

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)



Webinar on the Management of Extraneous Agents in IVMPs – Part 2

01 April 2020

Webinar on the “Management of Extraneous Agents in IVMPs”

Afternoon Session: Regulatory landscape

EMA guidelines (including needs for revision or elaboration of new guidelines) and Q&A document as a tool for harmonized assessment

1st April 2020

Esther Werner

Disclaimer

I attend this webinar as an individual expert and I express here my personal views.

The information contained in this presentation does not necessarily reflect the opinion or the position of the Paul-Ehrlich-Institut, the Committee for Veterinary Medicinal Products or the Immunological Working Party.

Agenda

- **EMA Guidelines**
- **EMA/CVMP/IWP/206555/2010-Rev.1 – guideline + annex 2**
- **EMA/CVMP/IWP/251741/2015 – reflection paper**
- **Ph. Eur. Approach - Management of Extraneous Agents in IVMPs**
- **Questions and points for considerations**
- **Next steps**

EMA Guidelines

EMA/CVMP/IWP/206555/2010-Rev.1

- **Guideline on requirements for the production and control of immunological veterinary medicinal products including Annex 2**
(The approach to demonstrate freedom from extraneous agents (into force 1st May 2017))

Draft revised guideline agreed by Immunologicals Working Party (IWP)	June 2015
Adoption by CVMP for release for consultation	9 July 2015
End of consultation (deadline for comments)	31 January 2016
Revised draft guideline agreed by IWP	19 October 2016
Adopted by CVMP	8 December 2016
Date for coming into effect	May 2017

EMA/CVMP/IWP/251741/2015

- **CVMP reflection paper** on methods found suitable within the EU for demonstrating freedom from extraneous agents of the seeds used for the production of IVMPs
which describes methods that have been evaluated by regulatory authorities within the EU and considered as suitable by CVMP, or by one or more NCA, for the detection of one or more EA on the lists contained in annex 2.

EMA/CVMP/IWP/206555/2010-Rev.1

- GL **originally adopted** by CVMP in June 2012 (into effect 1st Jan 2013) – replaced a couple of guidance documents.
- **Revision** to include a new annex (annex 2) entitled *“The approach to demonstrate freedom from extraneous agents as part of the production and control of immunological veterinary medicinal products for mammalian species and finfish”*.
- **Annex 2 replaced** the table of extraneous agents to be tested for in relation to the general and species-specific guidelines on production and control of mammalian veterinary vaccines (“7Blm10a” dated 1994).

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EMA/CVMP/IWP/206555/2010-Rev.1

Until May 2017

- **Specification for materials** that need to be tested at the various stages of production (European Pharmacopoeia - Ph. Eur.)
 - Substrates, seeds and raw materials, intermediate materials and final products.
- **Specification of agents** from which freedom needs to be shown
 - Mammalian vaccines in the CVMP table of EA (7Blm10a).
 - Avian vaccines in the Ph. Eur. (*Ph. Eur. general chapters 2.6.24, 2.6.25. and 5.2.2.*)
- **Tests divided into ‘general’ and ‘specific’ tests**
 - Mammalian vaccines in CVMP table.
 - Methodologies and requirements for cell cultures (eggs) and substances of animal origin in the Ph. Eur. (5.2.4., 5.2.5., monograph 0062, 2.6.1, 2.6.2., 2.6.7, 5.2.8)
 - Avian vaccines: specific tests in the Ph. Eur.

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EMA/CVMP/IWP/206555/2010-Rev.1

Revised EU approach – considerations behind it

- **Move away** from prescribing the test methodology that must be used for a particular agent or substrate
- **Move towards** describing the general approach in order to demonstrate suitability of tests applied to show freedom of the relevant substrate from specified EA
- Reservations on the concept of ‘general’ and ‘specific’ tests
- Flexibility for applicants - use of any suitable (validated) method
- Take into account progress made in development of new techniques
- Link between EA and cells
 - EA: Definition of what you want to detect
 - Cells: Identification of cells able to detect the virus you are looking for

