VICH, International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products.

WORKING GROUP: **Biologicals Quality Monitoring.**

TOPIC: ***Test on the presence of extraneous agents***

DRAFT TEXT

GUIDELINE

FOR THE TESTS

ON

THE PRESENCE OF EXTRANEOUS VIRUSES

IN VETERINARY VIRAL VACCINES

Date: MARCH 2002, VS.09

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1. INTRODUCTION.

The guideline describes the test methods used to ensure that vaccines are not contaminated with extraneous agents. Such test methods may be used for the starting materials, intermediate products and finished product dependent on the requirements to be met for new vaccines in order to get a marketing authorisation.

1.1 Objective of the guideline.

It is essential that vaccines for veterinary use are free of contaminants, notably viral agents. Potential sources of contamination are the viral strains, the cells and other 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 viruses are not present in veterinary vaccines nor in the starting 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 description of the test methods to detect the presence of extraneous viruses which shall be used on all materials of animal origin used in the production of veterinary viral vaccines, on intermediate and finished product.

Harmonisation of the test methods will be the first step in the harmonisation of the regulatory requirements for veterinary vaccines.

1.2. Background.

The materials used in the manufacture of veterinary viral vaccines include:

- Viral strains
- Cell substrates
- Starting materials of animal origin which may be used in the production of the active ingredient and / or in the assembly of the finished product.

This guideline deals specifically with the test methods to detect the presence of extraneous viruses.

Present methods of testing for extraneous viruses of substances of animal origin are described in the European Pharmacopoeia monograph 62 (1995), the USA Code of Federal Regulations 9CFR 113 , the Pharmaceutical Affairs Law and related laws in Japan nd the OIE Manual of Standards for Diagnostic Tests and Vaccines.

1.3. Scope of the guideline.

The scope of the guideline is to provide guidance on the methods to determine the presence of extraneous viruses in:

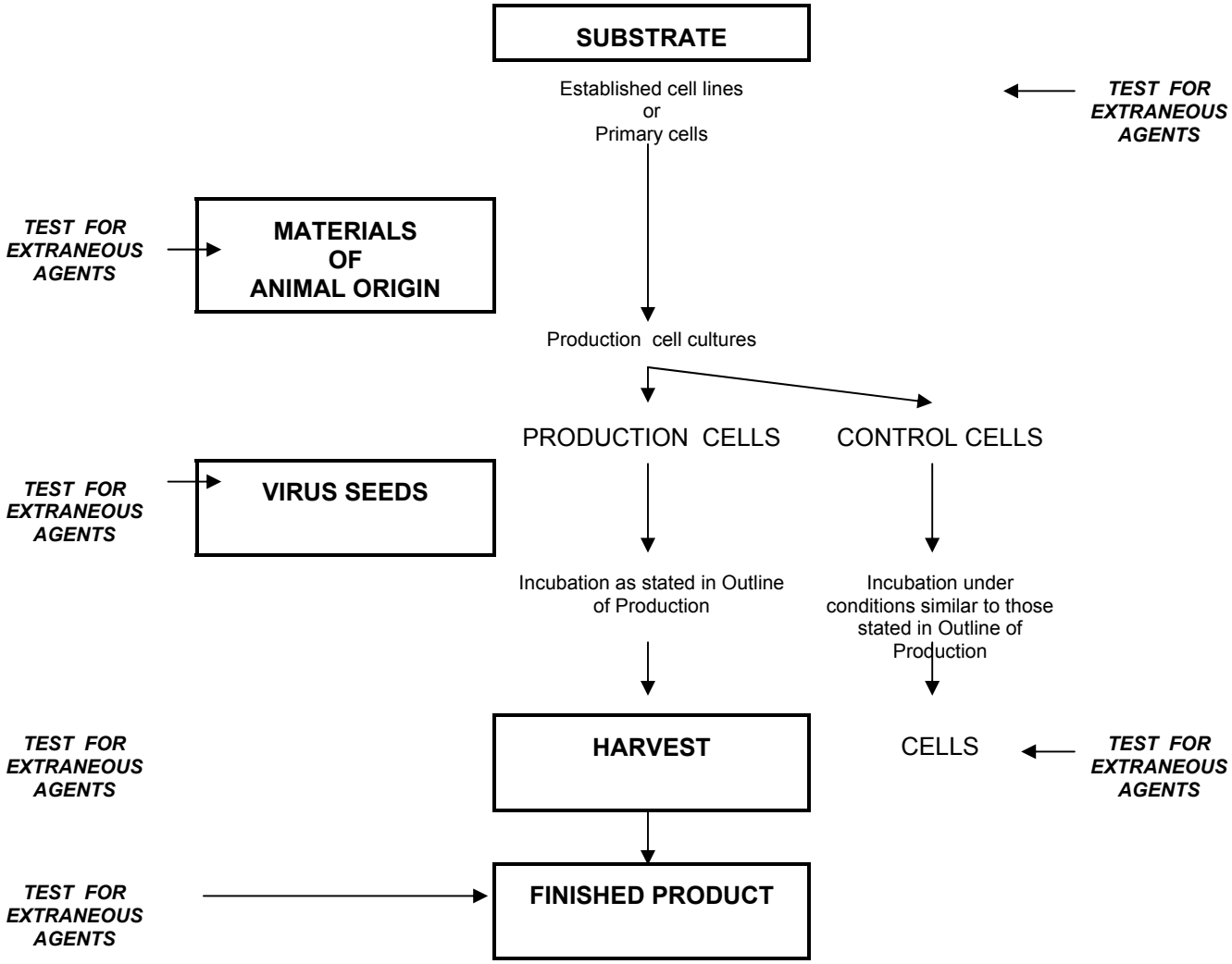
- Master Seed Virus c.q. Working Seed Virus
- Master Cell Seed c.q. Working Cell Seed
- Bovine serum
- Other starting materials of animal origin
- Intermediate products (in-process controls)
- Finished product

