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Guideline on the use of bovine serum in the manufacture of human biological medicinal products

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This guideline replaces Note for Guidance on the use of bovine serum in the manufacture of human biological medicinal products (CPMP/BWP/1793/02).

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Executive summary

This guideline outlines the general principles that should be applied to the control of the quality and safety of bovine serum used during the manufacture of human biological medicinal products. The original guideline (CPMP/BWP/1793/02) was adopted by CPMP in July 2003 and came into operation in October 2003. This revision affects mainly Sections 7.3.3 and 7.3.4 where the testing requirements for BVDV and anti-BVDV antibodies have been revised to be in accordance with the requirements applied for the production of immunological veterinary medicinal products (EMEA/CVMP/743/00-rev 2.).

1. Introduction (background)

The CHMP/CVMP Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products ('TSE Note for Guidance', ref. 1) recommends that when a manufacturer has a choice between the use of ruminant or non-ruminant materials, the use of non-ruminant material is preferred. However, it is recognised that some material of ruminant origin, namely bovine serum, may be an essential ingredient of the culture media of cells used in the production of many medicinal biological products.

Different risks are associated with the use of such raw materials. Indeed, the nature and quality of the bovine serum used in the manufacturing process can profoundly influence the quality of the finished product. In particular, the potential presence of extraneous agents in bovine serum represents a risk to the safety of the biological medicinal product. Consequently, it is strongly recommended that a risk analysis, taking into account the quality and properties of the serum batches and the impact of these sera on the quality of the finished product, be conducted by the manufacturers of biological medicinal products prior to use. In this guideline emphasis is made on the control of viral contaminants. The guideline should be read in conjunction with the Ph. Eur. monograph on bovine serum (2262).

2. Scope

This guideline outlines the general principles that should be applied to the control of the quality and safety of bovine serum used during the manufacture of human biological medicinal products including vaccines and biotech products. This includes serum used directly during production cell growth and serum used during cell growth prior to a production phase, for example in the growth of cells prior to vaccine production. Limited use of serum, such as in the establishment of a master cell bank, during cryopreservation or during a developmental phase, such as during transfection in the generation of genetically engineered cells, also falls within the scope of this guideline.

Recommendations included in this guideline can also be taken into consideration when bovine serum is used in the control of a medicinal product, since the quality of the serum included in the nutritive media for cells within a quality control procedure can play a major role in the reliability of the results obtained.

Viral safety and other testing may be carried out by the serum supplier, by the medicinal product manufacturer (serum user), by a contract laboratory or by more than one of these. This guideline is not intended to proscribe which party(ies) should carry out the testing but rather to define the testing which should be performed. It is the responsibility of the marketing authorisation applicant (MAA) of the medicinal product to ensure that testing is carried out to the required standard as it is the MAA who is ultimately responsible for the safety and quality of the medicinal product. Compliance with the TSE Note for Guidance (ref. 1) must be demonstrated.

Notwithstanding the above, efforts should be made to reduce the use of bovine serum or to replace its use with material of non-animal origin.

3. Legal basis

This guideline has to be read in conjunction with the introduction and general principles (4) and part I of the Annex I to Directive 2001/83 as amended.

4. Types and sources of serum

The most common type of serum used in the manufacture of a human biological medicinal product is foetal bovine serum (FBS). Foetal bovine serum is obtained from foetuses harvested in abattoirs from healthy dams fit for human consumption. There may be use of other bovine sera, such as new-born calf serum (obtained from calves under 20 days old) or donor bovine serum (obtained from animals less than 36 months old by repeat bleeding of donor animals from controlled standing herds).

As herd health status for exotic diseases is usually defined through the health status of the country of origin, the Office International des Epizooties (OIE) code should be followed to ensure freedom at source from exotic diseases (e.g. foot-and-mouth disease (FMD), Bluetongue). As the OIE code does not necessarily consider the emergency use of vaccines (live or inactivated) against exotic diseases, the exclusion of collection of serum from animals from the regions where such vaccination is performed, should be considered. Animals from which serum is sourced at time of slaughter have to pass ante- and post- mortem inspection and must be declared “fit for human consumption”.

For donor bovine serum, the health status of the donor herd should be well defined and documented. It is recommended not to vaccinate these herds against Bovine Viral Diarrhoea (BVD), in order to prevent any impact of vaccine-derived antibodies on the herd health control strategy.

Serum should be collected according to specified protocols by personnel trained in these procedures. Serum from other source animals (e.g. horse) is also used occasionally. Although this Note for Guidance does not apply specifically to such sera, the principles contained within are applicable.

