BVDV White Paper

Don Knowles, VMRD - Steve Doelger & Rosemary Versteegen, ISIA

Serum is used by industries such as biotechnology, animal and human pharmaceuticals, research, and diagnostics. Fetal Bovine Serum, bovine serum, and bovine serum products are critical nutrient rich supplements frequently used in cell culture systems. Serum may be contaminated with various adventitious agents that may increase risk for use in cell culture systems. To minimize the risk of contaminating agents, collection and manufacturing and treatment processes have very rigorous.

Introduction

Bovine Viral Diarrhea Virus (BVDV) is considered one of the most significant infectious diseases in the livestock industry worldwide due to its high prevalence, persistence and clinical consequences [(1)]. It is also considered one of the most significant potential contaminants in mammalian cell culture due to its ability to reproduce in cells without cytopathic effect.

Transmission of BVDV occurs both by contact with infected cattle (horizontally) or from the dam to the calf (vertically) [(2)]. Virus can be transmitted via direct contact, bodily secretions and through items that can carry the infection such as soil and clothing. BVDV can persist for some time in a cool and moist environment, especially when there is abundant organic material. Following viral entry and contact with the mucosal lining of the mouth or nose, replication occurs in epithelial cells. BVDV infection is frequently gradual, with a wide manifestation of non-specific clinical signs including fertility issues, decreased milk production, pyrexia, diarrhea and fetal infection. Although clinical signs are generally mild, especially in previously exposed or vaccinated cattle, a severe acute form of BVD may occasionally occur. Such outbreaks are characterized by high morbidity and mortality [(3)].

Intrauterine BVDV infection of the dam either just prior to conception or during the first 18 days of gestation results in future delayed conception and an increased interval from calving to conception. If the embryo is attached, infection during days 29–41 can result in embryonic infection and/or subsequent embryonic death. BVDV can cross the placental barrier in cattle, thus, the fetus is exposed to the virus from the dam's exposure [(4)]. If a fetus is exposed to the virus between 80 and 150 days of gestation, prior to the development of an independent immune system, the fetus can become "persistently infected" (PI). This results in the animal being infected throughout its life with no ability to produce an immune response to the strain with which it is infected. PI animals are the most important reservoirs of the virus, continuously excreting a viral load thousand times that shed by acutely infected animals [(5)]. In addition, persistently infected dams always produce persistently infected fetuses and calves [(6)]. These animals often fail to thrive and are smaller than their peers, however they can occasionally appear normal.

Viral Classification

BVDV is an RNA virus. It is a member of the *Pestivirus* genus, belonging to the family *Flaviviridae* [(7)]. Three BVDV genotypes are recognized, based on the nucleotide sequence of the 5'untranslated (UTR) region; BVDV-1, BVDV-2 and BVDV-3 (HoBi-like virus). BVDV-1 and BVD-2 have been further grouped into subgenotypes with BVDV-1 having a greater number of these than BVDV-2. BVDV strains are also divided into distinct biotypes (cytopathic or non-cytopathic) according to their effects on cell cultures [(8)].

1. Non-cytopathic (ncp) viruses can induce persistent infection and have an intact NS2/3 protein.

2. Cytopathic (cp) biotypes, formed via mutation of non-cytopathic (ncp) biotypes, induce apoptosis in cultured cells. In cp viruses the NS2/3 protein is either cleaved to NS2 and NS3 or there is a duplication of viral RNA containing an additional NS3 region.
3. A new strain (BVDV 3, HoBi-like or atypical pestivirus) has been isolated from cattle, water buffalo and FBS from South America. It is currently thought that this strain is prevalent across Brazil [9]. It has also been found in cattle in other parts of the world such as Thailand and Italy [10].

Eradication of BVDV

The mainstay of eradication for this disease is the identification and removal of PI animals. Further incidence of the disease can then be prevented by vaccination and high levels of biosecurity, supported by continued surveillance. Scandinavian countries led the way in BVDV eradication implementing an eradication program almost 20 years ago. It took all these countries approximately 10 years to reach the final stages of eradication, and this program continues to require ongoing monitoring of all cattle herds within each country to be effective [11,12].

