

## Virus (antigen)

### **VI (Virus isolation)**

Definitive (gold standard) test proving both presence and viability of virus. Virus isolation is performed using *in vitro* cell culture (and embryonated eggs for avian viruses). Virus isolation is laborious, expensive and slow – results are generally not available for 1-2 weeks after submission. Aseptic collection at an early stage of disease and proper handling of samples (continuous cold chain) is critical for VI. Poor sample handling may lead to false negative results.

Possible on most clinical samples (tissues, blood, secretions) although materials such as urine, faeces and semen prove very difficult due to their toxicity for cell cultures.

### **PLA (Peroxidase linked assay)**

The peroxidase-linked assay is used primarily at CVRL for the detection of BVD virus by isolation. The test is slow (~5 days) but is highly sensitive and specific. Submit at least 1ml of whole blood (0.5 ml of serum) for each PLA requested (no anti-coagulant). All PLAs are conducted in quadruplicate, the test requiring infectious virus. Results are reported as negative, positive or toxic (requiring a rebleed). Any inconclusive results may be further tested with an antigen ELISA (BVD) for confirmation.

### **FAT (Fluorescent antibody test)**

The FAT is a rapid, specific diagnostic test for detecting viral antigen in tissues (thin sections) and cell smears. FAT is extremely dependent on the quality of clinical sample submitted and for best results very fresh specimens are required. Intact cells are required so freezing and thawing of samples is detrimental to FAT. Specific instructions for the preparation of FAT are given on the submissions page.

### **EM (Electron microscopy)**

Electron microscopy is an ideal method to directly visualize viruses. For definitive diagnosis, the viruses must have morphological features which are sufficiently distinct to permit unique identification. EM is a nonspecific test with somewhat low sensitivity (requiring lots of virus) and is expensive and slow, being very labour-intensive. EM can be performed on tissue (thin sections), tissue homogenates and secretions / excretions. Screw top containers should be used when submitting the latter. The quality of the sample is of utmost importance in order for the test to work correctly. Although extremely useful, the role of EM in many cases has been superseded by molecular methods of viral detection.

### **PCR (Polymerase chain reaction)**

PCR is a process whereby a target portion of viral nucleic acid (either RNA or DNA) is replicated many million-fold, providing results which are highly specific and highly sensitive. Detection of the amplified product indicates whether a sample is positive for the target virus (genome). A wide range of samples such as tissues, excretions, secretions, blood, serum, and biopsies may be submitted for PCR analysis. It is best practice to submit clinical samples taken aseptically from individual animals, taking great care to avoid cross-contamination. Use only clean receptacles during collection. Refrigeration and rapid dispatch to the laboratory will greatly enhance the quality of nucleic acid and thence the subsequent results from PCR analysis.



## Serology (antibody)

### **SNT (Serum neutralisation test)**

The serum neutralisation test is the accepted definitive serological test. The demonstration of seroconversion or a 4-fold increase in titre in paired sera provides proof of recent infection. A high antibody titre in a single sample may also be significant. Ideally, serum should be removed from the clotted blood samples within 24 hours to avoid haemolysis. The SNT is a slow (~ 5 days) and labour intensive test. Submit at least 2ml of whole blood (1 ml of serum) for each SNT requested (no anti-coagulant). Results will be reported as titres, usually with two-fold step-wise dilutions. In disease outbreaks, to assist clinical interpretation, compare titres in clinically affected Vs unaffected animals, or acute Vs convalescent. Remember serum samples from calves under 6 months old and pigs under 3 to 4 months old commonly contain high levels of maternally derived colostral antibody.

### **ELISA (Enzyme linked immunosorbant assay)**

The ELISA is a reliable, sensitive technique, well suited to testing many samples very quickly. ELISA can be used to detect serum antibody (antibody ELISA) or the presence of virus (antigen ELISA). Antigen ELISA will detect both inactivated and infectious virus. Ideally, serum should be removed from the clotted blood samples within 24 hours to avoid haemolysis. Submit at least 1ml of whole blood (0.5 ml of serum) for each ELISA requested (no anti-coagulant). All ELISA test results are interpreted using thresholds or cut-off values and reported either as optical densities (OD), percentage positivities (PP%), sample/positive (S/P) ratios or percentage inhibitions (PI%). Remember serum samples from calves under 6 months old and pigs under 3 to 4 months old commonly contain high levels of maternally derived colostral antibody. Most ELISAs detect IgG antibody and not IgA, IgM and IgE.

### **AGID (Agar gel immunodiffusion)**

Agar gel immunodiffusion is a sensitive assay for the detection of viral-specific antibody, often used in conjunction with antibody ELISA. The AGID is based on the formation of lines of precipitation at the meeting points of specific antibody and soluble antigen, both allowed to diffuse through an agar base. Ideally, serum should be removed from the clotted blood samples within 24 hours to avoid haemolysis. The AGID is specific, inexpensive and reasonably fast (overnight) but experience is required for reading the results. Submit at least 1ml of whole blood (0.5 ml of serum) for each AGID requested (no anti-coagulant). It is best to submit a batch of samples (>5) from different suspect animals when basing diagnosis on the AGID test.



### **HAI (Haemagglutination Inhibition Test)**

The Haemagglutination test is a well established assay for the detection of viral-specific antibody in serum. Some viruses can cause erythrocytes of mammals or birds to agglutinate or clump together. Examples include influenza, parainfluenza, flaviviruses and some picornaviruses. If however specific antibodies against the test virus are added, they bind out the virus and prevent (or inhibit) haemagglutination – this is the basis of the HAI test. The specificity of the HAI test depends on the virus being tested, some allowing virus strain identification (influenza) but others cross-reacting between viral species (flavivirus). The test is inexpensive and reliable but reading requires experience. For best results avoiding abnormal agglutination patterns, **fresh** samples are needed. Ideally, serum should be removed from the clotted blood samples within 24 hours to avoid haemolysis. Results are usually reported as HAI titres.

