8 December 2016
EMA/CVMP/IWP/74071/2016
Committee for Medicinal Products for Veterinary Use (CVMP)

Overview of comments received on 'Guideline on requirements for the production and control of IVMPs' (EMA/CVMP/IWP/206555/2010-Rev.1) including Annex 2 on the approach to demonstrate freedom from extraneous agents

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

Stakeholder no.	Name of organisation or individual
1	European Pharmacopoeia Group 15V
2	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	Group 15V has taken note that avian extraneous agents are out of the scope of the Guideline. For the future, it would be easier for the users to have all the lists for extraneous agents in the same location. This could be discussed at a later stage.	**Accepted.** The comment was noted.
2	IFAH-Europe welcomes the opportunity to comment on this draft guideline. We appreciate that in this third draft version of Annex 2 to Guideline EMA/CVMP/IWP/ 206555/2010 the comments provided by IFAH-Europe earlier have been taken into consideration. In our view, the Annex 2 in its whole has become a balanced document to which we only have a few comments (see below at 'Specific comments'). This document clarifies EU expectations with regards to purity testing. It highlights the need for a risk assessment which is very welcome for the good management of seed testing. Even if the approach departs from the classical specific and non-specific approach the basic laboratory work remains the same; and this may be taken forward at VICH with some collaborative intents. Looking ahead to the future: introduction and implementation of Annex 2 to this guideline and of reflection paper EMA/CVMP/IWP/251741/2015 ('*CVMP reflection paper on methods found suitable within the EU for demonstrating freedom for extraneous agents of the seeds used for the production of IVMPs'*) 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	**The general comment on support of Annex 2 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.

Stakeholder no.	General comment (if any)	Outcome (if applicable)
	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 issues proposed to be discussed in the third document are presented by IFAH-Europe in a separate letter to the CVMP/IWP that accompanies this comment.	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
142-146	1	Comment: Has the future ban of mercury been taken into consideration? The recommendation to refrain from the use of toxic preservatives (e.g. mercury containing substances) is missing. Was this done intentionally? Proposed change (if any):	**Not accepted.** The comment was noted and the issue needs to be discussed separately.
181	1	Comment: 'viruses' should be replaced by "micro-organisms" (cf. chlamydia) Proposed change (if any): "For ~~virus~~ micro-organisms grown in eggs, each batch of clarified virus harvest shall …"	**Accepted.**
262	1	Comment: In the light of 3Rs, the following modification is proposed. Proposed change (if any): "Mortality as an evaluation parameter in vaccination-challenge studies should be avoided whenever possible; humane endpoints have to be respected. Moribund animals should be humanely killed."	**Accepted.**
390–423	1	Comment: This chapter reflects only in part the state of the art. Tissue culture methods are described extensively, whereas methods using molecular technologies are described only in a few sentences. Methods using molecular techniques are in some cases the only method suitable for the detection of ext.	**Partly accepted.** The chapter was revised.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		agents (e.g. Papillomaviruses, RD-114...). This justifies a more extended chapter in the text. Proposed change (if any): change the order of the methods (to start with the description of the genome detection methods -see lines 418-421- and have cell cultures methods at the end of the paragraph).	
398	1	Comment: Is there proven scientific evidence for the sentence “Whenever one cell culture is used to detect several agents, the use of one agent as positive control is sufficient to demonstrate the suitable growth characteristics of the cell culture”? As long as not proven, it shouldn’t be stated. Proposed change (if any): sentence deleted.	Partly accepted. Paragraph is revised.
430	1	Comment: Equine flavivirus may be added to the list Proposed change (if any):	**Not accepted.** Horses can be infected by several flaviviruses: Mainly West Nile virus (WNV – is included), but also Kunjin virus (very close to WNV in Australia), Murray Valley encephalitis virus (Australia), Japanese encephalitis virus, Usutu virus (Europe etc.). However, biologically and epidemiologically, none of them can characterised as an "equine flavivirus", because they are viruses of other species (e.g. birds), only "accidentally" affecting horses.
341	2	**Comment:** We propose to differentiate between atlantic salmon and other finfish. **Proposed change:** This annex is applicable to IVMPs for mammalian species, Atlantic salmon and other finfish.	**Accepted.**
365 *ff.*	2	**Comment:** We propose to include two additional types	**Partly accepted.**

