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Laboratory Diagnosis of Viral Infections

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Diagnostic Approaches

The basic approaches for laboratory viral diagnosis are the isolation of the virus, demonstration of the virus or some viral product in clinical specimens (direct methods), and detection and measurement of viral-specific antibodies (indirect methods). Each approach has its merits, but direct demonstration of the virus and/or viral products is the most effective and useful approach for routine diagnosis.

Demonstration Procedures

Direct methods include visualizing the virions by electron microscopy, detecting the viral genome using DNA probes, and detecting viral antigens by immunofluorescence. The latter procedure has been the most useful in diagnostic laboratories.

General Serologic Procedures

During the late stages of illness, testing the serum of infected animals for antibodies to specific viruses may be the only means of diagnosis. This may be accomplished through various serologic tests.

The serologic tests most often used in veterinary diagnostic laboratories to diagnose viral infections are: serum neutralization (SN) tests; hemagglutination inhibition (HI) tests; agar gel immunodiffusion (AGID) tests and enzyme linked immunosorbent assays (ELISAs). These tests are based on the fact that viral activity can be inhibited and/or viral proteins are bound by specific antibody. Dilutions of serum are tested, and results are reported as the reciprocal of the highest dilution in which antiviral activity is noted. Ideally, the results of serum collected during the acute phase of illness are compared with results of serum collected during convalescence (two serum collections 14 - 21 days apart). Diagnosis is confirmed if a four-fold increase in titers is noted between these paired samples.

Results from single (non-paired) serum samples are more difficult to interpret. For viruses causing an acute and self-limiting infection, positive results only mean that the animal has been exposed, either naturally or through vaccination. Interpretation is made easier by testing a percentage of those animals that were sick vs. those that were not, because higher titers are usually more indicative of recent infections. For viruses causing persistent or latent infections (e.g. herpesviruses and retroviruses), positive serology means the animal is a potential carrier of the virus.

Positive results of regulatory tests are always meaningful regardless of the antibody titer. For this reason, other serologic tests more suited to standardization and test kit form, have been developed. Examples are the agar gel immunodiffusion test (AGID), also known as the Coggins test, for equine infectious anemia and the ELISA and LA tests for pseudorabies. The results of these tests are reported as either positive or negative.

Discussion of the various diagnostic procedures employed follows.

Virus Isolation

Use: Isolation and identification of virus.

The acute phase of illness is the best time to demonstrate and isolate viruses. As disease progresses antibody develops, virus shedding is reduced, and virus is cleared from tissues.

Clinical manifestations of disease generally guide the selection of appropriate clinical specimens, such as nasal and ocular swabs from upper respiratory tract infections, feces from enteric infections, blood from systemic infections, etc.
Viruses require living cells in order to replicate. In the laboratory, living cells are usually provided as cell cultures, whose cells have been obtained by enzymatic digestion of animal tissues. The cells are cultivated on glass or plastic surfaces.
When clinical specimens containing virus are inoculated onto susceptible cell cultures, the virus (if viable) replicates and often produces a characteristic pattern of cell pathology, characterized by changes in cell appearance, called cytopathic effect (CPE). In some instances, the virus replicates without any discernible effect on the cells and must be demonstrated by special stains that reveal viral inclusion bodies or by fluorescent antibody (FA) tests to detect viral antigens. The time required isolating viruses ranges from less than 24 hours to as long as several weeks.