While challenge studies indicate that inactivated (killed), as well as attenuated (live), vaccines prevent fetal infection under experimental conditions, the efficacy of vaccines under field conditions has been questioned. The birth of PI calves into vaccinated herds suggests that such vaccines may not stand up to the challenge presented by the viral load of PI cattle in the field [12,13].

BVDV in FBS.

BVDV occurs in herds worldwide, and the overall incidence appears to be consistent globally. It is impacted by cattle density and, to a lesser degree, by season. Persistently infected cattle range from 1 to 2% in a herd [14]. One liter of fetal bovine serum can be collected from several animals. Manufactured lots of serum are pooled and are usually around 2000 liters.

The incidence of BVDV in serum follows basic principles of statistical binomial probability:

- If a pool of 1000 animals is used at 1% incidence, then the pool will be positive 100% of the time
- If a pool of 100 animals is used at 1% incidence, then the pool will be positive 60% of the time
- If a pool of 20 animals is used at 1% incidence, then the pool will be positive 20% of the time

Hence it is possible, to reduce the BVDV burden in FBS by reducing the number of animals in the pool. Small pools can also be pretested for BVDV and selected to further reduce the risk of contamination. Such methods, while effective, are costly.

Post-manufacturing treatment

Many methodologies have been used in the attempt to reduce the viral burden in serum. Gamma irradiation, the method of choice, (provides post-manufacturing viral load reduction for BVDV due to the susceptibility of the virus: 30kGy will result in 10^6 reduction in viral load(ref). Considering that gamma irradiation targets the viral genome, potentially causing strand breaks and other types of damage to the nucleic acids, it is essential *not* to use nucleic acid-based analytical tools for determining virus titer (*e.g.*, polymerase chain reaction [PCR]). Although viruses will be completely inactivated (rendered non-infectious), nucleic acid testing methods will still be able to detect fragments of the viral nucleic acids, making it impossible to interpret the study results. Because of this, cell-based infectivity methods must be used for virus titrations. Virus reduction titers are then analyzed using assays such as $TCID_{50}$, (the concentration at which 50% of the cells are infected when cell cultures are inoculated with a diluted solution of virus containing fluid), PFU (the number of virus

particles capable of forming plaques in cell cultures) , and fluorescent focus assay (FFA). Such methods should be validated at least as to specificity and sensitivity (limit of detection), and any method limitations should be well understood.

There are numerous tests for BVDV but the most common are: [15].

1. BVDV Virus Isolation - Virus isolation has been the "gold standard" for BVDV detection. This involves the incubation of BVDV susceptible cells in the presence of a serum sample and the subsequent analysis of the cells for BVDV.
2. BVDV PCR Detection – Serum samples are treated to extract RNA which is then converted to DNA. The DNA is replicated up to 50 times and then quantified.
3. BVDV Antibody Detection – The presence of BVDV immunity is detected by BVDV antibody enzyme linked immunoassays (ELISAs). Such ELISAs do not diagnose active infection but detect the presence of antibodies produced by the animal in response to viral infection.
4. BVDV antigen detection or antigen capture - This assay detects BVDV Erns antigen directly and is the basis for PI detection.

A comparison of the strengths and weaknesses of these methodologies is shown in Table 1.