SCHEMATIC OUTLINE

OF THE STAGES OF PRODUCTION AT WHICH TESTING OF VETERINARY

VIRAL VACCINES FOR THE PRESENCE OF EXTRANEOUS VIRUSES MAY BE

REQUIRED



2. MAMMALIAN VIRAL VACCINES PRODUCED IN ESTABLISHED CELL-LINES.

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2.1. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN VIRUS SEEDS.

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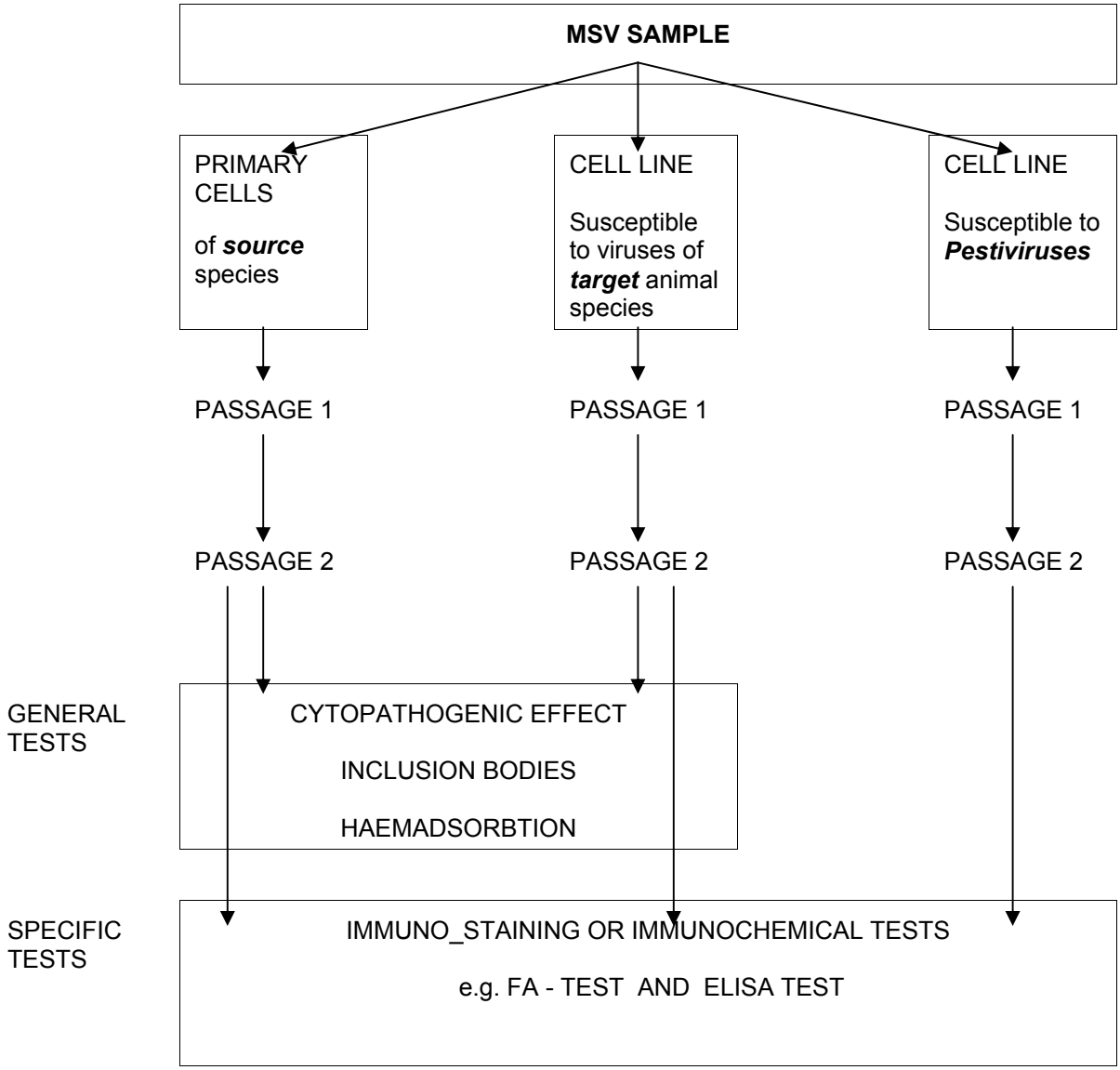
2.1. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN VIRUS SEEDS

SCHEMATIC OUTLINE OF TEST FOR EXTRANEOUS VIRUSES

MASTER SEED VIRUS



2.1.1. *General*.

Samples of all viral seed materials are tested to the extent possible for the presence of extraneous viruses that may originate from:

- the source species of the material.
- from those animal species to which the seed material may have been exposed to e.g. during passaging.

and also

- for the presence of possible contaminating viruses to which the target animal species is susceptible to infection.

NOTE:

> The extent of testing for specific viruses that potentially may have been introduced by exposure of the MSV to materials of a different origin than that of the MSV will depend on the available documentation on the origin, history and treatment of these materials.

