Learning from Experience

Reviewing known historical incidents of virus contamination in biopharmaceutical products helps to identify areas where manufacturers can further strengthen control measures to minimise risk.

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Recent examples of virus contamination in GMP manufactured biopharmaceutical products (for example Porcine circovirus (PCV) and Vesivirus 2117) has intensified the focus of authorities keen to identify the best ways for manufacturers to ensure the virus safety of biopharmaceutical products (1). Many of the regulations relating to virus safety were based on historical lessons learnt by the biopharmaceutical industry.

This article will focus on examples of exogenous virus contamination introduced into biopharmaceutical products either through the use of contaminated materials in the manufacturing process or from sources yet to be identified (but presumed to be exogenous).

Virus Contamination in Whole Tissues

Most incidences of virus contamination in biopharmaceutical products have originated through the use of poorly characterised materials. The level of characterisation which can be performed on any material is directly related to the complexity of the system being used, and in this regard whole human or animal-derived tissues present the greatest challenge.

Unlike recombinant cell lines, which can be prepared as a master cell bank (MCB) and extensively characterised, whole tissues often present a number of issues. The greatest risk stems from the fact that source materials are normally sourced from multiple donors for the manufacture of a single batch of product. Only a single contaminated donor is required to contaminate the whole batch. Furthermore, living systems present difficulties with regards to unknown or newly emerging infectious viruses.

Table 1 lists some of the better known examples of virus contamination originating through the use of contaminated human or animal tissues as the source material. Some of these contamination events have resulted in fatal consequences to the recipients of the products (for example CJD contaminated growth hormone). In this context, *in vitro* systems offer an attractive alternative since they provide an opportunity to detect and identify (and thereby exclude) possible contaminating viruses prior to their use in manufacture.

Adventitious Virus Agents

By far the largest single root cause of virus contamination in recombinant biopharmaceutical systems has been the use of animal-derived components. Despite attempts to reduce or limit the use of animal-derived components in the handling of recombinant cell lines or in media formulations, it has not always been possible to eliminate their use completely. Even where serum is not used directly, many formulations still use purified factors derived from, for example, serum, which may still present a risk for introducing virus contamination into the system.

Table 2 lists some of the more frequently occurring contaminants identified in animal-derived components (primarily bovine or porcine). The risk posed by such potential contamination to human health is not easy to define, and will depend on the zoonotic potential of the contaminating virus as well as the potential dose present. For the purposes of risk minimisation in biopharmaceutical products, absence of any contaminating virus is the ultimate goal, and therefore the primary determinant for risk will be the titre of virus present in the start material. The potential to grow in the production cells can also play a role, and will be discussed later in this article.

Sourcing and Testing

Current regulations for the use of animal-derived components in biopharmaceutical products stipulate a number of sourcing, testing and manufacturing measures that should be implemented to minimise any potential risk from virus contamination:

- Animals should be subject to ante- and post-mortem inspections by qualified veterinarians
- Operators should be trained to collect the source material (reduction in cross-contamination resulting from unhygienic practices)
- Start materials prior to any purification are tested according to, for example, FDA 9CFR guidelines for the testing of animal components (or equivalent EMEA CPMP/BWP/1793/01 guidelines on testing of bovine serum) (2). These tests include a general observation for cytopathic or haemabsorbing viruses as well as specific end points for known contaminants
- It is recommended that bovine serum be subjected to a virus inactivation through a minimum gamma-irradiation dose of 35 KGy

In combination, these measures would be considered to be state of the art for minimising the

Table 1: Exogenous virus contamination where whole animals were used as source material

Year/timeframe	Product type/company	Production system	Contaminating agent	Ref
1930s	Louping ill sheep vaccine	Sheep brain	Scrapie	9
1960s	Poliovirus vaccine	Primary monkey cells	Simian virus 40 (SV40)	10
1960s	Yellow fever virus vaccine	Eggs or primary chick embryo fibroblasts	Avian leucosis virus (ALV)	10
1960s-1970s	Human growth hormone	Cadaveric pituitary gland	Creutzfeldt-Jakob disease (CJD)	9
2006	Marek's disease vaccine	Eggs	Avian leucosis virus (ALV)	11
2000s	Urokinase	New born human kidney cells	Reovirus	10

potential for contamination in animal-derived components.

