Diagnosis of diseases in livestock: Do's and not do's

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Introduction

Successful diagnosis of a disease in food-producing animals depends in many times on proper collection and handling of specimens, as well as careful clinical observation and evaluation of the diseased animals. During the period required to complete the appropriate laboratory tests, an awareness of the potential problems of disease spread to animals at risk and the available palliative treatments is essential. Because most microbial-induced diseases in herds or flocks cannot be effectively treated once the problem is established, management through prevention and control of the disease must be instituted to offer the maximum protection to animals at risk. Thus, the course of disease management for infectious diseases is oftentimes a difficult judgment decision. This paper presents a brief general guide to specimen collection and laboratory methods used for diagnosis of diseases in production animals.

PATHOLOGY

General Guidelines for Field Necropsy

When collecting tissues from necropsied animals, the history, clinical signs, and gross lesions should determine which tissues are collected. The following sampling recommendations and reminders may be helpful in optimizing the diagnostic information that can be obtained.

Gross lesions

ALWAYS submit representative samples of ANY gross lesions. For example, oral erosions/ ulcers are often described on submission forms but less often submitted, especially in suspect

BVD-mucosal disease cases. Fixed ulcers are an excellent sample for detecting BVD virus by immunohistochemistry (IHC).

Skin

Skin lesions should be submitted fixed and fresh. Nonlesional skin from dead bovines is useful for determining BVD virus persistent infection status. Ear (pinna) or coronary band are preferred sites (see discussion under 'Special Tests').

Tongue

Formalin-fixed tongue is useful for detecting generalized myopathies, especially in fetuses (Neospora caninum) and neonates. Muscular diaphragm is also useful for this purpose.

Brain

Sever the cranial cervical spinal cord as far caudally as possible when removing the head. Sagittally section brain and submit one-half in formalin and one-half chilled. Be sure to include cerebellum and brainstem in addition to cerebrum. Brain should always be submitted from animals found dead without gross lesions in the thoracic or abdominal viscera.

Thymus

Fixed and fresh thymus are useful for detecting BVD virus in cattle and PRRS virus in pigs.

Trachea

In general, formalin-fixed and fresh trachea is only useful if gross lesions are present. In cattle, hemorrhages are common but nonspecific in animals with respiratory disease. Pooled exudate from lung is easily wiped away to reveal a smooth, glistening (normal) mucosal surface. This feature distinguishes pooled exudate from an adherent fibrinonecrotic membrane, such as is typical of IBR virus.

Lung

Submit both formalin-fixed and fresh tissue samples from at least four different areas: cranial, hilar, middle, and caudal lung. More extensive sampling greatly increases the probability of

diagnosis. Fresh portions of lung should be as large as possible. The entire lung (less the parts collected for formalin fixation) should be submitted, if possible. If lung lavage for PRRS virus isolation is desired, submit one entire (unsampled) lung. Also, include both fixed and fresh enlarged lymph nodes.

Heart

Open all chambers and examine all valves. If lesions are identified, submit formaliin-fixed and fresh (if infectious agents are suspected) samples. If no lesions or evidence of heart failure are identified, submit a fixed portion *of papillary muscle from the left ventricle.*

Liver, kidney, spleen

Since these organs have many potential uses for the diagnosis of many different infectious agents and diseases, please submit formalin-fixed and fresh samples in all cases.

Intestines

When sampling the intestinal tract, always begin at the ileocecal junction and work toward the stomach. Examination of multiple areas is often required, since lesions and/or agents are usually not uniformly distributed throughout the intestinal tract. Place 3 widely spaced 1-2 cm segments of ileum, 3 segments of jejunum, and one segment of duodenum in formalin, making sure that the formalin contacts the mucosal surface. Cecal contents should be collected for virology, and a portion of cecum should be fixed. Samples of several areas of spiral colon should be placed in formalin so that both ascending and descending loops will be included. As noted above, also submit any gross lesions. If the animal is of small size, the entire remaining intestinal tract may be submitted chilled. Otherwise, representative loops of ileum, jejunum, and colon should be submitted. Also, include fixed and fresh enlarged lymph nodes.

Stomach

Submit any lesions in formalin. In ruminants, submit one piece of fixed abomasum even if no gross lesions are present.

