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

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Final

CONCEPT PAPER

for a Guideline on Test on the Presence of Extraneous Viruses in veterinary viral vaccines

1. Introduction

Three following guidelines on extraneous viruses (EV) testing have been discussed for a long time in Biologicals Quality Monitoring-Expert Working Group (BQM-EWG).

(1) Cell-based methods for testing virus seeds, cell seeds and other starting materials of animal origin for the presence of EV,

(2) General principles for detection of EV in veterinary vaccines and defining the testing of seeds and materials of animal origin, and

(3) A list of EV that need to be covered.

For GL (1), there have been discrepancy how to proceed with the shortened version (2016) provided by EU or with the earlier, more detailed version (2012, updated 2015) between EU and others. This topic has been discussed for nearly twenty years in VICH.

To compromise some discrepancies among the members, JMAFF has been proposing a simple concept for regrouping the specific testing into a few common testing groups.

2. Problem statement

There have been arguments between the parties on the concepts for EV testing as follows;

1) Specific test for selected viruses on risk-based approach supported by the EU, and
2) General test based on non-specific test methodology supported by rest of the party.

Although these two concepts seem very different in appearance, we have found some commonalities between them as described in the "discussion" in this paper and couple of documents presented at the past SC meetings. As we cannot reach the harmonization without resolving the issue, we should continuously explore the way to recognize the commonalities between them.

3. Impact for public health, animal health and animal welfare

Harmonization of extraneous virus testing required for all vaccine products will facilitate vaccine availabilities throughout the VICH regions and other countries.

4. Anticipated benefit

This concept will be valuable for both regulatory authority and industry to reduce the overall burden for EV testing required for vaccine products.

5. Discussion

According to the JMAFF proposal presented at the previous VICH Steering Committee meeting in Tokyo [*VICH/IN/17036, 6 Nov. 2017*], we found specific testing, taken from the EU response document [*EMA/CVMP/9917/201, 15 Feb. 2018*], could be categorized into a couple of common testing groups with a cell substrate and detection method as

discussed in the previous JMAFF proposal [*VICH/IN/18006, 01/06/2018]* (see appendix for detail).

In this paper, we further examined the possible categorization of suitable culture substrates and detection methods in other species shown in "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*].

The tests listed in the tables below as well as those in the previous documents relating to this concept paper (see Appendix) are all drawn from the above-mentioned CVMP reflection paper. The conclusion that these tests are suitable for demonstrating freedom from extraneous viruses was based on evaluation of data submitted to EU regulators. Regulators from other regions may not have seen (all of) these data and may therefore not be in a position to support the EU conclusions on the suitability of the various methods. In addition, regulators from regions other than the EU will have experience with tests for extraneous viruses of particular relevance to their regions and not included in the CVMP reflection paper.

As we only have a list of tests found suitable in the EU, the grouping concept envisaged in this concept paper would be of particular relevance to the EU. However, similar lists of methods found suitable for demonstrating freedom from extraneous agents could be compiled for each of the other regions.

It is not intended to generate a list of standard tests that would be required in all VICH regions. Rather, the purpose of generating and publishing such regional lists would simply be to allow companies to select methods that are known to be accepted in multiple regions[1]. Where testing for absence of a given virus is considered necessary, alternative culture substrates/test methods to those included on the lists could be selected by a company where those substrates/test methods have been shown to be fit for purpose.

Porcine				
Common testing	**1. Extraneous agent(s)**	**2. Suitable culture substrates for amplification**	**3. Suitable methods of detection**	**Remarks**
Group 1: PK+CPE	Porcine adenovirus	PK, PK-15, SK, ST	CPE	
		FSK, MA104	IS	
	Porcine coronavirus - Transmissible Gastroenteritis Coronavirus/Porcine Respiratory Corona Virus	PK, PK-15, ST	CPE	
	Porcine enterovirus (incl. SVDV)	BHK-21, PK, PK-15, SK, ST	CPE	
	Swine herpesvirus - Aujeszky's disease virus	BEL, BSR, CK, CrFK, DK, FEA, FK, FLK, IPB3, MDCK, MDBK, PBEK, PEK, PK, PK-15, SK, ST, Vero	CPE, IS	
		MA104	IS	

[11] The fact that a test has been previously accepted in a region does not preclude the need for data demonstrating the method to be fit for purpose when used in the laboratory undertaking product testing.

