8 December 2016
EMA/CVMP/IWP/65876/2016
Committee for Medicinal Products for Veterinary Use (CVMP)

Overview of comments received on 'CVMP reflection paper on methods found suitable within the EU for demonstrating freedom from extraneous agents of the seeds used for the production of immunological veterinary medicinal products' (EMA/CVMP/IWP/251741/2015)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	Clean Cells
2	European Pharmacopoeia Group 15V
3	IFAH-Europe

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

1. General comments – overview

Stakeholder no.	General comment (if any)	Outcome (if applicable)
1	Equine, caprine and ovine agents in line with the 7BMI10A “guideline” could be added.	**Partly accepted.** It is recognised that the paper is not complete. Not all species are covered. Furthermore only a few companies contributed. It needs to be discussed how to proceed in the future with this reflection paper and further updates.
2	Group 15V and EDQM have expressed their concerns to IWP at multiple times before and would like to re-iterate these concerns. Rather than describing the methods in details, it would be of interest for the users that key performance criteria be given for these methods. These criteria would include, for example, sensitivity, specificity, robustness of the method, need positive and negative controls. Proposed change (if any): Not to proceed with this Reflection Paper, or at least to delete section 3 on viral detection methods.	**Partly accepted.** Section 3 on viral detection methods was revised. It needs to be discussed how to proceed in the future with this reflection paper and further updates.
3	IFAH-Europe welcomes the opportunity to comment on this reflection paper which has fruitfully mined the experience with extraneous agents testing of both marketing authorisation holders and competent authorities and fully supports this pragmatic approach. However, the paper is not yet complete. Not all species have been covered and for those species covered companies not having been able to contribute so far, should have the opportunity to share their data from EU-assessed and EU-approved seeds, and by doing so add some more examples of suitable cells and methods for extraneous agents testing to	**Partly accepted.** It is recognised that the paper is not complete. Not all species are covered. Furthermore only a few companies contributed. It needs to be discussed how to proceed in the future with this reflection paper and further updates.

Stakeholder no.	General comment (if any)	Outcome (if applicable)
	the tables in this paper. We would welcome either in this document or in a notification the description of the approach to take by companies that want to later participate to data collection for the work (an EMA contact and some basic rules for eligibility on natures of dossiers and procedure). This should also be the rules for any new cell or tests methods that were not described assessed at time of writing this guidance.	
3	Looking ahead to the future: introduction and implementation of this reflection paper and Annex 2 to guideline EMA/CVMP/IWP/206555/2010 ('*The approach to demonstrate freedom from extraneous agents as part of the production and control of IVMPs for mammalian species and finfish*') will have major effects and consequences. In IFAH-Europe's view, it is essential that the publication of the final versions of these two documents is accompanied by the publication of a third document describing how the first two documents should be used with regard to: - applicability to seeds for existing products, - applicability to seeds for new products where the seed is already in use for (an) existing product(s), - testing for extraneous agents in the new guidance additional to the ones in the existing guidance, validation of new test techniques, in particular tests based on nucleic acid amplification technology (NAT).	**The general comment on support of the reflection paper was noted.** The issues proposed to be discussed in a third document and presented by IFAH-Europe in a separate letter to the CVMP/IWP will be discussed separately.

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
78–82	1	Comment: Number of flasks should be reduced (in line with EP5.2.4) and haemadsorption step could be clarified e.g suppress “25-30 min and/ or one hour” Proposed change (if any):2 cell monolayers of at least 70 cm^2 are washed....One flask is incubated for at least 25-30 min at 2-8°C and the second flask is incubated for at least 25-30 min at 20-25°C	**Partly accepted.** Section 3 on viral detection methods was revised.
83	1	Comment: What does really mean possible confirmation? PCR can be performed for this aim. It is necessary to give a definition of appropriate: Is it depending of the targeted species for the vaccine or of the researched virus (eg CPV with pig red cells). This assay is supposed to be non specific and therefore with a limited number of variations. Proposed change (if any):	**Partly accepted.** Section 3 on viral detection methods was revised.
83	1	Comment: Is there a real interest of performing this assay (species of red cells should be considered) since PCR or IS could detect these viruses. Proposed change (if any): suppress the haemagglutination	**Partly accepted.** Section 3 on viral detection methods was revised.