Submission Guidelines

Submission of tissue specimen(s) is often the best method of obtaining the right diagnosis.

However, proper selection and preservation of samples is essential to make the most efficient and economical use of the laboratory. The two conditions that most frequently interfere with diagnosis are (1) post mortem autolysis, and (2) sample collection too late in the course of disease.

Fresh specimens should be large enough to demonstrate the lesion yet small enough to allow for rapid chilling. In some cases (e.g., bronchoalveolar lavage for PRRS virus isolation) submission of an entire organ may be preferred. Fresh samples should be packaged individually to prevent cross-contamination. Do NOT package fresh intestines with other tissues.

Specimens for histopathology should include slices of the appropriate organs, including the lesion, transitional zones, and adjacent grossly normal tissue, in 10% buffered formalin in a leak-proof, wide-mouthed solid container. DO NOT USE GLASS CONTAINERS. When in doubt, collect specimens from multiple organs, including brain. The pathologist can then select the most appropriate specimens for complete microscopic examination. Unless it is important that individual animals be examined and reported separately, specimens from each individual animal can be pooled in a single container - provided the specimens are small enough to maintain a 10:1 formalin:tissue ratio. The ratio of formalin:tissue must be at least 10:1 to allow adequate fixation.

A minimum of 48 hours is required for histopathology, 24 hours for fixation and 24 hours for processing. Consequently, specimens for histopathological examination should be collected and placed in formalin at the time of necropsy in order to minimize autolysis and generally allow results to be available the day after the specimen is received.

General guidelines for histopathologic examination/submission of formalin-fixed specimens are as follows:

1. Solid organs: 0.5 - 1 cm slices to include lesion and transitional areas between lesions and normal tissue.
2. Intestine: 1 - 2 cm lengths held open as they are immersed in formalin or flushed with formalin prior to immersion. Do NOT tie the ends.
3. Brain (including brain stem): immerse sagittal half of small brain in formalin. 'Bread-slice' larger brains for better fixation. Nerves can be pinned to a tongue depressor and immersed in formalin.
4. Tumors: immerse in 10% formalin. If greater than 1 cm, incise, leaving a base attachment

intact.

5. Impression smears and needle aspirates of tumors are helpful in a few situations. However, actual biopsies are usually required for definitive diagnosis. Stain and examine smears and aspirates in your practice laboratory and, if a diagnosis is not obvious (i.e., mastocytoma, abscess, etc.), biopsy the mass and forward both the biopsy and the smears to the reference laboratory.

Preparation of 10% neutral buffered formalin

Buffered formalin concentrates are available from several suppliers. These are ready for use after the addition of water. Non-buffered 10% formalin (1:9 of 37% formaldehyde:water) can be substituted for neutral buffered formalin in emergency situations. Do NOT submit samples in undiluted 37% formaldehyde.

Histochemistry and Immunohistochemistry

Various histochemical techniques are available to demonstrate agents/structures within histologic sections. These include the following:

Histochemical staining

Histochemistry demonstrates bacteria, fungi, cellular components, etc., by use of differential stains, e.g., a Gram stain for demonstrating bacteria or Giemsa stain for mast cell granules.

Immunohistochemistry (IHC)

IHC is used to demonstrate specific antigens within tissue sections. The location of agents (bacteria, viruses) can be visualized within the tissue in association with lesions. This technique is performed on formalin-fixed specimens. Results can be generally available 24 - 48 hours after receipt, providing the proper specimens are submitted in formalin.

Special Tests

Diagnosis of BVD virus persistent infection (PI)

Skin biopsy is a low cost method to detect cattle persistently infected with BVD virus. In PI cattle, BVD virus is present in epidermis and hair follicles throughout the skin. Ear (pinna) is a

convenient site and can be sampled with an ear notcher. Ear notch specimens (at least 1.0 x 0.5 cm) should be submitted in numbered (1, 2, 3, 4) snap-cap tubes (not in plastic bags) half-filled with 10% neutral buffered formalin and are processed for immunohistochemistry (IHC) to detect BVD viral antigen. If desired, positives can be retested in 30 days to confirm PI status, but they should be isolated until retesting, because all available evidence indicates that ear notch IHC positive calves are PI. This test offers several advantages:

1. Maternal antibody does not interfere with detection of BVD virus in skin.
2. Identifying PI's as early as possible allows them to be removed from the herd prior to the next breeding season.
3. Samples may be collected by producers as neonates are processed.
4. Samples do not require refrigeration and may be submitted once every 1-2 weeks during calving season.

