These will include morphological, cultural, biochemical, genetic and serological properties of the strain. The master seed lot and the working seed lot, where applicable, must be demonstrated to have identical profiles. Only a seed lot that complies with the following requirements may be used.

Identification. Each seed lot is identified as containing pure cultures of C. botulinum type A bacteria with no extraneous bacterial or fungal contamination.

Microbial purity. Each seed lot complies with the requirements for absence of contaminating micro-organisms. The purity of bacterial cultures is verified by methods of suitable sensitivity. These may include inoculation into suitable media and examination of colony morphology.

Phenotypic parameters. Each seed lot must have a known fatty acid profile, sugar fermentation profile (glucose, lactose, mannose, etc.) and proteolytic activity and must demonstrate relevant lipase, lecithinase and gelatinase activity.

Genetic purity. Each seed lot must have information on the toxin gene sequence and comply with requirements for the absence of other genes encoding other toxin serotypes.

Production of active toxin. A bacterial strain producing a high yield of active toxin, as determined by an acute toxicity assay, is suitable. Seed lots should demonstrate a capability of producing at least a minimum toxicity level appropriate for the manufacturing process and scale.

MANUFACTURER’S REFERENCE PREPARATIONS

During development, reference preparations are established for subsequent verification of batch consistency during production and for control of the bulk purified toxin and finished product. They are derived from representative batches of botulinum toxin type A that are characterised as described under Bulk Purified Toxin.

The reference preparations are suitably characterised for their intended purpose and are stored in suitably sized aliquots under conditions ensuring their suitability.

BULK PURIFIED TOXIN

C. botulinum type A strain is grown anaerobically, in suitable media, from which cultures are selected for step-up incubations under a suitably controlled anaerobic atmosphere through the seed culture and bulk fermentation stages to allow maximum production of toxin. The toxin is purified by suitable methods to remove nucleic acids and components likely to cause adverse reactions.

Only a purified toxin that complies with the following requirements may be used in the preparation of the final bulk. For each test and for each product, limits of acceptance are established and each new purified toxin must comply with these limits.

Residual reagents. Removal of residual reagents used in purification steps is confirmed by suitable limit tests or by validation of the process.

Nucleic acids. Removal of nucleic acids is confirmed by suitable limit tests or by validation of the process.

Immunological identity. The presence of specific type A toxin is confirmed by a suitable immunochemical method (2.7.1).

Specific activity. The specific activity is confirmed in a mouse model of toxicity or by in vivo/ex vivo methods validated with respect to the LD50 assay and expressed in mouse LD50 units per milligram of protein. Specific activity must not be less than $1 \times 10^8$ mouse LD50 units per milligram of protein for the 150 000 relative molecular mass neurotoxin and must not be less than $3 \times 10^6$ mouse LD50 units per milligram of protein for the 900 000 relative molecular mass neurotoxin complex.

Protein. The total protein concentration is determined by a suitable method. An acceptable value is established for the product and each batch must be shown to comply with the limits.

Protein profile. Identity and protein composition are determined by polyacrylamide gel electrophoresis (2.2.31) under reducing or non-reducing conditions or by other suitable physicochemical methods such as size-exclusion chromatography (2.2.30), comparing with suitable reference standards.

Total viable count. It complies with the limits approved for the particular product.

FINAL BULK

The final bulk is prepared by adding approved excipients to the bulk purified toxin. The solution is filtered through a bacteria-retentive filter. If human albumin is added, it complies with the monograph on Human albumin solution (0255).

FINAL LOT

The final bulk is distributed aseptically into sterile, tamper-proof containers. Uniformity of fill is verified during filling and the test for uniformity of content (2.9.6) is not required. The containers are closed so as to prevent contamination.

Only a final lot that is within the limits approved for the particular product and is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

pH (2.2.3). The pH of the reconstituted product is within ± 0.5 pH units of the limit approved for the particular product.

Water: not more than the limit approved for the particular product.
IDENTIFICATION
The presence of botulinum toxin type A is confirmed by a suitable immunochemical method (2.7.1).

TESTS
Sterility (2.6.1). It complies with the test for sterility.
Bacterial endotoxins (2.6.14): less than 10 IU per vial.

