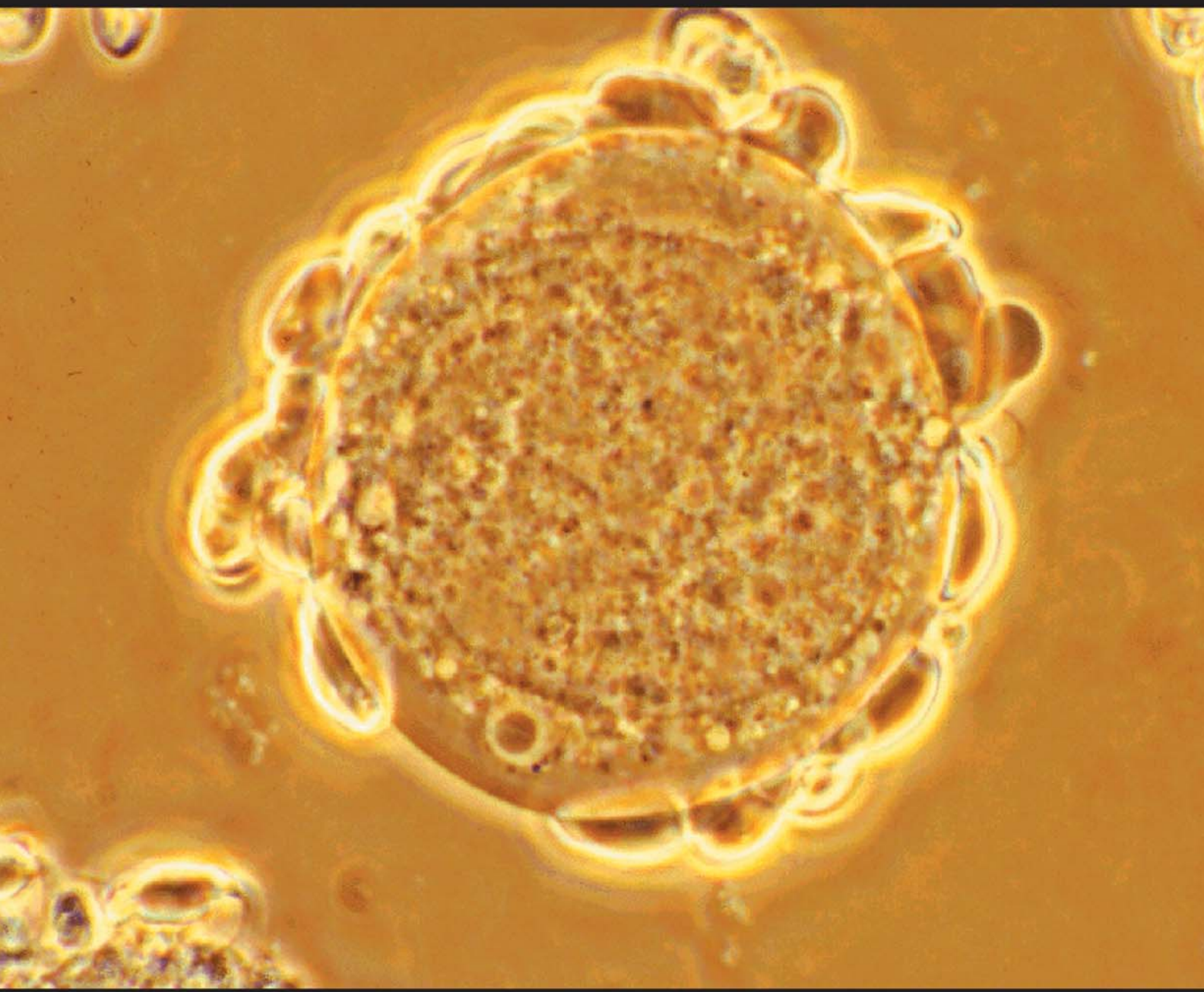
A Publication of
The Williamsburg BioProcessing Foundation