It is important, however, to understand that sourcing and testing alone cannot assure the absence of virus contamination in biopharmaceutical products for the following reasons:

- The limit of sensitivity for current cell culture and PCR-based tests cannot assure viral sterility for a product no matter how much it is tested. The volumes tested are significantly lower than the volumes used in manufacturing, and it is always important to consider the total potential load of the virus that might be introduced into a bioreactor, particularly where that virus is capable of infecting and amplifying in the production cells
- Cell culture or *in vivo* based tests cannot detect all known potential contaminants. Many viruses do not replicate in these systems, or maybe replicate without any visible effect on the cells or animals (for example PCV would not be detectable in standard cell culture test systems)
- Matrix inhibition (for example through the presence of antibodies) may mask potential contaminating viruses, and it is difficult to include interference controls in any test for every possible virus contaminant
- Even with testing, the possibility may exist for the introduction of virus contaminants from other sources (such as facility rodents as has been demonstrated for contamination with Mouse minute virus) (3)
- History has demonstrated that even tested products can still result in virus transmission events if the virus is infectious for humans (for example with human plasma derived products (4))

Virus Epidemiology

The human plasma industry has long recognised the power of virus epidemiology and its role in minimising the risk for virus transmission (5). It would even be fair to say that any risk minimisation strategy for components of animal or human origin which does not incorporate elements of virus epidemiology has not appropriately addressed virus safety risks. Without a proper understanding of both the likelihood of contamination along with the potential viraemic load, it is impossible to evaluate the potential risk posed by a particular virus.

As an example for bovine derived viruses, Table 3 provides a detailed list of the viruses of greatest significance for bovine serum sourced material (Table 3, page 82, has been abbreviated: see source link for full details). The virus prevalence in Europe, Australia and New Zealand in Table 3 has been based as far as possible on published reports. Where no reports exist, but it is considered likely that the virus is present (for example due to likely under-reporting for viruses of low economic importance) then as a worst case scenario it is assumed that the virus is present. For the purposes of categorising the risk from viraemia for the different viruses, the following classification system was used in Table 3:

- Level of viraemia – high: published evidence of high titre viraemia or where Arboviralvector transmission via insects has been confirmed
- Level of viraemia – medium: no evidence of Arboviral transmission via insect vectors but published evidence of low level viraemia

Table 2: Exogenous virus contamination incidences in recombinant biopharmaceutical products

Year/timeframe	Product type	Contaminating virus	Ref
Contaminants originating from contaminated animal-derived components (for example serum, trypsin)			
Continuous	Bovine serum	Bovine viral diarrhoea virus (BVDV)	12
Continuous	Bovine serum	Bovine polyoma virus (BPyV)	13
1990s	Bovine serum	Cache valley virus (CVV)	14
1990s	Bovine serum	Epizootic haemorrhagic disease virus (EHDV)	15
Continuous	Porcine trypsin	Porcine parvovirus (PPV)	16
1983 (identified 2010)	Porcine trypsin	Porcine circovirus (PCV)	1
Contaminants with an unclear source or aetiology			
Late 1990s, 2009	Recombinant CHO cells	Vesivirus 2117	1
1990s to present	Recombinant CHO cells	Minute virus of mice	3

- Level of viraemia – low: no evidence of Arboviral transmission or demonstration of significant viraemia

Where specific data was identified regarding possible virus titre during viraemia, this is included in the table in brackets (the units are either infectivity units per ml or PCR units per ml depending on the method used in the respective publication). It is important to realise however that the titres for viraemia should not be taken as the upper limit, and actual titres could be significantly higher (for example where animals are weak or immuno-compromised).

New Zealand Ecosystem

Table 3 highlights the example of the importance of the ecosystem and history of New Zealand in limiting the impact on viruses present, as well as the potential for emerging infectious zoonoses (6). Geographically, the islands are the most isolated and temperate in the world, and until recently had allowed the development of a unique native fauna in the absence of natural predators. Until the first human incursions to the islands some 700 years ago, the only native mammals were two species of bats, and thus the development of parasitic arthropods capable of spreading disease matched the limited ecosystem dominated by such terrestrial fauna. New Zealand's native fauna does not include hosts which are in other countries the recognised hosts for many human pathogens.