Fluorescent Antibody
Use: To detect viral antigen in clinical materials or in infected cell cultures.
The fluorescent antibody (FA) test detects viral antigens in infected cells with specific antiviral antibody that has been labeled with a fluorescent dye (fluorescein isothiocyanate). FA tests are performed on frozen sections of tissues, blood smears, tissue imprints, scrapings or in cell cultures. Results are available often in less than one hour, and they are accurate provided that the antibody is specific and specimens are in moderately good condition.
There are two basic types of FA techniques: direct (DFA) and indirect (IFA). In the direct procedure, the antiviral antibody is labeled. This labeled antibody is then used to detect viral antigens in cryostat sections of tissues, scrapings, blood smears, etc.
The IFA technique is a two-step procedure. The specimen to be examined is first reacted with an unlabeled antiviral antibody. After sufficient incubation time is allowed for antigen-antibody interaction (usually one hour or less), the specimen is washed and then incubated with a labeled antibody directed against IgG of the species in which the unlabeled antiviral antibody was prepared. This labeled anti-antibody will attach itself to the viral-bound unlabeled antibody, and if this occurs, fluorescence is observed and the test is considered positive.
There are advantages and disadvantages to both FA procedures. The DFA technique is quicker to perform and is most often used because of the availability of DFA conjugates. The IFA technique, on the other hand, is usually more sensitive and specific (if monoclonal antibodies are used); is more time consuming but only requires one labeled antibody if all antiviral antibodies are prepared in a single species.
Immunofluorescence or fluorescent antibody (FA) is the single most useful test for routine viral diagnosis. A number of FA conjugates are available commercially for detecting viruses. Conjugates that detect viruses of dogs and cats are available commercially.
An indirect procedure called Immunofluorescence Assay is used to detect and measure antibody. It involves infecting cell cultures with a virus, and then preparing "spot" microscope slides with these infected cells. Sera can then be tested for specific antibody by reacting them with the infected cells followed by a reaction with the anti-species IgG conjugate. Those spots that fluoresce (the result of serum antibody plus conjugated anti-species IgG binding) indicate sera that are positive for the presence of specific antibodies.

Immunoperoxidase
Use: To detect viral antigen in clinical materials and cell cultures.
The immunoperoxidase technique is similar in principle to the fluorescent antibody procedure. The difference is that the antibody is conjugated to an enzyme (horseradish peroxidase or alkaline phosphatase) rather than to a fluorescent compound. Although the enzyme is bound to the antibody, it remains active and, when provided with its substrate, reacts and gives a color reaction. This technique has the advantage over FA that it does not require a fluorescence microscope, and it is especially useful for locating viral antigens in histopathologic lesions.

Enzyme -Linked Immunosorbent Assay (ELISA)
Use: To detect antigen or antibody.
The sensitivity of ELISA is comparable to radioimmunoassay (RIA), which is similar in principle to the former.
A solid-phase system is used for most ELISA assays. For virus detection, specific antibody is first adsorbed to the surface of a polystyrene tube, microtiter plate, etc., and the sample containing the suspected virus is then added. If the virus is present, it binds to the adsorbed antibody. After washing, specific antiviral antibody labeled with an enzyme (usually alkaline phosphatase or horseradish peroxidase) is added. The labeled antibody reacts with the complex, creating a "sandwich effect". Following washing, the substrate for the enzyme is added, resulting in a color reaction. Some tests are visually interpreted, but greater sensitivity is obtained by spectrophotometric analysis.
An indirect ELISA procedure is used for antibody detection. Antigen is first adsorbed to a solid phase, followed by the addition of the test serum. After washing, an enzyme-labeled anti-gammaglobulin is added followed by addition of the enzyme substrate.
Variations of the standard ELISA include competitive ELISA for antibody detection, in which the enzyme labeled anti-gammaglobulin is replaced with enzyme-labeled antiviral antibody. The subsequent color development following the addition of the enzyme substrate is inversely proportional to the level of antibody present in the test sample. In other words, if specific antibody has been bound, the enzyme-labeled antibody will not bind. Thus, positive tests are those with no color

reaction or less than that of appropriate controls.
Another variation is the kinetic ELISA, which is used for the detection of antibody to canine Lyme disease, feline leukemia virus, feline infectious peritonitis, feline toxoplasmosis, and bovine herpesvirus 1. In the kinetic ELISA, the reaction is continuously monitored over a period of time, rather than being stopped after a predetermined amount of time.
Enzyme-linked immunosorbent assays (ELISA) and latex agglutination (LA) systems detect viral antigens by "capturing" them with specific antibody adsorbed to appropriate substrates.
These techniques provide for a rapid diagnosis and are often available for "in office" use. Commercially available kits include ELISA and LA kits for detecting rotaviruses in feces from a variety of animal species, and rapid ELISA kits for detecting canine parvovirus in feces and feline leukemia viral antigen in blood.