ASSAY
The potency of the reconstituted product is determined by an LD50 assay in mice or by a method validated with respect to the LD50 assay. The potency is expressed in terms of the LD50 for mice or relative to the reference preparation. For determination of the LD50, graded doses of the product are injected intraperitoneally into groups of mice and the LD50 is calculated by the usual statistical methods (5.3) from the mouse lethality in each group. A suitable reference preparation is assayed in parallel; the potency of the toxin is expressed relative to the reference or the value found for the reference is within suitable limits defined in terms of the assigned potency.

After validation with respect to the LD50 assay (reference method), the product may also be assayed by other methods that are preferable in terms of animal welfare, including 1 of the following:
1. endopeptidase assay in vitro;
2. ex vivo assay using the mouse phrenic nerve diaphragm;
3. mouse bioassay using paralysis as the end-point.

For these other methods, the potency is calculated with respect to a suitable reference preparation calibrated in mouse LD50 units.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated potency. The test may be repeated but when more than 1 test is performed, the results of all valid tests must be combined in the estimate of potency.

LABELLING
The label states:
- the number of units of toxin per vial with a statement that units are product specific and not applicable to other preparations containing botulinum toxin type A,
- the name and the volume of the diluent to be added for reconstitution of a dried product.

01/2008:2262

BOVINE SERUM
Serum bovinum

DEFINITION
Liquid fraction of blood obtained from the ox (Bos taurus L.) and from which cells, fibrin and clotting factors have been removed.

Different types of bovine serum are used:
- adult bovine serum obtained at slaughter from cattle that are declared fit for human consumption;
- calf serum obtained at slaughter from animals, fit for human consumption, before the age of 12 months;
- new-born calf serum obtained at slaughter from animals before the age of 20 days;
- foetal bovine serum obtained from normal foetuses from dams fit for human consumption;
- donor bovine serum obtained by repeated bleeding of donor animals from controlled donor herds.

This monograph provides a general quality specification for bovine serum. Various measures are applied during the production of bovine serum aimed at obtaining a product that is acceptable as regards viral safety. No single measure, nor the combination of measures outlined below can guarantee complete viral safety but they rather reduce the risk involved in the use of serum in the manufacture of medicinal products. It is therefore necessary for the manufacturer of a medicinal product to take account of this when choosing the serum for a particular use by making a risk assessment.

PRODUCTION
All stages of serum production are submitted to a suitable quality assurance system.

Traceability of serum is maintained from the final container to the abattoir of origin (for blood collected from slaughtered animals) or to the herd of origin (for blood collected from donor animals).

Further guarantee of the safety and quality of serum may be ensured by the use of a controlled donor herd. Where serum is obtained from such a herd, the animals are subjected to regular veterinary examination to ascertain their health status. Animals introduced into the herd are traceable as regards source, 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 all agents and antibodies from which the donor herd is claimed to be free. It may be necessary to test the animals in quarantine for freedom from additional agents, depending on factors such as information available on their breeding and rearing history. It is recommended that animals in the herd should not be vaccinated against bovine viral diarrhoea virus. Tests are carried out for any agent and/or antibody from which the herd is claimed to be free.

Serum is obtained by separation of the serum from blood cells and clot under conditions designed to minimise microbial contamination. Serum from a number of animals is pooled and a batch number is allocated to the pool. Appropriate steps are taken to ensure homogeneity of the harvested material, intermediate pools and the final batch. Suitable measures (for example filtration) are taken to ensure sterility or a low bioburden. Before further processing, the serum is tested for sterility or bioburden.

Primary and specific tests for viral contaminants are carried out as described below.

A step or steps for virus inactivation/removal are applied to serum intended for production of immunological veterinary medicinal products. Unless otherwise justified and authorised for a particular medicinal product, a step or steps for virus inactivation/removal are applied to serum intended for production of human and non-immunological veterinary medicinal products.

INACTIVATION
The inactivation procedure applied is validated with respect to a suitable representative range of viruses covering different types (enveloped, non-enveloped, DNA, RNA viruses). The optimal choice of relevant and model viruses depends strongly on the specific inactivation/removal procedure; representative viruses with different degrees of resistance to the type of treatment must be included. Bovine viral diarrhoea virus must be included in the viruses used for validation. Serum free from antibodies against bovine viral diarrhoea virus is used in part or all of the validation studies.