Table 1. Comparison of testing methods for BVDV

Testing Method	***Strengths***	***Weaknesses***
BVDV Virus Isolation	Required by many regulatory agencies	Can be manipulated to provide desired result. For example, use of cells less sensitive to BVDV can provide a false negative result.
	Can be developed to be extremely sensitive through selection of BVDV sensitive cells and viral concentration	BVDV Antibody can block the ability of cells to replicate BVDV
	Available from independent testing labs	Vey time consuming requiring weeks of incubation
PCR virus detection	Extremely sensitive, on the same level as some BVD isolation tests	Measures both infectious and non infectious
	Measures both infectious and non-infectious	
	Relatively quick	
BVDV antibody detection	Easy and inexpensive	Doesn't detect in PI calves
	Does not detect presence of the Virus	
BVDV antigen detection	Directly detects the Erns protein of BVDV	

Regulations

The presence of BVDV in serum is regulated by both government and pharmaceutical agencies. Because BVD is a worldwide disease with little difference in incidence rates between countries anywhere in the world, the restriction on the importation of animal serum based on the presence of BVDV to protect animal health in the importing country can be considered by some to be overly

regulated. However, many countries require that serum is tested for BVDV, prior to allowing importation.

The requirements for BVDV testing in the pharmaceutical arena are in general highly detailed and designed for maximum sensitivity. It is common practice to use BVDV Isolation Assays to protect cell lines in this sector, however, it must be noted that there are no standard reagents available for BVDV testing. Hence the selection of less sensitive cell lines and low titer antibodies can lead to erroneous negative results. Care should therefore be taken in the selection of testing laboratories.

BVDV Free Serum

The gold standard for BVDV activity is virus isolation, but as stated above there is no established standard of sensitivity for this assay. Serum screened in small batches can be pooled, creating a dilution effect that allows it to pass testing as "BVDV free" serum. This can perhaps be better described as BVDV free at the level of sensitivity of detection of the assay employed. True BVDV negative serum must test negative by both PCR and virus isolation methods. Raw serum can also be either gamma irradiated or treated with BVDV antibody to ensure a negative result in a virus isolation test. It should be noted that gamma irradiation does not produce true "BVDV free" serum. To avoid those factors that will complicate interpretation, several tests must be used in tandem to determine the true status of the serum and the means by which the serum has been rendered "BVDV free".

References

1. Fray; et al. (2000). The effects of bovine viral diarrhoea virus on cattle reproduction in relation to disease control. Animal Reproduction Science 60–61, 615–627
2. Ridpath; et al. (1994). Segregation of bovine viral diarrhoea virus into genotypes. Virology 205 (1), 66–74.
3. Stahl; et al. (2010), Atypical 'HoBi'-like pestiviruses—Recent findings and implications thereof. Veterinary Microbiology, Volume 142, Issues 1–2, 21 April 2010, Pages 90-93
4. Hult and Lindberg (2005) Prev Vet Med 72: 143–148
5. Rikula et al. (2005) Prev Vet Med 72: 139–142
6. O'Rourke (2002) Journal of the American Veterinary Medical Association 220(12): 1770–1772
7. Moennig and Liess (1995). Pathogenesis of intrauterine infections with bovine viral diarrhoea virus. Vet. Clin. Food Anim. 11:477–488.
8. APHIS Info Sheet (2007). https://www.aphis.usda.gov/animal_health/emergingissues/downloads/bvdinfosheet.pdf
9. Grooms; et al. (2005) Diagnosis of BVDV: A key component to a comprehensive BVDV control prigram. https://www.ars.usda.gov/ARSUserFiles/50302000/BVD2005/Produce2_Grooms_Hout.pdf
10. Methods for Detection of BVDV - Cornell University. https://ahdc.vet.cornell.edu/programs/NYSCHAP/docs/bvdtestnyschap07.pdf
11. Fenner (2011). Fenners Veterinary Virology. Elsevier, 4th edition.
12. Houe (1999). Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. Veterinary Microbiology, Volume 64, Issues 2–3, 1 January 1999, Pages 89-107
13. Brownlie; et al. (1987). Pathogenesis and epidemiology of bovine virus diarrhoea virus infection of cattle. Annales de Recherches Veterinaires 18 (2), 157–166.

14. Gillespie; et al. (1962). Cellular resistance in tissue culture, induced by noncytopathogenic strains, to a cytopathogenic strain of virus diarrhea virus of cattle. Proceedings of the Society for Experimental Biology and Medicine 110, 248–250.
15. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2008/pdf/2.04.08_BVD.pdf