<table>
<tr><th>Line no.</th><th>Stakeholder no.</th><th>Comment and rationale; proposed changes</th><th>Outcome</th></tr>
<tr><td></td><td></td><td>paragraph</td><td></td></tr>
<tr><td>89</td><td>1</td><td>Comment / Proposed change (if any):

The detection by immunostaining or ELISA can be replaced by the detection by PCR. The performance parameters and the validity of the PCR assay will be established according to the European Pharmacopoeia guideline § 2.6.21.</td><td>Partly accepted.
Section 3 on viral detection methods was revised.</td></tr>
<tr><td>114–119</td><td>1</td><td>Comment: this approach is different from that which is described in EP2.6.24

Proposed change (if any):</td><td>Partly accepted.
Section 3 on viral detection methods was revised.</td></tr>
<tr><td>158–168</td><td>1</td><td>Comment: All the virus mentioned below are detectable by PCR:<table><tr><td>Akabane virus</td><td>Feline Herpes virus</td></tr><tr><td>Alcelaphine herpesvirus 1</td><td>Feline immunodeficiency virus</td></tr><tr><td>BlueTongue Virus</td><td>Feline Panleukopenia virus</td></tr><tr><td>Bornavirus</td><td>Feline sarcoma virus</td></tr><tr><td>Bovine Adenovirus 2, 3,4,5,8</td><td>Foot-And-Mouth Disease Virus (all types)</td></tr><tr><td>Bovine coronavirus (Betacoronavirus 1)</td><td>Influenza Virus type A</td></tr><tr><td>Bovine enterovirus</td><td>Lumpy skin disease virus</td></tr><tr><td>Bovine herpesvirus 1,2,3,4</td><td>Ovine herpesvirus 2</td></tr></table></td><td>The comment is noted.
However the information included in the annex is based on available data from already EU-assessed and EU-approved seed. In the context of the preparation of this reflection paper and the annex no data are available from EU-assessed and EU-approved seeds using PCR.
It is recognised that the annex is not complete. Not all species are covered. Furthermore only a few companies contributed.
It needs to be discussed how to proceed in the future with this reflection paper and further updates.</td></tr>
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Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome

Bovine Leukaemia Virus	Porcine CircoVirus (1&2 et Bovine circovirus)
Bovine papillomaviruses 1 to 8	Porcine cytomegalovirus
Bovine papular stomatitis virus	Porcine epidemic diarrhea virus
Bovine Parainfluenza 3	Porcine Hemagglutinating Encephalomyelitis Virus
Bovine Parvovirus	Porcine Parvovirus
Bovine Respiratory Syncytial Virus	Porcine Respiratory Reproductive Syndrome Virus
Bovine Viral Diarrheoa Virus type 1 and 2	Pseudorabies Virus
Canine adenovirus 1 and 2	Reovirus 3
Canine Coronavirus	Rinderpest virus
Canine Distempervirus	Canine parainfluenza virus type 2
Canine oral papillomavirus	Pseudocowpox virus
Classical swine fever virus	Swine vesicular disease virus
Cowpoxvirus	Swinepox virus
Encephalomyocarditis Virus	Transmissible Gastroenteritis Virus
Epizootic hemorragic disease virus (+ BTV)	Vesicular stomatitis virus New jersey / Indiana
Feline Foamy Virus	Rabies virus

158 Table “Porcine”	1	Comment:	**Not accepted, but the comment is noted.**

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): 1) Classical swine fever virus 2) Add ST cells 3) CPE 4) Vesicular stomatitis virus 5) Add MDCK, CRFK, E. Derm, BHK-21 cells 6) CPE	However the information included in the annex is based on available data from already EU-assessed and EU-approved seed. The proposed culture substrates and methods of detection are not included in dossiers providing basis for this document. It is recognised that the annex is not complete. Not all species are covered. Furthermore only a few companies contributed. It needs to be discussed how to proceed in the future with this reflection paper and further updates.
160 Table “Bovine”	1	Comment: Proposed change (if any): 1) Reovirus 2) Add MDCK cells CPE	**Not accepted, but the comment is noted.** See comment above.
32	2	Comment: It's questionable if good laboratory practice allows the use of controls, which are different from the virus to be tested. Extrapolation to other agents is not acceptable if not based on data. Proposed change (if any): The agents used as positive	**Accepted.**