In the case of serum obtained from abattoirs, it is not usually possible to demonstrate freedom from diseases such as BVD. Only if the supplier can certify that all of the source animals originate from farms, which are part of a regional or national control programme for the disease concerned and that there is active surveillance for the presence of the disease, can such a claim be made when supported by appropriate testing of the serum itself.

The traceability of serum from final container back to the abattoir of origin is of prime importance and a clear audit trail must be demonstrable including records of volumes at each stage.

Traceability of serum from final container to farm of origin should be ensured for donor herds used to obtain Donor Bovine Serum.

5. Preparation of batches

A batch of serum may contain serum derived from any number of animals. Once designated and given a batch number, a batch shall not be mixed with other batches, unless reprocessed and re-assigned. Each batch of serum should be filtered to remove bacteria and mycoplasmas. Appropriate steps should be taken to ensure good homogeneity of the harvested material, intermediate pools/bulks and the final batch. The serum supplier must document the process for harvesting material, the formation and blending of intermediate pools/bulks and the production and processing of the final batch of serum.

Compliance with the TSE Note for Guidance (ref. 1) must be demonstrated. If a TSE Certificate of Suitability has been issued by EDQM via the process of Certification of Suitability of Monographs of the European Pharmacopoeia, no further data on traceability and methods of harvest need be included in the MAA.

6. Certificate of analysis

The certificate provided by the serum supplier should state the catalogue number, the batch number, the country of origin of the source animals, the final batch volume, the date of manufacture of the batch and the shelf life. The serum supplier should demonstrate and certify that the serum is exclusively of bovine origin. The content of serum proteins and the physico-chemical properties of the serum will also generally be indicated along with the results of cell growth tests.

No bacteria, fungi, mycoplasma or viruses should ultimately be detected in the final batch of serum (see also 7.3.5.). The stage at which definitive testing is performed for the purposes of certification is discussed under Section 7 below.

All tests should be performed preferably in compliance with the requirements of the European Pharmacopoeia; if not, all alternative tests should be validated according to appropriate standards. All operations performed by the serum supplier should be controlled by a suitable quality assurance system such as GMP or ISO 9000 and documented by a suitably qualified person.

7. Testing for adventitious agents

A representative sample from each batch of serum should be tested as follows. Such testing should be performed after filtration of the batch to remove bacteria and mycoplasma but before any steps, which have been introduced specifically to inactivate or remove viruses.

7.1. Bacterial and fungal sterility tests

Each batch of serum should comply with the tests for sterility of the European Pharmacopoeia Monograph.

7.2. Tests for the presence of mollicutes

Each batch of serum should comply with the tests for the presence of mollicutes as described in the European Pharmacopoeia (tests for Mycoplasma).

7.3. Tests for the presence of viral contaminants

Tests for viral contaminants should be carried out prior to any steps taken to inactivate or remove viruses. Consideration should be given to the volume of serum that is to be tested and all tests must be appropriately controlled and validated.

7.3.1. General tests

General tests for virus detection should be performed on at least two distinct cell lines, one of which should be of bovine origin, and should be validated and in compliance with appropriate guidelines. The cell lines chosen should be capable of detecting haemadsorbing viruses such as parainfluenza virus 3 and cytopathic agents such as infectious bovine rhinotracheitis virus (IBRV). Control cells should be cultivated with a serum previously shown to be free of virus contamination.

7.3.2. Tests for specified viruses

Tests for specific viruses will depend on:

- the ability of the general test(s) to detect specific viruses
- the current epidemiological situation in the country of origin.

Specific tests for the following viruses should be considered:

- bluetongue and related orbiviruses
- bovine adenovirus
- bovine parvovirus
- bovine respiratory syncytial virus
- bovine viral diarrhoea virus
- rabies virus
- reovirus 3

The MAA should provide the necessary justification for the range of viruses tested.

Generally, if an infectious virus contaminant is detected in a batch of serum, then the serum should not be used for the manufacture of a human biological medicinal product (with the exception of 7.3.3 and 7.3.5 below).

7.3.3. Recommendation for bovine viral diarrhoea virus (BVDV)

BVDV is a highly prevalent infection of cattle and its presence in bovine serum cannot completely be avoided except in serum from specifically controlled donor herds or from cattle from BVDV-free geographic areas. In any case the presence of BVDV in a batch of serum should be tested before any viral inactivation/removal treatment is performed by an accepted assay for infectious virus. The assay should be suitable to detect cytopathogenic as well as non-cytopathogenic BVDV strains and staining of cell cultures with fluorescent antibody (FA) is recommended. Direct RT-PCR has limited value in the detection of infectious virus. The level of contamination, if present, should be quantified and must be below the level that has been shown to be effectively inactivated in the validation tests for inactivation treatment. If BVDV is detected, the serum must be re-tested for infectious virus after any inactivation/removal step(s) and used only if no infectious virus is detected, taking into account the comments below on anti-BVDV antibodies.