	Swinepox virus	PK, PK-15, SK, ST	CPE	At least 5 passages are needed
		embryonated eggs	embryo lesions (pock on CA membrane)	
	Vesicular stomatitis virus	BHK-21, PK, PK-15	CPE	
		embryonated eggs	embryo death	
	Encephalomyocardits virus	BHK-21, PK, SK, ST, Vero	CPE	
	Foot-and-mouth disease virus	BHK-21, CTY, IB-RS-2, IPB3, MDBK, PK	CPE, ELISA	
	Bovine viral diarrhoea virus (cytopathic)	BEL, BHK-21, BT, CK, EBK, EBTr, FBLP, FBT10, IPB3, MDBK, PK-15, SCP	CPE or cytopathic strains	
Group 2: PK+IS	Bovine viral diarrhoea virus (non-cytopathic)	BEL, BHK-21, BT, CK, EBK, EBTr, FBLP, FBT10, IPB3, MDBK, PK-15, SCP	IS for non-cytopathic and cytopathic strains	
	Porcine parvovirus	MA104, PK, PK-15, SK, ST	IS	
	Porcine circovirus, type-1 and type-2	CCL-33, PK, PK-15, PS, SK, ST	IS	
	Classical swine fever virus	IPB3, PK, PK-15	ELISA, IS	
Group 3: MA104+IS	Porcine reproductive respiratory syndrome virus	MA104, PAM, PLM	IS	EU strains do not grow in cells other than macrophages.
	Porcine rotavirus	MA104	IS	Requires trypsin to grow in cell culture. Therefore, no need for testing when trypsin is not used.
Group 4: MDCK+IS	Rabies virus	BHK-21, BSR, BT, DK , EBK, FK, FLK, FSK, MA104, MDCK, MDBK, ST, Vero	IS	
	Influenza virus	embryonated eggs		
		MDCK	IS	
Group 5: Vero+IS	Porcine coronavirus - Porcine Epidemic Diarrhea Virus	Vero	IS	Requires trypsin to grow in cell culture. Therefore, no need for testing when trypsin is not used.
Group 6: PLM+CPE, IS	Swine herpesvirus - Porcine cytomegalovirus	PLM	CPE, IS	Does not grow in cells other than macrophages.

Bovine				
Common testing	**1. Extraneous agent(s)**	**2. Suitable culture substrates for amplification**	**3. Suitable methods of detection**	**Remarks**
	Akabane virus	BEL, CK, FLK, MDBK	CPE	
		Vero	IS	

