On the journey for great diagnostics – Tips and tricks to let the magic happen

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Abstract

Diagnostic medicine is an integral part of bovine medicine and veterinary production animal medicine. These proceedings will discuss the standard of care related to diagnostic medicine. Strategies will be presented for minimizing diagnostic errors in the critical thinking process associated with making diagnostic decisions, as well as in the logistics of sample handling and requesting testing. Common diagnostic errors will be highlighted with suggestions on how to avoid them. Recommended resources for bovine veterinarians are included.

Key words: bovine, cattle, diagnostic standard of care

Résumé

La médecine diagnostique est une partie intégrante de la médecine bovine et de la médecine vétérinaire des animaux de production. La présentation abordera la qualité des soins en médecine diagnostique. Des stratégies seront présentées pour minimiser les erreurs diagnostiques dans le processus de pensée critique associé aux prises de décisions diagnostiques de même que dans la logistique de la manipulation des échantillons et de la requête de tests. Les erreurs diagnostiques les plus fréquentes seront soulignées avec des suggestions pour les éviter. Des ressources recommandées pour les vétérinaires bovins sont incluses.

Introduction: The Diagnostic Standard of Care

There are many working definitions of "standard of care." From the *New England Journal of Medicine*, 2004, we have: "the quality of care that would be expected of a reasonable practitioner in similar circumstances."[8] And we also have a statement from a legal resource specific to veterinary malpractice, "The exercise of the care and diligence as is ordinarily exercised by **skilled veterinarians.**"[3]

When is careless or uninformed attention to sample collection, sample handling, or diagnostic test choice an issue impacting standards of veterinary care? From the perspective of diagnostic laboratory standards, originally established for human medicine, accredited veterinary diagnostic laboratories are required to provide specific guidance on samples which are appropriate for carefully validated tests. When guidelines for sample submission are not followed, we are required to document departures from normal or specified conditions for the specimen, contact the submitter and, if a decision is made to test the sample, document that the condition or suitability of the sample did not meet the specifications and may have affected the results. Laboratory diagnosticians would not meet diagnostic standards every time we did not follow these requirements. And I would argue that veterinarians are not meeting the standards of care for their patients every time they participate in uninformed or careless diagnostic sampling or testing.

How might poor diagnostic testing have an impact on the veterinary care being provided? Excluding the need for treatment or control measures based on faulty negative test results for particular conditions or diseases is the most obvious example of an area of concern when diagnostic results are based upon poor diagnostic choices, samples or processes. Whether considering individual animal care or population health and disease concerns, we all want the information related to diagnosis to be reliable. Yet diagnostic labs receive incomplete paperwork, unidentified samples, inappropriately handled samples, and samples unsuitable for particular tests on a daily basis. I would contend that we witness diagnostic malpractice daily.

Diagnostic errors are largely preventable, may result in patient harm, and reflect a breakdown in our healthcare system and/or clinical reasoning.[9] In addition, veterinary medicine is very complex and production animal medicine doubly so, incorporating the concepts of individual animal medicine with population medicine needs is somewhat analogous to the separate field of public health. Also, veterinarians are often faced with a need to perform complex diagnostic decision-making in the face of resource limitations, a high degree of uncertainty, and limited time. There are also considerations of human health and safety relative to the ability to acquire some diagnostic samples.

Certainly, high-quality diagnostic laboratories address the quality of their testing by adopting formal quality control systems for training, test validation, test performance, result reporting, and other important functions. Some features of those quality systems include regular internal and external audits, proficiency testing, and documentation of errors with corrective and preventive actions adopted to prevent future occurrences. Laboratory quality control is beyond the scope of this talk other than to suggest that veterinarians should always use laboratories that demonstrate attention to quality.

Minimizing Diagnostic Error

How can veterinarians minimize their own diagnostic errors? Veterinary colleges teach a formal process of diagnostic reasoning. It consists largely of developing a problem list, and working on both a diagnostic plan to define the problems more clearly as well as a treatment plan to ameliorate the problems. The latter 2 typically require the clinician to outline a differential diagnosis, and then diagnostic tests or procedures, as well as treatments, are systematically applied. Seasoned clinicians often speed through the process in their head and often land on a plan that addresses the most pressing needs. However, skipping steps can allow even the best clinician to forget something, or allow their inherent bias to omit something that should be in consideration.