If during one or more of the preparatory stages of the testing for extraneous agents an additional process is used e.g. adsorption it will be necessary to demonstrate that this process does not affect the sensitivity of the test and the test results.

In exceptional cases and when justified inoculation/serology tests in sero negative animals may be used .

2.1.2. Samples

Normally the samples shall be taken from the Master Seed Virus. However, there may be instances in which it should be permitted to use material obtained by a further passage of the MSV. This may be the case when e.g. it is necessary to update the testing of the MSV for a vaccine which is already on the market and of which the quantity of MSV has become very limited, This material should have the lowest possible passage level necessary to obtain sufficient material for the test and not exceed 5 passages of that MSV.

The Master Seed Virus shall - when necessary - be neutralised with a monoclonal antibody or a polyclonal specific antiserum containing sufficiently high levels of neutralising antibody to the virus present in the Master Seed Virus. When a polyclonal specific antiserum is used, it shall be free of antibodies against those viruses for which presence the Master Seed is tested. To that end , such an antiserum will be prepared, wherever possible, with an antigen that

- is not derived from any passage level of the virus isolate giving rise to the Master Seed Virus.
- has been passaged on different cells
- has been adequately purified.

If this is not possible the antiserum may be prepared with an earlier isolate of the virus concerned.

NOTE: If the MSV cannot be completely neutralised, alternative validated methods to eliminate the MSV may be used when duly justified.

2.1.3. Substrates.

The substrates used for testing the presence of extraneous agents shall consist of one or more types of sensitive cells, provided that the three following conditions are met:

- primary cells of the source species or, when justified, cells that are sensitive to a suitable range of viruses that may be present in the source species;
- cells sensitive to viruses pathogenic for the target species for which the vaccine is intended;
- cells sensitive to pestiviruses. The sensitivity of these cells shall be demonstrated by the use of positive controls prepared with a non-cytopathic strain of . Bovine Viral Diarrhoea virus.

Where relevant, other cells or substrates e.g. eggs, sensitive to other relevant viruses that may potentially be present in the virus seed ,as a result of passaging the virus strain in cells of other animal species shall be used.

The test methods using embryonated eggs are described in the Guideline for Testing for Presence of Extraneous Viruses in Avian Viral Vaccines.

2.1.4. Test method

2.1.4.1. Preparation of the substrate cells.

The monolayers of cells of the appropriate sensitivity to be used in the test shall cover an area of at least 70 cm^2 **.**

2.1.4.2. Inoculum.

An inoculum of 1 ml of the MSV shall be used to inoculate at least 8 monolayers of suitable cells with a surface of 20 cm^2 each.

> **Note:** The number of monolayers depends on the number of different types of erythrocytes used for haemadsorbtion testing.

2.1.4.3. Procedure.

2.1.4.3.1. General tests to detect presence of extraneous viruses.

2.1.4.3.1.1. Observation of cell cultures.

All cell cultures are maintained for at least 21 days, during which at least 2 subcultures are made at 7 days intervals, unless the cells do not survive for this length of time, when the subcultures shall be made on the latest day possible.

They are examined as follows:

- All cultures shall be observed at least every second working day
- At the end of the last subculture

- monolayers are examined for cytopathogenic effect in accordance with the method described in section 2.5.1.

- monolayers are examined for haemadsorbtion in accordance with the method described in section 2.5.2.

2.1.4.3.2. Tests to detect presence of specific viruses.

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

2.1.4.3.2.1. Preparation of the substrate cells.

The monolayers of cells of the appropriate sensitivity to be used in the test shall cover an area of at least 70 cm^2.

The monolayers are maintained in culture for a total of at least 21 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.

