VIRBAC Laboratories

Viral inactivation related to steam sterilisation of biological products

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Introduction

The aim of this report is to present an analysis of virus sensitivity to steam sterilisation. Since data on virus inactivation related to this particular treatment are not precisely described in great details in the literature, we decided to provide an additional bibliographic analysis focused on general heat sensitivity of a large spectrum of viruses from different families. In addition, a viral inactivation validation study concerning steam sterilisation (according to European Pharmacopoeia general chapter 5.1.1 requirements) of model viruses is afforded. This study has been performed for Virbac by a specialized Contract Research Organization (CRO).

The first part of the report therefore summarizes the literature-described sensitivity to heat treatment of a large range of virus families representing the different virus subclasses. This bibliographical analysis can easily be extrapolated to steam sterilisation effect since the heating conditions described for this analysis are far less drastic than the combination of time and temperature recommended by the European Pharmacopoeia (general chapter 5.1.1.) to achieve sterilisation (121°C for 15 minutes).

In the second part of the report, viral inactivation validation study results concerning steam sterilisation of viruses chosen according to their known resistance to physical treatment are also provided to strengthen bibliographic data.
Based on this approach, and assuming that viruses from the same nature share similar sensitivities to heat treatments, the data collected for a representative set of viruses included in this investigation can be transposed to the whole range of known viruses. In fact, in this study we have looked at model viruses usually described as the “type species” of each family. Moreover, the several model viruses were chosen in order to the best cover all virus families. Therefore, sensitivity to heat treatment of extraneous viruses included in the table of extraneous agents to be tested for in relation to the general and specific guidelines on production and control of mammalian veterinary vaccines (7Blm10a guideline) can be assessed.
The following assessment covers the bovine, ovine/caprine, porcine, canine, feline, equine sections of the table of extraneous agents (according to the updated annex II: table of extraneous agents to be tested for in relation to the guideline on requirements for the production and control of IVMPs (draft document EMA/CVMP/IWP/105112/2011) and the avian viruses listed in Ph. Eur. general chapter 2.6.24.

Bibliographic analysis

Viral extraneous agents can be divided into two different subclasses depending on absence or presence of the peplos (envelope) that is generally linked to the resistance of the viruses and their environmental stability. Most enveloped viruses are relatively highly susceptible to physical and chemical treatment. In contrast, naked viruses are more resistant. These different natures are usually taken into account in classical virus removal validation studies for the choice of model viruses to be used.
In order to perform relevant extrapolations, our bibliographical analysis is based on viral structure criteria with viruses considered to be representative of a broad range of virus families.

1. Naked (non-enveloped) viruses

Single-stranded DNA viruses

Parvoviridae can be used as model viruses. Indeed, they are well known resistant naked ssDNA viruses. It has been demonstrated in the literature that parvoviruses are inactivated by heat. Indeed, thermal sensitivity of canine parvovirus was demonstrated with a 2.5 Log or 5 Log reduction of the viral titre after 4 h at 80°C, or 2 minutes at 100°C, respectively ([1]*Mc Gavin, 1987).*

Circoviviridae are naked ssDNA viruses that are very resistant to inactivation treatment. However, heat inactivation data for porcine circovirus-2 have been established and demonstrate a 5.6 Log reduction of the viral titre after a 5-second virus exposure to 95°C ([2]*Emmoth, 2004*).

Viruses of the *parvoviridae* and *circoviridae* families are therefore sensitive to heat treatment.

Double-stranded DNA viruses

Adenoviridae family viruses can be used as model viruses. In the literature, data on type V human adenovirus indicate an 8 to 8.9 Log reduction of infectivity after heat treatment at 70°C for 20 min ([3]*Maheshwari, 2004*). Moreover, in the same study, experiments performed on human adenoviruses demonstrated that these viruses are totally inactivated by a 54°C treatment for 50 minutes.

Papillomaviridae family viruses can also be considered as models since they are hardy viruses. Human papillomavirus type 11 infectivity has been shown to be inactivated after a 1-hour treatment at 100°C in a SCID murine model ([4]*Bonnez, 1994*).