January/February 2003
ISSN 1538-8786

BioProcessing JOURNAL

Advances & Trends In Biological Product Development



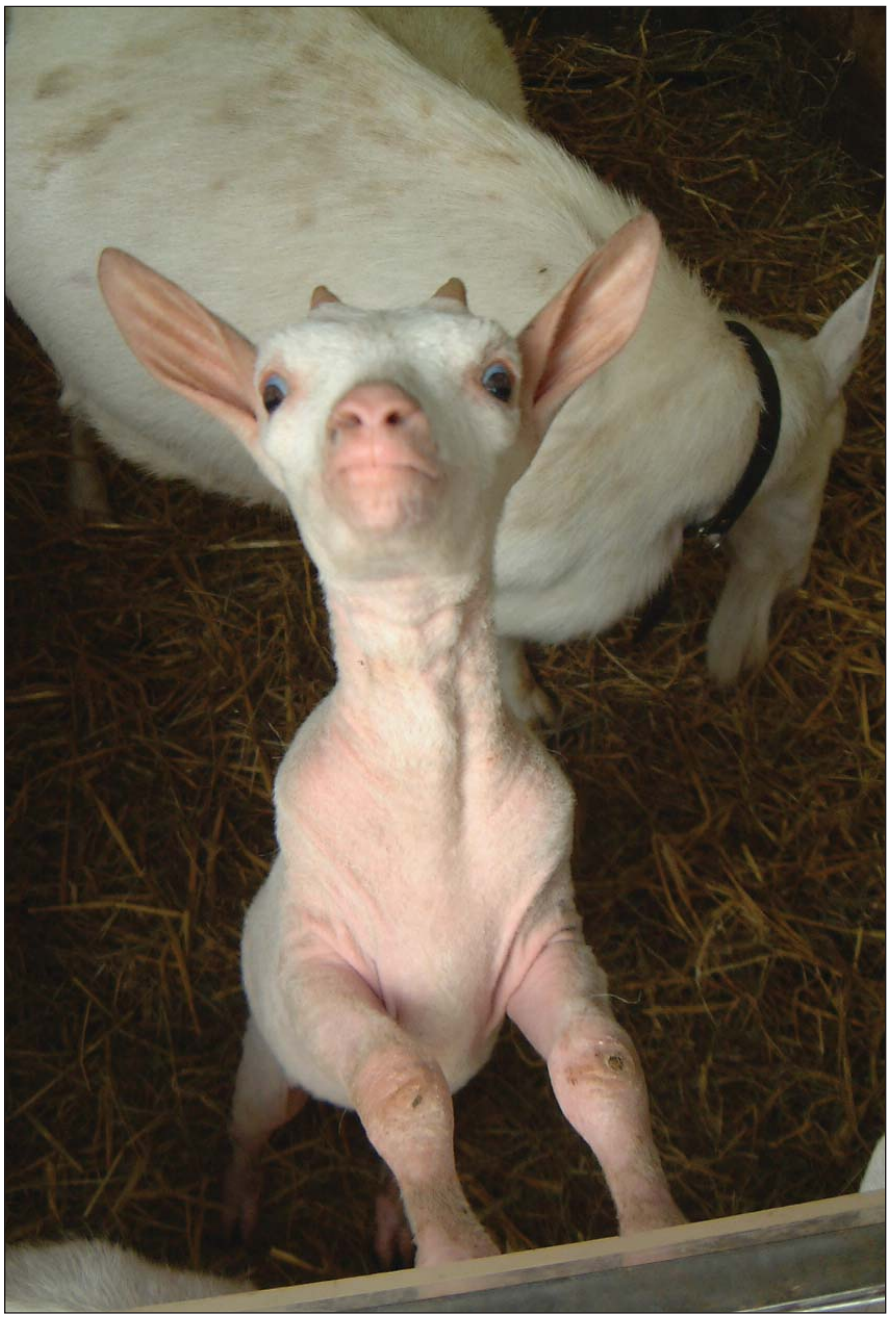
Vol. 2/No. 1

www.bioprocessingjournal.com

TECH REVIEW

Assuring Viral Safety in Products Produced in Milk from Transgenic Animals: What Can Be Learned from Ascites-Derived Products

BY KATHERINE F. BERGMANN AND LEONARD J. SCHIFF



Martin de Wit

Various systems are used for production of biopharmaceuticals, including bacteria, yeast, mouse ascites and animal cell culture. Each production system has its own set of risk factors for infection by viruses and their potential transmission in the final product. Viral contamination in products can arise from the animals themselves, from environmental sources, from the starting cells, or from materials introduced during the production and purification procedures. Methods have been developed for the prevention and control of these risks. The strategy used to minimize the risk of viral contamination combines several levels of viral safety that are outlined in Table 1.

One of the newer production systems is based on expression of products in the milk of transgenic animals. As with any new system, it is useful to ask whether strategies developed previously for assuring virus safety can be useful in the design of strategies for preventing and controlling risks in newer systems. This article evaluates the extent to which the approaches taken with the ascites production model can be useful in development of safety strategies in transgenic animal production.