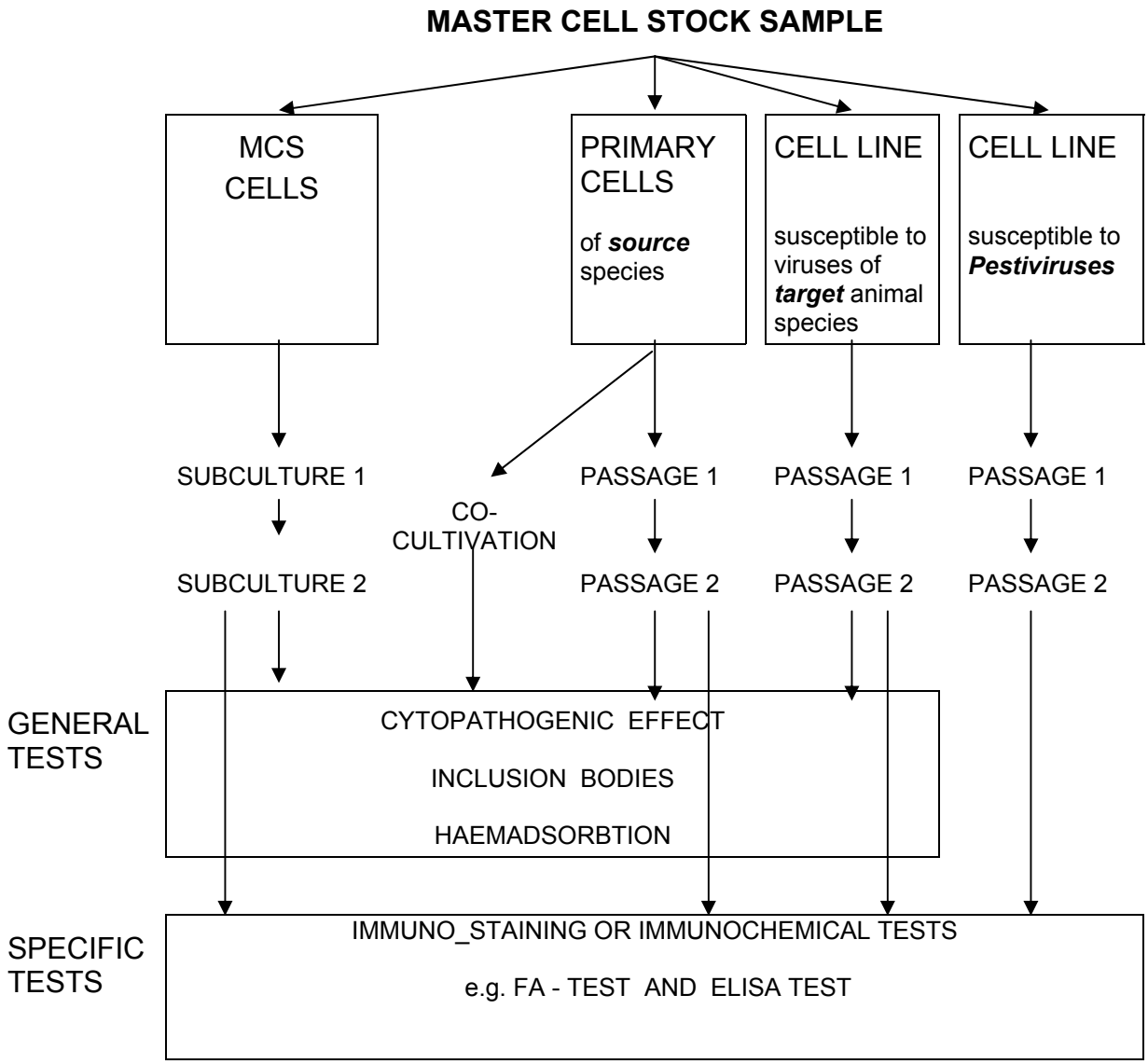
2.1.4.3.2.2. Examination for specified viruses.

This shall be done in accordance with section 2.6.

2.2. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN CELL STOCKS

SCHEMATIC OUTLINE OF TEST FOR EXTRANEOUS VIRUSES.

MASTER CELL STOCK.



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2.2.1. General.

The cell stock is tested to the extent possible for the presence of extraneous agents. Tests should be carried out for possible contaminating viruses that may originate from:

- the source species of the cells.
- from materials of animal origin used in the preparation of the Master Cell Stock.

and also

- for the presence of possible contaminating viruses to which the target animal species is susceptible to infection. The extent of testing for specific viruses that may have been introduced by exposure of the MCS to materials of animal origin will depend on the available documentation on the origin, history and treatment of these materials.

2.2.2. Samples

The size of the sample shall depend on the number of cells present in the cell seed material but shall contain at least 1×10^5 cells per ml. or sufficient cells to produce a monolayer of at least 70 cm^2
Alternatively monolayers with a total surface of at least 70 cm^2 may be used.

The size of the sample taken in the case of suspension cultures of non anchorage dependent cells shall be at least 1×10^5 cells per ml. or at least 1 ml. of the suspension culture, whichever sample contains the largest number of cells.

2.2.3. Substrates

The substrates used for testing the presence of extraneous agents shall consist of at least one or more different types of sensitive cells provided that the 3 following conditions are met:

- primary cells of the source species, provided they are more sensitive than available non-primary cells ,except in the case of testing well-known established cell lines of simian origin. In the latter case the test may be done on another established cell line of simian origin.

- cells sensitive to viruses pathogenic for the target species cells

- cells sensitive to pestiviruses. The sensitivity of these cells shall be demonstrated by the use of positive controls prepared with a non-cytopathic strain of Bovine Viral Diarrhoea virus.

2.2.4. Test method.

The test to determine the presence of extraneous agents in cells consists of three different stages:

- the test on subcultures of the MCS.
- the test on passaged cultures of the MCS
- the test using co-cultivation of the MCS

2.2.4.1. The test on the subcultures of the MCS

2.2.4.1.1. Preparation of the cell cultures.

The Master Cell Stock is used to establish monolayers in suitable culture vessels.

The monolayers to be used in the test shall cover an area of at least 70 cm^2 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. The monolayers shall be confluent for not more than 70% at moment of inoculation. The medium used shall be that described in the relevant Outline of Production.

2.2.4.1.2. Procedure.

At least 2 subcultures are made of the monolayers with an interval of 7 days or as frequently as may be necessary for suitable growth. The cell-cultures are maintained for at least 21 days during which period the cultures are examined regularly for cytopathogenic effect.

At least 7 days after the initiation of the last subculture the monolayers are examined:

- for cytopathogenic effect in accordance with the method described in section 2.5.1.
- for haemadsorbtion in accordance with the method described in section 2.5.2.
- for the presence of specific extraneous agents in accordance with the method described in section 2.5.6.

2.2.4.2. **The test on the passaged cultures of the MCS.**

2.2.4.2.1. Preparation of the cell cultures.

The Master Cell Stock is used to establish monolayers with a surface of at least 140 cm^2.

In the case of non-anchorage dependent cells suitable alternative methods have to be established.