Latex Agglutination (LA)
Use: To detect antigen or antibody.
LA tests are similar in principle to bacterial agglutination in that latex particles coated with antibody will agglutinate when mixed with the corresponding antigen and thus identifying it.
Conversely, the latex particles can be coated with antigens and used to detect antibody. These tests are easy to perform and provide results within minutes. Commercial kits for "in office" use are available for the detection of antibody to some diseases and for detection of some viruses.

Electron Microscopy (EM)
Use: To demonstrate viruses in clinical samples.
In the technique of negative contrast EM, distilled water lysates of clinical specimens are "stained" with a solution of heavy atoms. This technique is primarily used for the examination of those clinical specimens expected to contain a large number of viral particles, such as feces (coronaviruses, rotaviruses, and parvoviruses) and vesicular and pox-like lesions (herpesviruses and poxviruses). Specimen preparation and EM examination usually can be completed within 30 minutes.

Immunoelectron Microscopy
Use: To demonstrate and identify viruses.
The negative staining technique of electron microscopy referred to earlier for the demonstration of viruses is also useful for identification. The virus is reacted with immune serum, resulting in clumping that can be seen when viewed under the electron microscope.

Virus Neutralization (VN)
Use: To detect and measure antibody.
Virus neutralization is the most widely used method of detecting and measuring antibodies to viruses of veterinary importance. This test is considered the most reliable of all serologic procedures, being less prone to variation and less subjective in its interpretation.
The principle of the test is based on the fact that the demonstrable replication and activity of the virus whether it is cytopathic effect (CPE) in cell culture, clinical signs, lesions, or death in embryonated eggs and animals can be inhibited by specific viral antibody.
VN tests are almost always performed using cell cultures. Stock viruses for use in the tests are previously grown, aliquoted, and stored at ultra-low temperature. These viruses are titrated several times to determine the amount of virus present. Dilutions of test serum are made in microtiter plates, followed by the addition of an equal volume of virus suspension diluted to contain approximately 100 to 300 infective doses (an infectious dose is the minimum number of virus particles needed to establish infection). Following an incubation of serum + virus 1 - 2 hours at room temperature (some systems used 37°C or even 4°C), indicator cell cultures are added. Plates are sealed, incubated at 37°C, and observed daily for development of viral CPE. The presence of specific antibody in the test serum inhibits the production of this CPE.
The VN test is also used to identify unknown viral isolates in essentially the same manner as described above. The only difference is that the antibody is known and the virus is unknown. If a specific antibody inhibits the development of CPE of the unknown virus, then identification is accomplished.

Hemagglutination Inhibition Test
Use: To detect and measure antibody.
The hemagglutination inhibition (HI) test is similar in principle to VN tests except that the viral activity being inhibited is hemagglutination. HI tests are quite sensitive and highly specific, and are particularly useful for measuring antibody to those hemagglutinating viruses that either replicate poorly in cell culture or produce little or no discernible CPE. Examples of such viruses are the Type A influenza viruses of most animal species, Newcastle disease virus of birds, and porcine parvovirus.
HI tests are usually performed in microtiter plates. Dilutions of test sera are made, followed by the addition of an equal volume of virus suspension diluted to contain approximately 4 to 8 HA units (a HA unit is the highest dilution of the viral sample that causes complete hemagglutination). The appropriate RBC suspension is then added and the plates are gently mixed and allowed to incubate for 1 - 2 hours at 4°C (for most viruses). If the specific antibody is present in the test serum,

agglutination of RBC will be inhibited and the RBCs will settle out in a well-defined "button". Agglutinated cells, in contrast, will settle out in a thin layer over the entire bottom of the test well, or in a rough, irregularly fringed button. Test sera often contain nonspecific inhibitors of agglutination and must first be adsorbed with RBCs prior to testing.

