

VICH, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products.

WORKING GROUP : Biologicals Quality Monitoring.

TOPIC: *Test on the absence of extraneous viruses*

PRELIMINARY DRAFT

GUIDELINE

FOR THE TESTING OF BIOLOGICAL PRODUCTS

ON

THE ABSENCE OF EXTRANEIOUS VIRUSES

TABLE OF CONTENTS.

INTRODUCTION.

1. Objective of the guideline
2. Scope of the guideline.
3. Background.
4. General principles.
5. Glossary

PROPOSED TEXT OF GUIDELINE.

1. Definition of samples to be used for testing.
2. General test procedure.

Annexes:

1. List of extraneous agents
2. Precise description of the specific diagnostic test methods.
3. Present national requirements and proposal for harmonisation.

INTRODUCTION.

1. Objective of the guideline.

It is important that biological products for veterinary use are free of contaminants, notably viral agents. Potential sources of contamination are the viral and bacterial strains used for the production of the active ingredient(s) and the starting materials of animal origin used in the production of the active ingredient and / or in the assembly of the finished product. Consequently it is necessary to demonstrate that extraneous agents are not present in biological products nor in the materials required for their manufacture, through the use of accepted testing procedures and sampling methods and subject to the limitations of the test

The purpose of the guideline is to provide a framework for establishing the tests for extraneous agents which should be undertaken on all materials of animal origin used in the production of veterinary immunological products for use in mammals. It shall also provide precise information on the method and conditions of the tests to determine the absence of extraneous viruses in these substances.

2. Scope of the guideline.

The scope of the guideline is the test for the absence of extraneous viruses in biological products for veterinary use, intended for mammals and to the starting materials of animal origin used in the manufacture of these products.

NOTE: The test for the absence of extraneous viruses in immunological veterinary medicinal products used in poultry are not considered in this document because of the differences between the two categories of product. It may also be considered to include products used for fish.

3. Background.

Note: *It is open for discussion whether or not the background information needs to be as detailed as described. If a detailed description of the backgrounds of the test is provided, this may help in the correct interpretation of the tests. In the present document it is given to increase awareness that the freedom of extraneous agents has to be ensured by a complex of measures of which the test on extraneous viruses forms only a part, although a critical part.*

The materials used in the manufacture of biological products for veterinary use can be divided into two main categories:

1. Viral and bacterial strains and mammalian cell substrates used in the production of the active ingredients.
2. Starting materials of biological origin used in the production of the active ingredients and/or in the assembly of the finished product.

At this moment substances of animal origin e.g. serum, trypsin and serum albumin, are essential in the manufacture of veterinary immunological products, either as ingredients of culture media etc. or as added constituents of vaccines or diluents. It is however recommended to reduce, wherever practicable, the use of substances of animal origin.

Normally, certain restrictions may be placed upon the use of such substances to minimise the risk associated with pathogens that may be present in them e.g.: their use is not generally acceptable except where they are sterilised by a suitable, validated method.

Where the use of such substances has been shown to be essential and sterilisation is not possible, it will be required to test and monitor the source animals for freedom from infectious agents and/or test these substances for the absence of contaminants. In the case of inactivated vaccines, the method used for inactivation of the vaccine strain may also be validated for inactivation of possible contaminants from substances of animal origin.

Present methods of testing for extraneous agents of substances of animal origin are described in the European Pharmacopoeia monograph 62 (1995) and in the Code of Federal Regulations 9CFR 113. The Japanese test requirements were provided by Dr. Itoh. An analysis of these requirements and the differences between them was made by Brian Roberts (Schering-Plough UK) and Francois Lacoste,(Merial, France) and the results are shown in Annex 1.

In addition attention shall be given to the OIE Manual of Standards for Diagnostic Tests and Vaccines.

Note: No references to the exact name of the documents in which the Japanese requirements are described are known at this stage.

4. General principles.

All viral seed materials and cell substrates must be thoroughly tested for freedom from extraneous agents, as described below. All bacterial seeds must be tested for purity. Tests should be carried out for possible contaminating agents from the source species of the material and the target animal for the finished product.

