The Veterinary Diagnostic Laboratory: Effective Use of Available Services

Louis E. Newman, *DVM, PhD*
Veterinary Diagnostic & Investigational Laboratory
University of Georgia
Tifton, Georgia

How can **you** help the diagnostic laboratory help **you**? Three primary themes are emphasized throughout this discussion: (1) Appropriate Samples, (2) Quality Samples and (3) Signalment and History. These three themes are applicable in all sample regimens and discussions that follow.

A. General

Inappropriate Samples

The most difficult problem faced by diagnostic laboratories and the greatest impediment to providing the help the practitioner wants and needs is **submission of inappropriate samples!** Example: Prior to death a cow exhibits CNS signs and the brain is submitted to the diagnostic laboratory. What if there are no lesions in the brain or the brain lesions require examination of additional organs? It is not difficult to include sections from all major organs and this can enhance the pathologist's ability to provide the diagnostic assistance desired. **A veterinarian should always ask the questions, "Are the samples submitted appropriate for the diagnostic workup requested? Are specimens included that allow examination for all differential diagnoses?"**

Quality of Samples

There are important things that can be done routinely to enhance the quality of samples sent to the laboratory:

1. Use 10% **buffered** formalin. Unbuffered formalin, alcohol and water are **not** satisfactory. These solutions all result in poorly fixed tissues and/or artifacts.

2. Cut all tissues **except brain** approximately 1 cm thick. Thicker tissues are less likely to fix satisfactorily.
   a. Brain tissue is the one exception. Cut the brain longitudinally down the middle and put one-half in buffered formalin. Do not make any other incisions into the brain. (Submit the other half of the brain as fresh tissue.)
   b. Include the natural margins of excised tissues or biopsies: skin, pleura, capsule, serosa, lesion border. This area frequently provides the pathologist with valuable information.

3. Use sharp knives, sharp scissors and handle the tissues carefully; crushing creates artifacts.

4. Immediately place tissues into a volume of buffered formalin that is ten times the volume of tissues to be fixed. Autolysis begins immediately; **do not delay immersing tissues in formalin.** After tissues have fixed several hours or overnight, pour off most of the formalin to reduce weight. Leave just enough formalin to keep tissues moist.

5. Collect a second set of tissues for microbiology, virology and/or toxicology. Fresh tissues should be chilled immediately. They should not be frozen except under special conditions. Fresh tissues should be significantly larger than fixed tissues: 3 to 6 cm thick. One-half the brain can be left intact with no incisions through it. Be certain all specimens from the digestive tract are in separate containers.

6. Shipping. When shipping specimens be sure the shipper will deliver the next day. Keep the specimens cool; include ice packs in the package. Always take steps to prevent leakage. Whirl-Pak bags seem to work best for most practitioners, but heat sealed bags and plastic jars with tight sealing lids are also acceptable for fresh tissues as well as formalin-fixed tissues. Zip-Lock bags are not satisfactory to contain fluids; they pop open under pressure. Zip-Lock bags can be used to group together several Whirl-Pak bags. Written forms and histories encased in a separate Zip-Lock bag will ensure that they arrive dry and legible.

Shippers often set leaky packages aside to deal with later. Diagnostic laboratories often get calls in the evening regarding leaky packages that arrived 12 to 16 hours earlier. The shipper (veterinarian) is considered liable for damages resulting from accidents or infections caused by leakage. In one situation a leaking package resulted in seven UPS employees and five diagnostic laboratory personnel requiring rabies post-exposure prophylaxis at a cost of $900 per person. Remember, any container may leak; double package samples. Wrap containers in newspaper or other absorbent material so bloody fluid won't leak to the outside. Encase the specimens and absorbent material in a large plastic bag inside the box or styrofoam cooler that is to be shipped. If you must use Zip-Lock bags for specimens, be sure to double them. Use one Zip-Lock bag inside a second to reduce the likelihood of popping open.

Signalment and History

The history is extremely important. The diagnostician is blind as far as the case is concerned. He can only "see" what the history reveals. Include breed, sex, age, any clinical signs observed and whether there are any other animals affected. Include the vaccination history. Age can play a significant role in determining the diseases or conditions considered in the differential diagnosis. The differential diagnoses will be different for animals that are 2 weeks, 6 weeks, 12 weeks or a year of age. Up to one-third of the cases arrive at the laboratory without indicating the age of the animal. Provide the diagnostician with information concerning what you think is the problem and what you specifically want done. A good history results in the diagnostician becoming much more involved. It will be worthwhile in difficult cases to telephone the pathologist on duty to discuss the problem. Most laboratories are staffed by several veterinarians; their collective thinking may enhance the diagnostic process.