Diagnosis of anaplasmosis using blood smears

Air-dried smears of blood collected in EDTA should be submitted. Smears should be made immediately after blood is collected. Parasites disassociate from RBCs over time and, for that reason, we prefer receiving smears rather than whole blood in EDTA. If serum is available, serology is probably more reliable than examination of blood smears for diagnosis of anaplasmosis.

BACTERIOLOGY

Submission Guidelines

General guidelines for submission are found in the Pathology section. If you are requesting bacteriology tests, remember to keep samples moist, cool, and send by overnight transport. The following are important points to keep in mind:

1. Tissues should be VERY fresh and collected aseptically.
2. Collect samples prior to antibiotic treatment.
3. Submit generous portions of tissue or several milliliters of pus, exudate, or feces.
4. Avoid swab submissions whenever possible.
5. Submit samples individually in separate bags or jars with correct and clear identification.

6. Maintain samples at refrigeration temperature (4 degrees C) and send with ice packs. Freezing is generally not recommended.

Anaerobic Cultures

1. Care in collection is essential. Do not contaminate the samples with surfaces which have resident anaerobic bacteria. Exposure to air for more than 20 minutes can be detrimental.
2. Samples from animals that have been dead longer than 4 hours are usually unsuitable.
3. Tissues and liquid exudates are recommended (ship in anaerobic pouches or tubes).
4. Swabs are not acceptable unless shipped in proper containers. Special collection devices and transport tubes with reduced oxygen environment are available.

Milk

1. Collect milk in sterile snap cap or screw cap tubes. Do not ask owner to collect milk samples without first providing training in proper sampling technique.
2. Cool samples before submitting to the laboratory, and mail with ice packs. Samples may also be frozen without altering recoverability of pathogens.

Blood

1. Not normally used for recovery of animal pathogens because bacteremia is intermittent. Call the laboratory for recommendations.

Urine

1. Collect by cystocentesis (best), catheter, or mid-stream catch. Submit a 3 ml sample.

Skin Lesions

1. To collect from pustules or vesicles, disinfect surface with alcohol, allow to dry, and aspirate material with syringe and needle.
2. Pluck hair from lesion and scrape edge of lesion when ringworm in suspected. Submit hair, skin scrapings, scab material, and toe nails.

SEROLOGY

Submission guidelines

The following guidelines are provided in order to expedite testing and shorten turn-around time.

1. Provide 2 ml of serum per assay requested. Submit serum only. Never submit blood. Even if you use serum separation tubes, serum must be poured off into plastic snap cap tubes. To avoid leakage, be sure to 'double snap' the caps on tubes.
2. Number the tubes in consecutive order 1 through 'X' matching the tube number with the tube number on a submission form. Use permanent marker to identify tubes. Grease pencil marks are easily rubbed off and labels fall off during the heating process necessary for some assays.
3. Place the tubes in consecutive numerical order in cardboard boxes designed to hold snap-cap tubes. Do NOT submit in bags.
4. Keep samples from each case or client separate. Save a portion of each serum sample in your freezer.
5. Keep a copy of the paperwork for your files.
6. Optimize the interpretation of test results by collecting acute and convalescent samples. Paired sera (acute and convalescent) should be submitted together. Alternatively, comparing serum antibody levels in affected vs unaffected animals can assist in interpretation.

Abortion Serology - General Comments

Fetal serology has not proven to be of much value in abortion diagnosis. In sheep, the diagnosis of toxoplasma abortion can be accomplished by detection of antibody in fetal fluids. Fetuses are rarely capable of producing antibody until the last trimester.

Serological analysis of maternal serum can aid in the diagnosis of abortion under limited circumstances with some diseases. The lack of antibody can be considered evidence ruling out a given disease. It is more difficult to establish guidelines that point toward a diagnosis.