As indicated above, for other starting materials e.g. serum, the first consideration should always be to minimise the risk through avoiding the use of materials that could be contaminated with extraneous agents. Where this is not possible, it is preferable to source the material from a known source, with the animals tested and monitored for freedom from some or all of the infectious agent in question and then conduct a minimal range of check tests on the substance. Where the source is not well controlled, the material has to be subjected to a validated inactivation procedure and /or tested for freedom from extraneous agents. In all cases, as a minimum, the source animal species and the country or possibly countries of origin of these source animals must be known as it is not possible to assess the risks and the testing that may be required without this information.

The following aspects of materials of biological origin have to be critically evaluated:

Source:

It is important to carefully evaluate what are the risks of contamination and the risks for the proposed recipient species. Account must be taken of the animal diseases occurring in the country of origin of the animals used as a source of the substance, the potential infectious diseases occurring in the source species and the proposed recipient species.

The risk related to the animal diseases occurring in the country of origin of the substances and to the potential infectious diseases occurring in the source species taken into relation to the proposed recipient species must be carefully evaluated. The strictest possible selection criteria must be applied, in particular for substances for use in products intended for the same species and for substances of bovine, caprine, ovine, porcine and equine origin.

Preparation:

Substances of animal origin must be prepared from a homogenous bulk designated with a batch number. A batch may contain substances derived from as many animals as desired but once it has been defined and given a batch number no material may be added to it.

All batches of substances shall be shown to be free from contaminants as described below and / or shall be subjected to a validated inactivation procedure.

Inactivation:

The inactivation procedure chosen shall be shown to be capable of reducing the titre of certain potential contaminants in the substance concerned by at least 10^6 . If this reduction in titre cannot be shown experimentally, kinetic studies for the inactivation procedure must be carried out and shown to be satisfactory, taking into account the possible level of contamination. The list of potential contaminating organisms that the

procedure must be shown to be capable of inactivating must be appropriate to the particular species of origin of the substance. The inactivation of the most hardy agent can be considered as adequate. The evidence for the efficacy of the procedure, which must relate to the current circumstances, may take the form of references to published literature or experimental data generated by the manufacturer.

NOTE: The following text as mentioned in Directive 81/852/EEC can also be considered:

If the presence of adventitious agents is detected or suspected, the corresponding material shall be discarded or used in very exceptional cases only when further processing of the product ensures their elimination and/or inactivation. Elimination and/or inactivation of such adventitious agents shall be demonstrated

The overall testing regime used should consist of a series of sufficiently sensitive validated methods providing a high probability of detecting an agent if it is present. This objective will be achieved by applying tests of a general nature that may be expected to detect a broad selection of agents which are complemented by specific tests to detect individual agents for which there is a particular risk of occurrence. These agents - only of viral nature - are listed in Annex 2.

Any batch of substance found to contain living agents, that should not be present in the substance, is unsatisfactory and is either discarded or, when justified, reprocessed and shown to be satisfactory.

5. Glossary

CELL-SEED SYSTEM (CELL-BANK SYSTEM)

A system whereby successive final lots (batches) of a product are manufactured by culture in cells derived from the same master cell bank (master cell seed). A number of containers from the master cell bank (master cell seed) are used to prepare a working cell bank (working cell seed).

CELL LINES

Cultures of cells that have a high capacity for multiplication *in vitro*. In diploid cell lines, the cells have essentially the same characteristics as those of the tissue of origin. In continuous cell lines, the cells are able to multiply indefinitely in culture and may be obtained from healthy or tumoral tissue.

CONTROL CELLS

A quantity of cells set aside, at the time of virus inoculation, as uninfected cell cultures. The uninfected cells are incubated under similar conditions to those used for the test

BATCH (FINAL LOT)

A collection of closed, final containers or other final dosage units that are expected to be homogeneous and equivalent with respect to risk of contamination during filling or preparation of the final product. The dosage units are filled, or otherwise prepared, from the same final bulk vaccine, freeze-dried together (if applicable) and closed in one continuous working session. They bear a distinctive number or code identifying the final lot (batch). Where a final bulk vaccine is filled and/or freeze-dried on several separate sessions, there results a related set of final lots (batches) that are usually identified by the use of a common part in the distinctive number or code; these related final lots (batches) are sometimes referred to as sub-batches, sub-lots or filling lots.

MASTER CELL SEED (MASTER CELL BANK)

A culture of cells distributed into containers in a single operation, processed together and stored in such a manner as to ensure uniformity and stability and to prevent contamination. A master cell bank (master cell seed) is usually stored at - 70 °C or lower.