Polyomaviridae are representative of small naked dsDNA viruses. Human poliomyelitis virus 1 is inactivated after a 15 seconds treatment at 71°C ([5]*Kaplan, 1952*) or by 6.5 Log after a 2-hour treatment at 60°C ([6]*Nasiri, 2010*).

Viruses of the *adenoviridae, papillomaviridae* and *polyomaviridae* families are therefore sensitive to heat treatment.

Single-stranded RNA viruses

Picornaviridae are representative for other ssRNA viruses. Picornaviruses as foot and mouth disease virus and swine vesicular disease virus are sensitive to heat. Indeed, heat treatment at 65°C for 2 minutes reduces swine vesicular disease infectivity by 6.7 Log ([7]*Turner, 1999).* Moreover, the foot and mouth disease virus is inactivated by a 30-minute treatment at 70°C or by temperatures above 50°C *([8]Foot and mouth OIE - Disease Card 2009).*

Another family (*caliciviridae*) represented by porcine vesicular exanthema virus can be considered. The latter is inactivated by heat treatment at 64°C for 30 minutes ([9]*Denholm, 1996*).

Viruses of the *picornaviridae* and *caliciviridae* families are therefore sensitive to heat treatment.

Double-stranded RNA viruses

Reovirus-3, epizootic hemorrhagic disease virus and bluetongue virus are members of the *reoviridae* family, a family that can be a model for dsRNA viruses. Heat treatments at 60°C are able to inactivate reoviruses ([10]*Sofer, 2003*) and bluetongue viruses (15 minutes) ([11]*OIE Bluetongue, 2009*).

Viruses of the *reoviridae* family are therefore sensitive to heat treatment.

2. Enveloped viruses

Double-stranded DNA viruses

Several virus families can be representative models for dsDNA viruses.

The *herpesviridae* family contains Aujeszky's disease virus (pseudorabies). The virus infectivity can be reduced by 4.3 Log after 16 minutes at 60°C ([12]*Borovec, 1998*) and by 7.1 Log after a 30-minute treatment at 60°C ([13]*Chang, 2010*).
Studies with other herpesviruses (herpes simplex virus types 1 and 2) showed that heat treatments at 56°C for 10 minutes reduce their viral titres by 5 Log *(*[14] *Croughan, 1988*).

Concerning the *poxviridae* virus family, even if the virion of the poxviruses is an enveloped particle, due to the low lipid content of its envelope it has an enhanced resistance to inactivation treatments as compared to other enveloped viruses. Nevertheless, the common inactivation procedures - thermal, chemical, and/or irradiation - are usually effective against pox viruses *(*[15]*Rheinbaben, 2007*).
For example, studies on monkey pox virus showed that heat treatment at 60°C for 10 minutes can reduce its infectivity by 5 Log *(*[16]*Rouhandeh, 1967*). Moreover, data on sheep pox and goat pox viruses that belong to the same family, revealed the susceptibility of these viruses to a 55°C treatment during time periods from 60 minutes to 2 hours depending on the strains ([17]*OIE sheep pox and goat pox*).
Finally, Lumpy skin virus disease (belonging to the *poxviridae* family) is, according to OIE data, susceptible to a 30-minute treatment at 65°C ([18]*OIE-lumpy skin disease*).

African swine fever virus is also an enveloped DNA virus from the *asfaviridae* family. Its sensitivity to heat is confirmed by The Center for Food Security and Public Health *(*[19]*African swine fever OIE - CFSPH 2010)* which states that the meat must be heated to at least 70°C for 30 minutes to inactivate the virus while 30 minutes at 60°C is sufficient for serum and body fluids.

Viruses of the *herpesviridae, poxviridae* and *asfaviridae* families are therefore sensitive to heat treatment.

Single-stranded RNA viruses

Several virus families can be representative models for ssRNA viruses.

Classical model virus is Bovine Viral Diarrhoea (BVD) virus (*flaviviridae* family, pestivirus genus). Its infectivity can be reduced by 1.9 Log after 16 minutes at 60°C ([12] *Borovec 1998*).

