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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE (CVMP)

REVISED GUIDELINE ON

REQUIREMENTS AND CONTROLS APPLIED TO BOVINE SERUM USED IN THE PRODUCTION OF IMMUNOLOGICAL VETERINARY MEDICINAL PRODUCTS

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This guideline replaces the revised guideline on Requirements and Controls applied to Bovine Serum (Foetal or Calf) used in the production of Immunological Veterinary Medicinal Products (EMEA/CVMP/743/00-Rev.1)

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REVISED GUIDELINE ON REQUIREMENTS AND CONTROLS APPLIED TO BOVINE SERUM USED IN THE PRODUCTION OF IMMUNOLOGICAL VETERINARY MEDICINAL PRODUCTS

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EXECUTIVE SUMMARY

This Guideline outlines the tests and inactivation treatments, which should be applied to bovine serum to ensure acceptable quality and to minimise the risks of transmitting infectious diseases. It should be noted that this Guideline is advisory by nature and should be read in conjunction with the requirements for substances of animal origin in the European Pharmacopoeia and relevant EU guidelines.

1. INTRODUCTION (BACKGROUND)

Starting materials of animal origin are necessary for the production of immunological veterinary medicinal products (IVMPs). Attempts should be made to reduce the use of bovine serum in the production of IVMPs and whenever possible the use of non-ruminant materials is preferred.

It is however recognised that materials of animal origin, including bovine serum are still essential ingredients of the cell culture media used in the production of many IVMPs. Different risks are associated with the use of such starting 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 consequence, 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 vaccine manufacturers prior to use. This should result in a number of batches of bovine serum being selected for the production of different types of vaccines.

2. SCOPE

The presence of extraneous agents in bovine serum certainly represents a major risk to the quality of the finished product. Therefore this Guideline will focus mainly on the risk due to extraneous agents
Testing before and after inactivation may be carried out by the serum supplier, by the manufacturer, by a contract laboratory or by more than one of these. This Guideline is not intended to prescribe which parties should carry out the testing but rather to define the testing which should be done at each stage of processing and the relevant quality standards that apply. It is recognised that not all serum suppliers, contract laboratories or manufacturers will necessarily possess the expertise and facilities required to perform all of the testing specified. The tests should be carried out in accordance with GMP, GLP or ISO 9001 principles; it is the responsibility of the manufacturer to ensure that the testing is carried out to the required standard. It is therefore strongly recommended that the testing is performed by the manufacturers themselves. Where this is not practical, testing can be devolved to the serum supplier or a contract laboratory; but responsibility for providing the necessary data to demonstrate compliance with the relevant quality standards rests with the manufacturer. It is essential that all available information regarding the quality of the serum be known by the vaccine manufacturer to enable the manufacturer to conduct a risk assessment on the use of the serum. It is essential that IVMP manufacturers manage the potential risk posed by the use of bovine serum by using selected batches of bovine serum for the production of different types of vaccines. The selection of these serum batches must be based on a risk analysis taking into consideration the biological properties of the serum and the intended use of the IVMP.

Considering the risk of pestiviruses in bovine serum, the highest risk will be with live and inactivated vaccines indicated for use in pestiviruses susceptible species (cattle, small ruminants and pigs). Of these, the greatest risk is associated with the use of live vaccines in pregnant pestiviruses susceptible females. Manufacturers should take into account that the production of the vaccine virus in pestivirus vaccines may be influenced by interfering BVD antibodies in the bovine serum used for production.

3. LEGAL BASIS

This Guideline concerns the application of Title II of Annex I to Directive 2001/82/EC as amended with a view to addressing the data requirements that need to be fulfilled for each new batch of bovine serum used in the manufacture of immunological veterinary medicinal products.

4. MAIN GUIDELINE TEXT

4.1. SOURCE

There are a range of bovine sera used by veterinary IVMP manufacturers (see also definitions):

- Adult Bovine Serum
- Calf Serum (under 12 months)
- New-Born Calf Serum (under 20 days)
- Foetal Bovine Serum
- Donor Bovine Serum (up to 36 months)

4.2. PREPARATION OF BATCHES

Products of animal origin should be prepared in a homogeneous manner, designated with a batch number.

4.3. ASSAYS AND CONTROLS TO BE CARRIED OUT EITHER BY THE VACCINE MANUFACTURER OR UNDER THEIR RESPONSIBILITY.

A number of samples from each batch of serum should be used for the following tests.

4.3.1. Bacterial and fungal sterility tests

The serum batch complies with the requirements of the tests for sterility of the European Pharmacopoeia Monograph. It is recommended to carry out these tests after filtration of the batch and before inactivation.

4.3.2. Tests for the presence of mollicutes

The serum batch complies with the requirements of the tests for the presence of mollicutes as described in the European Pharmacopoeia Monograph (tests for Mycoplasma) applied to the culture method. It is recommended to carry out these tests after filtration of the batch and before inactivation.