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EMA/CVMP/IWP/206555/2010-Rev.1

Revised EU approach in

Annex 2 to guideline on requirements for the production and control of IVMPs

- **Annex 2 applicable to** IVMPs for mammalian species, salmonids and other finfish.
- **Annex 2 defines** the following:
 - **Substances, substrates, starting materials, intermediates and final products** for which testing is required.
 - **Criteria** by which the requirement to demonstrate freedom of a material from particular EA(s) may be waived (source of material, treatment applied, SPF status etc.).
 - **How tests may be shown to be suitable** for EA testing in terms of performance criteria (sensitivity, specificity, etc.).
 - **Lists of EAs per species**

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EMA/CVMP/IWP/206555/2010-Rev.1

List of extraneous agents

- **Reference list** which must be taken into account when considering which testing for EA is appropriate.
- Established in accordance with the existing knowledge at the time of writing this guideline.
- If scientifically justified, the list may be updated in the future.
- The presence of an EA on the list **does not mean that a test for this agent must be carried out.**
- **Justification** for not carrying out a test for a specific EA.

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EMA/CVMP/IWP/206555/2010-Rev.1

Risk assessment identifying those EA for which testing is necessary

- **Step 1:** Justification for not carrying out a test for a specific EA
 - **Definition of criteria** by which the requirement to demonstrate freedom of a material from particular EA may be waived (source of material, treatment applied, SPF status, biotechnological process etc.)
- **Step 2:** Implementation of tests for the detection of EA
 - Explanation how tests may be shown to be suitable for EA testing in terms of key criteria and performance characteristics (sensitivity, specificity, etc.)

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EMA/CVMP/IWP/251741/2015

CVMP reflection paper on methods found suitable within the EU for demonstrating freedom from extraneous agents of the seeds used for the production of IVMPs

- To be read in conjunction with Annex 2 of GL EMA/CVMP/IWP/206555/2010-Rev.1

- Testing of virus and cell seeds

- Methods of viral detection (classical cell culture methods, use of embryonated eggs, molecular methods, e.g. NAT)

- Annex: examples of suitable cells and methods for testing for freedom from a range of extraneous agents are shown.

Porcine			
1. Extraneous agent(s)	2. Suitable culture substrates for amplification	3. Suitable methods of detection	Remarks
Bovine viral diarrhoea virus	BEL, BHK-21, BT, CK, EBK, EBTr, FBLP, FBT10, IPB3, MDBK, PK-15, SCP	CPE for cytopathic strains IS for non-cytopathic and cytopathic strains	
Classical swine fever virus	IPB3, PK, PK-15	ELISA, IS	
Encephalomyocarditis virus	BHK-21, PK, SK, ST, Vero	CPE	
Foot-and-mouth disease virus	BHK-21, CTY, IB-RS-2, IPB3, MDBK, PK	CPE, ELISA	
Influenza virus	embryonated eggs MDCK	HAg IS	
Porcine adenovirus	PK, PK-15, SK, ST	CPE	
Porcine circovirus, type-1 and type-2	FSK, MA104 CCL-33, PK, PK-15, PS, SK, ST	IS IS	

- Currently limited to porcine, bovine, canine, feline, but work is ongoing with additional species – ovine, equine.

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Ph. Eur. Approach - Management of Extraneous Agents in IVMPs

The **new approach for EA testing** constitutes a move from a prescriptive to a scientifically sound and targeted approach.

- Restricted to **living replicative** extraneous agents.
- Covers **all materials** (master seeds, substrates - eggs, cells, etc.), materials are **starting materials** of animal or human use.
- Includes the **entire production process**, from the sourcing of raw materials to the final product stage.
- Allows the **use of any suitable culture or other fit-for-purpose method** capable of detecting specified extraneous agents but with a focus on in-vitro methods (for example, based on nucleic acid amplification technology).
- Reflects May 2017 revision to EMA/CVMP/IWP/206555/2010-Rev.1.

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Ph. Eur. Approach - Management of Extraneous Agents in IVMPs

The new approach for EA testing ...