2.2.4.2.2. Procedure.

2.2.4.2.2.1. Cell passage.

The following text is proposed:

The cells of the monolayers of each cell type used and supernatant are harvested and 50% of the cells and 50% of the supernatant are three times subjected to freeze-thawing and subsequently mixed with the remaining 50% of the cells and supernatant. 1 ml of the resulting material is inoculated on each of two monolayers of each of the cell cultures, with a surface of > 70 $cm^{2.}$,

to replace the following text:

The cells of the monolayers of each cell type used, are subjected to freezing-thawing (3 times). The material (1 ml) is then inoculated on each of two monolayers of each of the cell cultures, with a surface of > 70 $cm^{2.}$,

After incubation for at least 7 days the cells are collected and treated in the same manner. The material is inoculated into new monolayers of the same cell type and incubated for a period of 7 days.
At the end of this period of 14 days the monolayers are examined:

- for cytopathogenic effect in accordance with the method described in section 2.5.1.
- for haemadsorbtion in accordance with the method described in section 2.5.2.
- for the presence of specific extraneous agents in accordance with the method described in section 2.5.3.

2.2.4.2.3. Co-cultivation of the MCS

Text to be provided by PASTORET

NOTE:

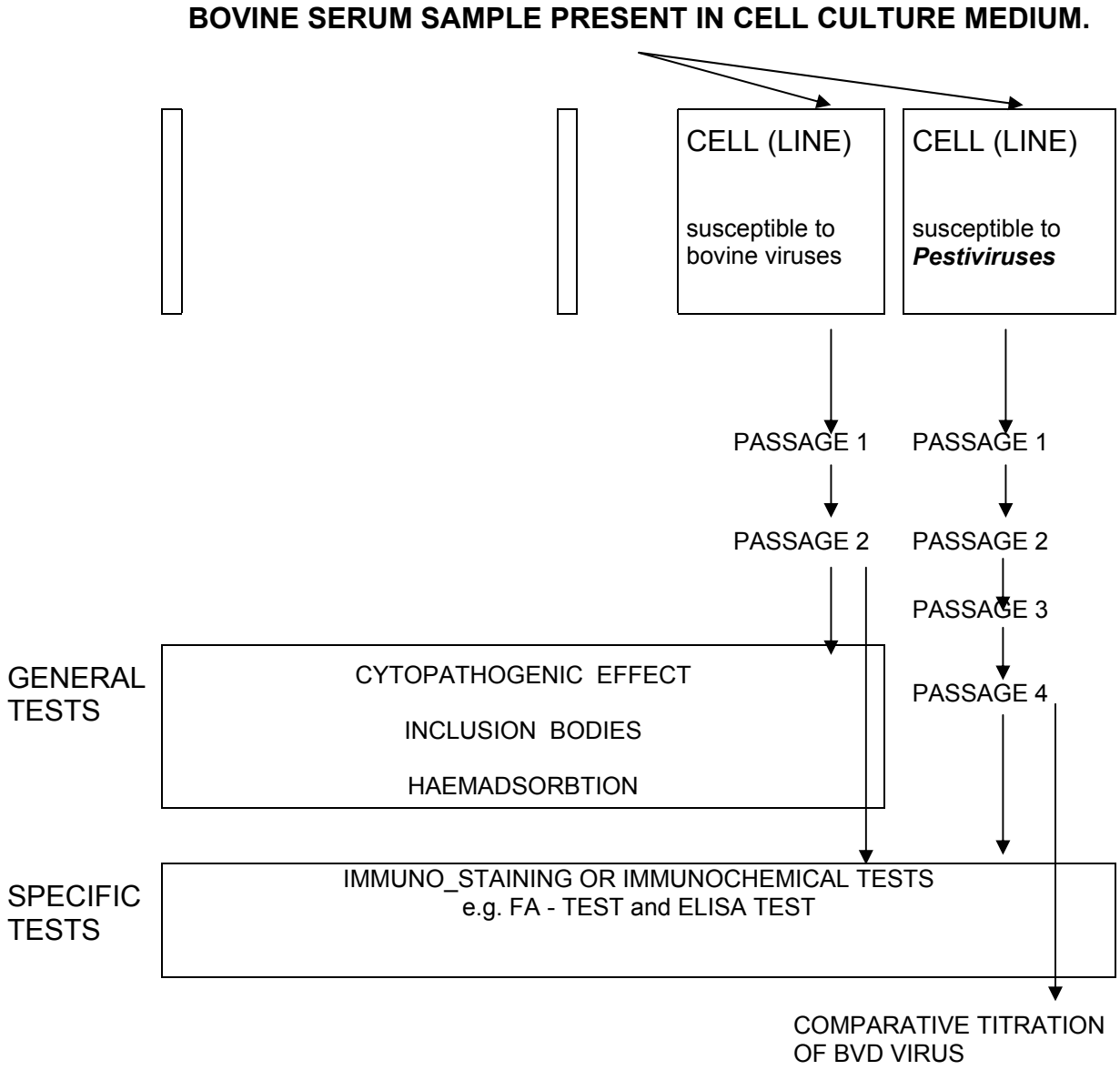
The EP states that the aliquots shall be inoculated at the cell cultures at any time up to 70 percent confluency. It seems therefore that it was the intention to use actively multiplying cells.

In the EP -General Texts section 5.2.3. "Cell substrates for production of vaccines for human use mention is made of co-cultivation. The process is described as follows:

"Co-cultivation. Co-cultivate intact and disrupted cells separately with other cell systems including human cells and simian cells. Carry out examinations to detect possible morphological changes. Carry out tests on the cell culture fluids to detect haemagglutinating viruses. The cells comply with the test if no evidence of any extraneous agent is found."

2.3. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN BOVINE SERUM

SCHEMATIC OUTLINE OF TEST



2.3. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN BOVINE SERUM.