Ely et al make a compelling case for the use of checklists to overcome these cognitive biases and mental shortcuts (heuristic errors) that lead to diagnostic errors.[2] The paper makes a case for 3 types of checklists. The first is a general checklist to prompt the clinician to optimize their clinical decision-making. I modelled the Example General Checklist, below, after the example provided in the paper. The second type of checklist is the more familiar differential diagnosis checklist. Assistance with differential diagnosis checklists comes from many resources, such as textbooks, training programs, and online resources such as the Cornell Consultant (http://consultant.vet.cornell. edu/). The third type is a checklist of common pitfalls or "cognitive forcing functions." I have developed an example of the third type, shown below, that might be appropriate to reduce diagnostic laboratory submission errors.

Example General Checklist

1. Did I obtain my own complete medical/production history?
2. Did I perform a focused and purposeful physical exam/necropsy/herd record review?
3. Did I generate initial hypotheses, and attempt to rule in/out with additional history, examinations and diagnostic tests?
4. Did I stop to consider the following:
   • Was I comprehensive?
   • Did I consider flaws of mental shortcuts?
   • Was I biased in any other way?
   • Do I need a diagnosis now or can I wait?
   • What is the worst-case scenario?
5. Did I embark on a plan, while acknowledging uncertainty and ensure a pathway for follow-up?

Example Common Pitfalls and Cognitive Forcing Functions for Diagnostic Submission

1. Am I trying to rule out the worst-case scenario? Am I trying to rule out all important scenarios?
2. Does the diagnostic testing requested reflect the problem list and differential diagnosis?
3. Will I be able to use the results of each of the tests I have requested for decision-making/treatment/ management changes?
4. Have I considered which test is the best test ("fit for the purpose") if more than 1 are available? Is there a reason to use more than 1 test for the same disease/condition?
5. Do I have the correct specimen for each test?
6. Have I collected every specimen that would be indicated? If not, can they be collected?
7. Have I handled each sample exactly according to the guidelines provided by the laboratory? Do I know what those guidelines recommend?
   • Appropriate attention to avoid cross-contamination during sample collection.
   • Serum separated from cells in clotted blood.
   • Plasma separated from cells in unclotted blood.
   • Whole unclotted blood submitted when required.
   • Slides freshly made and included for hemogram, cytology, fluid analysis.
   • Samples held at appropriate temperature.
   • Sample shipped to arrive within time frame to maximize sample integrity.
8. Will the diagnostic result be available in time to be useful? Consider both an acute problem and if the problem becomes chronic or persistent in a population.
9. If I do not need to test now, is there a reason to store a sample for potential testing later?
10. Are there regulatory considerations or public health considerations?
11. If I must consider a diagnosis of exclusion, have I been thorough enough?
12. Might there be legal or forensic ramifications of this problem or testing?
13. Are there any other examinations that should be performed or clinical history to consider prior to requesting this testing?

Most veterinary practices increase diagnostic test costs to the client by some percentage, often 100% or more, to cover their expertise and time. That additional fee should include the time taken to carefully review the samples required for a test or tests and how to properly handle them. It should also include the time to complete paperwork appropriately and ensure samples are properly identified and packaged. It may also include the cost of shipping or there may be a separate shipping fee. And it typically will include the archiving of diagnostic results and the interpretation of those results and subsequent clinical decision-making based upon those results.

To Test or Not to Test

A thoughtful and helpful discussion entitled Strategic Laboratory Sampling was circulated in April 2019 on the

AABP-L e-mail list.[4] I find the discussion regarding the reasons to perform laboratory testing and also how much testing to do very helpful. While the article was written in 1993, the principles are timeless and the discussion is presented in a very understandable way. A colleague of mine suggested that it should be required reading for DVM students going into production animal medicine.