MASTER SEED LOT

A culture of a micro-organism distributed from a single bulk into containers and processed together in a single operation in such a manner as to ensure uniformity and stability and to prevent contamination.

PRIMARY CELL CULTURES

Cultures of cells obtained by trypsinization of a suitable tissue or organs. The cells are essentially identical to those of the tissue of origin and are no more than five *in vitro* passages from the initial preparation from the animal tissue.

Note: This definition needs further discussion.

SEED-LOT SYSTEM:

A seed-lot system is a system according to which successive batches of a product are derived from the same master seed lot. For routine production, a working seed lot may be prepared from the master seed lot.

WORKING CELL SEED (WORKING CELL BANK)

A culture of cells derived from the master cell bank (master cell seed) and intended for use in the preparation of production cell cultures. The working cell bank (working cell seed) is distributed into containers, processed and stored as described for the master cell bank (master cell seed).

WORKING SEED LOT

A culture of a micro-organism derived from the master seed lot and intended for use in production. Working seed lots are distributed into containers and stored as described above for master seed lots.

PROPOSED TEXT FOR A HARMONISED GUIDELINE.

Note: *The following proposed text is only intended as a suggested format of the guideline*

1. DEFINITION OF SAMPLES TO BE USED FOR TESTING.

1.1. Seed materials.(virus and bacteria)

DISCUSSION POINT:

- The size of the sample shall be at least 10 times the MOI used in 1 production vessel.
- It could also left being not specified.
- It is also proposed to use the dose used in virus neutralisation tests (100 TCID₅₀)
- The sample shall be defined as a number of vials.

The Master Seed micro-organisms, to be used for the preparation of the active ingredient, shall be neutralized with a monospecific antiserum which is shown to be free of antibodies against the contaminating organisms to be tested for.

1.2. Cells

↳ not homologous

Normally the cells used in manufacture of veterinary vaccines shall be obtained from established cell-lines.

Note:

For most mammalian vaccines, the use of primary cells in production is not desirable.. If there exists no alternative, these cells must be obtained from a herd free from specified pathogens, with complete protection from infection or disease (for example, barriers, filters on air inlets, suitable quarantine before introduction of animals). Reference is made to the lists of infectious agents for different animal species.

The herd is shown to be free from relevant specified pathogens. All the breeding stock in the herd or flock intended to be used to produce primary cells for vaccine manufacture is subject to a suitable monitoring procedure (**to be defined**) including regular serological checks carried out at least twice a year and two supplementary serological examinations performed in 15 per cent of the breeding stock in the herd between the two checks mentioned above.

Wherever possible, particularly for mammalian cells, a seed-lot system is used. The maximum passage level of the cells beyond the MCS may be at least 20 but also more provided adequate data are provided to support this higher passage level.

Each master cell seed shall be examined.

The sample tested shall cover all the sources of cells used for the manufacture of the batch. No batches of vaccines manufactured using the cells may be released if any one of the checks performed produces unsatisfactory results.

The size of the sample shall depend on the number of cells present in the material but shall contain at least 1×10^5 per ml. or alternatively monolayers totalling at least 75 cm² (**to be defined**) are required. The size of the sample taken in case of suspension culture shall be defined, and shall probably be at least 2 ml – 5 ml

The cells are frozen and thawed at least three times and then centrifuged to remove cellular debris. The supernatant and / or the sediment is used in the actual test.

1.3. Other substances of animal origin.

Any solids are dissolved or suspended in a suitable medium (**to be defined**) in such a way as to create a solution or suspension containing at least 30 per cent w/v of the substance to be examined. If the substance is not soluble or where cytotoxic reactions occur, a lower concentration may be used. (**to be defined**)

The total quantity of the material to be used in the test shall be at least (**to be defined**)

2. GENERAL TEST PROCEDURE.

2.1. Substrates.

The substrates used for testing the absence of extraneous agents, either in seed materials, cells or in other substances of animal origin shall consist of at least three different types of sensitive cells and include at least primary cells from the same species as the substances to be examined. (**to be defined**)

- primary cells of the source species;*
- cells sensitive to viruses pathogenic for the species for which the vaccine is intended;
- cells sensitive to pestiviruses.