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		controls may be are those to be tested or other suitable agents. ~~but in~~ In any case they controls must be carefully chosen …	
36	2	Comment: It is understandable that handling of dangerous viruses should be avoided. Nevertheless danger of viruses is no argument to compromise the validity of tests. Proposed change (if any): sentence deleted	**Accepted.**
38–40 starting with "However…"	2	Comment: This is only valid, provided it is experimentally proven. Proposed change (if any): delete the sentence.	**Accepted.**
69–144	2	Comment: It is questionable to state such detailed. procedures. They may differ from lab to lab and from virus to virus. Proposed change (if any): to be deleted.	**Partly accepted.** Section 3 on viral detection methods was revised.
47–49	3	**Comment:** Please see also our point in the general comments regarding the updating of the document. The data should be provided in the same format as the existing bovine, porcine, feline and canine lists. **Proposed change:** The annex of the document is not complete yet. Tables for ovine/caprine, equine, rabbit, hamster, mouse, rat, primates (Vero cells) and finfish extraneous agents testing can be added,	**Partly accepted. But the comment is noted.** Lines 47-49 are revised as suggested in the second part of the proposed change. It is recognised that the paper is not complete. Not all species are covered. Furthermore only a few companies contributed. It needs to be discussed how to proceed in the future with this reflection paper and further updates.

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		provided that suitable test methods are available. Marketing authorisation holders possessing such data or having data additional to the information included in the tables already are invited to send these data in appropriate format ([…link…]) to […address…]. The annex of the document can be updated whenever necessary, in particular to take into account new extraneous agents or additional cells and techniques for which sufficient experience and/or validation data become available to justify their inclusion into the annex below.	
55	3	**Comment:** "content equivalent to 10 doses of vaccine per ml". This recommendation (which has existed in EU for decades) is not easily compatible with new technologies and can be difficult to implement for the inactivated approach. An option to use an alternative, such as 1ml of MSV as in current VICH proposal, would be welcome.	**Accepted.**
59–60	3	**Comment:** When a primate cell line (e.g. Vero) is involved, this would mean that tests should also be performed on primary primate monkey cells. However, for obvious reasons the availability of primary primate monkey cells has become very difficult. For the detection of the viruses listed in Annex 2 to guideline EMA/CVMP/IWP/206555/2010 for 'Primates (Vero cell)', primary primate monkey cells are not necessary. Therefore we propose to follow the existing practice and indicate that use of primary cells will not be necessary for tests to detect primate extraneous viruses. Similarly for primary cells for fish experience	**Partly accepted.** The 3Rs argument is not sufficient to not perform the necessary tests if it is the best way to detect an EA contamination. The use of primary cells is justified by the fact that in principle they would allow the multiplication of EA that are not listed in the table and perhaps not detected by the cell lines commonly used. As long as the Ph. Eur. maintains the test on primary cells, there is no possibility to delete it. Nevertheless, with regard to the use of