Moreover, regarding the *coronaviridae* family, a study on severe acute respiratory syndrome coronavirus (SARS-CoV) has shown that heat treatment at 65°C for 4 min is able to decrease viral infectivity by 4.5 Log ([20]*Darnell 2004*). Other studies on SARS coronavirus demonstrate that its viral load is decreased by 4.9 Log after a 2-hour treatment at 58°C ([21]Paga, 2007).
In the same way, another enveloped ssRNA virus from the *nidovirales* order, the porcine respiratory and reproductive syndrome virus (PRRSV, *arteriviridae* family), is sensitive to heat treatment (total inactivation after 45 minutes at 56°C) ([22]*Scott Williams consulting 2003*).

Porcine influenza (*orthomyxoviridae* family) is inactivated by heat treatment at 56°C for 60 min *([23]Spickler 2009*).

Akabane virus belongs to the *bunyaviridae* family. Akabane viruses are known to be inactivated by 4.5 Log after a 5-minute treatment at 56°C ([24]*Takahashi, 1978*).

Concerning the *paramyxoviridae* family, avian paramyxovirus is known to be inactivated by a 30-minute treatment at 60°C ([25]*OIE Newcastle disease*).

Rabies (*rhabdoviridae* family) is susceptible to heat (1 hour at 50° C) *([26]Rabies OIE - CFSPH 2009).*

Vesicular stomatitis viruses also belong to the *rhabdoviridae* family. Data provided by OIE stipulate that these viruses are efficiently inactivated by heat. Indeed, they are inactivated by heat treatment at 58°C for 30 minutes *([27]Vesicular stomatitis OIE - Disease card 2009).*

Bornaviridae represented by bornavirus are known to be inactivated by a 56°C treatment for 3 days ([28]*Ludwig, 2000*) but also after 3 hours at this same temperature (cell free virion) ([29]*Stramer, 2009*).

Togaviridae, another family of enveloped ssRNA viruses, are also sensitive to heat. Indeed, the infectivity of Sindbis virus, a model virus of this family, is decreased by 6.5 Log after a 30-minute treatment at 100°C ([30]*Stadler, 2006*).

Viruses of the *flaviviridae, coronaviridae, ortho-* and *paramyxoviridae, rhabdoviridae, bornaviridae* and *togaviridae* families are therefore sensitive to heat treatment.

For the *retroviridae* family, studies have extensively been performed on murine leukemia virus. At 56°C, murine leukemia virus load is known to be reduced by 3.6 Log ([10]*Sofer, 2003*). In addition, the human immunodeficiency virus (HIV) has also been shown to be

sensitive to heat. Indeed, a 2-hour treatment at 56°C induces a viral titre reduction by more than 4 Log *([31]Rossio, 1998*).
According to this latter result, virus belonging to the *retroviridae* family can therefore be considered as sensitive to heat treatment (see Table 2).

In this study, bibliographical data for viruses representative of a large panel of virus families have been collected. According to our analysis, literature data presented on a broad range of viruses of different nature provide evidence that viruses are effectively inactivated by heat treatments far less drastic than steam sterilisation (see Table 2). To complete this bibliographic approach and the drawn conclusions, actual experimental data concerning the sensitivity to steam sterilisation of model viruses known to be particularly resistant to physical treatment were produced and are presented below.

Experimental validation of steam sterilisation-induced viral inactivation

To support the previous bibliographical analysis and to confirm sensitivity of viruses to this tough treatment, experiments concerning the validation of the viral inactivation induced by the steam sterilisation were performed for VIRBAC by a specialized contract research organization (CRO).

The aim of the study was to assess the viral inactivation efficiency of steam sterilisation of an aqueous protein solution performed according to the European Pharmacopoeia requirements (≥ 121.1°C for 15 min).

The efficiency of viral inactivation by steam sterilisation was evaluated by:

- spiking the material to be treated with significant amounts of viruses from a suitable representative range of relevant or model viruses,
- carrying out this step under the defined conditions
- determining the resulting virus inactivation

A scale-down model of the heat steam sterilisation step conducted according to the European Pharmacopeia requirement (high temperature treatment of minimum 121.1°C for 15 min) was therefore studied.