Note: It has been remarked that using only bovine cells to detect pestiviruses may not be ideal. Pestiviruses affecting ruminants will grow well in bovine cells. Porcine pestivirus will grow better in porcine cells. It would therefore be better to use porcine cells for porcine pestiviruses

* The use of primary cells may be problematic. It might be considered to use defined diploid cell-lines which can be expected to have a similar sensitivity. It is also to be considered if it would be useful to specify under which conditions primary cells must be used and under what conditions alternative cell systems can be used instead. The production may be done using hollow fiber flow systems or using serum free media.

2.2. Cultivation of the substrate cells.

The monolayers shall be prepared in (*type of culture vessels to be defined*)

The monolayers to be used in the test shall cover an area of at least cm², (*to be defined*) and shall be prepared and maintained using medium and additives, and grown under similar conditions – as far as is feasible - to those used for the preparation of the vaccine. At least the culture media, the cell system and incubation temperature shall be mimicked.

The monolayers are maintained in culture for a total of at least 28 days. At least two subcultures are made at 7-day intervals, unless the cells do not survive for this length of time, when the subcultures are made on the latest day possible.

2.3. Examination of the cells.

The cells are observed regularly, at least every second day and a proportion of the cells is passaged at least twice. (*to be defined*) The frequency of observation may depend on the type of virus (slow growing or rapid growing).

The cells are observed regularly (*to be defined*) for 28 days for the presence of cytopathic effects. At the end of each 7 day period, a proportion of the cells are used for the following tests :

a. Detection of cytopathic viruses.

Two monolayers of at least cm² (*to be defined*) each are stained with an appropriate (*to be defined*) cytological stain. (e.g. HE or Giemsa stain) The entire area of each stained monolayer is examined for any inclusion bodies, abnormal numbers of giant cells or any other lesion indicative of a cellular abnormality which might be attributable to a contaminant. The method described in the EP would be acceptable.

b. Detection of haemadsorbent viruses.

Monolayers totalling at least cm² are washed several times with an appropriate buffer (*to be defined*) and a sufficient volume of a suspension of

suitable red blood cells (*to be defined*) added to cover the surface of the monolayer evenly. After different incubation times (*to be defined*) cells are examined for the presence of haemadsorption. The method described in the EP would be acceptable.

c. Detection of specified viruses.

For viruses which are unlikely to be detected by the general tests described above, specific test methods must be applied. These are indicated under the heading "Specific tests" in the relevant tables.

DISCUSSION POINT: *If an agent can be detected by a general test it shall not be necessary to use a specific test. This should be reflected in the tables. Only one test method shall be indicated.*

Tests are carried out for contaminants specific for the species of origin of the seed virus, the cells or the other substances and for the species for which the product is intended. Sufficient cells (*to be defined*) on suitable supports (*to be defined*) are prepared to carry out tests for the agents specified. The material shall be obtained after at least 2 passages and a surface of at least 4 sqcm shall be examined. Suitable positive controls and negative controls are included in each test. The cells are subjected to suitable tests, for example using immunochemical methods as e.g. fluorescein-conjugated antibodies or other techniques, especially those suitable for testing large number of samples. (*to be discussed*)

Note: It may be useful also to consider co-cultivation methods to detect extraneous agents

The use of experimental animals shall be minimised and if required the need to use animal tests shall be justified

ANNEX 1. LIST OF EXTRANEIOUS AGENTS

The extraneous agents which must be taken into account when considering which extraneous agents have to be tested for are listed in the tables, according to animal species.

The presence of an agent shall be determined as matter of principle The presence of an agent in the table does not necessarily mean that a specific test for that agent must be carried out, but a justification for not carrying out a specific test for an agent will be required.

The types of justification that can be given include:

- a) Disease does not occur in country of origin (and material could not have been contaminated by this agent, subsequently). Supporting documentary evidence must be provided.
- b) Substance in question cannot be contaminated with this agent, e.g. agent does not cross placenta.
- c) The applicant has shown that the general test can be shown to detect the specified agent, with equivalent sensitivity.

For points (a) and (b), these justifications may be given by referring to published literature.

In addition to consideration of relevance of the agents listed in the table, changes in the disease situation in the country of origin of materials and, particularly, new emerging disease shall be taken into account.

It has been remarked that TSE agents are not included in the list and that it might be necessary to apply such lengthy tests retroactively when the need for testing is justified.

Discussion points:

1. Inclusion of following text in respect of scrapie agent : Demonstration of freedom requires monitoring for absence of disease for at least two years and examination of brain material from culled animals by histology and/or examination for scrapie associated fibrils.
2. Table for Vero cells
3. Sensitivity of tests on Bovine Adenovirus subtype 2, which only grows on primary calf testes cells.
4. Borna disease virus can only be detected by intracerebral inoculation of rabbits or newborn rats

Table 1 Extraneous agents relevant for bovines and for material of bovine origin.