- Includes a reference to risk management.
- Provides an updated single reference list of EA to be considered in the risk assessment.

Risk management (identify – assess – control – review controls)

- Risk identification
- Risk assessment – risk of contamination of materials and FP
- Risk control – define and apply appropriate control measures

Aspects/concerns are raised that are considered to be outside the scope of Ph. Eur. texts (particular where matters for authorisation of IVMPs are concerned)

- **Proposal:** Prepare a Question & Answer document to address these concerns.

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Questions and points for considerations

- **Re-testing of well-established cell banks and master seeds - Applicability of revised/new guidance to seeds as starting materials for existing products or for new products where the seed is already in use for existing products.**

(How to manage material assessed in the past (seed or cell lines) and used again in a new product/application? Is additional assessment/testing needed?, Is re-validation of test methods requested? Should well-established cell banks used for production of vaccines, and master seeds be re-tested?)

- **Historical value of the previous requirements and detailed testing methods as mentioned in the Ph. Eur.: Can the “old” detailed protocols now available in the Ph. Eur. archives still be used?**

(What is expected with regard to fit-for purpose aspects of these tests methods? What documentation has to be provided to demonstrate adequate sensitivity and specificity?)

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Questions and points for considerations

- **Validation of new test techniques – expectation with regard to validation and documentation in the dossier.**

(What information should be provided e.g. on PCR tests (details of the primers used, justification of the cut-off limits, justification that the methods are able to detect relevant field virus strains. Clarification of what is meant by methods of “adequate sensitivity and specificity”. If a new method is used and a positive result is obtained, which measures can be taken to accept such “contaminated” materials? “viruses unable to multiply in vitro”: What does this mean and what are the consequences?)

- **Uncertainty regarding assessment based on a risk management approach.**

(What kind of justification for not carrying out a test for a specific agent might be considered suitable? Are bibliographic data acceptable?)

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Next steps

- Preparation and finalisation of the Question and Answer document until July 2020
- Need for revision of EMA/CVMP/IWP/206555/2010-Rev.1 – guideline + annex 2
- Need for revision and further completion of EMA/CVMP/IWP/251741/2015 – reflection paper
- Need for elaboration of new or further guidance?

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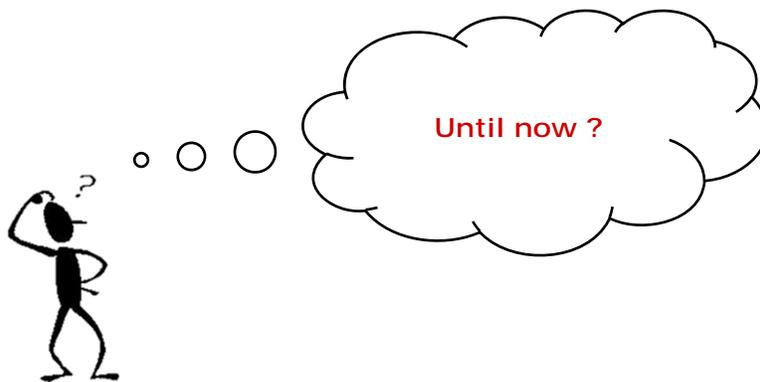
www.pei.de

Training on the new European Pharmacopoeia
approach to the 'Management of EA in IVMPs'

The particular case of old master
seeds used for the production of
new vaccines:
re-testing of "well-established" cell
banks and master seeds?

What can be used to justify no
re-testing?

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01.04.2020





Test requirements on extraneous agents (EA) mentioned in the past in different EU guidelines and guidances.