The authors visit the idea of needing to have a very clear idea for why any diagnostic testing will be performed. The assumption is that tests are performed to show something or verify something and that an action will follow. Therefore, if a test result will not be used to make a change or take an action, it probably should not be performed. The article suggests that when describing the problem, one should focus on defining a managerial issue(s) that can be changed and that the testing will determine what and how to make those changes. An exception to this process that the authors outline is when trying to develop a hypothesis for management-level changes, using laboratory testing for unusual necropsy or exam findings only after performing a sufficient number of necropsies or exams to establish a pattern. Other exceptions routinely encountered might include testing performed for regulatory purposes, public health purposes, or academic curiosity.

In addition, determining the underlying cause of a problem may require comparing a group or setting in which a problem is found with a similar setting in which the problem is not evident. The problem needs to be clearly and thoroughly defined or described. Efforts also need to be made to identify changes that could have caused or introduced some unwanted effect. Whether or not these steps are shared with the diagnostic laboratories in the clinical history or problem list, they should be followed. Certainly if the clinician anticipates requesting assistance from the laboratory in defining testing or interpreting test results, a good summary of the clinical history and/or a well-defined problem list, as well as a pertinent discussion of changes on the farm or in the population will need to be provided to the laboratory subject matter experts. When a veterinarian stands out as an excellent diagnostician, they probably mentally go through the exercise of these steps quickly and naturally. They may have a wealth of prior experiential knowledge that assists them in quickly recognizing abnormalities and patterns of abnormalities. That quick recognition or prior experience could also bias them away from some possibilities. Any clinician can train himself or herself to follow the steps needed to solve problems, potentially by adopting the aforementioned checklist approach. It can be applied to individual sick animals as well as to herd health nutrition and production problems.

Another question to consider is whether the cause of the problem must be determined. Empiric therapy is a choice in both veterinary and human medicine. In some cases definitive diagnosis might only be made postmortem. The notation "NYD" for not yet diagnosed, is made in human medical charts while other decision-making or treatment is occurring.[4] In addition, obtaining diagnostic samples is not always feasible or may be medically unsafe for the patient. Cost must also be weighed against benefit for both the individual animal and the population.

Sample Numbers

I have not included formulas for calculating sample numbers needed to detect something or determine if a herd is free of a particular disease, condition or attribute. That work has been presented in epidemiology texts, statistical classes, and is available in numerous online resources. I excerpted Table 1 from the previously discussed article "Strategic Laboratory Sampling."[4] This table allows you to use an estimate of prevalence within a herd, herd size, and the desired level of certainty of either 90, 95, or 99%, and determine the number of samples required to detect a particular condition.

Similar tables are also provided by the same authors for sampling strategies to estimate proportions of a herd affected with an attribute, and for sampling to estimate an average for a group, the example used being sampling to estimate the average whole blood selenium of a group of cattle.

Avoiding Common Diagnostic Errors

Internal, unpublished data from a single recent year (2016) at the Animal Health Diagnostic Center at Cornell University regarding just 2 basic laboratory tests documented frequent errors by submitters. For serum chemistry requests 40% of the bovine samples were shipped overnight or longer with the serum still on the cells. In contrast, the same error was only made in ~10 to 15% of small animal serum chemistry requests. For hemogram (CBC) requests, sending anticoagulant whole blood to arrive overnight or later, without freshly made blood smears, occurred at a similar rate. At today's test costs, the poorly prepared submissions would add up to approximately $98,000 in potentially erroneous testing for just those 2 test panels.

It is possible that the use of point-of-care testing capabilities during veterinary education rotations and in growing percentages of veterinary practices will have an impact on better understanding of diagnostic test use, sample handling, and other concerns that lead to frequent errors associated with "send-out" diagnostics.[7] Certainly, the individuals carrying out point-of-care testing need adequate training both in accomplishing the testing with appropriate samples, as well as the ability to manage quality control issues that similarly affect diagnostic laboratories, such as sample integrity, reagent integrity, equipment calibration, equipment maintenance, kit or reagent expiration dates, and other issues. Exposure of DVM students and graduate veterinarians to these rigors may make for better diagnosticians overall, with more awareness of avoidable errors, such as those that follow here.