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Table 1: Strategy to minimize viral risks in products produced in animals

- Monitoring of health status of host animals (colony viral surveillance and testing program) and good animal care practices
- Characterization of cell and transgenic banks
- Testing of source material and production stream for virus contamination
- Incorporation of steps in the purification process that can remove or inactivate viruses

Health Status of Host Animals, Monitoring, and Surveillance

All animals used for biopharmaceutical production should be from a controlled source and should be housed and maintained in a manner to minimize the possibility of viral infection. In most instances, specific-pathogen-free (SPF) mice are used for ascites production. The SPF mouse colonies are housed in environments that prevent exposure to vermin and insects that could carry infectious agents. Sentinel animal populations are housed with the production animals and are used for periodic health monitoring. The sentinel animals are evaluated using the Mouse Antibody Production (MAP) Test, which detects the presence of 16 murine viruses including several zoonotic viruses (Reovirus, Hantaan, and Lymphocytic Choriomeningitis).[1,2]

Animals used for transgenic production include rabbits, sheep, goats and cattle. Colonies of SPF rabbits can be maintained since they, like mice, are housed in controlled facilities. However, grazing animals cannot be considered to be SPF, since the nature of a farm does not allow the complete protection of animals from vectors of infectious diseases, such as rodents and insects. As a result, maintenance of the health of grazing animals may require more rigorous and extensive testing for infectious agents.[3] Sentinel animal populations are thus critical to health monitoring. There is not an accepted panel of species-specific viruses for each transgenic animal species used. Each manufacturer should evaluate the risks for the presence of viruses known or suspected to be infectious in the host species. Assays may need to be developed for pathogens considered to be significant (for example, polymerase chain reaction — PCR — assays).

Animals should also be tightly controlled and should be quarantined for an appropriate period before entering the herd. Sick animals, or animals that are positive for any infectious agent, even if not sick, should be removed from the herd. Animals should be vaccinated against pathogens (such as rabies and tetanus) wherever possible, and a herd surveillance and testing program should be in place for important viral agents (e.g. Caprine Arthritis/Encephalitis Virus, Caprine Herpes Virus, and contagious ecthyma for goats). Standard operating procedures should be in place to minimize human, animal, and vehicle vectoring of viral diseases into the production facility.[4]

Characterization of Cell and Transgenic Banks

Cell lines used for ascites production are characterized for the presence of endogenous and adventitious agents at both the Master Cell Bank (MCB) and Working Cell Bank (WCB) stages (Table 2).[5,6] Any virus contaminant should be quantified and identified, where possible, and the tropism for human cells should be established. The characterization of murine cell lines should include testing for species-specific viruses (MAP Test) and for retroviruses. The presence of other unidentified viruses is assessed using *in vitro* and *in vivo* assays for adventitious viruses. The *in vitro* assay involves the inoculation of material onto several indicator cell lines, and then the assay of the resulting cell cultures for several markers of virus infection, including cytopathology,

Table 2: Safety testing requirements for murine Master and Working Cell Banks used for ascites production

Master Cell Bank	Working Cell Bank
• Bacteriostasis and Fungistasis	• Bacteriostasis and Fungistasis
• Sterility (Bacteria and Fungi)	• Sterility (Bacteria and Fungi)
• Mycoplasma	• Mycoplasma
• Isoenzyme Analysis	
• Retroviral Infectivity Assays (XC Plaque and S+L- Focus)	
• Transmission Electron Microscopy	
• *In Vitro* Virus Assay — for detection of adventitious viruses (28 day assay)	• *In Vitro* Virus Assay — for detection of adventitious viruses (28 day assay)
• *In Vivo* Virus Assay — for detection of adventitious viruses (eggs, adult and suckling mice, and guinea pigs)	
• Mouse Antibody Production Test with LCM challenge	
• Bovine adventitious viruses (9 CFR)	

hemadsorption, and hemagglutination. In the *in vivo* assay, SPF animals (guinea pigs, mice, and embryonated chicken eggs) are inoculated with material and observed for signs of virus infection.

For production of a transgenic herd, a similar banking system may be used, consisting of a Master Transgenic Bank (MTB) and a Working Transgenic Bank (WTB).[3] These banks may consist of well-characterized animals, or possibly of frozen sperm and embryos from founder animals and their immediate offspring. As with the MCB and WCB, the purpose is to produce a consistent product over time. At present, there is no standard approach for virus testing. Therefore, manufacturers are expected to design an appropriate program for characterization of the MTB and WTB for the presence of endogenous and adventitious agents.