Complement Fixation Test
Use: To detect and measure antibody.
Complement fixation (CF) tests are most useful as an aid in the diagnosis of acute or recent viral infections, because they primarily detect IgM, the first immunoglobulin class to respond to infection.
The test entails the use of viral antigens, guinea pig complement, and an indicator system of "sensitized" sheep RBCs. Reacting with antibody directed against them sensitizes the sheep RBCs. This anti-sheep RBC antibody is referred to as hemolysin and is prepared in rabbits. The antigen and complement are each titrated and diluted. If no specific antibodies are present in the serum being tested, the complement is free to react with the sensitized RBCs, causing lysis. If sufficient antibody is present, the specific antigen-antibody complexes will have bound the complement and no lysis of the RBCs will occur.

Immunodiffusion
Use: To detect specific antibody or antigens.
The two most commonly used techniques of immunodiffusion are the double-diffusion plate system and immunoelectrophoresis. Both tests are conducted in a semi-solid medium usually agar or agarose. The essential difference between the methods is that in immunoelectrophoresis the antigen is electrophoretically fractionated prior to an overlay of antibody. In both methods, the antigen and antibody diffuse against each other forming a line of precipitation where they react.
The double-diffusion plate system is the more commonly used diagnostic test. The best-known example is the "Coggins test" for equine infectious anemia.
The immunodiffusion test can be made more sensitive by using a radioactive label, which will permit the detection of antigen-antibody reactions not visible to the naked eye. The radioactive label is usually iodine (^{125}I), and either the antigen or antibody can be labeled. Labeling occurs by ^{125}I coupling to the amino acid tyrosine. The tests are read by covering the plates or slides with an x-ray film that records the radioactive (precipitation) lines.

Protection Tests
Use: To identify viruses.
Protection tests are used for virus identification when other less involved means are not available. They involve the production of either active or passive immunity in an animal or animals followed by challenge with the agent in question. An example of a protection test once used is that for the identification of hog cholera virus. The test was carried out by injecting anti-hog cholera serum simultaneously with blood or spleen suspension from the animal suspected of having hog cholera. If the agent was hog cholera virus, the passive immunity provided by the anti-hog cholera serum would protect the pig from the challenge dose. The control, unprotected pig would come down with hog cholera.

Nucleic Acid Hybridization
Use: To detect viral DNA or RNA sequences in nucleic acid extracted from clinical specimens.

Nucleic acid hybridization consists of the following:
- The double-stranded nucleic acid of a virus is denatured with alkali to separate strands.
- The single strands of nucleic acid are attached to a solid support, usually a nylon or nitrocellulose membrane, to prevent the strands from reannealing. The nucleic acid attaches to the membrane by its sugar-phosphate backbone; the nitrogenous bases are thus projecting outward.
- A probe (single-stranded DNA or RNA molecule of known origin - containing the nucleotide sequence specific to that of the target virus - labeled with a radioactive atom or enzyme) is added to the membrane.
- Formation of hydrogen bonds occurs between complementary bases. Unreacted probe is removed by washing and hybridization is detected by an assay for the probe.

Different formats of solid-phase hybridization assays are employed:
Southern hybridization. This is a technique used to identify the presence of a specific DNA fragment.
Northern hybridization. This is used to detect specific RNA sequences.
Dot blot hybridization. This is procedurally similar to Southern and Northern, may be used both to detect DNA and RNA. The difference is that the nucleic acid is placed onto nitrocellulose in an apparatus that focuses the individual spots into concentrated areas, similar to a microtiter plate.
In situ hybridization. This principle of this technique is similar to Southern and Northern hybridization (detection of specific nucleotide sequences by a labeled probe), except rather than extracting the nucleic acid and transferring it to a nitrocellulose membrane, intact cells or tissue sections on a microscope slide are probed. This technique has little use in

routine diagnostic, yet is very valuable for research and pathogenesis studies.