B. Specimen Types

Swabs

If a swab/culturette is the appropriate sample to send and multiple testing in bacteriology, virology and electron microscopy is requested, then several swabs should be submitted. Swabs do need to be refrigerated and kept on ice packs during shipping. Some organisms, such as *Moraxella bovis*, are fastidious and do not ship well. In these cases the practitioner may have to streak plates "cowside" and send the plates to the laboratory. It is extremely difficult to process a swab without knowing the location from which it came. For example, eye swabs are processed differently from rectal swabs. Some laboratories will not process swabs unless the origin of the specimen is known.

Blood

Blood samples for clinical pathology and serology can be handled in ways that will lead to better laboratory results. One or two days in the mail can dramatically alter test results. A CBC should be performed as soon after collection as possible. As blood ages, especially at warmer temperatures, cell morphology changes, hemolysis occurs and intracellular constituents leak into the serum. Even when the sample does not appear hemolyzed, covert hemolysis and increased cell permeability occur; the warmer the temperature the more severe the cellular leakage. Serum constituents such as enzymes and hormones degrade as a result of prolonged handling and warm temperatures.

Hemolysis also affects some serology tests. Excessive hemolysis is very deleterious and may result in the inability to run some tests. The following steps will prevent or minimize artifacts and unreliable results from improper handling and prolonged shipping:

1. Make blood smears immediately. Air dry them. Keep them dry and at room temperature to prevent condensation and formation of water droplets on slides.

2. Refrigerate EDTA (purple top) tubes. Keep them refrigerated during shipment.

3. Allow red top tubes for clinical chemistry and antibody testing to clot at room temperature for approximately one hour, centrifuge and using sterile technique transfer the serum from the clot into sterile tubes. The biochemical values will remain stable for several days if the serum is removed from the clot and kept refrigerated during transit. In addition, if serum is kept sterile during separation from the clot it can be used for antibody testing over a much longer period of time and virus isolation for BVD can be attempted. It may be necessary to freeze sera for vitamin analyses to prevent deterioration.

Fluids

Fluids collected for cytology or bacterial culture are easily contaminated or overgrown by normal flora. Fluids should be placed in sterile containers and packed with ice packs immediately. In addition, unstained smears of fluid should be made at the time the sample is collected. Evaluation of cell morphology is extremely difficult if smears are not made until samples arrive at the laboratory. Smears should not be refrigerated and should be wrapped separately from materials that need to be kept cold during transit.

Ocular fluids are useful for nitrate, nitrogen, potassium, magnesium and sodium analyses. The intact

eye may be submitted to the laboratory, however, it is preferable to submit either aqueous or vitreous humor collected with a sterile needle and syringe and transferred to a sterile tube. The fluid or eye must remain refrigerated during transit.

C. Special Problems

Calf Scours

A diagnosis of calf scours often results in the desire to screen for numerous agents including rotavirus, coronavirus, infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), adenovirus, *Escherichia coli*, salmonella, clostridia, cryptosporidia and coccidia. In early cases when there are no dead calves, bacterial cultures and electron microscopy can be attempted on fecal samples. Fresh fecal samples can be collected in sterile tubes and kept refrigerated. When there are dead calves a complete set of samples can be submitted for fluorescent antibody testing on tissue sections, electron microscopy on feces and gut contents, aerobic and anaerobic bacterial cultures with antibiotic sensitivity testing of significant isolates and parasitologic and histopathologic examinations. Specimens required to perform these examinations include:

1. Multiple sections of small intestine, colon and all major organs in 10% buffered formalin for histopathology.

2. Multiple sections of fresh small intestine and colon in separate Whirl-Pak bags for microbiology.

3. A section of distal small intestine tied off for anaerobic culture.

4. Feces and content from distal small intestine for electron microscopy and parasitology.

5. Frozen intestinal contents if enterotoxin testing is requested.

Good samples from two or three properly chosen calves should result in identification of most agents the first time around and allow development of better strategies for control early in the course of the disease.

BVD

Bovine virus diarrhea infection continues to be a significant problem. Virus isolation from blood or serum is an effective tool to determine persistent BVD virus infection. The best sample is 20 ml of blood in heparin, but blood in EDTA and serum are also satisfactory. The samples must be refrigerated throughout transit and shipment. Virus isolation is effective because persistently infected cows are viremic. Acute and persistent infections can be differentiated by sending a second sample 7 to 10 days later. Isolation of BVD virus from paired samples usually indicates persistent infection. Knowing the genotype of the BVD virus or whether the strain is cytopathic makes little difference in how a herd is handled or vaccinated.