Table 3: Design and performance of a viral clearance study

• Select process steps	Steps selected should have a reasonable expectation of viral clearance, be easy to scale down, and be reproducible under process conditions. Examples include UV/gamma irradiation, chaotropic salts, pH inactivation, solvent/detergent treatment, heat treatment, chromatography, and filtration procedures.
• Scale down process steps	Scale to a convenient "bench" scale for efficient use of material and highest sensitivity.
• Select appropriate viruses	Select viruses resembling ones that contaminate or may contaminate the product (relevant viruses) and that represent a wide range of physiochemical properties (model viruses). Viruses should be selected that can be grown to a high titer and can be detected in an efficient and reliable assay system.
• Evaluate each step independently	Spike the starting product intermediate with a virus and perform the process step. Analyze each fraction for the amount of virus present.
• Calculate viral clearance	The log reduction value (LRV) is calculated for each step, and the values are summed over the steps in the entire process.

Virus Contamination in Source Material and the Production Stream

Ideally, bulk materials for biopharmaceutical production are free of microbial contamination. However, this is usually not possible for mouse ascites production since ascites cannot be collected in a sterile manner. Therefore, the unprocessed bulk material is commonly filtered through a 0.45 µm filter immediately after collection to remove microbial contamination. Like ascites, milk cannot be routinely collected from animals in a sterile manner, and may also need filtering. Owing to the presence of large amounts of lipid and casein micelles in the milk, it may be more difficult to design an efficient method for filtration at this stage.

Testing for endogenous and adventitious viruses can also be used to evaluate the presence of viruses in the bulk starting material.[5] Unprocessed bulk ascites is tested for specific murine viruses by the MAP test. The bulk material is also tested for the presence of retroviruses by transmission electron microscopy, reverse transcriptase activity, and infectivity assays for murine retroviruses. The presence of unidentified viruses is assessed with the *in vitro* assay for adventitious viruses.

Milk from transgenic production animals is also tested for virus contamination. Where possible, specific assays should be employed for viruses identified in a risk analysis. Retrovirus testing can also be performed using reverse transcriptase activity, or by PCR testing against the species-specific retrovirus. It may not be possible to assay retroviruses by transmission electron microscopy because of the presence of lipid and casein micelles in the milk. An *in vitro* assay for adventitious viruses can also be performed after selecting the indicator cells to be appropriate for the particular species.

Removal and Inactivation of Viruses during Product Purification

The assays described above cannot detect all of the possible viruses that could infect the production animals substrate. Therefore, a complementary strategy is used that evaluates the capacity of the purification process to remove and/or inactivate viruses that might be present in the product. This strategy, known as process evaluation for viral clearance, involves the deliberate spiking of a relevant or model virus into process intermediates, and the demonstration of its inactivation or removal during the subsequent processing steps. This strategy is outlined in Table 3, and is discussed in detail in references 6-8. As each product and its purification procedure are different, the design and performance of these studies will vary. The approach, however, is common to most products.

The extent of required testing will depend on the stage of product development. For ascites-derived products entering Phase I clinical trials, testing will usually include only one virus, a retrovirus, and only limited steps will be tested. For products entering Phase III clinical trials, a virus panel will be used that consists of three or four relevant or model viruses with widely varying properties. To evaluate the robustness of the results, duplicate testing will be performed for each step and virus. Additional process steps may be tested as well.

The major difference between products derived from ascites and those from milk of transgenic animals is the

choice of model viruses that should be used in the study. The viruses selected should be based on the viruses that could be expected to contaminate the product, and would therefore differ for each species. The full panel of tests will include duplicate studies with four relevant or model viruses that have widely varying properties. However, the regulatory agencies may expect to see a more complete study at early clinical stages, since there is relatively little experience with this production system.

Summary

In products derived from animals, assuring viral safety requires some strategies that are not necessary with those derived from cell culture. There are many similarities between the products derived from mouse ascites and those from the milk of transgenic animals, and these similarities are reflected in similar strategies for assuring viral safety. As discussed in this article, there are also significant differences between the production systems. In any production system, animal-based or not, there is a variety of risks for virus contamination. Some of these risks arise from viruses that cannot be assayed directly. These risks cannot always be prevented, but they can be minimized using a combination approach including system controls, monitoring and surveillance programs, specific and broad-based testing, and inactivation and removal strategies. This multifactorial approach is discussed in many regulatory documents, including the ones referenced in this article.[3,5,6,7,8] A thorough analysis of virus risks, plus a well-designed program for risk minimization and management, are essential steps in the path to licensure of any biotechnology product.

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