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		has shown testing on primary salmon cells does not provide information additional to that obtained by using non-primary piscine cell cultures. Also primary salmon cells appear not a very sensitive system to detect extraneous viruses, at least they appear non-permissive to important fish pathogens like SPDV and ISAV. There are no established methods for extraneous agents testing on primary piscine cell cultures. Finally, from a 3Rs viewpoint numerous animals have to be sacrificed to prepare the numbers of primary cells sufficient to meet Ph. Eur. requirements with regard to the number of cells (cm2) to be used in the tests. **Proposed change:** …taking into account that primary cells from the species must be included in all cases unless justified (i.e. ,for 3Rs reasons the testing for primates, fish extraneous viruses or where existing cell lines for other species are of good sensitivity).	primate primary cell lines, the exception seems appropriate.
75-76	3	**Comment:** A major cytopathic effect, i.e. cell lysis/cell degradation, should not be omitted here. **Proposed change:** Cell degradation, inclusions, giant cells or other abnormalities attributable to virus replication are examined over the whole surface of the cell layers.	**Partly accepted.** Section 3 on viral detection methods was revised.
80-82	3	**Comment:** This description must be made clearer. **Proposed change:** Separate cultures are incubated at 2-8°C and at 20-25°C for at least 25 minutes. The monolayers are regularly inspected for haemadsorption.	**Partly accepted.** Section 3 on viral detection methods was revised.
83-84	3	**Comment:** Haemagglutination can be used for the detection of many more viruses than the three mentioned here. It is suggested to omit the reference to the three viruses. If the reference is	**Partly accepted.** Section 3 on viral detection methods was revised.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		maintained, the standard virus nomenclature rules should be followed. **Proposed change:** Either: Detection of haemagglutination Or: Detection of haemagglutination (possible confirmation of the presence of canine parainfluenza 2 virus, canine parvovirus and ~~F~~feline panleukopenia virus)	
85-88	3	**Comment:** Haemagglutination can be used for the detection of many more viruses than the three referred to in lines 83-84. If the reference to these three viruses is maintained, it seems logical to indicate what erythrocytes are appropriate for these viruses. Alternatively in order to align with the other paragraphs in section 3 (e.g. ELISA and PCR) specific viruses should not be mentioned. **Proposed change:** A cell culture supernatant sample is put in haemagglutination microplates (conical bottom). An equivalent volume (e.g. 50 µl) of a suspension of erythrocytes of appropriate species in physiological buffer is added to each well. After an incubation period of at least 30 minutes at 4°C, the microplates are observed for haemagglutination. A positive control run in parallel should show complete haemagglutination.	**Partly accepted.** Section 3 on viral detection methods was revised.
90-91	3	**Comment:** Suitable fixation methods are not restricted to cold acetone alone. Paraformaldehyde is an example of a proven	**Partly accepted.** Section 3 on viral detection methods was revised.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		alternative **Proposed change:** …a total cell area of at least 6 cm^2 is fixed with cold acetone or by other suitable means…	
93-94	3	**Comment:** In contrast to the description of other methods, a description of the observation(s) to be made is lacking here. **Proposed change:** To add: The whole cell area is inspected for immunostaining specific for the virus to be detected.	**Partly accepted.** Section 3 on viral detection methods was revised.
96-98	3	**Comment:** This first part of the description is superfluous and disproportionally detailed compared to other sections. **Proposed change:** After the amplification passages of the test sample, …	**Partly accepted.** Section 3 on viral detection methods was revised.
102-106	3	**Comment:** 1. For conformity with Ph. Eur. 2.6.21, please replace “molecular methods” by “nucleic acid amplification techniques (NAT)”. 2. “Suitably validated” already implies that sensitivity (and specificity) must have been established. 3. The title of this reflection paper restricts its scope to seeds. Therefore reference to (other) materials of animal origin is not appropriate. 4. If PCR analysis is applied after an amplification procedure in cells that are susceptible to the seed virus, the seed virus must have been neutralised before the amplification procedure. 5. If the PCR analysis is applied directly on a virus seed, neutralisation of the virus seed is not necessary indeed.	**Partly accepted.** Section 3 on viral detection methods was revised.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		6. With regard to the requirements for the sensitivity of the PCR method when applied directly on the virus/cell seed: please see the accompanying separate letter. **Proposed change:** Detection by nucleic acid amplification techniques (e.g. PCR~~s~~) For the detection of selected agents, a suitably validated nucleic acid technique (NAT) can be applied either in cells after the amplification procedure, or directly on the cells/virus seeds or materials of animal origin, provided the sensitivity has been proven. For virus seeds neutralisation is not necessary.	
107 *ff.*	3	**Comment:** 1. The methodology for a test using embryonated eggs is given for the detection of a few specified extraneous viruses: influenza virus, swine pox virus and cowpox virus. The methodology for the tests using embryonated eggs for the detection of vesicular stomatitis virus (VSV), bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV) is not provided, although these tests are mentioned in the Annex as suitable methods for detection of these agents. 2. Citation from H. Mahnel in 'Virus infections of porcines', ed. M.B. Pensaert, 1989, Elsevier, Amsterdam, NL: "*Only porcine cell cultures are permissive for swinepox virus. Apart from pigs, no other animal species, including laboratory animals and chicken embryos are susceptible to the virus or suitable hosts for its growth (Shop, 1940; Mayr, 1959; Kasza et al., 1960; Cheville 1996; Meyer and Conroy, 1972)*". Inclusion of	**Partly accepted.** Section 3 on viral detection methods was revised.