NOTE: The method of testing of bovine serums is based on the "Guideline on the Requirements and Controls applied to Bovine Serum used in the Production of Immunological Veterinary Medicinal Products" (EMEA/CVMP/743/00 Final).

The method is included in the text to facilitate discussion.

2.3.1. General.

Bovine serum is considered as a material with a potential relatively high risk in respect of acting as a source of contamination for veterinary vaccines, notably with Bovine Diarrhoea Virus and is therefore subject to specific control tests. It shall be tested both for the presence of extraneous viruses and for the presence of antibodies against Bovine Viral Diarrhoea virus. It shall be demonstrated that the level of antibodies against BVDV does not interfere with the detection of a low level of BVD virus e.g. by validated spiking techniques

The tests shall be done before the serum is subjected to a sterilisation c.q. inactivation procedure. The material is tested by applying tests of a general nature that may be expected to detect a broad selection of agents, 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.

2.3.2. Testing of bovine serum for the presence of extraneous viruses.

These tests shall be capable of detecting viruses inducing viraemia and transplacental infection:

Bovine Adeno Virus
Bovine Viral Diarrhoea Virus
Parvo Virus
Bovine Respiratory Syncytial Virus
Reo Virus
Parainfluenza 3 Virus
Infectious Bovine Rhinotracheitis Virus
Other virus which do not occur in country of importation.

2.3.2.1. Samples.

NOTE: The sample size has to be determined. Since the serum has to incorporated in the culture medium the sample size will probably have to be related to the size of the cell culture used in the test.

2.3.2.2. Substrates.

NOTE: The substrate shall consist at least of bovine cells, sensitive to pestiviruses *notably non-cytopathic BVD virus*

2.3.2.3. Test method.

2.3.2.3.1. Preparation of the substrate cells.

The monolayers of cells of the appropriate sensitivity to be used in the test shall cover an area of at least 70 cm^2 .

Control cells cultivated with a bovine serum controlled and inactivated in line with the guideline shall be included.

2.3.2.3.2. Inoculum.

NOTE: The size of the inoculum has to be related to the serum concentration in the culture medium and the size of the cell culture.

2.3.2.3.3. Procedure.

2.3.2.3.3.1. General tests to detect presence of extraneous viruses.

Observation of cell cultures.

All cell cultures are maintained for at least 21 days, during which at least 2 subcultures are made at 7 days intervals, unless the cells do not survive for this length of time, when the subcultures shall be made on the latest day possible.

In the case of the cell cultures used to detect the presence of BVD virus at least 4 subcultures have to be made.

They are examined as follows:

- All cultures shall be observed at least every second working day
- At the end of the last subculture

  - monolayers are examined for cytopathogenic effect in accordance with the method described in section 2.5.1.

  - monolayers are examined for haemadsorbtion in accordance with the method described in section 2.5.2.

  - the presence of specific extraneous agents in accordance with the method described in section 2.5.3.

2.3.2.3.3.2. Specific tests to detect presence of extraenous viruses.

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

2.3.2.3.3.2.1. Preparation of the substrate cells.

The monolayers of cells of the appropriate sensitivity to be used in the test shall cover an area of at least 70 cm^2 .

The monolayers are maintained in culture for a total of at least 21 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.

In the case of the cell cultures used to detect the presence of BVD virus at least 4 subcultures have to be made.

2.3.2.3.3.2.2. Examination for specified viruses.

This shall be done in accordance with section 2.5.3.

NOTE: *Other test methods, e.g. PCR may be used, provided they are validated and shown to have a sensitivity and specificity at least equivalent to the other tests. It will also be required to clarify whether or not any nucleic acid detected originated from infectious particles.*

2.3.3. Comparative Titration Test for BVD virus.

This test shall be done after the serum has been subjected to a inactivation treatment.

Appropriate sensitive cells which have been grown with at least 3 subcultures, in the presence of the serum tested, are used, at the end of the observation period to titrate a reference strain of BVD virus.
A similar titration shall be done with cells grown in the presence of a previously controlled and inactivated reference bovine serum, prepared in line with the guideline.

The result of the titrations shall demonstrate that the serum does not have a significant inhibitory effect on the multiplication of BVD virus.

2.3.4. Test to detect antibodies against BVDV.

The samples are tested using a validated technique to detect BVD antibodies. The serum should either be free of BVD antibodies or it should be demonstrated that the level of antibodies present does not interfere with the detection of a low titre of BVD virus e.g. by validated spiking techniques.

The validation performed should clearly demonstrate what the limit of detection (LOD) of the testing method is in the presence of any given titre of antibodies and how this compares with the LOD when specific antibodies are not present. Ideally the titre of virus used for spiking should be equivalent to the lower limit of detection of the validated assay for BVD virus meaning that the LOD is not affected by the titre of antibodies present.

Where this is not possible the inactivation procedure shall have a sufficient safety margin to inactivate not only the titre of virus detected but also the additional titre "masked" by the presence of antibody

2.4. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN MATERIALS OF ANIMAL ORIGIN.

2.4.1. General.

The material is tested by applying tests of a general nature that may be expected to detect a broad selection of agents, 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.

2.4.2. Samples.

The sample size shall be justified on the basis of likelihood of contamination (origin and history) degree of purification applied, degree of concentration and availability. In the case of serum 15 % v.v. is recommended.

The material, when prepared for testing, shall be dissolved or suspended in a defined medium that is identical or at least related to the medium in which the substance will be used in the production process, provided this medium is compatible with the test system.