The techniques of restriction enzyme analysis, polymerase chain reaction, and microarray analysis are particularly useful and are discussed directly below.

Restriction Enzyme Analysis
Use: Identification of specific viruses.
Restriction enzyme analysis utilizes enzymes called restriction endonucleases to "profile" the genomic sequences of viruses. The presence of mutations and/or genetic variability at potential cleavage sites in some viral strains results in different patterns of fragments when they are separated in an agarose gel. This analysis is called restriction fragment length polymorphism (RFLP) and has been used to characterize and to compare among field isolates of a given virus.
The technique requires fairly large amounts of purified or partially purified viral DNA, a set of restriction enzymes to cleave the DNA, the ability to separate the resulting DNA fragments by electrophoresis, and a method of documenting the results.
Restriction cleavage of the genome of viruses with large genomes, such as cytomegalovirus, may yield 20 to 50 DNA bands, while viruses with smaller genomes, such as adenoviruses, usually yield only five to ten DNA bands. There is no easy way, nor is there generally a need, to correlate the pattern (missing or extra bands) with mutations at specific locations in the genome without extensive molecular hybridization studies or even sequencing of the genomes being compared.
A distinct limitation of the method is that the presence of a mutation cannot be detected unless that mutation happens to fall within the recognition sequence of the restriction endonuclease being used for digestion of the DNA.
The use of different restriction enzymes will optimize the probability of detecting mutations in a particular genome or portion of a genome.

Polymerase Chain Reaction
Use: Identification/detection of specific viruses or specific gene sequences.
The polymerase chain reaction (PCR), an in vitro method of DNA replication, is capable of amplifying DNA segments by more than a million-fold. A single copy of the viral genome - if present in the clinical material - is amplified, yielding millions of copies that can be readily detected by electrophoresis. This is accomplished by creating a reaction mixture that, in addition to sample DNA (potentially containing the target genome), contains two oligonucleotide primers that complement opposite ends of each strand of the targeted sequence, deoxynucleoside triphosphates, and a thermostable DNA polymerase (*Taq* polymerase).

The reaction mix is then subject to a temperature cycle in order to facilitate DNA replication and thus increase the amount of DNA. The following represents a typical cycle:

- The first step of the PCR cycle is to denature the sample DNA by heating the reaction mixture to 95°C.
- Secondly, the mixture is cooled to allow the primers to anneal to the target DNA.
- Thirdly, the mixture is warmed to 72°C to allow for DNA polymerization by the *Taq* DNA polymerase. The temperature cycle of the PCR is repeated, typically 35 to 40 cycles. Greater than one million copies of the DNA can be obtained in this manner.

Once the desired number of cycles is completed, the target DNA is separated by gel electrophoresis and analyzed by measuring DNA bands or by Southern hybridization with specific probes.
A number of PCR-based diagnostic tests are now available commercially.
PCR is applicable to the study of RNA viruses if reverse transcriptase is first used to make cDNA from the target RNA; called reverse transcriptase PCR. Other PCR alternatives are "real-time" PCR, which simultaneously analyzes the samples spectrophotometrically to visualize polymerization, and nested PCR, which uses a second set of primers within the region amplified by the first primer set. Either technique is helpful when viral nucleic acid levels are low. The efficacy of "real-time" PCR in clinical diagnostics has yet to be established.