Concerning acute or peracute BVD, it usually does not matter clinically whether Type I or Type II BVD virus is present. Both types have pathogenic strains and both have cytopathic and noncytopathic biotypes. Most laboratories cannot type the virus and at present biotyping would not provide additional benefit to the practitioner. In most outbreaks of acute or peracute BVD virus, improperly maintained vaccination schedules have been the root problem. Available vaccines given properly protect against **acute disease** but not necessarily against infection, reproductive problems or abortion. Unclotted blood for virus isolation is satisfactory for diagnosing persistent infection and diagnosing BVD in live animals with clinical signs of acute infection; however, it is not an appropriate specimen from animals that are not viremic and viremia does not persist very long during acute infection. When dealing with dead animals, clinical disease, abortions or repeat breeding problems selection of appropriate samples becomes extremely important. Paired serum samples may be essential. A significant rise in antibody titer may be the most valuable diagnostic aid in cases of abortion or repeat breeding.

Abortion

Abortion cases are a diagnostic challenge at best. Always include fresh and formalin fixed placenta and cotyledons with the fetus. In a field necropsy of a fetus collect sections of all major organs. In addition, sections of brain, skeletal muscle and heart are necessary for detection of neospora. Fetal stomach content, fetal thoracic fluid, fetal ocular fluid and dam's serum are also helpful. Best results are obtained by submission of the entire fresh fetus, placenta and serum from the dam.

Milk Samples and Mastitis

It is important to have a strategy in mind prior to sending milk samples to a laboratory. The costs of disruption of the milking process, labor, shipping and laboratory procedures are too great to sample without specific objectives in mind. Diagnostic laboratory support can be a significant asset in developing strategies for improving udder health and milk quality. However, the following questions may need to be answered first. How are the results going to be used? Can the dairyman actually do the things the veterinarian believes are required? Does the dairyman have the facilities to segregate? Does the dairyman have the facilities to set up

separate milking strings?

The type of results desired should be determined in advance. This means asking questions such as: Are major pathogens such as *Streptococcus agalactiae*, *Staphylococcus aureus* and *Mycoplasma spp.* the primary concern? Are environmental organisms of interest? Is treatment of clinical cases an important consideration? Are there concerns about sensitivity patterns of bacteria isolated? Is the problem an individual cow or herd problem? How important is turnaround time? Do *Str. agalactiae* results need to be available in 24 hours? What are the somatic cell counts? Have other samples been tested previously? Have bulk tank samples been submitted to a laboratory? Is anything being done to look at the milking equipment, milking routine and other management practices? It may be advantageous to talk to laboratory personnel or a knowledgeable person associated with the laboratory or university about a specific problem.

The milk sampling procedure is important. Has the person taking the samples been trained to collect aseptically? One to two milliliters of milk per sample is adequate. Collection of this amount lessens the time the vial remains open under the cow. Full tubes or tubes that overflow are more likely to contain contaminants. If three or more organisms are cultured from a milk sample, the results likely are not meaningful. Screw cap or snap cap tubes are preferable. Whirl-Pak or plastic bags should not be used; they leak and easily become contaminated. Alert the laboratory when large numbers of samples will be submitted so adequate media and required personnel will be available.

Freezing and batching samples collected over a two week or longer period may be effective in determining pathogen patterns from clinical mastitis cases. This results in the ability to deliver samples from several cases of clinical mastitis to the laboratory at one time. The only drawback to frozen samples is that somatic cell counts cannot be performed.

Find out who in the laboratory has an interest in mastitis, then talk to that individual about the laboratory's capabilities and your expectations. If specific milk quality testing is needed, find out whether the laboratory provides such tests as coliform counts, standard plate counts and pre- and post-pasteurization counts.

Toxicology

Toxicologic testing requires fewer tissues than other procedures; however, adequate quantities are essential. Stomach contents, liver and kidney are generally required. A whole kidney from a calf or half a kidney from an adult is not too much tissue. Submit 500 grams of liver, kidney and stomach contents either chilled or frozen. There are additional requirements for specific toxins. **If in doubt call the laboratory.**

D. New Developments

Immunohistochemistry has moved from the research laboratory to a fairly common diagnostic tool. The big plus concerning immunohistochemistry is that it can be done on the same formalin-fixed tissues used for histopathology. Once the decision to utilize immunohistochemistry is made, results can be available almost as fast as histopathology results. Most immunohistochemistry procedures utilize an indirect immunoperoxidase staining procedure and specific monoclonal antibodies. Different laboratories have different capabilities and are pursuing tests for different agents. One or more laboratories now have the ability to test for IBR, BVD, coronavirus, rabies, *Neospora spp.*, *Hemophilus somnus*, *Clostridium chauvoei*, *Campylobacter jejuni*, *Mycoplasma bovis* and leptospirosis.