A protein aqueous solution was used as representative aqueous model to assess the ability of steam sterilisation to inactivate suitable range of relevant model viruses, covering different types and sizes of potential adventious viruses (classically DNA/RNA, naked/enveloped, single/double strand). Murine leukemia virus (MLV) and bovine viral diarrhoea virus (BVDV) were used as models for enveloped RNA viruses whereas pseudorabies (Aujeszky's disease) virus (PRV) represented enveloped DNA viruses. Moreover, reovirus-3 (reo-3) and encephalomyocarditis virus (EMCV) were representative of naked RNA viruses, whereas human adenovirus 5 (Adv-5) and porcine parvovirus (PPV) were studied as models of naked DNA viruses.

The applied heat treatment is detailed in the table 1 below. Viral samples were tested before and after this treatment. An absence of measurable viral infectivious titre was considered as 'inactivated'

The results provided by the studies demonstrated that:

- heat treatment of naked viruses induces,
  - a titre reduction of at least 7.6 Log and 7.2 Log for both DNA virus Adv-5 and PPV, respectively, and
  - a minimal titre reduction of 7.4 Log for the RNA reo-3 virus and 7.3 for EMCV virus.

- heat treatment of enveloped viruses induces,
  - a titre reduction of at least 7.3 Log for BVDV , and 6.5 Log for MLV
  - a titre reduction of at least 6.5 Log for the PRV virus,

It has also to be noticed, that in each case, no virus was detectable in the sample at the end of the steam sterilisation process (mentioned in the Table 1 last column as inactivation: Yes, before the reduction titre)

According to these experimental results, the sterilisation step, applied on an aqueous protein solution used as a representative aqueous model was shown to be very effective in the inactivation of all the selected viruses (reductions >6 Log).

The Table 1 below summarizes the results obtained.

Table 1 : Validation of inactivation of model viruses by steam sterilisation

Virus	Sterilisation step parameters			F_0 value at the end of the cycle (min)	Inactivation/ Reduction factor (Log_{10})
	Duration (min)	Temperature (°C)	F_{0*} value at the end of the sterilisation step (min)		
MLV	15	121.1 – 124.0	27.7	**29.3**	**Yes ≥6.87 ± 0.30**
BVDV	15	121.2 – 124.0	29.7	**31.0**	**Yes ≥7.57 ± 0.23**
PRV	15	121.2 – 124.0	29.7	**31.0**	**Yes ≥6.76 ± 0.29**
Reo3	15	121.1 – 124.0	28.5	**29.8**	**Yes ≥7.70 ± 0.26**
AdV5	15	121.1 – 124.0	28.5	**29.8**	**Yes ≥7.88 ± 0.27**
EMCV	15	121.1 – 124.1	28.0	**29.5**	**Yes ≥7.58 ± 0.24**
PPV	15	121.1 – 124.0	28.8	**30.1**	**Yes ≥7.46 ± 0.26**

* calculating according to Ph.Eur 5.1.5

These experimental results are in accordance with those of the literature. According to all available data, steam sterilisation can be considered as an efficient way to inactivate viruses including the ones most resistant to heat inactivation.

Discussion

Based on the bibliographic data on heat sensitivity of a large range of viruses completed by experimental validation data on the sensitivity of model viruses to steam sterilisation, it can be concluded that the whole range of viruses included in the lists of bovine, ovine/caprine, porcine, feline, equine and avian extraneous viruses to be tested for are therefore sensitive to steam sterilisation. Indeed, the viruses cited in the different lists are within the virus families described in the present report and for these families robust data on representative viruses have been provided. The data show that steam sterilisation-induced inactivation of model viruses is efficient (able to reach more than 6 Log), and therefore meets the Ph. Eur. general chapter 5.1.1 requirements.
Altogether the studies presented in this assessment demonstrate that steam sterilisation can be considered as an efficient way to eradicate viruses from biological materials with a high level of confidence.
The data collected for this report are summarized in Table 2. Data on the heat sensitivity of model viruses based on both experimental and bibliographical analyses are presented. Additionally, Table 2 also provides an assessment of expected heat sensitivities of all the viruses listed in bovine, ovine/caprine, porcine, canine, feline, equine and avian lists of extraneous viruses). This supports the efficiency of steam sterilisation (Ph.Eur) for viral inactivation.