Radioimmunoassay
Use: To detect antigen or antibody.
Nowadays, radioimmunoassay systems are rarely used in veterinary diagnostic laboratories.
There are two basic radioimmunoassay (RIA) systems, liquid phase and solid phase. In the liquid phase system, the antigen-antibody complexes are precipitated by subsequent addition of anti-gammaglobulin. The precipitate is collected by centrifugation and dried. The amount of radioactivity in the precipitate compared to the total radioactivity is a quantitative measure of the antigen-antibody reaction. The labeling is done with ^{125}I (see Immunodiffusion), and anyone of the three components can be labeled.
In the solid phase system, the antibody is coated to the inside of a polystyrene tube and then reacted with antigen. Briefly, the specimen is added to a polystyrene tube previously coated with antiviral antibody. If the antigen is present, it attaches to the bound antibody. Following rinsing, ^{125}I labeled antiviral antibody is added, which reacts with the complex giving a "sandwich effect". The tube is washed and the amount of radioactivity is determined.

While the aforementioned techniques of antigen detection are used as the first approach to viral diagnosis, in many instances these techniques are not applicable because appropriate specimens are often not obtainable from live animals. Also, rapid antigen detection systems are not available for numerous viral diseases. In these instances, virus isolation is attempted.

Microarray Analysis
Use: Identification of specific viruses or specific viral sequences.
The development of microarrays has been fueled by the application of robotic technology to routine molecular biology, rather than by any fundamental breakthrough. Southern and Northern blotting techniques for the detection of specific DNA and mRNA species provided the technological basis for microarray hybridization.
The construction of microarrays involves the spotting of specific DNA sequences on a glass slide or silica chip via robotics. The glass slide may contain up to 50,000 genes. The slides are then exposed to fluorescently labeled source DNA. A computer monitors fluorescence on the slide, indicating where the labeled DNA has bound to a DNA sequence on the slide. As many DNA sequences can be present on a slide, it is possible for microarray analysis to test for multiple pathogens simultaneously. This is particularly important for bio-weapons detection and disease diagnosis. Several microarrays are commercially available, such as the CapitalBio_ SARSarrayTM-1.8 Detection System for identifying early stage infection by the SARS virus (see Chapter 24).
In addition to nucleic acid microarrays, protein microarray analysis is also being performed. In this case, one is looking for the presence of a particular protein.

Collection and Submission of Specimens

The laboratory diagnosis of clinical illness depends to a large extent upon the kind and condition of submitted specimens. It also depends upon the practicing veterinarian and laboratory working in close concert. Because many of the laboratory tests are for specific disease agents, an adequate clinical history must accompany all submissions. This will permit laboratory staff to perform additional tests if indicated.
General guidelines for the collection and submission of specimens are presented below. Most laboratories supply a specimen submission form that should be completed with the available pertinent information. In the absence of a form, the veterinarian should supply as complete a history as possible. Veterinarians should contact the diagnostic laboratory if they have any questions.

Animals
Live, sick animals are preferable to dead animals. Whenever possible, animals should be submitted directly to the diagnostic laboratory for complete necropsy examination. If a herd problem exists, more than one animal should be submitted. Bus and courier service may be used to ship small animals, provided they are packaged in leak-proof insulated containers with sufficient ice or cold packs. Do not freeze animals submitted for necropsy.

Tissues
To minimize contamination during necropsy, it is best to collect a routine set of tissues prior to thorough examination. Recommended tissues are lung, kidney, liver, spleen, small intestine, large intestine, and mesenteric lymph nodes. Brain tissue or head should also be collected if central nervous system disease is suspected. Other tissues containing abnormalities noted during the thorough examination should also be collected. A portion of these tissues should be placed in leakproof plastic bags and placed under refrigeration. While it is recommended that each tissue be placed in a separate bag, it is absolutely essential that intestine be separated from other tissues; otherwise bacteriologic examinations will be compromised.
Tissues should be brought directly to the laboratory, or shipped under refrigeration by over-night mail, bus, or courier service. Tissues collected during the latter part of the week should be frozen and shipped on Monday.
Since many viruses produce characteristic microscopic lesions, small pieces (1/4 inch thick) of each tissue should be placed in ten percent buffered formalin for histopathologic examination. An entire longitudinal half of the brain should be submitted. These samples should not be frozen.