Additional points to consider would include the following:

1. Paired serum samples (at the time of abortion and 2-3 weeks later) may provide more information (i.e., a 4 fold increase in titer) than a single sample. However, at the time of

abortion, the dam may have already been infected long enough to reach a peak titer.

2. As an alternative to paired samples, comparing mean antibody titers and percent seropositivity between affected and unaffected groups of animals may be helpful. Samples should include serum from approximately 20 aborting animals and at least an equal number of matched (as closely as possible by age and stage of gestation) unaffected animals.
3. With some diseases, many herds are endemically infected and a majority of the animals will have titers. Examples: parvovirus in swine, toxoplasma in sheep, BVD in cattle. Presence of antibody against these organisms in aborting animals is not sufficient evidence of cause. However, high titers may indicate recent infection.
4. Response to vaccination also can complicate interpretation of titers.

VIROLOGY AND MOLECULAR DIAGNOSTICS

Submission Guidelines

Successful isolation and/or detection of viruses in clinical materials depends largely on proper collection and handling of specimens. Care should be taken to protect the virus in specimens from environmental damage and maintain virus infectivity by using the proper transport system.

In general, specimens intended for virological testing should be collected as early as possible in the course of the disease, i.e., within the first 7 days after the onset of illness. Samples collected during the acute phase of viral infection usually contain adequate amounts of virus for detection in available assays. Samples collected later in the course of infection usually require more laboratory time and often yield poor or negative results. Since certain viral infections may predispose the host to secondary viral or bacterial infections, samples collected late in the disease process may lead to a misdiagnosis when secondary infection is involved.

The appropriate samples for virus detection include bodily fluids and secretions (e.g., nasal or conjunctival secretion, genital swabs, urine, saliva, vesicle fluid, semen, milk), feces, blood samples, and skin biopsies from infected, live animals (i.e., antemortem), and relevant tissues and organs from necropsied animals (i.e., postmortem). The collection of antemortem samples from sick animals should be based on clinical manifestation. For example, nasal or nasopharyngeal swabs should be collected from animals with respiratory diseases; fecal samples from animals with enteric diseases; cerebrospinal fluid (CSF), nasal secretion and feces from animals with CNS signs; vesicle fluid and biopsies from animals with skin lesions. The same principle can be applied to the

collection of postmortem samples. As a general practice, whole blood and serum should always be collected from animals with suspected viral diseases, regardless of clinical manifestations. For collecting whole blood, citrate is preferable to EDTA as an anticoagulant because EDTA may inactivate some viruses due to its chelating activity. Secondary lymphoid tissues (e.g., tonsil, lymph nodes, spleen) are always good specimens for viral diagnosis.

For best results in isolation and detection of viruses, clinical specimens should be aseptically collected, kept fresh, and transported immediately to the laboratory. If delays are unavoidable or any detrimental affects on virus in samples are anticipated during transport, samples should be refrigerated at 4 degrees C for no more than 2 days. For longer storage periods, freeze samples at -70 degrees C, but NEVER at 20 degrees C. Self-defrosting freezers in conventional refrigerators are not appropriate for storage. NEVER freeze whole blood samples. Ideally, frozen samples should be submitted on dry ice, but commercial refrigerant packs can be used if necessary.

Unbleached swabs (e.g., Dacron swabs are available from Baxter) are strongly recommended for collecting nasal and fecal swabs. Standard cotton swabs contain residual bleach that can inactivate viruses. Swabs MUST be prevented from drying. For that reason, swabs may need to be placed in a viral transport system. Ideally, swabs should be placed in a broth medium or balanced salt solution supplemented with 0.5% gelatin, serum, or bovine serum albumin, to protect the viability of viruses in transit plus antibiotics to prevent bacterial and fungal growth. Minimally, physiological saline or Lingo solution could be used on an emergency basis. Specimen collection and transport systems (e.g., Viral Culturette, Becton Dickinson) are available.

It is important to choose not only the most appropriate specimen, but also to collect an adequate amount of specimen for virological testing. Submit a minimum of 3-4 ml serum and 3-5 grams or 5 ml wet volumne of fecal material. Insufficient amounts of sample are a potential cause of inconclusive diagnosis or false-negative result.

