Fetal bovine serum (FBS) and other animal origin biological products can be contaminated with various adventitious agents. Viruses are of particular safety concern because of their biophysical properties: composition, size, and morphology (1, 2). Proper processing during manufacturing can minimize the risk of such agents being found in a final product such as FBS. Raw materials and final product testing for adventitious agents — including viruses — can further reduce the risk of introducing a contaminated product into a production facility or research project (3).

Bovine viral diarrhea virus (BVDV) is considered one of the most common viral contaminants of FBS, which is often used as a cell growth medium in the biotechnology industry. BVDV is classified as a pestivirus, family Flaviviridae, antigenically related to hog cholera virus (also known as the classical swine fever virus) and border disease virus of sheep. BVDV is an enveloped, positive sense ss-RNA virus, 30–50 nm in size. Cattle of all ages are susceptible to infection with the virus. Distribution is worldwide, and more than 80% of cattle possess antibodies against it. The virus spreads mainly by contact among cattle. Vertical transmission (in utero infection of a fetus from its dam) plays an important role in the epidemiology, distribution, and pathogenesis of BVDV (1, 4–6).

Types and genotypes. Bovine viral diarrhea virus exists as two types, cytopathic (CP) and noncytopathic (NCP) based on the presence or absence of cytological changes in cell culture; and two genotypes, numbered 1 and 2 based on genetic variations and antigenic distribution. Noncytopathic viruses produce no visible cytopathic changes in cell cultures, and infected cells generally appear normal. Cytopathic viruses, by contrast, cause cellular vacuolation and cell death (5).

Fetal infections and serum contamination. Noncytopathic viruses may cause little, if any, overt disease in cattle, and in utero infections between 90 and 120 days of pregnancy may establish a lifelong persistent infection (PI) in apparently normal newborns (5, 7). PI fetuses possess a very large amount of virus in their blood, but because they are not immunocompetent at that age, they show no antibody response. These PI fetuses are the principal source of FBS contamination. A high viremia ($10^3$–$10^6$) in persistently infected fetuses is very common. The frequency of persistently infected fetuses is believed to range between 0.2% and 2% (5). Serum obtained from such fetuses contains a high viral load although no neutralizing antibodies can be detected.

Fetal BVD virus infections at later stages of bovine pregnancy (second and third trimesters) trigger...
an immune response. When blood is collected from such fetuses, both BVD virus and specific anti-BVDV antibodies (virus–antibody complexes) are likely to be present in serum. Under typical practice, blood is collected from both PI and non-PI fetuses. Therefore, various variations for virus load and antibody concentrations are possible when large FBS pools are made.

**The prevalence of BVDV types.** Noncytopathic BVD virus capable of establishing persistent infection is the predominant type existing in nature and is isolated more frequently (80–90%) from cattle than are the cytopathic strains (8). By contrast, although cytopathic viruses appear able to cross the placenta and infect a fetus, they do not establish persistent infections and therefore have no mechanism of maintaining their presence in cattle population. That explains why detection of NCP virus is far more common in FBS.

**BVDV and cell culture.** The BVD virus can contaminate various types of cell and tissue cultures. One study demonstrated that 13 of 41 different cell lines obtained from ATCC (www.atcc.org) were inapparently infected with NCP virus (9). Presence of the virus was confirmed using polymerase chain reaction (PCR) and immuno-cytochemical procedures. The consequences of culture contamination differ for CP and NCP viruses. The NCP type causes no visible changes. A cell culturist is unlikely to observe visible problems, and the cells in culture would remain morphologically normal for generations.

For such inapparently infected cultures, the virus can be detected by PCR or immunostaining. Because the NCP virus is accountable for over 80% of natural BVDV infections, it is therefore the principal BVDV strain encountered in FBS. It poses minimal concerns to most research applications, but it is an important concern for the manufacture of biological products. The CP type is of concern to all because it causes cytopathic changes in cell culture, destroying the cells. But the CP virus accounts for less than 10–20% cases of natural animal and fetal infections, so it is therefore a low-profile contaminant of FBS.

**Diagnostic Approach**

Various diagnostic technologies are available for virus detection in FBS. The following are the most commonly accepted testing tools for FBS and other bovine sera:

- Detection of live virus by testing protocols such as those described in Title 9 of the US Code of Federal Regulations (or the EMEA’s Committee on Veterinary Medical Products regulations, for example);
- Detection of viral RNA by nucleic acid amplification technologies (RT-PCR or QPCR as described below); and detection of virus-neutralizing antibodies.

**The 9 CFR testing protocol** for BVDV is currently considered the “gold standard” for live virus detection in FBS and other bovine sera. This protocol requires that a sample be tested in susceptible cells of bovine origin and in Vero cells for at least 21 days. During that period, the cells are regularly inspected for morphological or cytopathic changes (CPE), and they are typically split and subcultured after every seven days. At the end of the assay, sample cells are stained using a specific anti-BVDV fluorescent antibody (FA staining) and examined under the fluorescent microscope along with appropriate positive and negative controls (10).

Results are interpreted at the end of each passage. Absence of CPE throughout the assay indicates an absence of the CP BVD virus in the test article. Absence of the specific cell fluorescence at the end of the assay indicates an absence of the noncytopathic (NCP) BVD virus.