- General requirements for the production and control of live mammalian bacterial and viral vaccines for veterinary use (GRLMV)
 - General requirements for the production and control of inactivated mammalian bacterial and viral vaccines for veterinary use (GRIMV)
 - Specific requirements for the production and control of live and inactivated viral and bacterial vaccines
- | EA | Test | Reference | Year |
|--------------------------|--------------------------|--------------------|------|
| Adenovirus | Adenovirus | EMA/CHMP/1000/2009 | 2009 |
| Arbovirus | Arbovirus | EMA/CHMP/1000/2009 | 2009 |
| Bacterial | Bacterial | EMA/CHMP/1000/2009 | 2009 |
| Bordetella | Bordetella | EMA/CHMP/1000/2009 | 2009 |
| Brucella | Brucella | EMA/CHMP/1000/2009 | 2009 |
| Campylobacter | Campylobacter | EMA/CHMP/1000/2009 | 2009 |
| Clostridium | Clostridium | EMA/CHMP/1000/2009 | 2009 |
| Coccidia | Coccidia | EMA/CHMP/1000/2009 | 2009 |
| Cryptosporidium | Cryptosporidium | EMA/CHMP/1000/2009 | 2009 |
| Deformed wing virus | Deformed wing virus | EMA/CHMP/1000/2009 | 2009 |
| Distemper | Distemper | EMA/CHMP/1000/2009 | 2009 |
| E. coli | E. coli | EMA/CHMP/1000/2009 | 2009 |
| Enterovirus | Enterovirus | EMA/CHMP/1000/2009 | 2009 |
| Equine coronavirus | Equine coronavirus | EMA/CHMP/1000/2009 | 2009 |
| Equine herpesvirus-1 | Equine herpesvirus-1 | EMA/CHMP/1000/2009 | 2009 |
| Equine influenza A virus | Equine influenza A virus | EMA/CHMP/1000/2009 | 2009 |
| Equine herpesvirus-2 | Equine herpesvirus-2 | EMA/CHMP/1000/2009 | 2009 |
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- Table of extraneous agents to be tested for in relation to the general and species specific guidelines on production and control of mammalian veterinary vaccines (so-called Table 1)
→ some justification types mentioned
 - Replaced by
EMA/CVMP/IWP/206555/2010 - Guideline on requirements for the production and control of immunological veterinary medicinal products (replacing general and specific requirements)
→ in Rev. 1 - Introduction of a list of EA



Test requirements on extraneous agents (EA) mentioned in the past in different Ph. Eur. monographs and chapters

- 0062 Vaccines for veterinary use
- 5.2.5 Substances of animal origin in the production of IVMPs
- 5.2.4 Cell cultures
- 5.2.2 SPF flocks
- 5.2.13 Healthy flocks
- 0030 Immunosera
- 2.6.24 Avian vaccines – EA testing in seeds
- 2.6.25 Avian vaccines – EA testing in final product
→ with listed avian EA
- Different specific monographs

TEST FOR EXTRANEAS AGENTS USING EMBRYONATED HEN'S EGGS	TEST IN CHICKEN EMBRYO FIBROBLAST CELLS
<p>Use a test substance, diluted if necessary, containing a quantity of neutralised virus equivalent to at least 10 doses of vaccine in 0.2 ml of inoculum. Suitable antibiotics may be added. Inoculate the test substance into 3 groups of 10 embryonated hen's eggs as follows:</p> <p>group 1: 0.2 ml into the allantoic cavity of 10 fresh eggs</p> <p>group 2: 0.2 ml into the chorio-allantoic cavity of 10 fresh eggs</p> <p>group 3: 0.2 ml into the yolk sac of 10 fresh eggs</p> <p>Incubate the eggs in groups 1 and 2 at 37°C and in group 3 at 25°C for 7 days. Observe the eggs daily. At the end of the 7th day, examine the eggs for signs of infection. Examine monographs for details.</p> <p>2.6.24. Extraneous agents. Neutralise the vaccine strain with a suitable antiserum specific for the virus and inoculate into cell cultures known for their susceptibility to virus pathogens for culture. Carry out at least 3 passages and maintain the cultures for 14 days. The vaccine complex with the leaf if no cytopathic effect develops and there is no sign of the presence of haemadsorbing agents. Carry out a specific test for persistence.</p>	<p>Prepare 7 monolayers of primary or secondary chicken embryo fibroblasts, from the 1st to the 7th passage, each monolayer having an area of about 25 cm². Inoculate 2 monolayers in negative controls and treat them in the same way as the 5 monolayers inoculated with the test vaccine, as described below. Renew the culture medium when the cells reach confluence. Inoculate 0.1 ml of test vaccine into each of 10 of the monolayers. Allow adsorption for 1 h and add culture medium. Incubate at 37°C for 10 days, subculturing at 4 to 6 day intervals. Each cell and fluid from all 5 monolayers after carrying out a 1:2 dilution of pooled material into each of 10 freshly prepared fibroblast cells, each monolayer having an area of about 25 cm². For passage grow the cells also in a suitable culture medium. Examine the cells from each of the monolayers, test A.</p> <p>Prepare seven 20 ml suspensions of the MRC-5 cell line or another cell line of appropriate sensitivity to the virus. Inoculate about 1 ml of culture, inoculate 1 ml of test vaccine into each of 10 of these flasks. Incubate at 37°C for 10 days. Examine the cell cultures for signs of infection. Renew the cell cultures for a total of at least 24 days, giving 4 passages at 4 to 6 day intervals. During the incubation the presence of infection must be established in a suitable culture during 10 passages. The culture fluids becoming of an appearance with the control cultures. In the case of the test cultures, the cells microscopically for cytopathic effect. In the case of the test cultures, the cells microscopically for cytopathic effect. In the case of the test cultures, the cells microscopically for cytopathic effect.</p>