Feces
Feces should be collected from acutely ill animals and placed in leakproof containers. While well-saturated swabs are adequate for many individual virologic examinations, several milliliters or grams of feces permit a more complete diagnostic work-up including bacteriologic and parasitologic examinations. Samples should be submitted to the laboratory using cold packs as coolant.

Swabs
Nasal and ocular swabs are useful for isolating viruses from animals with upper respiratory-tract infections. Genital infections may also be diagnosed by examining swabs collected from the reproductive tract (vagina, penis mucosa, etc.). These swabs should be collected from acutely ill animals and placed directly into screw-capped tubes containing a viral transport medium. The sampling of several animals in different stages of the illness increases the likelihood of isolating the

causative agent. Swabs are also useful for the sampling of vesicular lesions. Fresh vesicles should be ruptured and the swab saturated with the exuding fluid. Two swabs should be collected, one for virus isolation and one for electron microscopy. The swab for virus isolation should be placed in viral transport medium and the swab for electron microscopy should be placed in a screw-capped tube containing one or two drops of distilled water. Scab material from the more advanced lesions should also be submitted.
There are several commercially available viral transport media that help maintain the viability of viruses during shipment to the laboratory. Most of these transport media are balanced salt solutions containing high protein content and antibiotics to prevent bacterial overgrowth. Many diagnostic laboratories provide their own version of transport medium to practicing veterinarians upon request.

Slides
A number of infectious diseases can be diagnosed by examining slides prepared from blood and tissues. Blood smears are used for diagnosing feline leukemia, while blood smears and conjunctival scrapings are used to diagnose canine distemper. Conjunctival scrapings are particularly useful for diagnosing herpesvirus and chlamydial infections in cats. Imprints made from liver, spleen, and lungs are especially useful for diagnosing chlamydia and herpesvirus infections of psittacine birds. Slides should have sufficient cells to allow thorough examination but should not be so thick as to cause difficulty in staining. A conjunctival scraper or some other device (blunt end of scalpel blade) should be used to scrape the conjunctiva; cotton swabs are not adequate. Matted eyes should be cleaned and flushed prior to scraping the conjunctiva. Tissue imprints should be made by lightly touching the microscope slide with fresh cuts of tissue previously blotted with a paper towel to absorb some of the blood.
Slides should be air-dried and sent to the laboratory in slide holders to prevent breakage. Several slides permit a more thorough diagnostic work-up, including cytologic examinations.

Serum
Blood samples should be collected in sterile tubes containing no anticoagulants. These should be submitted to the laboratory in specially designed Styrofoam holders to avoid breakage. Blood samples should not be frozen or allowed to overheat. If samples cannot be delivered to the laboratory within a reasonable time, serum should be removed and refrigerated or frozen.

Glossary
Aliquoted: Divided (as a solution) into equal parts.
Primers: These are short stretches of DNA or RNA used as starting points for nucleic acid synthesis. A primer hybridizes with a template strand of nucleic acid and provides a 3' hydroxyl end for the initiation of synthesis. The primers delimit the region that will be amplified. In PCR, two (sometimes more) synthetic oligonucleotide primers (about 20 nucleotides each) complementary to regions on opposite strands flank the target sequence; the 3' hydroxyl ends are oriented to each other. In PCR the target sequence in a sample is about 100 to 2000 bp in length. Designed primers and arbitrary ("off-the-shelf") primers are used.
Regulatory tests: These are tests conducted under the auspices of official disease control agencies in the interest of controlling important infectious animal diseases.
Restriction endonucleases: These are enzymes derived from bacteria that recognize and cleave specific DNA sequences.
Restriction fragment length polymorphism: These reveal many differences in DNA sequences among individuals of a species. RFLPs result from cleavage of DNA by restriction enzymes (see above), separation of the fragments by gel electrophoresis and visualization of bands (DNA fragments) by staining with fluorescent ethidium bromide.
Taq polymerase: This is the DNA polymerase used in PCR. It is derived from the bacterium Thermus aquaticus that lives in hot springs; the polymerase's thermostability makes PCR possible.