**Detection of viral RNA** is accomplished by reverse transcriptase polymerase chain reaction (RT-PCR). Real-time quantitative PCR (QPCR) is now used by many diagnostic and commercial testing labs (11). The QPCR technology is based on amplification of any tiny amount of the viral RNA that might be present in a test article. It has an exquisite sensitivity, detecting a very small amount of viral RNA and therefore a very low viral load in the serum.

QPCR can detect live virus, dead virus, or fragments of broken viral nucleic acids. That can lead to an incorrect interpretation of results: Detection of nucleic acid sequence does not confirm the presence of infectious virus. Although it is a great diagnostic tool in a clinical diagnostic laboratory (where it is interpreted in conjunction with patient history and other valuable clinical information), QPCR as a standalone assay has not been widely accepted for testing commercial products of animal origin because of

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**QPCR can detect live virus, dead virus, or fragments of broken viral nucleic acids — which can lead to incorrectly interpreted results.**

**Typical virus panels**

A typical virus panel used for FBS clearance studies includes tests for BVDV, infectious bovine rhinotracheitis (IBR, also known as bovine herpes virus 1), parainfluenza 3 (PI3), adenovirus, porcine parvovirus, bovine enterovirus, and reovirus 3. A typical virus panel used in downstream virus clearance studies tests for herpes simplex virus (HSV-1), minute murine virus (MMV), murine leukemia virus (MLV), PI3, reovirus 3, and simian virus 40 (SV-40).


**A NEW DETECTION ASSAY**

**CITATION**

**ABSTRACT**
Recently, a new approach of upstream detection of BVD virus in raw, unprocessed FBS has been described. This is a sandwich enzyme-linked immunosorbertent assay (S-ELISA) designed to detect presence of BVD virus in the serum, using a commercially available diagnostic kit (Syracuse Bioanalytical, Syracuse, NY).

In this study, the S-ELISA kit that uses raw (unprocessed) FBS as the testing sample was evaluated for upstream BVDV testing. Pooled FBS samples were tested: (n = 84), each representing a 3-L FBS sublot. Thirty serum samples originating from persistently infected (PI) calves that had been confirmed by virus isolation (VI) as BVDV positive and another 30 samples previously confirmed by VI as BVDV negative were also evaluated. Of the 84 field samples, the S-ELISA detected 13 (15.5%) BVDV-positive specimens. When these 13 positive samples were tested by VI and immunofluorescent assay, 11 (84.6%) were positive and 2 (15.4%) were negative. The S-ELISA was positive for all 30 PI samples (100%) and negative for all 30 negative samples (100%).

These data indicate that the new kit is a relatively reliable diagnostic tool and can be considered for upstream detection of BVDV-contaminated raw FBS pools. Although the S-ELISA cannot replace 9 CFR virus testing, it can be efficiently implemented at early manufacturing steps to identify and segregate positive sublots. This approach would considerably reduce the chance of virus entering the manufacturing plant, as well as minimize the percentage of contaminated final FBS products in commercial use.

**SOLUTIONS TO BVDV CONTAMINATION**
Several options can reduce the risk of FBS contamination with BVDV (and some other bovine viruses). Although thorough virus testing following 9 CFR requirements is a good way to detect live virus in a test sample, it cannot guarantee an absolute absence of the virus. This is due to a limited detection threshold, the small amount of sample tested to represent a large lot size, an uneven virus distribution within the serum, and the frequent presence of blocking antibodies in the serum. With that in mind, it is important to consider virus inactivation steps that minimize the risk of getting live virus into the product.

**Virus clearance.** Various inactivation and removal technologies are available to inactivate BVDV and other viruses: ultraviolet (UV) light, pasteurization, chemical treatment, and so on. The following are some commonly used methods.

*Gamma irradiation* at dose range 30–45 kGy can inactivate at least $7 \log_{10}$ of the BVDV present along with many other animal viruses (IBR, PI-3, adeno, parvo, reovirus, and bluetongue, to name a few). This is the only terminal (final container) sterilization technology available today (13).

*Heat inactivation* at 56 °C for 30 minutes inactivates about $5 \log_{10}$ of BVDV and several other important bovine viruses, with exception of parvovirus (14). A combination of gamma irradiation and heat inactivation can guarantee very high level of viral safety: $10–14 \log_{10}$ (13).

*Nanofiltration* technologies (40–50 nm pore size filters) can be used to remove larger and some medium-size viruses from FBS. Ultraviolet (UV) irradiation and pH treatments can also be used for successful BVDV inactivation (15, 16).

It is important to understand that the BVD virus is a ubiquitous worldwide agent. It has evolved a perfect survival strategy by establishing persistent animal infections through intraurotional infection and by crossing the interspecies barrier into pig, deer, and sheep. Thus it would be very difficult to eradicate BVDV from the global cattle population. The way FBS is manufactured, it is nearly impossible to guarantee a product that is absolutely virus negative. A solution to the problem this presents in biomanufacturing is not simple but can be achieved through the maintenance of high manufacturing standards,
appropriate virus testing, and implementation of validated virus clearance steps during the manufacturing process.

References