Any solids are dissolved or suspended in a suitable medium 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 when justified. In case of very scarce ingredients a minimum sample size may be used.

2.4.3. Substrates.

The test shall be done with suitably sensitive cell cultures, including primary cell cultures of the same species of origin as the ingredient to be tested and VERO cells. The monolayers shall have a surface of at least 70 cm^2

2.4.4. Test method.

At least 2 subcultures are made of the monolayers with an interval of 7 days. The cell-cultures are maintained for at least 21 days during which period the cultures are examined regularly for cytopathogenic effect.

At the end of the incubation period the last subculture shall be examined :

- for cytopathogenic effect in accordance with the method described in section 2.5.1.
- for haemadsorbtion in accordance with the method described in section 2.5.2.
- for the presence of specific extraneous agents in accordance with the method described in section 2.5.3.

2.4.4.1. Preparation of substrate cells.

The monolayers of cells of the appropriate sensitivity to be used in the test shall cover an area of at least 75 cm^2 .

2.4.4.2. Inoculum.

2.4.4.3. Procedure.

At least 2 subcultures are made of the monolayers with an interval of 7 days. or as frequently as may be necessary for suitable growth. The cell-cultures are maintained for at least 21 days during which period the cultures are examined regularly for cytopathogenic effect.

At least 7 days after the initiation of the last subculture the monolayers are examined :

- for cytopathogenic effect in accordance with the method described in section 2.5.1.
- for haemadsorbtion in accordance with the method described in section 2.5.2.
- for the presence of specific extraneous agents in accordance with the method described in section 2.5.3.

2.4. TEST FOR THE PRESENCE OF EXTRANEOUS VIRUSES IN INTERMEDIATE AND FINISHED PRODUCTS.

2.4.1. General.

In the case that all master seed, master cell stock and starting materials of animal origin were tested for the presence of extraneous agents and the manufacturing and testing processes were done under internationally acceptable conditions of Good Manufacturing Practice, it will not be necessary to test the intermediate and finished product for extraneous agents.

NOTE:

- ***This is in accordance with the General monograph of the EP on veterinary vaccines. However, it states as well that in the monograph of a specific vaccine a test for extraneous agents may be required.***
- ***The EU document "General Requirements for the Production and Control of Live Mammalian Bacterial and Viral Vaccines for Veterinary Use" requires a test for the presence of extraneous virus by "appropriate and suitably sensitive methods". A quantity of 10 doses of vaccine shall be tested.***
- ***The EU document "General Requirements for the Production and Control of Inactivated Mammalian Bacterial and Viral Vaccines for Veterinary Use" states that a serological test may be required following inoculation of a double dose of vaccine .***

In the case that master seed, master cell stock and starting materials of animal origin were not tested for the presence of extraneous agents even if the manufacturing and testing processes were done under internationally acceptable conditions of Good Manufacturing Practice, the tests should be done to detect the presence of specified relevant agents.

In the case that the starting materials of animal origin were not tested for the presence of extraneous agents, it will be necessary to test the finished products for all those agents. Since there are agents which are unlikely or impossible to detect during the tests done on the finished product, it will be also necessary to test the appropriate intermediate products for those agents.

In Japan, where testing of starting materials is not required, the finished product is tested for extraneous viruses by inoculation of samples of the finished product on the following mammalian origin cell substrates:

Cell type	Passage level		Number of subpassages	Test methods		
	Primary	Secondary		c.p.e.	Haem-adsorbtion	
Porcine Kidney	+	+	1	+	Guinea pig Goose Chicken	
Swine Testicle	+	+	None	+		
Bovine Testicle		+	1	+ (BVD)		
Canine Kidney	+	+	1	+	Guinea pig	

Cat Kidney	+	+	1	+	Porcine	
Guinea Pig	+		None	+	Guinea pig Goose Chicken	

NOTE: In addition the following chicken origin substrates are used:

- Embryonated Chicken Eggs
- Chicken Kidney Cells
- Chicken Embryo Cells

These will not be considered here because they will be dealt with in the guideline for the testing of avian vaccines.

It is not clear whether all cell substrates are used for each vaccine or that certain substrates are selected for specific vaccines.

Additional tests:

- An intracerebral Inoculation of suckling mice
- A specific test to determine the absence of Classical Swine Fever.

In the case the starting materials of animal origin are not tested the results of the testing of the finished product shall provide the same degree of assurance that extraneous agents are not present. In principle this can only be the case if the test methods are identical as described below:

2.4.2. Samples

The vaccine virus shall - when necessary - be neutralised with a monoclonal antibody or a polyclonal specific antiserum containing high levels of neutralising antibody to the vaccine virus.

When a polyclonal specific antiserum is used, it shall be free of antibodies against those viruses for which presence the vaccine is tested. To that end , such an antiserum will be prepared, wherever possible, with an antigen that

- is not derived from any passage level of the virus isolate giving rise to the vaccine virus.
- has been passaged on cells different from those used at any stage of the passaging of the virus isolate giving rise to the vaccine virus
- has been adequately purified.

If this is not possible the antiserum may be prepared from an earlier isolate of the virus concerned.

2.4.3. Substrates

The substrates used for testing the presence of extraneous agents shall consist of one or more types of sensitive cells, provided that the three following conditions are met:

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

- cells and/or other substrates e.g. eggs, sensitive to viruses which potentially are present in any of the starting materials of biological origin used for vaccine production.
- cells sensitive to pestiviruses. The sensitivity of these cells shall be demonstrated by the use of positive controls e.g. Bovine Viral Diarrhoea virus.