And from now ?
(from 01.07.2020)



Test requirements on extraneous agents (EA) are in future mentioned in the fully revised Ph. Eur. chapter 5.2.5.

- All requirements for EA testing of veterinary vaccines are covered by one chapter (5.2.5).
- Any detailed information and method descriptions are deleted = reference to 5.2.5

2-1-3-2-6. Absence of extraneous viruses. General requirements for managing the presence of extraneous viruses in master seed lots are given in general chapter 5.2.5.

3-9. Extraneous agents (5.2.5). The vaccine is free from extraneous agents.

Absence of contaminating Extraneous viruses. The cells must not be contaminated by viruses. General requirements for managing the presence of extraneous viruses in cells are given in general chapter 5.2.5. Management of extraneous agents in immunological veterinary medicinal products; suitably sensitive tests; including those prescribed below, are carried out. In light of the result of the risk assessment, testing may be reduced or omitted.



According to Ph. Eur. 5.2.5 (including lists of EA provided in Annex I)

- ✓ Materials → seeds, substrates, substances used for the production of vaccines
= cell seeds (e.g. cell lines, primary cells)
= master seed (e.g. virus seeds)



- ✓ The risk of contamination of cell seeds and master seeds with EA must be assessed - **RISK ASSESSMENT**
- ✓ The specific lists of EA which may be present in specific cell seeds/master seeds are part of the risk assessment.
(Annex I does not preclude additional EA from being considered, if necessary)

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“Well-established” / existing seeds : re-testing?

- ✓ The new/revised Ph. Eur. requirements do apply retroactively.
- ✓ Re-testing is not required for existing cell seeds/master seeds currently used in approved products.
- ✓ For new products and for introduction of new cell seeds/master seeds the new/revised requirements need to be fulfilled.
- ✓ If an existing cell seed/master seed is to be used in a new product, a full risk assessment should be provided.
 - Historical/existing data based on older methods can generally be used.
 - Re-testing may only be necessary in part and combined with risk assessment.
 - EA to be tested are those which could not be excluded by justification.



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“Well-established” / existing seeds : re-testing?



If an existing cell seed/master seed is to be used in a new product:

- ✓ Test methods performed historically for existing cell seed/master seed are in most cases found acceptable.
→ those methods can still be used with the new approach → fit for purpose !
- ✓ Re-validation of historically used test methods is generally not required.
→ fit for purpose !
- ✓ Additionally, new developments in methodology should be taken in consideration.
- ✓ Documentation for historically performed tests should be provided.
- ✓ Justification for all specific EA not tested should be provided and supported by reliable independent sources and scientific evidence.
→ EA included in the new guideline.
- ✓ In case new EA have been identified since the original tests were performed, these should be subject to risk assessment or testing, if not excluded by risk assessment.

