

The Use of the Diagnostic Laboratory in Bovine Practice

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I want to look for a few minutes at the subject from where I sit.

In Maryland we have a central full-service laboratory at College Park in the Washington suburbs with a staff of about 20, a laboratory at Frederick in the cattle area with a staff of seven, one at Centreville with a staff of four, a small laboratory at Oakland with a staff of one and a half, and one at Salisbury in the chicken country with a staff of nine. The total laboratory staff includes nine veterinarians—three pathologists, two poultry pathologists, one virologist, one clinical pathologist and two generalists. We are in the planning stages of a new central laboratory containing 20,000 square feet which should be completed, hopefully, in 1981. Our total laboratory budget is about \$840,000. We believe we are rendering reasonable laboratory support to our food animal agriculture, and for a state the size of Maryland, our financial contribution is quite good.

Each laboratory has a veterinarian-director, and as state veterinarian, each director answers to me. To my desk come the complaints from practitioners and owners about our service, reports and results, and complaints from our staff about unreasonable and unusual requests, quality of samples and other things. We conscientiously try to look into all that seem justified and respond accordingly. From this experience a few things stand out, that, if resolved or not allowed to happen, would result in significant improvement of service to you, the practitioner.

First, over half the complaints I see would have been avoided had there been sufficient dialogue between the practitioner and the laboratory at the time the samples were delivered. Too often the submitter assumes the laboratory will automatically do so and so, and the laboratory assumes the submitter wants so and so done, when in reality they both are wrong.

Second, the quality of the samples received too often precludes a meaningful examination being performed, completely negating any results. Here are a few examples that have come to my attention in recent weeks and months: milk samples for culture and sensitivity received in such containers as an open plastic tube covered with Scotch tape and in a syringe case closed with masking tape; tissues for histopathology received frozen or having been frozen, and tissues for histopath received partly autolyzed; serum samples that are cytotoxic for tests that involve the use of live cells.

So before we get into specifics, let me give you a few hints that will help you get better results from your laboratory.

First, get to know, in some way, some of the people responsible for the operation of the laboratory. Now I grant you this will take a little time and effort on your part and the laboratory's part. But have you ever considered how much money you and your client have invested in the samples you send in? \$5? \$500? \$5,000?

Try to regard the laboratory people as your professional associates. When you send in some diagnostic samples from a difficult case or an animal for necropsy, pick up the phone and discuss it with them, or at least send in a full and complete history. Tell them honestly and completely what treatment has been given, and when. It is not very productive of laboratory time, or very good use of your money, to do a lot of blood chemical determinations on a freshly dead animal that had received 1,000 ml of Ca-P-Mg solution an hour previously. Laboratory personnel, like you, have tremendous egos that must be reinforced regularly, like yours. If you remember that, it will pay you.

Second, the quality of the results you receive will in most cases be directly proportional to the quality of the sample submitted. If you send in a useless sample, the results will be equally useless. If you send in a sample from a case not representative of the problem, the results will be misleading. We will go into this in a bit more detail later.

Third, don't expect the laboratory to make a diagnosis for you on most cases or samples submitted. It is their job to report findings and interpret them. It is your job to make the final diagnosis. I grant you some pathologists think theirs has to be the final infallible word, but believe me, it is not always. They too report what they saw and their opinions. *Don't abdicate your responsibility.*

Fourth, try to give the laboratory some direction based upon what we hope were careful clinical observations and deductions. For example, there are sitting in my office a bunch of samples that were sent in two or three weeks ago, and I expect I'll soon discard them. They include ground corn, hay, lime, urea molasses mixture, water, and scrapings from a tank where the molasses was fed.

The request is to check for "poisons" since three or four animals had died some months previously. Shall we check for aflatoxins? nitrates? heavy metals? chlorinated hydrocarbons? other insecticides? her-

bicides? look for poisonous plants? We could very quickly spend \$1,000 and several weeks and be completely on the wrong track. Chances are, anyway, there is nothing wrong with the material.

Fifth, When you receive some reports, we like to assume it is your responsibility to communicate with your client. The laboratory tries to avoid communication directly with your clients. But too often we get phone calls and complaints a week or two after reports have been sent out, from irate animal owners because they have heard nothing from the material submitted. And this puts everyone in a difficult position.

One function of the diagnostic laboratory is to assist the animal owner in the diagnosis and control of disease. Most, but not all, of this help is provided through the veterinarian. Most laboratories can restrict samples accepted, except those submitted by practicing veterinarians. The rationale for this policy is generally that *you* should be able to more capably select proper samples and interpret findings. But please realize that most laboratories are at least partly supported by public funds, and there are continuing pressures to change this philosophy. And those trying to bring about such change constantly remind us of their *tax-paying status*.

The diagnostic laboratory is also an integral and vital part of regulatory programs. It serves as a second line of defense behind you, the practitioner, against exotic disease introductions and is the source of vital observations about disease trends and pathogens that are emerging and becoming important to the livestock industry.

Now let's get a little more specific. First, we want to discuss virus diagnosis.