Insufficient information. Failure to include sufficient clinical history with diagnostic submissions limits the lab's ability to screen for proper sample collection or test selection.

Table 1. Sample size required to detect attribute in a population with defined level of certainty

Prevalence	1%	1%	1%	5%	5%	5%	10%	10%	10%	25%	25%	25%	50%	50%	50%	75%	75%	75%
Certainty	90%	95%	99%	90%	95%	99%	90%	95%	99%	90%	95%	99%	90%	95%	99%	90%	95%	99%
N = 10	10	10	10	10	10	10	10	10	10	6	7	8	3	4	5	2	3	4
20	20	20	20	19	20	20	14	16	18	7	9	11	4	5	6	2	3	4
30	30	30	30	24	26	29	16	19	23	8	9	13	4	5	7	2	3	4
40	40	40	40	28	31	36	17	21	27	8	10	14	4	5	7	2	3	4
50	50	50	50	30	35	42	18	22	29	8	10	14	4	5	7	2	3	4
60	59	60	60	32	38	47	19	23	31	8	10	15	4	5	7	2	3	4
70	68	70	70	34	40	51	19	24	33	8	10	15	4	5	7	2	3	4
80	76	79	80	35	42	54	20	24	34	8	10	15	4	5	7	2	3	4
90	84	87	90	36	43	57	20	25	35	8	10	15	4	5	7	2	3	4
100	91	96	100	37	45	59	20	25	36	8	10	15	4	5	7	2	3	4
125	106	114	122	38	47	64	21	26	37	8	11	16	4	5	7	2	3	4
150	118	130	143	39	49	68	21	26	38	8	11	16	4	5	7	2	3	4
175	128	144	163	40	50	71	21	27	39	8	11	16	4	5	7	2	3	4
200	137	155	180	41	51	73	21	27	40	8	11	16	4	5	7	2	3	4
225	144	166	196	41	52	74	21	27	40	8	11	16	4	5	7	2	3	4
250	151	175	210	42	53	76	21	27	41	8	11	16	4	5	7	2	3	4
275	156	182	223	42	53	77	22	28	41	8	11	16	4	5	7	2	3	4
300	161	189	235	42	54	78	22	28	41	8	11	16	4	5	7	2	3	4
400	175	211	273	43	55	81	22	28	42	8	11	16	4	5	7	2	3	4
500	184	225	300	43	56	83	22	28	42	8	11	16	4	5	7	2	3	4
600	191	235	321	44	56	84	22	28	43	9	11	16	4	5	7	2	3	4
700	196	243	336	44	57	85	22	28	43	9	11	16	4	5	7	2	3	4
800	200	249	349	44	57	85	22	28	43	9	11	16	4	5	7	2	3	4
900	203	254	359	44	57	86	22	29	43	9	11	16	4	5	7	2	3	4
1000	205	258	368	44	57	86	22	29	43	9	11	16	4	5	7	2	3	4
Infinite	229	298	458	45	59	90	22	29	44	9	11	17	4	5	7	2	3	4

Prevalence = lowest prevalence in population of interest detectable at defined certainty level using sample size given. Certainty = level of certainty of detection desired. N = number of individuals in population of interest. Computations based on: Cannon RM and Roe RT, Livestock Disease Surveys: A Field Manual for Veterinarians. Australian Bureau of Animal Health, Department of Primary Industry. Australian Government Publishing Service, Canberra, 1982.

It also limits the ability to suggest additional testing options. And it may not allow the lab to provide a test interpretation that makes sense for the clinical problem encountered. In the long run, it also limits the ability of the laboratory to provide summary diagnostic testing data to veterinarians related to clinical conditions. Since defining the problem and considering the differential diagnosis are important parts of the pre-testing process, providing a summary of that critical thinking in the medical record or on submission forms should not be excessive.