Group 1: MDBK+CPE	Alcelaphine herpesvirus (= malignant catarrhal fever virus – African form)	BEL, CK, FLK, MDBK	CPE	
	Bovine adenovirus (subgroup 1)	BEL, CK, CT, FBTy, FLK, IPB3, PBEK, MDBK	CPE, HAd, IS	
		BT, EBK, FBLP	IS	
	Bovine coronavirus	CK, FLK, MDBK, PBEK, PK-15, SKP	CPE, HAd, IS	
		BT, EBK	IS	
	Bovine herpesvirus	CK, EBTr, FLK, IPB3, MDBK, PBEK, SKP	CPE	
		BT, EBK, FLK	IS	
	Bovine papular stomatitis virus	CK, FBTy, MDBK, PBEK	CPE	
	Bovine parainfluenza virus 3	BEL, CK, EBTr, FLK, IPB3, MDBK, PBEK	CPE, HAd, IS	
		Vero	CPE, HAd	
		BT, EBK	IS	
	Bovine parvovirus	CK, EBTr, FLK, IPB3, MDBK, PBEK	CPE, HAd, IS	
		FBT-10	CPE	
		BT, EBK, FBLP	IS	
	Bovine respiratory syncytial virus	BEL, BFDL, BHK-21, CK, MDBK,	CPE, IS	
		IPB3, Vero		
		BT, EBK, FBLP	IS	
	Bovine viral diarrhoea virus (cytopathic)	BEL, BHK-21, BT, CK, EBK, EBTr, FBLP, FBT10, IPB3, MDBK, PK-15, SCP	CPE for cytopathic strains	
	Cowpox virus	BEL, CK, CrFK, EBTr, FEF, FK, FLK, MDBK, PBEK, Vero	CPE, IS	
		embryonated eggs	embryo lesions (pock on CA membrane)	
		BSR, FEA	IS	
	Lumpy skin disease virus	CK, IPB3, MDBK, PBEK, Vero	CPE	
	Pseudocowpox virus	BHK-21, CK, MDBK	CPE	
	Reovirus	BEL, CK, MDBK	CPE, IS	
		BT, DK, FBLP, FK, Vero	IS	
	Rinderpest virus	CK, MDBK, Vero	CPE	
	Swine herpesvirus 1 (= Aujeszky's disease virus)	BEL, BSR, CK, CrFK, DK, FEA, FK, FLK, IPB3, MDCK, MDBK, PBEK, PEK, PK, PK-15, SK, ST,Vero	CPE, IS	
		MA104	IS	
	Vesicular stomatitis virus	BEL, BHK-21, CK, CTY, IB-RS-2, MDBK, PK, Vero	CPE, IS, ELISA	
		FBLP	IS	
		embryonated eggs	embryo death	
Group 2: MDBK+IS	Bovine viral diarrhoea virus (non-cytopathic)	BEL, BHK-21, BT, CK, EBK, EBTr, FBLP, FBT10, IPB3, MDBK, PK-15, SCP	IS for non-cytopathic and cytopathic strains	
	Epizootic haemorrhagic disease virus	BHK-21, MDBK, Vero	IS	
		embryonated eggs	embryo death	

	Rotavirus	BT, CK, EBK, MDBK	IS	Requires trypsin to grow in cell culture. Therefore, no need for testing when trypsin is not used.
	Rabies virus	BHK-21, BSR, BT, DK, EBK, FK, FLK, FSK, MA104, MDCK, MDBK, ST, Vero	IS	
	Bovine leukemia virus	BHK-21, CK, FBL, FLK, IPB3, MDBK	IP, IS	
Group 3: BHK-21+IS	Bluetongue virus	BHK-21	ELISA, IS	
		embryonated eggs	embryo death	
		BK, BT, FBLP, FK, Vero	IS	
	Bovine enterovirus	BHK-21, CK, Vero	CPE, IS	
		BT, EBK	IS	
Group 4: CK+CPE	Bovine rhinovirus	CK	CPE	
Others	Bovine papilloma virus	this virus does not grow in cell culture		
	Jena virus (Norwalk-like)	this virus does not grow in cell culture		
	Ovine herpesvirus 2 (= malignant catharral fever virus – European type)	this virus does not grow in cell culture		

Feline				
Common testing	**1. Extraneous agent(s)**	**2. Suitable culture substrates for amplification**	**3. Suitable methods of detection**	**Remarks**
Group 1: CrFK+CPE	Cowpox virus	BEL, CK, CrFK, EBTr, FEF, FK, FLK, MDBK, PBEK, Vero	CPE, IS	
		BSR, FEA	IS	
		embryonated eggs	embryo death	
	Feline calicivirus	CrFK, FEF, FK, IRC	CPE, IS	
		FEA	CPE	
	Feline coronavirus	CrFK, FEF, FK, IRC	CPE, IS	Type-II feline coronaviruses induce CPE onvarious feline cell lines. Type-I feline coronaviruses only replicate in feline macrophages.
	Feline herpesvirus 1	CrFK, FEA, FEF, FK, IRC	CPE	
	Swine herpesvirus 1 (= Aujeszky's disease virus)	BEL, BSR, CK, CrFK, DK, FEA, FK, FLK, IPB3, MDCK, MDBK, PBEK, PEK, PK, PK-15, SK, ST,Vero	CPE	
		MA104	IS	
	Feline panleucopenia virus	CrFK, FK, IRC	CPE (+ HAg)	Additionally, a haemagglutination test may be performed to improve reading of the CPE.