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What can be used to justify no re-testing?



Examples for types of justification for not performing a test for a specific EA (or disease) in cell seeds/master seeds:

- Non-occurrence in the country/geographical area of origin at the time of isolation/recovery of the seeds.
→ by official data (e.g. OIE, official websites)
→ by bibliographical references/published literature
- Non-occurrence in the source species and target species involved.
→ by bibliographical references/published literature
→ by independent expert statements
→ by information/data on a likely infectivity in the source organ or tissue
→ by results of any test for EA already available
- Non-occurrence in the herd of origin (i.e. SPF status).
→ by documentary evidence for the SPF herd status (SPF certificate indicating control methods used and freedom of respective EA)

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What can be used to justify no re-testing?



Examples for types of justification for not performing a test for a specific EA in cell seeds/master seeds e.g. based on specific properties:

- specific EA cannot be present in the seed in question (e.g. agent does not cross placenta or does not produce viremia).
→ by bibliographical references/published literature
- specific EA cannot grow in some systems or under some specific conditions (e.g. the EA does not grow/replicate in cell culture or does not grow in the absence of trypsin)
→ by bibliographical references/published literature
→ by internal data/results already available

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What can be used to justify no re-testing?



Examples for types of justification for not performing a test for a specific EA in master seeds :

- specific agents can be inactivated using validated methods, or removed by the production process, where applicable
→ by bibliographical references
→ by documentary evidence/results regarding effectiveness of any treatment

For master seeds derived by recombinant DNA techniques

- exclusion of specific EA from the source species and target species because of implemented biotechnological processes
- in any case, a risk assessment including also the materials of animal origin that were/are used to produce the rDNA-derived active substance, and a thorough justification

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Summary



New requirements regarding EA – based on risk management / risk assessment (Ph. Eur. 5.2.5 including EA lists provided in Annex I)

“Well-established” / existing seeds : re-testing?

- New Ph. Eur. requirements - retroactively.
- Re-testing - not required for seeds currently used in approved products.
- New products / new seeds - new requirements to be fulfilled.
- Existing seeds used in new products - risk assessment needed.

What can be used to justify no re-testing?

- Different types of justification:
 - e.g. geographical exclusion – official references
 - e.g. bibliographical references/published literature
 - e.g. data/statements for certain aspects
 - e.g. results of any test for EA already available/historically performed tests
 - e.g. specific inactivation/treatment processes

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Thank you
for your
attention!

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Webinar on the “Management of Extraneous Agents in IVMPs”

Afternoon Session: Regulatory landscape

Historical value of the previous requirements and detailed testing methods as mentioned in the Ph. Eur.: Can the “old” detailed protocols now available in the Ph. Eur. archives still be used?

1st April 2020

Esther Werner

Disclaimer

I attend this webinar as an individual expert and I express here my personal views.

The information contained in this presentation does not necessarily reflect the opinion or the position of the Paul-Ehrlich-Institut, the Committee for Veterinary Medicinal Products or the Immunological Working Party.

Questions and points for considerations

- **Historical value of the previous requirements and detailed testing methods as mentioned in the Ph. Eur.: Can the “old” detailed protocols now available in the Ph. Eur. archives still be used?**

(What is expected with regard to fit-for purpose aspects of these tests methods? What documentation has to be provided to demonstrate adequate sensitivity and specificity?)

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Historical value of the previous requirements and detailed testing methods as mentioned in the Ph. Eur.: Can the “old” detailed protocols now available in the Ph. Eur. archives still be used?

Situation

- Detailed descriptions of the test methods for EAs in materials which are used during the manufacture of IVMP were referred to in Ph. Eur. monograph 0062, chapter 5.2.4, chapter 2.6.24 and chapter 2.6.25. – **now deleted**
- New approach on managing EA is now described in chapter 5.2.5 and chapter 2.6.37.
- Detailed protocols for the methods deleted from the current Ph. Eur. edition, will be **available in the Ph. Eur. archives** (<https://pheur.edqm.eu/app/arch/search/>).
- Current tests have been validated through use for decades.
- Revised requirements for EAs will apply also to already authorized products.