Wrong sample submitted. Sample requirements for testing are often fairly specific and substitutions may not be appropriate. The substitution of plasma for serum for antibody detection may not even be appropriate for all tests. Laboratories may also be limited in their ability to handle certain samples within a particular testing platform. For example, some laboratories only have nucleic acid extraction methods for blood and cannot perform extractions on tissues for PCR tests, even if they are biologically relevant samples. Always check for appropriate samples in laboratory guidelines, and attempt to collect the highest priority specimens indicated by the lab. If optimum samples are not available, it may be worthwhile to contact the laboratory regarding acceptable substitutions. For example, pleural fluid, pericardial fluid or peritoneal fluid is often a suitable substitute for serum, especially from postmortem carcasses when it may be impossible to collect unclotted heart blood or the serum from clotted heart blood is of poor quality due to advanced hemolysis.

Sample degradation. Failure to maintain specimen integrity for the testing requested may result in sample rejection without testing, or render results inaccurate or uninterpretable. Specific sample handling guidelines are provided by the laboratory to maximize sample integrity. Samples requiring refrigeration should be chilled during all aspects of storage and shipping, e.g. tissues for culture. The age of sample is critical for some testing, such as hemograms or assays for volatile compounds like ammonia. Hemolysis negatively affects many tests, including colorimetric assays, as well as any testing where the release of RBC intracellular compounds will alter results, such as serum chemistry analysis for glucose or potassium. For all testing which is not

routinely performed by a particular clinician or their support staff, sample handling guidelines should be reviewed, ideally prior to sample collection.

Freezing, or repeated freeze/thaw cycles, may degrade samples for certain analyses, while freezing and shipping on dry ice to absolutely prevent thawing might be recommended for other assays. Some compounds are degraded by exposure to light, and diagnostic results on inadequately protected samples, such as for vitamin E analysis on serum samples, will not be reliable. Exposure to moisture or condensation is a common problem that degrades blood smear slides or cytology smear slides shipped with freezer packs or other refrigerant. Leaking 10% neutral buffered formalin within a package also degrades exposed slides.

For some specialized testing, some sample handling requirements during shipment are hard to achieve, for example, samples that are required to be rapidly frozen and then shipped for next day delivery with dry ice. If testing requiring difficult sample handling is important, consider collecting the samples and getting them into appropriate storage and figuring out the shipping logistics at a later date. For example, troponin testing may be important for defining exposure to toxic levels of ionophores in currently unaffected herdmates of dead animals. Blood can be collected and serum separated, frozen and held indefinitely in the freezer. In the event that a case becomes a legal case with a farm trying to recover damages, those frozen samples can be retrieved for appropriate testing later.

Omission of microbiological transport media when its use is indicated, or its incorrect use, may result in failure to detect a pathogen when the bacteria, fungus, or virus was present in the original specimen.[1,10] This omission may also result in an inability to provide related secondary testing such as antimicrobial sensitivity or special typing or genotyping PCR, which typically require isolates. Transport media may provide nutrients or may have protective properties, such as buffers. Swabs submitted for bacterial culture testing should always be submitted in an appropriate bacterial transport media. Tissue samples often do not require transport media for submission. Swabs submitted for PCR testing should not be submitted in gel transport media that may interfere with PCR testing.

Failure to submit freshly made blood smear slides with the whole unclotted (anticoagulant, EDTA, heparin) blood for all hemogram requests except those accomplished quickly at point-of-care settings will negatively impact results. Age-related changes in cell morphology may mask underlying conditions or lead to erroneous conclusions or an inability to interpret the hemogram. While it is less common in production animal medicine to consider hemograms as a routine testing choice for many problems, when they are considered and requested, there is often a very serious problem, often with a lot of uncertainty due to a lack of defining patterns, and the clinician is hoping that baseline hematology will help guide further diagnostics. Poor results in that setting are particularly problematic.

Likewise, *failure to separate serum or plasma from cells, or delayed separation* will result in changes to various chemistry parameters due to continued cell metabolism, e.g. glucose depletion, elevations in potassium, even when hemolysis is minimal. Blood chemistry testing to establish changes against expected baselines is also not as routine in production animal medicine as in more individual-animal medicine settings. Certainly individual animals may benefit from consideration of blood chemistry changes when planning treatment and assessing potential recovery, and also when providing a prognosis. In addition, a poorly defined herd problem may be clarified in some instances with blood chemistry testing. Reliable recognition of unexpected patterns will depend on generating reliable results.