		CrFK	IS	
Group 2: FEA+CPE	Feline foamy virus (feline syncytia forming virus)	FEA, FEF	CPE	
		CrFK, IRC	IS	
	Feline leukemia virus	CrFK, FEF	ELISA	
		CrFK, IRC	IS	
		C81, FEA, QN-10	CPE	S+L- cells are transformed by infection with FeLV or replication-competent FeSV.
	Feline sarcoma virus	FEA, QN-10	CPE	S+L- cells are transformed by infection with FeLV or replication-competent FeSV.
		CrFK	ELISA	
Group 3: MYA-1, Q201+ELISA	Feline immunodeficiency virus	MYA-1, Q-201	ELISA	
Group 4: MDCK+IS	Rabies virus	BHK-21, BSR, BT, DK, EBK, FK, FLK, FSK, MA104, MDCK, MDBK, ST, Vero	IS	
Group 5: HEK293+RT-PCR	Feline endogenous retrovirus	HEK293	RT-PCR for RD114 virus: sense primer:5'-ccattcctgccattgatcatta-3' antisense primer:5'-ggtgattcccagtccagctagt- 3'	

6. Recommendation (action plan, issues to be addressed, mandate, etc.)

As shown above, grouping into common testing would be possible in some major species. In order to maximize the potential value of such grouping we propose that the VICH BQM-EWG develops a document listing cell culture-based methods found suitable for demonstrating freedom from extraneous viruses on a region by region basis. For confidentiality reasons, and to make the project manageable, each region should be responsible for drawing up its own list. The work should initially focus on limited high priority animal species, e.g., canine, bovine and porcine. The EWG should also comment on the potential to group extraneous viruses with a view to minimizing the amount of testing required.

7. Timetable/ Milestones

2020	Focusing on single animal spieces (porcine), EWG member of each country/region develops a document listing cell culture based methods suitable for demonstrating freedom from extraneous viruses.
2021	The EWG develops a comparison table based on the regional lists, then initiate discussion on the potential grouping of EV-testing.
2022	The EWG develops a draft guideline.
2023	The EWG presents first draft to the SC.

Appendix

Test on the presence of extraneous viruses in veterinary viral vaccines -JMAFF response to the EU document with its regulators' view

JMAFF would like to express our gratitude to the EU for its detailed consideration on the JMAFF proposal presented at the previous VICH Steering Committee meeting in Tokyo [VICH/IN/17036, 6 Nov. 2017], resulted in a response document showing the EU regulators' view [EMA/CVMP/9917/201, 15 Feb. 2018].

We thoroughly examined the document and the list of extraneous agents for canine vaccine with suitable culture substrates and methods of detection (Table 1). We found these specific testings could be categorized into a couple of common testing groups with a cell substrate and detection method as discussed in the previous JMAFF proposal (see appendix for detail).

Table 1. Virus-specific tests and suitable cell substrate / detection methods, taken from the EU response document [EMA/CVMP/9917/201, 15 Feb. 2018]

Canine			
1. Extraneous agent(s)	**2. Suitable culture substrates for amplification**	**3. Suitable methods of detection**	**Remarks**
Canid herpesvirus	DK, MDCK	CPE	
Canine adenovirus	DK, MDCK	CPE, HAd	
Canine coronavirus	A-72, CrFK, DK, IRC, MDCK	CPE	
Canine distemper virus	A-72, DK, MDCK, Vero	CPE	
Canine oral papilloma virus	No known cell culture replication		
Canine Parainfluenza 2 virus	DK, MDCK	CPE (+ HAg), IS	Additionally, a haemagglutination test may be performed to improve reading of the CPE.
	CrFK, Vero	CPE (+HAg)	
Canine parvovirus	CrFK, DK, FEF, IRC, MDCK	CPE (+ HAg)	Additionally, a haemagglutination test may be performed to improve reading of the CPE.
	CrFK	IS	
Rabies virus	BHK-21, BSR, BT, DK, EBK, FK, FLK, FSK, MA104, MDBK, MDCK, ST, Vero	IS	
Swine herpesvirus 1 (= Aujeszky's disease virus)	BEL, BSR, CK, CrFK, DK, FEA, FK, FLK, IPB3, MDCK, MDBK, PBEK, PEK, PK, PK-15, SK, ST, Vero	CPE, IS	
	MA104	IS	