Additionally, it has to be noticed that the European Pharmacopoeia (general chapter 5.1.1.) recommends steam sterilisation at 121°C for 15 minutes to achieve sterilisation. This Ph.Eur steam sterilisation is also considered as a rigorous inactivation step to remove and / or inactivate viruses in the European Pharmacopeia (general chapter 5.1.7.). It is true that Ph. Eur. general chapter *Viral Safety* (5.1.7.) does apply to all medicinal products except immunological veterinary medicinal products since for IVMPs the subject is addressed into more detail in European Pharmacopoeia monograph 0062 and general chapter 5.2.5. Nevertheless it is obvious that if 5.1.7 identifies steam sterilisation as an effective inactivation method for human viruses this is equally true for veterinary viruses because the physical properties of human viruses are not different from those of similar viruses infecting animal species.

Table 2: Reported heat sensitivity of model viruses

TYPE	FAMILY	MODEL VIRUSES				EXTRAPOLATION TO OTHER VIRUSES
		DATA ORIGIN	ASSESSED VIRUS	CONDITIONS	OUTCOME	
Naked ssDNA	Parvoviridae	Biblio	Canine parvovirus (C)[1]	2 min, 121°C	5 Log	Bovine parvovirus (B), Porcine parvovirus (P), Canine parvovirus type 2 (C), Feline panleucopenia virus (F), Duck and goose parvoviruses (A)
		Validation	Porcine parvovirus (P)	15 min, 121°C	7.2 Log	
	Circoviridae	Biblio	Porcine circovirus type-2 (P)[2]	5 sec, 95°C	5.6 Log	Porcine circoviruses (P), Chicken anaemia virus (A)
Naked dsDNA	Adenoviridae	Biblio	Human type V adenovirus	20 min, 78°C	8 Log	Bovine adenoviruses (B), Caprine adenoviruses (O/Ca), Porcine adenoviruses (P), Canine adenoviruses (C), Equine adenoviruses (E)**, Avian adenoviruses (A), Egg drop syndrome virus (A), Avian infectious haemorrhagic enteritis virus (A)
		Validation	Human type V adenovirus[3]	15 min, 121°C	7.61 Log	
	Papillomaviridae	Biblio	Human papillomavirus type 11[4]	1h, 100°C	Complete*	Bovine papillomavirus (B)**, Ovine papillomavirus (O/Ca)**, Canine oral papillomavirus (C)**
	Polyomaviridae	Biblio	Human Polyomyelitis virus 1[5]	15 sec, 71.1°C	Complete*	Bovine polyoma virus (B)**, Goose haemorrhagic polyomavirus (A)
			Human Polyomyelitis virus 1[6]	2h, 60°C	6.5 Log	

*Complete inactivation refers to bibliographical data indicating a total inactivation of the virus by the treatment but for which no quantitative indication (in Log reduction factor) is provided.
**Virus added in the new lists : updating of annex II : table of extraneous agents to be tested for in relation to the guideline on requirements for the production and control of IVMPs (draft document EMA/CVMP/IWP/105112/2011)
(number) refers to the bibliographic reference
Legend : B: bovine viruses , O/Ca: Ovine/caprine viruses, P: porcine viruses, C: canine viruses, F: feline viruses, E: equine viruses, A: avian viruses

TYPE	FAMILY	MODEL VIRUSES				EXTRAPOLATION TO OTHER VIRUSES
		DATA ORIGIN	ASSESSED VIRUS	CONDITIONS	OUTCOME	
Naked ssRNA	Picornaviridae	Biblio	Swine vesicular disease virus (P)[7]	2 min, 65°C	6.7 Log	Bovine enterovirus (B) **, Bovine rhinovirus (B) **, Teschen virus (P), Porcine enterovirus (P), Duck hepathitis virus type I (A)
			Foot and mouth disease virus (B, 0/Ca, P)[8]	30 min, 70°C	Complete*	
		Validation	Encephalomyocarditis virus(P)	15min, 121°C	7.34 Log*	
	Caliciviridae	Biblio	Porcine vesicular exanthema virus (P)[9]	30 min, 64°C	Complete*	Jena virus (B) **, Hepatitis E (hepevirus)(P)**, Feline calicivirus (F)
Naked dsRNA	Reoviridae	Biblio	Reovirus (B)[10]	60°C	Complete*	Reovirus 3 (B), Epizootic hemorrhagic disease virus(B, O/Ca), Rotavirus(B), Porcine rotavirus (P), African horse sickness virus (E), Equine encephalosis virus (E)**, Equine rotavirus (E)**, Avian orthoreovirus (A)
			Bluetongue virus (B, O/ca)[11]	15min, 60°C	Complete*	
		Validation	Reovirus (B)	15 min, 121°C	7.44 Log	