When it is necessary to ship a specimen, use a leak-proof container (e.g., tubes, plastic bags) enclosed in a second watertight container containing absorbent material. Ideally, the specimen container should be placed in a Styrofoam box with commercial refrigerant packs or dry ice. Avoid using wet ice because it will melt and leak from the package. Dry ice is preferable if transport requires more than 3 days.

Conventional Virological Assays

Virus isolation (VI)

Virus isolation consists of two steps - the attempted recovery of virus and identification of the

isolate. Isolation is attempted using *in vitro* cell culture. Identification of the isolate is done using immunofluorescence microscopy, electron microscopy, or molecular techniques. Although isolation of virus followed by identification is considered to be the definitive diagnosis, VI is often laborious, expensive, and time consuming. Results are not generally available for 1-2 weeks after submission. Therefore, VI should primarily be attempted under specific circumstances:

1. When other detection methods fail or when trying to isolate virus(es) from previously unrecognized diseases.
2. If there is no other detection method of similar or greater sensitivity.
3. If the virus is required for other purposes, such as differentiation (e.g., RFLP, sequencing), characterization (e.g., typing), and/or for production of autogenous vaccines.

Aseptic collection and proper handling of clinical specimens is critical for VI. Although certain viruses (e.g., parvovirus, circovirus) can withstand harsh environmental changes, this is not true for most viruses, particularly enveloped viruses. Virus isolation can be done on most clinical specimens, including biopsy and necropsy tissues, blood, secretions, and excretions. However, some clinical materials, e.g., urine, feces and semen, are difficult to work with because they are toxic to cell cultures.

The quantity of sample required for VI is usually more than that needed for rapid diagnostic assays so collect and submit the adequate amount of sample. To avoid unnecessary expense, be specific in your VI requests. Refer to specimen selection guidelines in the User's Guide or call the laboratory for advice.

Electron microscopy (EM)

Electron microscopy is useful for the direct visualization of viruses. For arriving at a definitive diagnosis, EM requires that the target virus have morphological characteristics sufficiently distinct so as to make identification of the virus possible.

EM is particularly effective for clinical specimens from which virus isolation cannot be attempted because of cytotoxicity problems, i.e., urine, feces, and intestinal contents, or for the identification of viruses that cannot be propagated in vitro. For certain viruses, EM has been replaced by newer diagnostic methods such as antigen-capture ELISAs.

EM is a nonspecific test with rather low sensitivity (i.e., requires plenty of virus). The nonspecificity of the test may be useful under certain circumstances as, for example, in investigating unusual enteric diseases. EM can be also done on tissues (thin section) to detect the

presence of certain viruses, such as poxvirus. However EM on tissue thin sections is laborious and time consuming (10 - 14 days).

Fluorescent antibody examination of frozen tissue section (FATS)

FATS is a rapid, specific laboratory diagnostic test for detecting viral antigen in tissues and cell smears. The test utilizes virus-specific antibody labeled with a fluorochrome. When viewed with a fluorescence microscope, complexes of viral antigen and labeled antibody appear as fluorescent green areas. For best results, FATS requires very fresh clinical specimens. Freezing and thawing tissues can be detrimental to the test. If autolysis of tissues is anticipated, other antigen detection methods, such as immunohistochemistry (IHC) on formalin-fixed tissues, should be considered.

It is important to recognize that VI and EM are nonspecific tests that can detect a variety of viruses. In contrast, FATS and IHC are very specific tests that, because they are use virus-specific reagents (e.g., antibody), only detect the targeted virus. Furthermore, FATS and IHC cannot be performed on certain specimens, such as serum or feces.

Antigen-capturing ELISA (AgELISA)

AgELISA is a rapid, sensitive laboratory diagnostic test for detecting virus or viral antigen(s) in a variety of clinical materials, e.g., tissues, serum, secretions, and excretions. The assay is a variant of the ELISA format in which virus-specific antibody is used to coat the surface of the plates instead of antigen. Therefore, virus or antigen in clinical specimen is captured by antibody. The use of virus-specific monoclonal antibody in AgELISAs provides high specificity. The presence of antibody-antigen complexes is subsequently visualized by a colorimetric reaction. In general, color development is proportional to the amount of viral antigen present in the specimen. One advantage of the AgELISA format is that it detects both infectious and inactivated viruses; however, expense is a consideration.