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Historical value of the previous requirements and detailed testing methods as mentioned in the Ph. Eur.: Can the “old” detailed protocols now available in the Ph. Eur. archives still be used?

- **Detailed protocols for methods considered to be “historically validated”** deleted from the Ph. Eur., available in the **Ph. Eur. archives** only.
- Testing protocols may still be used provided they have been **shown to be fit-for purpose** (i.e. suitable sensitivity, specificity, repeatability and use of appropriate controls).
- Proposal **using model viruses for validation** (e.g. porcine parvovirus for validation of trypsin irradiation). Is this an acceptable approach?

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Historical value of the previous requirements and detailed testing methods as mentioned in the Ph. Eur.: Can the “old” detailed protocols now available in the Ph. Eur. archives still be used?

- **New chapter 2.6.37 ‘Principles for the detection of extraneous viruses in IVMPs using culture methods’**
 - Keeps existing methods (cell cultures, chicken materials such as embryonated eggs & primary cells), but in less detail.
 - CVMP reflection paper – guidance of suitable cells/substrates and methods of detection for the listed cells and substrates for specific extraneous agents (historic data).

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Historical value of the previous requirements and detailed testing methods as mentioned in the Ph. Eur.: Can the “old” detailed protocols now available in the Ph. Eur. archives still be used?

- What is expected with regard to **fit-for purpose** aspects of these tests methods? - i.e. suitable sensitivity, specificity, repeatability and use of appropriate controls.
- What documentation is needed for authorisation?
- *“The results of the tests are acceptable if the method has been demonstrated to provide adequate sensitivity and specificity for the detection of the targeted extraneous agent.”*
 - Clarification of what is meant by methods of “adequate sensitivity and specificity”.
 - What is expected to be “adequate sensitivity and specificity” for the detection of the targeted EA?

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Historical value of the previous requirements and detailed testing methods as mentioned in the Ph. Eur.: Can the “old” detailed protocols now available in the Ph. Eur. archives still be used?

- **Discussion is ongoing with regard to the requirements for using detailed testing methods for EA described in previous editions of Ph. Eur.** 
Old methods: simply accept without justification (it was accepted like that for many years) or provide justification or include positive control?
- See also presentation on ‘Management of extraneous agents in IVMPs Validation of new techniques – Expectation with regard to validation and documentation in the dossier.’

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Where are we going? Validation of new tests

- What does it mean a “fit for purpose method”?
- What is expected from method validation?
- What are the key parameters to be checked?

Validation of new techniques

- In principle, concepts of VICH GL1 (Validation: definition) and VICH GL2 (Validation: methodology) are applicable to methods for detection of EA
 - ⇒ demonstrate that the test is suitable for its intended purpose
 - ⇒ test results obtained are of relevance
- Analytical procedures for biological products may be approached slightly differently
 - ⇒ not all validation characteristics of the guidelines may be necessary



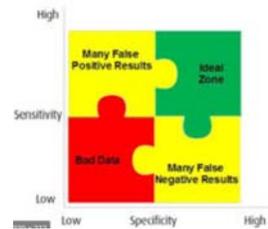
Validation of new techniques

Which parameters are important? **Detection of EA = qualitative test**

– Specificity : distinguish unequivocally an extraneous agent in the presence of components that may be expected to be present, without false positive or negative results.

– Sensitivity : detect the presence of the test agent enabling as accurate as possible a measurement.

⇒ LOD = measure of the analytical sensitivity of the method = lowest amount of EA in a sample that can be detected but not necessarily quantitated as an exact value.



Validation of new techniques - Specificity

What is expected to be “adequate specificity” for the detection of the targeted EA?