*Complete inactivation refers to bibliographical data indicating a total inactivation of the virus by the treatment but for which no quantitative indication (in Log reduction factor) is provided.
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TYPE	FAMILY	MODEL VIRUSES				EXTRAPOLATION TO OTHER VIRUSES
		DATA ORIGIN	ASSESSED VIRUS	CONDITIONS	OUTCOME	
Envelopped dsDNA	Herpesviridae	Biblio	Aujesky's disease virus (B, O/Ca, P, C)[12]	16 min, 60°C	4.3 Log	Bovine herpes Virus, alcephaline herpes virus (B) **, Caprine herpes virus(O/Ca)**, Ovine herpes virus 2 (O/Ca, B)**, Swine herpes virus 1 (P, B, O/Ca, C, F) **, Canine herpes virus type 1 and 2 (C), Feline herpes 1 (F), Equine herpes virus (E), Avian infectious laryngotracheitis virus (A), Marek's disease virus (A), Duck enteritis virus (A)
			Aujesky's disease virus (B, O/Ca, P, C) [13]	30 min, 60°C	7.1 Log	
			Herpes simplex virus type 1 and 2[14]	10 min, 56°C	5 Log	
		Validation	Porcine Pseudorabies Virus (P)	15 min, 121°C	6.47 Log	
	Poxviridae	Biblio	Monkey pox virus[16]	10 min, 60°C	5 Log	Cowpox (B, F), Vaccinia (B), Bovine popular stomatitis (B), Pseudocowpox viruses (B), Orf virus (O/Ca)**, Swine pox (P)
			Sheep pox and goat pox (O/Ca)**[17]	60 to 120 min, 55°C	Complete*	
			Lumpy skin disease virus (B)[18]	30 min, 65°C	Complete*	
	Asfaviridae	Biblio	African swine fever virus (P)[19]	30 min, 70°C	Complete*	

*Complete inactivation refers to bibliographical data indicating a total inactivation of the virus by the treatment but for which no quantitative indication (in Log reduction factor) is provided.
**Virus added in the new lists : updating of annex II : table of extraneous agents to be tested for in relation to the guideline on requirements for the production and control of IVMPs (draft document EMA/CVMP/IWP/105112/2011)
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TYPE	FAMILY	MODEL VIRUSES				EXTRAPOLATION TO OTHER VIRUSES
		DATA ORIGIN	ASSESSED VIRUS	CONDITIONS	OUTCOME	
Envelopped ssRNA	Flaviviridae	Biblio	Bovine Viral Diarrhoea (B, O/Ca, P)[12]	16 min, 60°C	1.9 Log	Tick borne encephalitis virus (B, O/ca) **, Wesselbron virus (B, O/Ca) **, Border disease virus (O/Ca)**, Classical swine fever virus P), Japanese encephalitis virus (E, P)**, West nile virus(E)**
		Validation	Bovine Viral Diarrhoea (B, O/Ca, P)	15 min, 121°C	7.34 Log	
	Coronaviridae	Biblio	Severe acute repiratory syndrome (SARS) coronavirus (CoV) [20]	4 min, 65°C	4.5 Log	Bovine coronavirus (B), Porcine respiratory coronavirus (P), Haemagglutinating encephalomyelitis virus (P), Transmissible gastroenteritis virus (P), Canine coronavirus (C), Feline coronavirus (F), Avian infectious bronchitis virus (A) Equine arteritis virus (E)**
			SARS-CoV[21]	2 h, 58°C	4.9 Log	
			Porcine respiratory and reproductive syndrome virus (P)[22]	45 min, 56°C	Complete*	
	Orthomyxoviridae	Biblio	Porcine influenza (P)[23]	60min, 56°C	Complete*	Equine influenza virus(E)**, Avian influenza A
	Orthomyxoviridae bunyaviridae	Biblio	Akabane virus (B, O/ca)[24]	5 min, 56°C	4.5 Log	Rift valley fever (B, P) Cache valley virus (B, O/Ca)**, Nairobi sheep disease virus (O/Ca)**
	Paramyxoviridae	Biblio	Avian paramyxovirus((A) (Newcastle disease virus) (A)[25]	30 min, 60°C	Complete*	Parainfluenza 3 (B), Bovine respiratory syncitial virus (B), Ovine respiratory syncitial virus (O/Ca)**, Peste des petits ruminants virus (O/Ca)**, Nipah virus (P) **, Canine parainfluenza 2 virus (C), Canine distemper virus(C), Hendra virus(E)**, Turkey rhinotracheitis virus (A) Avian paramyxovirus type III (A)

