Diagnostic Virology

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Methods for Viral Detection

Virus isolation (VI) has been the gold standard for viral detection but because of the expense and time involved is becoming less popular. Tissue specimens, swabs or serum can be used for virus isolation depending on the virus suspected. Virus isolation is recommended for herpesviruses, bovine viral diarrhea virus (BVDV), feline calicivirus, and enteroviruses. Virus isolation is not a suitable test for viruses that do not replicate easily in cell culture.

Antigen detection tests are a good way of detecting viruses in clinical samples. The most common of these is the fluorescent antibody test performed on frozen tissue sections. A similar test is the immunoperoxidase (IP) test usually performed on formalin-fixed tissues. Other antigen detection tests, such as the ELISA tests, are those that capture antigen on some sort of solid support and presence of the viral antigen is detected by a visible color change. These tests are quick, specific, sensitive and thus, preferred when sufficient viral protein is present. Such antigen detection tests are available for many but not all viruses and false negatives may occur if the wrong tissue is used, insufficient antigen is present, advanced tissue decomposition or hemolysis is present. Antigen detection tests such as the ELISA are recommended for canine parvovirus, bovine coronavirus, rotaviruses, and bovine respiratory syncytial virus.

Antibody detection tests, such as serum neutralization (SN), complement-fixation (CF) and hemagglutination-inhibition (HI), are indirect means of inferring the presence of viral antigen by measuring antibody levels in serum, i.e. a titer. These tests are preferred for sale, import and export.

Nucleic acid detection is the latest technique for "finding" viruses. Of the various methods used for this purpose, the polymerase chain reaction (PCR) is the one currently most favored. While this test is rapid and very sensitive, it requires meticulous laboratory technique and those performing the test must be very careful to avoid contamination by foreign nucleic acid and thus causing false positive results. Appropriate controls, both positive and negative controls, are critical to obtaining accurate results. The strength of the PCR is its exquisite sensitivity but this is also its downfall because only tiny amounts of contaminating nucleic acid will be inadvertently amplified and cause false positive results. PCR is one of the better techniques for identifying PRRS virus in serum samples.

Electron microscopy (EM) has been the workhorse for diagnosis of viral diarrhea in all species. Most enteric viruses have a unique shape and size and can be easily identified by EM. It is most accurate during the early acute phase of the disease when large amounts of virus are being shed. An advantage to EM is that it will pick up multiple infectious agents, e.g. rotavirus and coronavirus or parvovirus and coronavirus. At least 4-5 mls of feces should be submitted; chilled but not frozen.

Histopathology. For some viral diseases the lesions are specific enough for a definitive diagnosis, examples include canine distemper, inclusion body rhinitis, pox virus infections. With many other viral diseases that lesions will be sufficiently characteristic to at least be suggestive or to confirm the role of an agent detected by other means, e.g. do the lesions fit the "bug".

BVD Diagnosis

For many years at Kansas State, Dr. Harish Minocha has focused a large part of his research on the problem of bovine virus diarrhea virus (BVDV). Dr. Minocha's research has focused primarily on the basic nature of the virus. More recently, Dr. Sanjay Kapil in the Diagnostic Laboratory has been studying BVD virus with the focus more on rapid diagnostic methods.

BVD Tests Offered at Kansas State Diagnostic Lab

1. Bovine virus diarrhea virus isolation - Performed on tissues or from buffy coat of whole blood.
2. Bovine viral diarrhea virus isolation from serum - Can use serum for routine virus isolation (VI) or for the microplate virus isolation test for herd screening.
3. Bovine viral diarrhea fluorescent antibody tests - Performed on frozen, unfixed tissue and also used to identify non-cytopathogenic BVD virus in cell cultures.
4. Bovine viral diarrhea immunoperoxidase test - IP is a form of immunohistochemistry test that can be used on formalin-fixed tissue.
5. Bovine viral diarrhea virus serum neutralization (SN).
6. Bovine viral diarrhea PCR on serum.
7. Bovine viral diarrhea ELISA on serum - New test still under development.

The selection of the proper tests for the correct reasons is paramount for success in diagnosing a BVDV problem.

1. Bovine viral diarrhea virus isolation

Virus isolation has been the "gold standard" for BVDV detection and will continue to be an important diagnostic test. This test is done primarily on tissue samples, whole blood or swabs (if using swabs be sure they are for virus isolation and are made from Dacron, or are calcium alginate free cotton since regular cotton tip swabs may be inhibitory for some viruses). Specimens for VI may be from acutely infected animals as well as from persistently infected animals. BVD virus is stable at room temperature for several days in serum. Shipment of specimens on ice packs is satisfactory but hemolyzed blood is not satisfactory due to toxic affects on cell cultures, likewise, fecal samples are not satisfactory for BVDV isolation due to the heavy contamination and presence of substances toxic to the cell cultures used to grow the virus. A number of studies have found 1-3% of serum samples will be positive for BVD virus. About 70% of BVD isolates in the North Central USA are the non-cytopathogenic type. The incidence of BVD type I versus type II is not yet known in Kansas.

2. Bovine viral diarrhea virus isolation from serum

Persistently infected immune tolerant carriers will usually have high levels of virus

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in the serum. This is the basis for the microplate virus isolation test for herd screening. The microplate virus isolation test is formatted to provide an economical way to screen herds for persistently infected animals. The economy of the test comes from the use of serum and being able to test many samples at the same time. A minimum of 20 samples is necessary to make the test economical. This test is a little less sensitive than virus isolation from the buffy coat of whole blood in acutely infected, immune competent animals since the latter will usually not have as high a level of serum viremia as immune tolerant persistently infected animals. Also, this test is not as accurate on animals less than three months of age because of colostral interference and is not considered appropriate for export testing or qualifying animals for AI centers.

3. Bovine viral diarrhea fluorescent antibody (FA)

This is an antigen detection test performed on frozen, unfixed tissue, as contrasted with the immunoperoxidase test which can be used on formalin-fixed tissues. The FA test is more rapid than the immunoperoxidase test since the tissues don't have to be fixed. It is most useful for detecting acute infections from necropsy specimens. Swabs or impression smears are not good specimens for FA. The FA test is also used within the laboratory to detect BVDV antigen in cell cultures.

4. Bovine viral diarrhea immunoperoxidase test

The immunoperoxidase test is an immunohistochemistry procedure for antigen detection that is able to detect viral antigen in formalin-fixed tissue. It is usually performed on tissues taken at necropsy or fixed tissues submitted for histopathology. It is generally quite sensitive and accurate as long as the tissues are not autolyzed.

5. Bovine viral diarrhea virus serum neutralization

This is the only test routinely used to detect and quantify serum antibodies specific for BVDV. In the classical use of this test, acute and convalescent sera are tested to determine whether a recent infection has occurred. A 4-fold or more rise in titer indicates recent infection. This test is also useful on single serum samples from a group of animals to determine the immune status of a herd or the presence of virus within an unvaccinated herd. Current vaccines, both killed and modified live can elicit high enough antibody responses that differentiation from natural exposure is difficult. At the Kansas State Diagnostic Laboratory the serum neutralization results for BVD virus reflect type I antibodies unless type II is specifically requested.

6. PCR detection of virus in serum

This test is used on serum samples and has high sensitivity to detect viral antigen. It does not detect antibodies. At the Kansas State Diagnostic Laboratory, three pairs of primers are used to cover both type 1 and type 2 and also cytopathic and non-cytopathic BVD virus.

If you have questions regarding the best test to request and the proper specimens to submit for a particular problem, please call the laboratory for assistance (785-532-5650).