2.4.4. Test method

2.4.4.1. Preparation of the substrate cells.

See: Tests for MSV and MCS

2.4.4.2. Inoculum.

A quantity of virus, or serum-virus mixture, equivalent to that present in .....doses of vaccine is used.

2.4.4.3. Procedure.

See: Test for MSV and MCS.

2.5. GENERAL TEST METHODS.

The sensitivity of the cells has in principle to be demonstrated using positive controls.

NOTE: The manner in which positive controls are included has to be clarified

2.5.1. Presence of cytopathogenic effect,

Monolayers of at least 12 cm^2 each are stained with an appropriate cytological stain e.g. Haematoxylin-Eosin or MayGrunwald-Giemsa stain. The entire area of each stained monolayer is examined for signs of cytopathogenic effects and inclusion bodies, abnormal numbers of giant cells or any other lesion indicative of a cellular abnormality that might be attributable to a contaminant.

2.5.2. Haemadsorbtion
Monolayers totalling at least 70 cm^2 are washed several times with an appropriate buffer. A sufficient volume of a suspension of appropriate red blood cells, but at least including chicken and guinea pig erythrocytes, are added to cover the surface of the monolayer evenly. After incubation for 25-30 minutes at +2 - +8 º C and incubation for 25-30 minutes at 20-25 ºC the monolayers are washed and than examined for the presence of haemadsorption.
Where appropriate the test shall be done using other temperatures and conditions.

Details on technical aspects of the haemadsorption test are summarised in the table below.

		EU	USA	JAPAN	VICH
Cell surface (sqcm)		70	>6 and at least 7 days from last subculture	20	
Type of erythrocytes		Not specified			

	Chicken		yes	yes	
	Guinea pig		yes	yes	
	Goose			yes	
Concentration of erythrocyte suspension		Not specified	0.2	0.1%	
Mixture /separate		Not specified	Mixed or separate	Separate	
Incubation time and temperature (C)		Not specified			
	+4		30	60 min	
	+20-25		30 (if +4 C test is negative)		
	+37			30 min	

2.6. SPECIFIC TEST METHODS

Sufficient cells on suitable supports (e.g. micro-plates or slides) are prepared to carry out tests for the agents specified. The material shall be obtained after at least 2 passages and a total surface area of at least 6 cm^2 shall be examined. Suitable positive controls and negative controls are included in each test. The presence of specific viruses shall be determined by means of immune staining technique using appropriate controls.

The sensitivity of the cells has in principle to be demonstrated using positive controls. A distinction has however to be made between the sensitivity of the cells as demonstrated by infecting the cells with the relevant viruses and the sensitivity as demonstrated with pre-prepared infected cells. In the latter case it is in fact the specifity of e.g. the conjugate which is determined.

2.7. OTHER TESTS METODS.

Other techniques as e.g. Transmission electronmicroscopy and PCR can be used in specific cases.

Inoculation of sero negative animals followed by serology may be used specifically for the detection of BVD virus.

GLOSSARY

BATCH (STARTING MATERIAL OF ANIMAL ORIGIN)

The total quantity of the material mixed in a single container and identified by a **unique** serial number.

BATCH (FINAL LOT) VACCINE

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.

CELL-SEED SYSTEM

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

CELL LINES

Cultures of cells that have a high capacity for multiplication *in-vitro* and can be subcultured continuously.

CELL PASSAGE

The result of an *in-vitro* process whereby cells are replicated, either by being transferred from one container to another or provided with additional growth surface or volume intended to result in renewed or expanded cell growth.

CELL PASSAGE

The result of an *in-vitro* process whereby the cells and supernatant are harvested and 50% of the cells and 50% of the supernatant are three times subjected to freeze-thawing and subsequently mixed with the remaining 50% of the cells and supernatant. The resulting material is inoculated onto fresh monolayers of the same cell type.

CONTROL CELLS

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

GENERAL TESTS,

General tests are tests based on characteristics common to different types of viruses e.g. haemagglutinins

.**MASTER CELL SEED**

A collection of aliquots of cells for use in the preparation of the product, 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. Master cell seed is usually stored at temperatures of - 70 °C or lower.

MASTER SEED LOT

A culture of a micro-organism for use in the preparation of the product, 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

Primary cell cultures are cultures of cells essentially unchanged from those in the animal tissues from which they have been prepared and being no more than 10 *in-vitro* passages to the test level from the initial preparation from the animal tissue. The first *in-vitro* cultivation is regarded as the first passage of the cells.

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 virus. For routine production, a working seed virus may be prepared from the master seed virus.

SPECIFIC TESTS

Specific tests are tests based on specific characteristics of a virus or group of viruses as detected by e.g.immunostaining

SUBCULTURE:

A subculture is prepared by collecting the cells which are than used to prepare a new monolayer or culture.

SUBCULTURE.

The result of an *in-vitro* process whereby cells are replicated, either by being transferred from one container to another or provided with additional growth surface or volume intended to result in renewed or expanded cell growth.

VIRUS PASSAGE

The result of an *in-vitro* or *in-vivo* process whereby a virus is replicated, either by inoculation of non-infected cells, embryos or animals or performing a cell passage in the case of persistently infected cells.

WORKING CELL SEED

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

WORKING SEED VIRUS

A collection of aliquots of a micro-organism derived from the master seed virus and intended for use in production. Working seed virus is distributed into containers and stored as described for master seed virus.