*Complete inactivation refers to bibliographical data indicating a total inactivation of the virus by the treatment but for which no quantitative indication (in Log reduction factor) is provided.
**Virus added in the new lists : updating of annex II : table of extraneous agents to be tested for in relation to the guideline on requirements for the production and control of IVMPs (draft document EMA/CVMP/IWP/105112/2011). (number) refers to the bibliographic reference
Legend : B: bovine viruses , O/Ca: Ovine/caprine viruses, P: porcine viruses, C: canine viruses, F: feline viruses, E: equine viruses, A: avian viruses

TYPE	FAMILY	MODEL VIRUSES				EXTRAPOLATION TO OTHER VIRUSES
		DATA ORIGIN	ASSESSED VIRUS	CONDITIONS	OUTCOME	
Envelopped ssRNA	Rhabdoviridae	Biblio	Rabies (B, O/ca, P, C, F, E)[26]	60 min, 50°C	Complete*	Vesicular stomatitis (B), New jersey and Indiana virus (B), Bovine parainfluenza 3 virus (B), Bovine ephemeral fever virus (B), Rinderpest virus (B)
			Rabies (B, O/ca, P, C, F, E) (internal data)	1min, 80°C	8 Log	
			Vesicular stomatitis (B, P, E)[27]	30 min, 58°C	Complete*	
	Bornaviridae	Biblio	Bornavirus (B, O/Ca, E) **[28]	3 days, 56°C	Complete*	
			Bornavirus (B, O/Ca, E) **[29]	3 h, 56°C	Complete*	
	Togaviridae	Biblio	Sindbis Virus[30]	30 min, 100°C	6.5 Log	Equine infectious anemia virus (E), Equine encephalomyelitis alphavirus (E)
	Retroviridae	Biblio	Murine leukemia virus[10]	56°C	3.6 Log	-Bovine leukemia virus (B), Bovine endogenous retroviruses (B) **, Ovine/Caprine endogenous retrovirus(O/Ca)**, Caprine arthritis encephalitis virus (O/Ca)**, Ovine pulmonary Adenocarcinoma virus (O/Ca)**, Porcine endogenous retrovirus (P) **, Porcine retrovirus (P) **, Canine endogenous retrovirus (C), Feline endogenous retrovirus (F) **, Feline foamy virus (F), Feline immunodeficiency virus (F), Feline leukemia virus (F), Feline sarcoma virus (F), Equine endogenous retrovirus (E)**, Avian leukosis virus (A), Avian reticuloendoteliosis virus (A)
			Human immunodeficiency virus [31]	2h 56°C	4 Log	
		Validation	Murine leukemia virus	15 min, 121°C	6.57 Log	

*Complete inactivation refers to bibliographical data indicating a total inactivation of the virus by the treatment but for which no quantitative indication (in Log reduction factor) is provided.
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Conclusions

According to these current experimental and bibliographical data, heating and particularly steam sterilisation can be considered as a very efficient way to sterilize a biological material. Therefore, materials that are effectively submitted to Ph.Eur sterilisations (5.1.1) by steam treatment, during the manufacturing process, can be considered as free from extraneous viruses with a high level of confidence.
The literature and experimental data presented on a broad range of (model) viruses of different nature provide ample evidence that viruses are effectively inactivated by steam sterilisation in accordance with Ph.Eur. general chapter 5.1.1.

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