The introduction of exotic terrestrial mammals followed shortly after the first human incursions and these introductions resulted in the concomitant introduction of ectoparasites capable of arthropod mediated disease transmission, including the dog, cat and rat flea, various human and animal louse parasites, various mosquito species and a limited number of tick species. Despite these incursions, the potential for the introduction of zoonotic diseases was limited due to a number of factors:

- The transport of only healthy stock from a limited number of animal sources (primarily the UK, Australia and Chile)
- The strict quarantine rules in New Zealand (one of the strictest in the world) which in large part have been in place since the late 1800s (7)
- The extended sea voyage necessary for the transport of animals in the 1800s from Europe, the Americas or Australia. This in itself served as a form of enforced quarantine, and thus only those diseases which could survive as a persistent or chronic infection were introduced
- The absence in New Zealand of certain insect vectors responsible for significant Arboviral disease spread in other countries (for example *Culicoides spp*)

The combination of the above factors, along with the virgin nature of the New Zealand ecosystem prior to the introduction of these exotic species, has resulted in a significantly lower than average number of zoonotic agents of concern – a fact apparent from the list presented in Table 3.

New Zealand is free from all the major epidemic diseases of animals (8) (OIE: Office International des Epizooties, List A). Of the viruses identified in Table 3 as being present in at least one of the three areas evaluated (Europe, Australia and New Zealand), 14 are absent or unreported from New Zealand. Furthermore, New Zealand maintains a strict surveillance programme administered by the Ministry of Agriculture and Fisheries (MAF), which actively monitors for both circulating and exotic diseases within the islands.

Risk Minimisation Strategies

As discussed in the preceding sections, the risk of virus contamination in animal-derived components used in biopharmaceutical manufacturing depends on the following factors:

- The risk of virus contamination originating from the source country. Not all countries are necessarily considered to demonstrate an equal risk
- The viraemic titre of the virus in the infected animal must be considered. Veterinary inspections would not be expected to catch all infected animals (for example animals are often asymptomatic). Where titre viraemia is high, the risk will be proportionally greater
- Regulatory guidelines require the testing of animal-derived material for specific viruses prior to use in medicinal products. Test cell lines however would not be susceptible to all possible virus contaminants, and therefore some contaminants might be missed. Manufacturers are encouraged to evaluate and apply alternative technologies for those viruses where cell culture tests are known to fail

Table 3: Bovine viruses of concern and their presence in Europe, Australia and New Zealand (abbreviated version)

Virus or Family	Virus Family	Zoo-notic?	Present in?			Level of Viraemia (titre)	Ref
			EU	AUS	NZ		
Akabane viruses	*Bunyaviridae*	No	×	✓	×	High	17, 18
Aujeszky's disease virus	*Herpesviridae*	No	×	×	×	Low	8, 17
Adenovirus virus	*Adenoviridae*	No	✓	✓	✓	Medium (10^3)	17, 19
Bluetongue virus	*Reoviridae*	No	✓	✓	×	High (10^5)	17, 20, 21
Borna disease virus	*Bornaviridae*	?	✓	✓	×	Low	17, 22
Bovine calicivirus	*Caliciviridae*	No	✓	✓	✓	Low	17, 23
Bovine corona virus	*Coronaviridae*	No	✓	✓	✓	Low	17
Bovine herpesviruses	*Herpesviridae*	No	✓	✓	✓	Low	17, 24
Bovine leukaemia virus	*Retroviridae*	No	✓	✓	✓	High	17, 25

For complete table please visit: www.virusure.com/downloads/publications/

- In order to prepare for potentially high prevalence or highly viraemic viruses, it is recommended to adopt an approach of routinely (for example, yearly as is done in the plasma products industry) evaluating the epidemiology for animal-derived viruses in the area from which the animal-derived components are sourced. Viruses that show an emerging or high sero-prevalence coupled with high level viraemia can then be evaluated on an individual basis. It can be argued strongly that implementation of such an approach could have identified and prevented the recent PCV contamination incidences in vaccines

It is also possible to include robust steps into the manufacturing processes both for animal-derived components (such as prior to use in manufacturing) as well as for the manufactured product itself. As demonstrated through the contamination incidences with MMV and Vesivirus 2117, which demonstrate no clear aetiology, the possible introduction of unknown (and possibly non-detected) viruses from unusual sources is ever present, and where such contaminants go undetected the only effective control would be via robust virus inactivation or removal. In addition, highly robust inactivation technologies are known to significantly reduce and control the risk for viruses below the limit of detection of cell culture or PCR based assays – manufacturers should not rely on testing alone to ensure virus sterility for a product.

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