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Bovine Viral Diarrhea Virus (BVDV) in Bovine Serum **WHITE PAPER**

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BVDV in bovine serum

BVDV is a common infection of cattle and transmission is reviewed in reference 1. When susceptible cattle are infected with BVDV from other cattle (horizontal transmission), they excrete virus for a short time before their immune response controls the virus infection. However, if a cow is infected while pregnant the fetus can become infected (vertical transmission). Fetuses infected at 80-125 days of age before they are capable of making an immune response can be born persistently infected, antibody negative and become life-long virus shedders (2). Fetuses infected later in gestation usually make an immune response including serum neutralizing (SN) antibody and control the virus (2). Persistently infected cattle which obtain the infection as a fetus secrete more virus than those with transient infections obtained by horizontal transmission and are more effective at spreading the virus (1).

Fetal bovine serum (FBS) batches are made by pooling serum from individual fetuses creating a risk that sera containing BVDV from infected fetuses will be added to the batch. There is also the possibility that sera from fetuses infected with BVDV later than 125 days of gestation that have SN antibody and have controlled the infection will be added to the batch. Since BVDV SN antibody is generally broadly cross reactive with other strains particularly within type 1 or type 2 strain groups (reviewed in reference 3), its presence in a batch may neutralize any added BVDV. Similar considerations are applicable for calf and adult bovine serum batches, except that the probability of adding sera with antibody that would neutralize any added virus is higher than with fetal sera. However, without assaying each serum used in a batch for BVDV and SN antibody, it is difficult to know what is being added and to predict possible outcomes. In any case, it is necessary to determine if batches of FBS, calf serum and adult bovine serum have infectious BVDV and SN antibodies. Serum batches without SN antibody are needed for the actual growth of BVDV and related pestiviruses such as classical swine fever virus. Those serum batches with detectable SN antibody generally do not have infectious BVDV (ABR results) and the presence of SN antibody may contribute to assuring virus safety of the product (4). There is a concern about SN antibody partially neutralizing BVDV (5), although if any infectious virus is present it should be detected with current tests. A further concern is that SN antibody may mask the detection of BVDV (6) the presumption being that SN antibody dissociates during use leaving infectious virus; however, there is no published evidence that this actually occurs. The detection of BVDV in cells grown in very large scale applications with bovine serum batches which tested negative for BVDV before use could be due to partial neutralization or masking by SN antibody; however such a finding could also be due to the inability of test procedures to detect very small amounts (a concentration of less than 1 infectious unit per sample volume tested, for example, 15 ml as EMEA requires) of infectious virus that may be present in large quantities of a serum batch. Based on these and other considerations, recommendations have been made that whenever possible bovine serum should be irradiated with a minimum dose of 30 kGy (equivalent to 3 Mrad) (5).

ABR tests that detect BVDV

The primary ABR test for BVDV detection that meets EMEA guidelines specified in references 5-7 is EM-AOP-BOV.

If BVDV is detected by EM-AOP-BOV in a bovine serum batch, then the BVDV titer should be determined before inactivation treatment. This is to ensure that the titer is below the level that has been shown to be effective in validation tests for the selected inactivation treatment. For serum batches with very low BVDV titers, ABR uses a test that involves concentration of virus by ultracentrifugation before titration (QUANT-AOP-BOV). For serum pools with higher titers, the ABR test for BVDV titration is VT-AOP.

After inactivation treatment, a serum batch should be tested again for BVDV. ABR uses the EM-AOP-BOV test and retest results should be negative for BVDV.

ABR tests that detect BVDV SN antibodies

The ABR validated tests for detecting BVDV SN antibodies are SN-AOP-BOV, ALPHA-AOP-BOV, and VR-AOP-BOV. Even though all 3 of these tests meet the EMEA guidelines specified in references 5-7, ABR does not recommend using the VR-AOP-BOV test.

The guidelines state that results from tests to detect BVDV antibodies are used to perform risk assessment of antibodies in bovine serum on the partial or complete neutralization of possible virus present in the serum and on the validation process.

The SN-AOP-BOV is a standard BETA SN test with constant virus and dilutions of serum. The results are expressed as serum neutralization titers. A titration of the constant amount of virus used (back titration) is performed with each test and this information and the titer can be used to calculate the amount of virus neutralized by 1 ml of test serum. The calculated results are similar to those obtained with the ALPHA-AOP-BOV SN test, but because of sensitivity differences, SN-AOP-BOV is not as effective when SN amounts are low.

The ALPHA-AOP-BOV SN test is performed with dilutions of virus and constant amounts of serum. The results are expressed as the amount of virus (TCID₅₀) neutralized by 1 ml test serum. The amount of virus neutralized by a serum is useful in assessing risk associated with the presence of these antibodies on virus presence, viral safety associated with the presence of the antibodies and influence of the antibodies on the validation processes. In addition, the ALPHA-AOP-BOV SN test is more sensitive than the SN-AOP-BOV test allowing the determination of the amount of virus neutralized when SN antibodies amounts are low.