4.3.3. Tests for the presence of viral contaminants

4.3.3.1. General and Specific Tests

The combination of general and specific tests to be carried out should be capable of detecting viruses inducing viraemia and transplacental infection such as Bovine Adenovirus, BVD Virus (see below), Parvovirus, Bovine Respiratory Syncytial Virus, Reovirus, Parainfluenza 3, IBR and those responsible for diseases exotic to Europe (such as Bluetongue). Methods such as PCR and RT-PCR can usefully be used to increase the probability of detecting viral contaminants. If such tests are used they should be demonstrated to have a sensitivity and specificity at least equivalent to the conventional tests. In addition, the Manufacturer should be able to clarify whether or not any nucleic acid detected originates from infectious particles.

4.3.3.2. Tests to detect Bovine Viral Diarrhoea virus

These tests should be carried out, a first time, before the inactivation treatment to assess the infectious titre of Bovine Viral Diarrhoea Virus potentially present to ensure it is below the level that has been shown to be effectively inactivated in the validation tests for inactivation treatment. Secondary tests should be performed after the inactivation treatment at which time no virus should be detected in the final serum batch. These tests could be omitted if no virus is detected before inactivation treatment.

The serum to be tested for virus isolation is incorporated in a nutritive medium used for cultivation of bovine cells sensitive to Pestiviruses. After 3 passages of the cells inoculated, an immuno-cytochemical technique is applied to the cells with a reference serum monospecific (polyclonal or a pool of monoclonal antibodies) for BVD virus. It is recommended to perform such an immuno-cytochemical technique on microplates to increase the number of clones of cells, which are observed in the wells. If virus is isolated from the serum tested, it has to be titrated directly from the bovine serum batch to ensure it is below the level that has been shown to be effectively inactivated in the validation tests for inactivation treatment.

In addition to the tests recommended in this Guideline, other validated methods such as PCR and RT-PCR can usefully be used to increase the probability of detecting viral contaminants. If such tests are used they should be demonstrated to have a sensitivity and specificity at least equivalent to the conventional tests. In addition, the Manufacturer should be able to clarify whether or not any nucleic acid detected originates from infectious particles.

Control cells are used for each test, cultivated with a bovine serum controlled and inactivated.

4.3.3.3. Tests to detect BVD antibodies

The samples are tested using a validated technique to detect BVD antibodies. The tests to detect BVD antibodies are important to be carried out to perform a pertinent risk assessment in regard to the potential impact of the presence of BVD antibodies in bovine serum on partial or complete neutralisation of possible virus present and on the validation process.

4.4. INACTIVATION TREATMENT

Due to the risk associated with the use of bovine serum in contaminating the finished IVMP, it is absolutely necessary, in addition to controls performed on each batch of serum, to inactivate the serum by validated and efficacious treatments for increased reduction of potential undetectable organisms. For validation, the rationale of the choice of the viral strains must be indicated, including representatives of different viral families (enveloped or naked viruses, DNA or RNA viruses) and representatives of viruses with different degrees of resistance to various types of treatments. The following viruses may be used for the validation of the inactivation procedure: BVD virus, IBR virus, one of the bovine enteroviruses, bovine adenovirus, one of the Reoviruses (REO) and Porcine Parvovirus (PPV). A check for pestiviruses must be included.

The titration of the chosen viruses should be carried out (before inactivation treatment) after incubation at 37°C for 1 hour with the serum which will be submitted to the inactivation treatment. It is recommended to use gamma radiation as a means of obtaining a safe but biologically active product. In consequence, the validation study has to determine the consistency and efficiency of the process while maintaining the product performance.

For inactivation by irradiation, the validation study has to

determine the optimal temperature,
establish a standard packaging configuration,
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,
set specific time limits in relation to the dose received,
determine the minimum and maximum radiation exposure or dose received by the product itself and
f) 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. Inactivation of the bottled serum batch with a minimum guaranteed dose of 30 kiloGray (kGy) is an efficacious treatment to inactivate most micro-organisms and/or viruses present (30 kGy is equivalent to 3 Mrad).

For inactivation by means other than the application of minimum of 30 kGy to the serum in each bottle, the validation studies undertaken must be suitable to demonstrate the extent to which the process to be applied is appropriate, effective and reproducible.

DEFINITIONS

Adult Bovine Serum: bovine serum is derived from bovine blood collected post mortem from cattle that are declared fit for slaughter and for human consumption.
Calf Serum (under 12 months): produced in a similar way to Adult Bovine Serum.
New-Born Calf Serum (under 20 days).
Fœtal Bovine Serum: obtained from fœtuses from cattle declared fit for slaughter and for human consumption.
Donor Bovine Serum (up to 36 months): this is produced by repeat bleeding of donor animals from controlled standing herds. It is recommended that these herds are not vaccinated against BVD

REFERENCES

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

Annex I: Flow chart of tests to be carried out at the different stages of the process of production of Bovine Serum.

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Tests to be carried out at the different stages of the process of production of Bovine Serum