AGENT	SPF HERD	CELLS AND SEEDS	
		GENERAL TESTS	SPECIFIC TESTS
Adenovirus , subgroups 1 and 2	+	+	
Akabane virus	+		+
Aujeszky's Disease virus	+	+	
Bluetongue virus	+		+
Epizootic haemorrhagic Disease virus	+		+
Bovine Corona virus	+	+	
Bovine ephemeral Fever virus	+		
Bovine Herpes virus type 1, 2 and 4	+	+	
Bovine Leukaemia virus	+		+
Bovine Papilloma virus	+		
Bovine Parvo virus	+	+	
Bovine Papular Stomatitis virus - pseudocowpox virus	+		
Bovine Respiratory Syncytial virus	+		
Bovine Rota virus	+		
Bovine Viral Diarrhoea virus	+		+

Cowpox virus , vaccinia virus	+	+	+
Foot and Mouth Disease virus types A,O,C, Asia 1, SAT 1, SAT 2, SAT 3	+		+
Lumpy Skin Disease virus	+	+	
Malignant Catarrhal Fever (African form) virus	+		
Malignant Catarrhal Fever (European form) virus	+		
Parainfluenza 3 virus	+	+	
Coxiella burnetti (Q-fever)	+	+	
Rabies virus	+		+
Rift Valley Fever virus	+		+
Rinderpest virus	+	+	
Vesicular Stomatitis Virus (Indiana and New Jersey)	+	+	

Table 2 Extraneous agents relevant for ovines and caprines and of material of ovine or caprine origin.

SPECIES	AGENT	SPF HERD	CELLS AND SEEDS	
			GENERAL TESTS	SPECIFIC TESTS
O + C	Adenovirus , subgroups 1 and 2	+	+	
O + C	Akabane virus	+		+
O + C	Aujeszky's Disease virus	+	+	
O + C	Bluetongue virus	+		+
	Epizootic haemorrhagic Disease virus	+		+
O + C	Bovine Herpes virus type 1, 2 and 4	+	+	
O	Bovine Leukaemia virus	+		+
O	Bovine Papilloma virus	+		
O	Bovine Viral Diarrhoea virus	+		+
O + C	Cowpox virus , vaccinia virus	+	+	+
O + C	Foot and Mouth Disease virus types A,O,C, Asia 1, SAT 1, SAT 2, SAT 3	+		+
O + C	Parainfluenza 3 virus	+	+	
O + C	Rift Valley Fever virus	+		+
C	Caprine Arthritis Encephalitis virus	+		+

C	Caprine Herpes virus	+	+	
O	Border Disease virus	+		+
O	Borna Disease virus	+		+
O	Ovine Pulmonary Adenomatosis virus	+		
O	Louping Ill virus	+		+
O	Nairobi Sheep Disease virus	+		+
O	Ross River virus	+	+	
O	Scrapie	+		
O + C	Ecthyma contagiosum virus (ORF virus)	+	+	
O + C	Maedi Visna virus	+		
O + C	Peste des petits ruminants virus	+	+	

O : Ovine
C : Caprine

Table 3 Extraneous agents relevant for porcines and of material of porcine origin.

AGENT	SPF HERD	CELLS AND SEEDS	
		GENERAL TESTS	SPECIFIC TESTS
African Swine Fever virus	+		+
Aujeszky's Disease virus	+	+	
Bovine Viral Diarrhoea virus	+		+
Classical Swine Fever virus	+		+
Encephalomyocarditis virus	+	+	
Foot and Mouth Disease virus types A,O,C, Asia 1, SAT 1, SAT 2, SAT 3	+		+
Haemagglutinating encephalomyelitis virus	+	+	
Transmissible Gastro Enteritis virus and Porcine Respiratory Corona virus	+	+	
Porcine Adeno viruses	+	+	
Porcine Cytomegalo virus	+		+
Porcine Epidemic Diarrhoea virus	+		
Porcine Entero viruses (Incl. Teschen-Talfan virus)	+	+	
Porcine Influenza virus	+		+
Porcine Parvo virus	+		+
Porcine Respiratory and Reproductive Syndrome virus	+		+