It has become apparent that the laboratory will be able to perform a much more meaningful service when it becomes recognized that samples must be collected early during the acute stages of disease rather than as an afterthought, when the disease process has far progressed and the animal is convalescent or dead. Necropsy material is a poor source of viruses—first because the concentration and location of the virus changed substantially during the advanced stages of illness and second because viruses *generally* do not live very long in a cadaver. Further, serologic tests are of little value in many virus diseases unless paired samples are received—an acute and a convalescent sample.

It is appropriate to look at a few examples:

IBR. Depending on the concentration of virus-swabs taken in the acute stage early in an outbreak—can be confirmed positive by virus isolation as early as 24 hours after receipt at the laboratory, with a maximum of 21 days.

BVD. Virus isolation should take 1-2 weeks.

PI3. Virus isolation should take 1-2 weeks. Laboratories using FA techniques will often have a diagnosis in 4-48 hours.

Coronavirus and Rotavirus. With good fecal samples may only take a few hours, depending on the

techniques used (electron microscopy).

Two *exceptions* to the above are:

(1) *Bovine leukemia*, where serum is the preferred sample for those laboratories doing the AGID test, which should be complete in 48-72 hours.

(2) *Abortion*, where most laboratories prefer to get the entire fetus and placenta.

When any of these are suspected—IBR, BVD, PI3—and your laboratory has virus isolation capabilities, take samples for virus isolation and blood samples very early in the course of the disease. If virus isolation is positive, you have your answer. But if there is no virus growth in about 21 days, and you still have suspicions, then take convalescent blood samples for confirmation.

To obtain serum samples of optimum quality to be used for tests for PRV, IBR, BVD, PI3, enteroviruses, parvoviruses:

Use sterile, chemically clean tubes. Vacutainers are preferred. Brucellosis tubes are unsatisfactory because they invariably contain chemical contaminants.

You need 2-3 ml serum.

DO NOT rinse your syringe, if you are using one, with a chemical disinfectant between samples. Use sterile saline and try to get it all out of the syringe.

Let clot in a slanted position at room temperature for 1-2 hours, then chill and keep cold. Avoid delays in transport to the laboratory.

The manner in which swabs or other samples are transported **IS VERY IMPORTANT!** Swabs must **ALWAYS** be put in transport medium immediately and kept there until sample reaches the laboratory. For optimum results, the sample should be frozen immediately and kept frozen until it reaches the laboratory.

The type of transport media and method of transport will be dependent on how far you are from the laboratory and what media your laboratory prefers. Check with them and determine preferences. Most laboratories will supply transport medium at no cost.

Fluorescent microscopy has now become a much-used tool in laboratory diagnostic work. When your laboratory has the appropriate conjugate, most diagnoses by this method can be completed in 1-6 hours. Currently, the method has been adapted to just about all the clostridia, rabies, IBR, TGE hog cholera, pseudorabies, parvoviruses, and several others. Fresh tissue is required.

When it comes to *bacterial diseases*, time does not permit us to go into detail about what specific tissue to submit for each disease. But we can make some important generalities. If you are doing the necropsy in the field, always submit tissue from the kidney, spleen, liver and lung. If there are specific lesions elsewhere, submit them as well. It is very important to send in the brain if there are any CNS signs.

Each tissue should be packaged separately to avoid cross-contamination. Two sets of each tissue should be submitted, one for histopathology, and one for

culture. The set for culture can be frozen, or immediately refrigerated and kept refrigerated. One of the problems of the bacteriologist is always overgrowth of contaminants. Samples carefully taken and handled can do much to minimize this problem.

Samples for *histopathology* must be put in formalin as soon as possible. The tissue should be cut in sections not exceeding 1/4 inch in thickness and placed in 10 times its volume of formalin until fixed. After 24-48 hours this volume can be reduced. Be certain tissues for histopath *do not get frozen*.

One of the best packaging methods currently available for specimens is the plastic whirl pack. They are cheap, relatively sterile, easy to carry, and almost unbreakable. Our branch laboratories use them exclusively for transporting both tissues in liquid and frozen tissue. Just be sure the pack is whirled several times during the closing process.

Probably *neonatal calf diseases* produce the biggest single bovine entity encountered by the laboratory. The laboratory prefers to get the entire cadaver, as soon after death as possible, or an animal in a moribund condition. They can easily be transported in the trunk of a car. A fresh representative animal will often give them the opportunity to check for enteric and other viruses. And too often the laboratory gets the 10th or 15th calf that dies when they could have done a much better job had they received the first, second or third animal, or preferably all three of them, before the outbreak became complicated with secondary infections that throw them off the track and confuse the diagnosis.

In summary, to realize the full potential of any diagnostic laboratory:

Establish a meaningful liaison with the laboratory: learn what services they provide, what are their limitations, and find out who you can talk to, on a professional basis, to discuss mutual requirements and desires.

Submit appropriate samples in good condition from representative cases.

Submit a complete and accurate history.

Carefully evaluate laboratory findings and correlate them with your clinical findings to arrive at a final diagnosis.

Questions and Answers

Dr. Bechtol: I know I will be able to take some of these ideas back home and utilize the diagnostic laboratory much better. Are there any questions that you would like to ask either of the speakers?

Question: Do any of the laboratories in Maryland have the capability to check for vibriosis?

Answer: Yes, sir, we have a couple of laboratories that regularly check for vibriosis. I believe they prefer their material submitted in saline-fresh material, fresh swabs from the female, prepuce washings from the male.



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