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		of justification: Ad g): See comment to line 438: Finfish agents. Ad h): See accompanying separate letter. **Proposed change:** g): For finfish: disease/agent does not occur in the source or target fish species involved. Available literature or expert view to support this should be provided. h): If a seed has been satisfactorily tested earlier for the agents listed in Eudralex 7BIm10a: agent would have been detected in the (general) tests performed earlier (e.g. by CPE in one of the cell types used). Literature to support this must be provided.	The proposal to add g) For finfish is accepted. The addition of h) is not supported. This issue will be discussed separately in connection with the letter presented by IFAH-Europe to the CVMP/IWP.
373-375	2	**Comment:** The wording will allow authorities to require validation of the tests (serological for example) used to guarantee the SPF status of the herd (which should be avoided as SPF status is being handled and audited) - We suggest the following rewording : **Proposed change:** b) Disease/agent does not occur in herd of origin (i.e. specific pathogen free (SPF) status). Supporting documentary evidence must be provided for the SPF status of the herd. SPF certificate showing that the herd is free of the respective extraneous agent will be accepted without further documentation.	**Partly accepted.** The reference if SPF animals are used was added. However if non-SPF animals they need to be tested and shown to be free from a defined list of infectious agents.
395	2	**Comment:** Robustness is mentioned as one of the key criteria for test suitability. According to VICH GL 1, “robustness of an analytical procedure is a measure of	**Accepted.**

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage". Robustness is an important criteria for validation of routinely used methods, and does not strictly speaking appear to apply here. Instead, repeatability (intra-assay precision) appears to be a better criteria here. **Proposed change:** The suitability of test methods used to detect extraneous agents is an essential prerequisite. The following aspects are identified as key criteria for test suitability: defined method, sensitivity, specificity, repeatability of the method and need for positive and negative controls.	
401-403	2	**Comment:** The last part is too vague ("considered" and "qualified laboratories") and may open to unnecessary questions. The principle should be: when a cell line has been shown to be suitable to support the growth and allow detection of an extraneous agent, it can be used in any laboratories being able to carry out such kind of testing. **Proposed change:** "Alternatively, test methods described in the document "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 for mammalian species and finfish" (EMA/CVMP/IWP/251741/2015) can be used when implemented by experienced laboratories	**Partly accepted.** It is accepted to replace considered by use. The addition 'without further validation' is not supported. If the test method mentioned in the CVMP RP (not described in detail) will be implemented by qualified/experienced laboratories it needs to be shown that the method works. Each testing laboratory must demonstrate their suitability to perform the relevant test.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		without further validation.	
407-408	2	**Comment:** Cell systems and detection methods for specified extraneous viruses have been and are selected for their known suitability for primary isolation of these viruses. In practically all cases, laboratory adapted strains can automatically be detected well in/by these systems/methods too. **Proposed change:** Their suitability for the detection of field (wild) strains of specified agents should be known.	**Accepted.**
409-411	2	**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 section 2 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 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	**Partly accepted.** 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 primate primary cell lines, the exception seems appropriate.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		cultures. Finally, from a 3Rs viewpoint numerous animals have to sacrifice 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:** Hence, except for the testing for primate and fish extraneous viruses, suitable primary cells must be part of the cell types selected for the detection of extraneous viruses not excluded by step 1.	
411	2	**Comment:** for clarity addition of primary in all case can be done. Nevertheless being primary those cells come from live animals that need to be used for testing. In view of 3Rs if sensitivity is shown equivalent and as doable under Ph. Eur. rules, cell lines can replace them. It may be worth mentioning this in this text. **Proposed change:** the detection of extraneous viruses not excluded by step 1. For 3Rs reason, replacement of such primary cells on a case by case basis is acceptable (when data demonstrating sufficient sensitivity are provided).	**Not 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.
413-416	2	**Comment:** Mycoplasma testing is relevant for all species. Mycobacterium testing is only relevant for a few species. In the earlier draft version of this Annex 2, mycobacterium testing was indicated for ruminant species only. We advise to specifically mention that here as well. **Proposed change:** Mycoplasma tests should be	**Not accepted.** The original sentence is kept. Mycobacteria can be found in other species than ruminants.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		implemented for all species and mycobacterium tests for ruminant species, whenever relevant. A thorough justification must be provided for the complete or partial omission of these testing.	
418	2	**Comment:** "Antigen" and genome detection: it is not necessarily the 'antigen' that may be found but viral elements. **Proposed change:** Extraneous agent specific elements and genome detection.	**Partly accepted.** Extraneous agent specific elements were added. Antigen was kept.
421	2	**Comment:** differentiation should be more precise. **Proposed change:** method for differentiation between replicative, live or killed agents.	**Accepted.**
422-423	2	**Comment:** Where the Ph. Eur. does not provide a method for mammalian/fish extraneous agents testing by serology, it would be helpful if some guidance is given here. The method suggested below largely follows the 'Test for extraneous agents using chicks' in Ph. Eur. 2.6.24. **Proposed change:** Detection of an agent may also be based on detection of corresponding antibodies. In this case, appropriate serological methods should be used. An example of a suitable animal immunisation protocol would be: Animals seronegative for and sensitive to the extraneous agent(s) to be detected are immunised with the test article at least twice, with 2-6 weeks interval, using a 5- to 10-fold higher dose for the second immunisation. Blood collection and serum preparation occurs a similar period after the second immunisation. In case of a test article expected to be	**Not accepted.** It is not foreseen to include an example of a suitable animal immunisation protocol.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		pathogenic for the animals to be immunised, the immunisations with non-treated test article can be preceded by immunisation with inactivated (and adjuvanted) test article to raise protection against the pathogenic effects.	
427 and lines below	2	**Comment:** tables of extraneous agents refer to either specific *Brucella* (such as *Brucella suis* for porcine), or to all *Brucella* species (*Brucella spp*. such as for Bovine). Likewise, Leptospira, Helicobacter and Chlamydia are all referred to as "spp.". This may raise unnecessary concerns and questions, regarding species of micro-organisms that are not relevant to the respective animal species. **Proposed change:** consider restriction of *Brucella, Leptospira, Helicobacter* and *Chlamydia* to the relevant species only (ie, species relevant for the specific animal species, such as *Brucella suis* for porcine), and not to all species within the same genus.	**Not accepted.** There is no need to be too specific for bacteria agents as the methods to detect them are general ones. Furthermore, it will avoid the updating if a new serotype/serogroup … of bacteria has to be included. For example, any specific test to identify each leptospira will not be requested. Another example: For sheep «Brucella» changed to «Brucella melitensis». However, it is know that sheep are affected by B. melitensis and B. ovis. Also, it was found that sheep are infected with B. abortus when live near infected cattle.
438	2	Finfish list of agents **Comment:** 1. "Finfish" comprises as many species as all other vertebrate species combined. The list of finfish agents is not made species-specific, however. It would make sense to sub-divide "finfish" into economically relevant species for which vaccines are developed, *e.g.:* Atlantic salmon, rainbow trout, common carp, red seabream, etc. 2. "Betanodavirus" is indicated here as one virus species, but in fact this comprises a very large	**Partly accepted.** Differentiation between Atlantic Salmon and other finfish has been included.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		group of different viruses each with their own group of susceptible fish species. For “iridovirus” two representatives are listed: epizootic haematopoietic necrosis virus and red sea bream iridovirus. On the other hand, four rhabdoviruses are listed: spring viraemia of carp virus and perch rhabdovirus (both belonging to the *Vesiculovirus* genus of the *Rhabdoviridae* family), infectious haematopoietic necrosis virus and viral haemorrhagic septicaemia virus (both belonging to the *Novirhabdovirus* genus). 3. If the “finfish” list of agents is not further organized, addition of an extra type of ‘Justification for not carrying out a test for a specific agent´ is required. See also comment to line 365. **Proposed change:** To add to “Step 1: Justification for not carrying out a test for a specific agent”: g) For finfish: disease/agent does not occur in the source or target fish species involved. Literature evidence or expert view should be provided.	
438	2	**Comment:** EMA/CVMP/388694/2014 “Guidance on the classification of veterinary medicinal products indicated for minor use minor species (MUMS) / limited market” lists salmon as a major species. Recommendation is made to differentiate between Atlantic Salmon and other finfish in the list of extraneous agents. **Proposed change:** Creation of an extraneous agent list for the major species Atlantic salmon, which includes agents known	**Accepted.**