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		the test using embryonated eggs for the detection of swinepox virus therefore seems questionable, both here and in the Annex. 3. The last sentence of the description of the test using embryonated eggs for swinepox virus has linguistic flaws. See below. 4. The cowpoxvirus is tested by CAM route not the IV route (error in original data that were double-checked during comment phase of this draft text. 5. Both for the test for swinepox virus and for the test for cowpox virus it is indicated what should be seen if the virus is absent. However, as these methods are described for virus detection, the signs to be seen when the virus is present should be indicated. **Proposed change:** Please re-examine this section. For 3.proposals: After 7 days of incubation, the eggs are chilled by overnight refrigeration. The chorioallantoic membranes are removed and inspected for pox lesion and the appearance of the embryos. For 4.proposals: Poxvirus - inoculation of the product on test. And delete all previously existing text on cowpox.	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
124	3	**Comment:** adjusting wording to current practice **Proposed change:** is added. The microplate is horizontally	**Partly accepted.** Section 3 on viral detection methods was revised.
155	3	**Comment:** For some viruses, the tables also refer to embryonated eggs as suitable substrates for amplification. **Proposed change:** Based on available data from already EU-assessed and EU-approved seeds, the cells and other substrates, as listed in…	**Accepted.**
168-182	3	**Comment:** Please see attachment for some questions and remarks with regard to the listed cells/cell lines.	**Not accepted, but the comment is noted.** However the information included in the annex is based on available data from already EU-assessed and EU-approved seed. The proposed culture substrates and methods of detection are not included in dossiers providing basis for this document. It is recognised that the annex is not complete. Not all species are covered. Furthermore only a few companies contributed.

ATTACHMENT: Comments to lines 168-182

Cells	Questions, remarks
▪ A-72 = canine fibroblast cell line	
▪ BEL = bovine embryo lung cell line	
▪ BFDL = bovine fetal diploid lung cell line	▪ = FBL ?
▪ BHK-21 = baby hamster kidney cell line	
▪ BT = bovine turbinate cell line	
▪ C81 = feline S+L- fibroblast cell line	▪ S^+L^-
▪ CCL-33 = porcine kidney cell line	▪ = PK-15
▪ CK = primary calf kidney cell	
▪ CrFK = Crandell-Rees feline kidney cell line	
▪ CT = primary calf testis cell	
▪ CTY = calf thyroid cell line	
▪ DK = primary dog kidney cell	
▪ EBK = embryonic bovine kidney primary cell	▪ = PBEK ?
▪ EBTr = embryonic bovine trachea cell line	
▪ FBL = foetal bovine lung cell	▪ = BFDL ?
▪ FBT10 =	▪ = **Foetal Bovine Testes cells**
▪ FBTy = primary fetal bovine thyroid cell	
▪ FEA = feline embryo fibroblast cell line	
▪ FEF = primary feline embryo fibroblast	▪ ~~primary~~ feline embryo fibroblast cell line
▪ FK = primary feline kidney cell	
▪ FLK = foetal lamb kidney cell	
▪ FSK = primary fetal swine kidney cell	
▪ HEK293 = human embryonic kidney cell line	
▪ IB-RS-2 = porcine kidney cell line	
▪ IPB3 = bovine lung cell line	
▪ IRC = cat kidney cell line	
▪ L929 = murine fibrosarcoma cell line	
▪ MA104 = monkey african green kidney cell line	▪ African green monkey kidney cell line*)
▪ MDBK = Madin-Darby bovine kidney cell line	
▪ MDCK = Madin-Darby canine kidney cell line	
▪ MYA-1 = feline lymphoid cell line	
▪ PAM = porcine alveolar macrophage	▪ = PLM ?
▪ PBEK = primary bovine embryo kidney cell	▪ = EBK ?
▪ PEK = pig embryo kidney cell line	

Cells	Questions, remarks
▪ PK = primary kidney cell	▪ primary pig kidney cell
▪ PK-15 = porcine kidney cell line	
▪ PLM = porcine lung macrophage	▪ = PAM ?
▪ PS = porcine kidney cells	
▪ Q-201 = feline S+L- lymphoid cell line	▪ S^+L^-
▪ QN-10 = feline S+L- fibroblast cell line	▪ S^+L^-
▪ SCP = sheep choroid plexus cell line	
▪ SK = primary swine kidney cell	▪ ~~primary~~ swine kidney cell line
▪ SKP = sheep kidney primary cell	
▪ ST = swine testis cell line	
▪ Vero = african green monkey kidney cell line.	▪ African green monkey kidney cell line

- 'MA' in 'MA104' stands for: 'Microbiological Associates'
- S^+L^- = sarcoma virus positive, leukaemia virus-negative
- 'CCL-33' is catalogue no. of American Type Culture Collection

NOTE: If lists for more species are included, it should be considered to divide this explanatory table of cell name abbreviations in listings per species.