Molecular Virological Assays

In general, samples appropriate for conventional microbiological evaluation (i.e., detection of viruses) are also suitable for molecular diagnostic tests. This includes excretions and secretions, feces, blood, serum, and biopsies from infected, live animals (i.e., ante mortem), and relevant tissues/organs and washings (e.g., bronchial lavage) from necropsied animals (i.e., post mortem).

For best results, clinical specimens should be collected aseptically from individual animals. Care should be taken to avoid cross contamination. Samples from different animals can be pooled for

molecular assays if cost is a concern, but it is recommended that clients consult with diagnostician prior to pooling samples. Alternatively, samples can be pooled in the lab.

Several molecular diagnostic methods are available for detection or differentiation of viruses, including polymerase chain reaction (PCR), in situ hybridization (ISH), fingerprinting, and sequencing. Some, but not all, molecular techniques first require isolation of the target agent.

Polymerase chain reaction (PCR)

PCR is a process by which a portion of viral nucleic acid (DNA or RNA) is replicated a million times or more. Detection of this amplified product (amplicon) indicates that the sample is positive for the target virus.

Proper amplification relies on a set of two short synthetic oligonucleotides (primers). Good test performance requires that primers bind only to the corresponding nucleotide sequences of the viral genome and nothing else. Thus, the specificity of the assay and accuracy of the results depend upon the design of PCR primers.

Theoretically, a PCR-based assay is capable of detecting one copy of the viral genome. However, depending upon the target agent, type of sample, and condition of sample, diagnostic sensitivity often is not as sensitive as anticipated and, depending on the circumstances, conventional assays may provide equivalent test performance. Also, PCR is expensive relative to most other diagnostic tests and positive results do not always have biological significance, i.e., PCR reacts with inactivated viruses, as well as infectious viruses.

There are several types of PCR assays.

RT-PCR is used to detect target RNA from clinical specimens. Reverse transcription (RT) of RNA is required to make complementary DNA for further amplification. This assay is most frequently used for specific detection of RNA viruses.

Nested PCR is a PCR done in two steps, a primary PCR reaction and a nested reaction. The primary (or first) reaction uses a set of primers to generate a product that serves as the template for the nested (or second) reaction. The nested reaction uses a set of PCR primers specific for a region within the amplified product from the first reaction. Therefore, the nested reaction often serves as a confirmation for the specificity of the PCR products amplified in the primary reaction.

Multiplex PCR is a PCR designed to detect more than one target sequence in a single PCR reaction. The assay uses two or more sets of primers. Each set of primers is specific for a

different target sequence. The assay is most commonly used for simultaneous detection of multiple viral genes and differentiation of genotypes or subtypes of related microorganisms.

Fluorogenic ("Real-time") PCR combines PCR amplification and detection into a single step. The basic principle of real-time quantitative PCR is the detection of target sequences using a fluorogenic 5' nuclease assay (often called 'TaqMan'). The advantages of this system include high reproducibility, the capability of handling large numbers of samples, the potential for quantitative results, and decreased turnaround time. The disadvantages include high instrument cost and the requirement for technical proficiency.

Hybridization

Hybridization procedures rely on base-pairing between a labeled DNA or RNA oligonucleotide (probe) and the complementary nucleotide sequences of the target genomic DNA or RNA. Since there is no amplication step, hybridization-based assays are generally less sensitive than PCR-based assays. Although most constituents for hybridization are commercially available, specific probes for each microorganism have to be generated by the lab performing the test.

***In situ* hybridization** is used for the detection of specific microorganisms in tissues. Depending on the protocol, ISH utilizes a labeled DNA, cDNA, or RNA probe that corresponds to a specific portion of pathogen's genome. Various methods are used to detect the binding of the probe to the agent. In the VDL, the presence of the target microorganism is detected by a colorimetric reaction that can be seen under a light microscope. The advantange of this method is that the location of the pathogen can be assessed in the context of the histological lesions.

Molecular Assays for Differentiation and Genetic Characterization

Differential PCR

Differential PCR can sometimes be used to distinguish closely related targets. Differential PCR is done either in a multiplex format using two or more sets of primers or by running two separate PCR assays. Refer to the Table of Molecular Assays for a list of differential PCRs.