- Differentiate an EA from similar organisms or other interference from matrix elements that could have a positive or negative effect on the assay value.
- Detect relevant EA representing temporal and geographical diversity (field virus strains).
- Validation assay adapted to the method of detection on a case by case basis

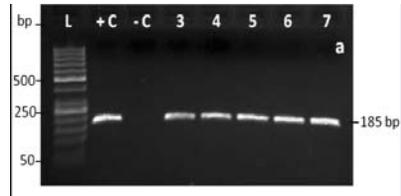
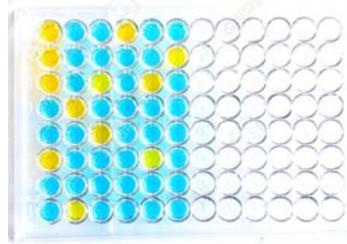
⇒ ELISA assay: specificity = testing other relevant microorganisms and showing that there is no cross-reaction.

⇒ PCR assay: details of the primers and probes - specificity to detect extraneous agents investigated by comparing the chosen sequences with sequences in published data banks: no major homology found with sequences unrelated to the extraneous agent,

Validation of new techniques - Specificity

What is expected to be “adequate specificity” for the detection of the targeted EA?

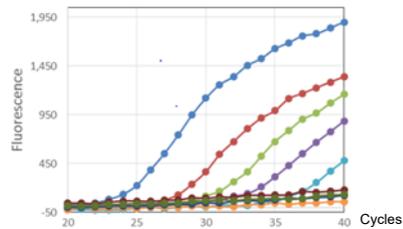
- Whatever the method developed, use of quality control samples (positive controls (including positive controls around the cut off to confirm test performance)/negative/internal controls) in each run to validate the results and evaluate test performance.



Validation of new techniques - Sensitivity

What is expected to be “adequate sensitivity” for the detection of the targeted EA?

- For the LOD: no acceptable cut-off can be defined a priori.
- Justification of the cut-off limits provided with relevancy to the test method used for the particular agent being tested (in a relevant matrix) and the target species (literature references and/or the minimum infectious dose if available from known challenge studies).
- Relevance of the results obtained in the validation assay assessed with regard to the risk of contamination by live extraneous agents.



Validation of new techniques

- If a new method, for which limited validation data or experience exists, gives a positive result
 - ⇒ Confirmation using another relevant/reliable method (e.g. cytopathic effect, haemadsorption, immunostaining)
 - ⇒ Appropriate method for differentiation between live (replicative/non-replicative) or killed agents
 - ⇒ Biological significance of confirmed positive result must be discussed in terms of potential contamination by a particular extraneous agent.

Validation of new techniques

- If a positive result is confirmed, then the general requirements of Ph. Eur. 5.2.5 Management of extraneous agents in immunological veterinary medicinal products will apply:
 - ⇒
 - any master seed lot (after processing, if relevant) found to contain extraneous agents of any kind, other than the species and strain stated, is unsuitable for vaccine production;
 - any substrate (after processing, if relevant) found to contain any extraneous agent shall be discarded or used only in exceptional and justified circumstances;
 - any batch of substance (after inactivation or processing, if relevant) found to contain any extraneous agents shall be discarded or used only in exceptional and justified circumstances; to be accepted for use, further processing must be carried out that will ensure elimination or inactivation of the extraneous agent in the final product, and it shall then be demonstrated that the elimination or inactivation has been satisfactory;
 - unless otherwise prescribed, any final product found to contain any extraneous agent shall be discarded.

Validation of new techniques- Conclusion

100% specificity and sensitivity of the method not possible: maintain a balance between specificity and sensitivity with respect to the potential negative impact of the agent tested

⇒ justify the proposed parameters of the methodology with regard to the foreign agent to be tested. Test controls should always be included.

Development of new techniques of detection of EA is encouraged

⇒ collaboration and dialogue between industry and regulatory authorities necessary to facilitate the development, standardisation and regulatory acceptance of new techniques for the detection of EAs in veterinary medicinal products.



Thank you for your attention!



Before containment: Previous office
French Agency for Veterinary Medicinal Products



Containment: Current office – Home
sweet home...