The VR-AOP-BOV test qualitatively detects SN antibody to BVDV very effectively and is as sensitive as the ALPHA-AOP-BOV test. However, the results are expressed as a neutralization index which is a less common measurement for SN antibody than either ALPHA-AOP-BOV and SN-AOP-BOV tests. Further, the VR-AOP-BOV test is not effective at determining the amount of virus neutralized (TCID₅₀/ml) by a serum because of discrimination (precision) problems near the neutralization index cut-off.

ABR comparative titration test

The ABR comparative titration test is CT-AOP-BOV.

The CPMP guidelines (6) on detection of BVDV and on the use of validated tests to detect antibodies to BVDV are similar to the CVMP guidelines (5). The major difference in the two documents is that the CPMP guidelines still include a serum inhibitory test (Reference 6, section 5.3.4) in which cells permissive for BVDV are grown with at least 3 subcultures in the presence of test serum and then used to titrate a reference strain of BVDV. The test is intended to detect any non cytopathic BVDV present in a test serum by inhibiting the reference strain, and therefore the test serum should not significantly ($>2 \log_{10}$) inhibit the growth of the reference BVDV strain. The serum inhibitory test was in the original CVMP guidelines and referred to as a comparative titration test; however the comparative titration test was removed from the current CVMP 2005 guidelines (5). Removal of the comparative titration test was considered a welcome major change in comments on the draft (7) which preceded the actual change (5).

It is not known if CPMP will follow the CVMP guidelines and remove the serum inhibitory test (same as the comparative titration test) from their next revision. ABR offers the comparative titration test (CT-AOP-BOV) so that clients can meet the CPMP guidelines for testing, but recommends that CPMP remove the test from their guidelines. This recommendation is based on the published results of J.W. Black (8), the previous President of ABR and on the unpublished results obtained at ABR since relocation to Pullman, WA. Both these results demonstrate that the serum inhibitory test (comparative titration test) is an unreliable test for the detection of non cytopathic BVDVs which are easily detected by the EM-AOP-BOV test.

Recommendations for preventing BVDV in bovine serum batches

1. Whenever possible bovine serum batches should be irradiated with a minimum dose of 30 kGy (equivalent to 3 Mrad) (5).
2. If irradiation destroys cell growth promoting properties of serum batches, an option is to obtain calf and adult sera for pooling from BVDV-free cattle. If BVDV-free cattle are not available, individual sera, particularly fetal sera, could be screened for BVDV before pooling into batches.
3. If serum batches are to be used in applications that do not involve the need for actual growth of BVDV and related pestiviruses such as classical swine fever, then adding sera, fetal or otherwise, with BVDV SN antibodies to a serum batch can increase the probability of neutralizing any BVDV that might be present contributing to viral safety.

Recommendations for SN antibody information about bovine serum batches

1. Use the ALPHA-AOP-BOV test to measure SN antibody. The results are expressed as the amount of virus (TCID₅₀) neutralized by 1 ml test serum and the test is more sensitive for detection of SN antibody than the SN-AOP-BOV SN (beta) test.
2. If serum batches are needed without SN antibody such as for growth of BVDV and related pestiviruses such as classical swine fever, BVDV SN antibody negative FBS or adult bovine sera may be used. However, individual sera should be screened for SN antibody before pooling if negative pools cannot be identified.
3. If serum batches are to be used in applications that do not involve the need for actual growth of BVDV and related pestiviruses such as classical swine fever, then adding sera, fetal or otherwise, with BVDV SN antibodies to a serum batch can increase the probability of neutralizing any BVDV that might be present contributing to virus safety.

Recommendation for changes in regulatory guidance

ABR recommends that EMEA CVMP remove the serum inhibitory test which is the same as ABR comparative titration test (CT-AOP-BOV) from their guidelines.

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About ABR, Inc.

For 25 years ABR, Inc. (American BioResearch) has served the global pharmaceutical and biotechnology industries with world-class quality assurance and industry-leading turnaround time. The Team of Scientists at ABR is dedicated to providing accurate, timely, and precise results, while ensuring a high degree of customer service. In addition, ABR continues to design and develop virus detection protocols to meet evolving needs and changing regulations

ABR's standard assays satisfy United States 9th Code of Federal Regulation (9CFR), European Medicines Agency (EMA), CVMP and CPMP regulations. Additionally, ABR operates under Good Laboratory Practice (GLP) as defined in the Twenty-first Code of Federal Regulations (21CFR), Part 58. Recently ABR has added sterility testing for bacteria, fungi, and mycoplasma. ABR has the capability to customize studies for clients needs in ever changing regulatory environments.

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