7.3.4. Detection of anti-BVDV antibodies

Anti-BVDV antibodies in bovine serum may mask the detection of BVDV in an infectious virus assay. Therefore, a validated test should be employed to detect such antibodies and an assessment made of their impact on the partial or complete neutralisation of any infectious BVDV virus that may be present in the serum, and of their impact on virus detection, and on the validation of the viral inactivation process. An impact on virus detection may be particularly critical in the case of residual infectivity potentially remaining after inactivation treatment. The assessment should therefore take into account the estimated residual virus in the product (virus burden before inactivation treatment vs. virus clearance by inactivation treatment).

7.3.5. Other bovine viruses

Methods based on the detection of viral DNA suggest that bovine polyoma virus (BPyV) is a common contaminant of bovine serum. Serum manufacturers and users are encouraged to apply infectivity assays for BPyV and to investigate methods for the inactivation/removal of BPyV in order to limit or eliminate infectious virus from batches of serum[1].

Serum suppliers and users should also be aware of emerging bovine viruses and are similarly encouraged to investigate the presence of such agents in bovine serum and to take appropriate action to eliminate or reduce the presence of any novel virus in serum.

Viruses are part of the risk analysis described in Section 1 of this Guideline.

8. Tests for toxicity-cell growth

An appropriate cell line should be used for testing each batch of serum for cell growth. The cell line chosen may depend on the intended use of the serum. These tests should be performed using the final batch of serum after any viral inactivation step or other processing.

9. Viral inactivation

Due to the risk of viral contamination associated with the use of bovine serum, it is strongly recommended, in addition to direct testing for viruses, to inactivate the serum by a validated and efficacious treatment. The use of non-inactivated serum should be justified[2].

Reference should be made to the CPMP Note for Guidance on Virus Validation Studies: the Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses (ref. 2). For validation of the inactivation/removal step(s), the rationale for the choice of viral strains must be indicated. Representatives of different viral families (enveloped or non-enveloped viruses, DNA or RNA viruses) and representatives of viruses with different degrees of resistance to the type(s) of treatment used should be included. BVDV should be used in the validation along with a serum batch that is free of detectable antibodies against BVDV.

For validation of inactivation/removal, virus should be spiked into the serum, incubated at 37°C for 1 hour and titrated before the inactivation/removal step is performed. For an inactivation step, kinetics of inactivation should be measured.

Gamma irradiation is the most commonly used method for viral inactivation of serum as a means of obtaining a safe but biologically active product. However, other validated approaches are acceptable. Whatever the process, the validation study has to determine the consistency and effectiveness of the process while maintaining serum performance.

When inactivation by irradiation is employed, the virus validation study should include a validation of the irradiation process:

a) determine the optimal temperature,

[1] The EMA acknowledges that current infectivity assays for bovine polyoma virus are difficult to interpret and are not widely available. As a consequence, it is not the intention of the EMA to require, for the time being, testing of bovine serum for bovine polyoma virus. The statement on bovine polyoma virus has been included to inform serum manufacturers and users about BPyV and forewarn that the Authorities may review their position when a suitable infectivity assay or more information about contamination events becomes available,

[2] It is understood that the non-inactivated serum meets the same criteria as the inactivated serum when tested for sterility and absence of mycoplasma and viral contaminants.

b) establish a standard packaging configuration,

c) establish a representative distribution of dosimeters capable of assessing the effective dose reached in the mass of the product whatever its position during the treatment,

d) determine the minimum and maximum radiation exposure or dose received by the product itself,

e) establish a radiation dose range that protects product integrity while maximising inactivation of microbial contaminants. The validation study must therefore demonstrate the actual dose received throughout the mass of the serum.

10. Dossier requirements for Marketing authorisation application / variations

All data relating to the content of the certificate required by the serum user, the identification of different sources, the tests and controls carried out on each batch of serum, the name of the company performing the controls, the criteria of acceptance or rejection of the batches by the serum supplier and the serum user should be included in the marketing authorisation dossier.

A change in serum supplier or a change in any inactivation/removal process should be the subject of a variation for which appropriate comparability data for the drug substance / drug product will be required as appropriate (ref. 3). When changing from non-irradiated to irradiated serum or when a new irradiation plant (addition or replacement) is introduced, the variation application should also include relevant information on the virus validation study as mentioned in section 9.

References

1. Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and Veterinary Medicinal Products (EMA/410/01 rev. 3)

2. Note for Guidance on Virus Validation Studies: the Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses (CPMP/BWP/268/95)

3. Note for Guidance on Biotechological/biological products subject to changes in their manufacturing process, ICH Q5E (CPMP/ICH/5721/03)