Restriction fragment length polymorphism (RFLP)

RFLP analysis is a molecular differential technique that, to a limited extent, can distinguish

between two viruses at the genomic level. RFLP is based on the fact that restriction enzymes recognize specific nucleotide sequences and cut the genome at that location. In general, the RFLP procedure consists of isolating the target microorganism from a clinical specimen, extracting DNA or RNA, digesting the nucleic acid material with restriction enzymes, and gel electrophoresis of the resulting products. The pattern of fragments observed on the gel is used to characterize or compare isolates. In general, RFLP requires virus isolates; however, RFLP using PCR products instead of native nucleic acid has recently been developed. This method provides faster turnaround since it is not necessary to isolate and propagate virus.

RFLP is useful for differentiating minor differences at the strain level that normally cannot be detected by any antigenic assays, such as tissue immunoassays, serology, and enzyme immunoassays. RFLP analysis is rapid and less expensive than sequence analysis, but RFLP will miss many of the genetic differences that are revealed by sequencing.

Sequence analysis

Sequence analysis is a molecular tool for use in characterizing the genetic information of microorganism in detail. Sequencing provides a list of the nucleotides in the viral genome in the order in which they appear. Comparison of the genetic information makes complete differentiation between viruses possible. Either partial or whole genomic sequencing can be done, depending upon the size of genome and purpose of the analysis. In addition, RFLP patterns and amino acid composition can be predicted from sequence data. One disadvantage of sequencing is the expense of conducting the analysis.

Submissions for Rabies Examination

The entire animal, chilled (not frozen), should be delivered by private carrier. Alternatively, submit the intact head, properly sealed to prevent leakage, and identified as a rabies suspect. According to the Centers for Disease Control and Prevention (CDC) guidelines, the cerebrum and cerebellum are REQUIRED for the appropriate diagnostic evaluation of rabies virus infection.

Guidelines for Packaging Specimens

The goals of packaging are to protect the specimens from temperature extremes (freezing and heating) and to protect persons who may come into contact with the package from exposure to

infectious agents. For this reason, it is extremely important to prevent leakage of specimens. Leak-proof specimen containers, abundant icepacks with chilled fresh specimens, and an insulated leak-proof transport container lined with a plastic bag are required.

1. Label all samples with the owner's name in waterproof marker. Please do not use stick-on labels as they often come off. Be sure the contents of the box, as well as the outside of the box, are identified.
2. If multiple cases are submitted in one box, package each case separately to ensure that all samples are assigned to the proper case. This is especially important if the package includes multiple serology submissions.
3. Place tubes in serum shipping boxes (i.e., brucellosis boxes) or similar boxes with dividers to separate tubes from one another. Do NOT package loose blood tubes in crumpled paper or styrofoam bits.
4. Enclose sufficient ice packs to preserve the quality of fresh tissues. This should be at least a 2:1 ratio of ice to tissues. If available, insulated styrofoam-lined containers should be used.
5. Plastic leak-proof jars can be used for formalin-fixed tissues. Whirl-Pak† bags are excellent for holding fresh tissues. Squeeze the air out of the bag, then fold the end over several times before bending over the tabs. Double-bagging tissues improves the biosecurity of the specimen.
6. If inside packages have the potential to leak, line the box with sealed plastic bags to prevent leakage. Pack with absorbent materials to soak up spills should they occur. All specimens should be packed in a manner to avoid leakage or breakage, and to withstand the trauma of mailing.
7. All specimens should be properly labeled and accompanied by a proper submission form. Please provide all information requested on the form: animal identification, age or body weight, gender, date of onset of disease, major clinical signs, days of gestation if samples originate from abortions, herd size, number of animals affected or dead, date of collection, animal source and location, vaccination history, and differential diagnosis. This latter information is essential for the selection of the most sensitive test system in the laboratory when samples are submitted by mail.

Conclusion

For the diagnosis of an infectious disease in a mammal or bird host, a systematic approach should be developed from the initial observation and examination of the diseased host to the collection of specimens and submission of materials. Further, acknowledge of the temporal constraints in receiving a laboratory confirmation of a causative agent and the interpretation and validity of laboratory results is essential for the management of the disease.