Polymerase Chain Reaction (PCR) and DNA probes are being utilized in diagnostic laboratories. These tests are expensive in terms of equipment, space, time and technical training necessary for personnel. Therefore, most of the DNA PCR techniques are still in the research laboratory. One weakness of this technique is its extreme sensitivity. PCR amplification makes multiple copies of a DNA sequence. Contamination by minute amounts of amplified DNA fragments can serve as PCR templates and lead to false positives and confusing results. Johne's Disease is the only disease for which a test is commercially available and present in a significant number of laboratories. Other bovine viruses that can be amplified with the Polymerase Chain Reaction include Foot and Mouth Disease, Bluetongue, BVD, Bovine Coronavirus and Bovine Leukemia. Many laboratories are involved in *Escherichia coli* diagnostic research. DNA PCR techniques are being used to detect verotoxin Type I and Type II, enterotoxins (both heat stable and heat labile), fimbrial genes and genotypes. Heat labile and heat stable enterotoxin probes recently have become available commercially. Researchers are also developing probes for *Salmonella*, *Leptospira* and *Clostridium perfringens*. PCR technology has great potential to assist in the diagnosis of problematic bacteriology cases. Just as fluorescent antibody testing and electron microscopy are much faster than virus isolation, PCR technology will be much faster and better for some bacterial pathogens.

Other techniques used in diagnostic biotechnology include Northern and Southern Blots. Southern Blots are used to detect the presence of DNA after PCR amplification. Both technologies involve electrophoresis of nucleic acids. The Southern Blots electrophorese DNA; Northern Blots electrophorese RNA. To complete

the blot, the nucleic acid is transferred to nitrocellulose or nylon filters where an appropriate probe is applied to the blot. One example of a commercially available test that utilizes a Southern Blot is the Johne's Disease probe.

Western Blot is another procedure that may be applied for the detection of bacterial or viral proteins. After electrophoresing the proteins, antibodies are used to analyze the interaction or response to the protein bands. Western Blot is used as a confirmatory test when there are discrepant results between two other tests, for example when the ELISA and AGID tests for Equine Infectious Anemia disagree. Western Blots are used in research routinely and are used diagnostically at a few laboratories.

E. Laboratory Issues

Turnaround Time

There are three major factors that affect turnaround time:

1. How long does it take samples to get to the laboratory? (Several carriers will deliver the next morning.)

2. How long does it take to run the tests and write the reports. (In one laboratory, 25 percent of the cases are reported in 24 hours and 75 percent within three days; the final reports on 90 percent of necropsy cases are in the mail within four working days.)

3. How long does it take the report to get to the practitioner? (Fax machines are cheap, reporting is immediate, nothing gets lost in the mail and costs are less than mailing). **Every practitioner who uses a diagnostic laboratory should have a fax machine.** Some laboratories also allow access to cases by computer.

Cost

The total cost of diagnostic services includes packaging, shipping and laboratory fees. Fees vary from laboratory to laboratory and can have a dramatic effect upon the selection of appropriate samples. Total laboratory costs will influence sample selection; however, appropriate samples in adequate quantities must be submitted to obtain the optimum benefit from available services.

F. Summation

1. Undirected sampling often does not yield meaningful results. Take the time to direct samples toward a potential solution. Ask the questions that will result in collection of appropriate samples.

2. Plan ahead concerning how results will be used when deciding what specimens to send to the laboratory.

3. Do not limit post mortem examinations to one or two animals when there are disease outbreaks or several deaths. Conduct post mortem examinations on one or more animals that are typical of the condition, but have not yet been treated, in addition to dead animals. Do not choose the runt of the group to sacrifice.

4. Serology can often help with a diagnosis, but acute and convalescent sera are usually necessary.

5. **The diagnostic laboratory has one primary function—to serve the veterinarian and his clients. Do not hesitate to call the diagnostic laboratory when assistance is needed. Remember, the telephone can be one of the most valuable diagnostic tools in a practitioner's arsenal. Use it.**