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		to be present or cause disease in this species of fish. SALMONIDS <u>Viral agents:</u> Infectious salmon anemia virus (ISAV) Salmon alphaviruses Infectious pancreatic necrosis virus (IPNV) Infectious haematopoietic necrosis virus (IHNV) Viral haemorrhagic septicaemia virus <u>Bacterial agents:</u> Aeromonas salmonicida (Ph.Eur 2.6.1 may be used provided the suitability of the Ph. Eur. 2.6.1 method to detect this agent is demonstrated). Fish-pathogenic Francisella spp. Flavobacterium psychrophilum Piscirickettsia salmonis Renibacterium salmoninarum Vibrio anguillarum Other FINFISH <u>Viral agents:</u> Betanodavirus Channel catfish virus Epizootic haematopoietic necrosis virus (EHNV) Koi herpes virus Oncorhynchous masou virus Perch rhabdovirus Red sea bream iridovirus Spring viraemia of carp virus (SVCV) Viral haemorrhagic septicaemia virus (VHSV) <u>Bacterial agents:</u> Aeromonas salmonicida (Ph.Eur 2.6.1 may be used provided the suitability of the Ph. Eur. 2.6.1 method to detect this agent is demonstrated)	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Edwardsiella ictaluri Fish-pathogenic Francisella spp. Flavobacterium psychrophilum Piscirickettsia salmonis Renibacterium salmoninarum Vibrio anguillarum	