As indicated in Table 2, MDCK seems to be a suitable culture substrate for all the viruses listed except for papilloma virus which has no known cell culture replication. As for the detection method, seven viruses out of eight can be detected by observing CPE on MDCK cell and a subsequent haemagglutination improving the reading in some viruses, while

Rabies virus needs immunostaining on infected MDCK cells. Taking into account these, a Canine vaccine sample can be tested for its extraneous viruses contamination by only three common testings, i.e., Group 1, MDCK+CPE; Group 2, MDCK+IS and Group 3, other methods such as PCR. Additional cells in the table can optionally be used in a situation when MDCK is not assumed appropriate for detection.

Table 2. Possible grouping of extraneous virus testing (conversion from the Table 1)

Canine				
Common testing	**1. Extraneous virus**	**2. Suitable culture substrates for amplification**	**3. Suitable methods of detection**	**Remarks**
Group 1: MDCK+CPE	Canid herpesvirus	DK, MDCK	CPE	
	Canine adenovirus	DK, MDCK	CPE, HAd	
	Canine coronavirus	A-72, CrFK, DK, IRC, MDCK	CPE	
	Canine distemper virus	A-72, DK, MDCK , Vero	CPE	
	Canine Parainfluenza 2 virus	DK, MDCK	CPE (+ HAg), IS	Additionally, a haemagglutination test may be performed to improve reading of the CPE.
		CrFK, Vero	CPE (+HAg)	
	Canine parvovirus	CrFK, DK, FEF, IRC, MDCK	CPE (+ HAg)	
		CrFK	IS	
	Swine herpesvirus 1 (= Aujeszky's disease virus)	BEL, BSR, CK, CrFK, DK, FEA, FK, FLK, IPB3, MDCK, MDBK, PBEK, PEK, PK, PK-15, SK, ST, Vero	CPE , IS	
		MA104	IS	
Group 2: MDCK+IS	Rabies virus	BHK-21, BSR, BT, DK, EBK, FK, FLK, FSK, MA104, MDBK, MDCK, ST, Vero	IS	
Group 3: Others	Canine oral papilloma virus	No known cell culture replication	PCR?	

If this simplified grouping would be acceptable among VICH region, it will be valuable for both regulatory authority and industry to reduce the overall burden for extraneous virus testing. JMAFF therefore proposes that the VICH Biological Quality Monitoring

The Expert Working Group could start to investigate the feasibility of dividing viruses into several common testing groups. It is also recommended to limit the scope within high priority animal species, e.g., canine, bovine and porcine, at the beginning stage for keeping the scale of the task to be manageable. Detection methods for emerging viruses such as HoBi and Seneca Valley viruses should also be considered separately.

A conversion methodology presented by JMAFF at the 36th VICH Steering Committee meeting in Tokyo

As shown in Table 1, each virus can be tested in combination with certain cell substrate (cell line or primary cells) and a detection method (CPE, HA, Immunostaining, etc.). If we can find mutual cell substrate and detection method for several different viral agents, these viruses can be categorized into several testing groups. In this example, twelve viruses ("a" to "l") are categorized into six testing groups ("A" to "F"). The Table 1 is therefore can be converted into general testing grouping as shown in Table 2.

Table 1. Virus-specific tests and grouping by cell substrate / detection methods.

Virus*	Cell substrate#	Detection methods†	Testing group
a	1	1	A
b	2	2	B
c	3	3	C
d	1	4	D
e	2	5	E
f	3	6	F
g	1	1	A
h	2	2	B
i	3	3	C
j	1	4	D
k	2	5	E
l	3	6	F

*Should be adapted from the "List of extraneous virus that need to be covered (Jan 2017)"
#Cell line or primary cells
†Cytopathic effect, haemoadsorption, immunochemical methods, PCR, etc.

Table 2. General grouping of extraneous virus testing

Common testing group	Cell substrate	Detection method	Virus group
A	1	1	a, g
B	2	2	b, h
C	3	3	c, i
D	1	4	d, j
E	2	5	e, k
F	3	6	f, l