Cross-contamination. Samples collected from sequential animals without appropriate attention to asepsis or changing gloves creates the possibility that tissues may be cross-contaminated. Cross-contamination also occurs when individual samples are pooled in the same container, such as multiple fresh necropsy tissues, or when leakage occurs during shipment. Cross-contamination is a serious consideration, because it usually affects multiple samples, potentially from multiple animals, and may preclude some or any of the testing needed or render the results unusable.

Failure to collect aseptic swabs will lead to the dreaded "contaminated" result. Aseptic collection is accomplished from non-sterile samples such as postmortem tissues by sterilizing the surface with heat (searing) or a chemical (antiseptic; sterilant), and using sterile instruments to open the tissue via this sterilized area to collect an interior sample. Sufficient contamination will mask many primary bacterial pathogens, since there is not always a selective media that will suppress contaminants and allow pathogens to be cultured successfully.

Selecting tests that are not "fit for the purpose." Failure to understand when to use pathogen detection tests and when to use antibody detection tests commonly leads to improper test selection. It requires the submitter to recognize what the selected test targets. It also requires a basic understanding of disease pathogenesis with some consideration of how long the pathogen is expected to be present in the animal, and which tissues are most likely infected. Attempts to use pathogen detection tests beyond the time during which pathogen presence is expected are not likely to be useful. Pathogen detection tests, using a specimen expected to contain the pathogen, based upon known disease pathophysiology, is always most appropriate in acutely infected animals.

In addition, the clinician needs to consider how early detectable antibody development would be expected, as well as whether there are likely to be confounding post-vaccination antibodies or maternal antibodies present. Antibody titers are usually not helpful in acutely ill animals at all, except when IgM detection tests are available. Interpreting antibody titers at a single point in time to definitively diagnose current or recent infection is often impossible, especially when vac-

cines against the pathogen in question are routinely used or when the pathogen is commonly encountered by animals that may remain healthy. Rare exceptions are when the presence of an antibody has been proven to define a carrier infection state, such as with bovine leukosis virus infection. Acute and convalescent serology testing generally only allows the diagnosis to be made in retrospect or in a chronically affected animal. If the animal does not survive, the convalescent sample is not available, and if the clinician did not remember to save an acute specimen, it may not be available at the later time point that it would be useful.

Failure to include histopathology. Omitting histopathology on a comprehensive or appropriate set of tissues when investigating the cause of death is a frequent diagnostic error. Histopathological changes may be the only source of clues to defining a pattern of abnormalities associated with a problem. The histologic lesions may also allow us to interpret other testing more effectively, such as the finding of a bacterial pathogen that may also be a commensal or contaminating organism and whether it is associated with expected pathogenic changes. Another example would be histologically determining that there is a severe inflammatory infiltrate and changes consistent with bacterial pneumonia in a lung that is culture-negative from an animal treated aggressively with antibiotics.

Failure to include histopathology testing may not allow a clinician to understand that they have made other diagnostic errors as well. For example, lesions may point the clinician in a direction that was omitted from the differential diagnosis, for example considering toxic, metabolic, parasitic or fungal causes of morbidity or mortality.

CSF as a multipurpose sample. Failure to consider using CSF (cerebrospinal fluid) for many testing modalities is an infrequent issue encountered in bovine medicine, as CSF collection is not very common antemortem outside of a hospital setting. However, CSF collection is very invasive in live animals, and if collected, the samples should be used for as many useful diagnostic tests as possible. Many normal animals are serologically positive for antibodies to agents which may be important in the differential diagnosis of neurologic diseases, e. g. *Toxoplasma*, *Sarcocystis neurona* (EPM), *Neospora,* West Nile Virus. Finding antibody levels higher than those found in serum may be definitive for central nervous system (CNS) involvement. If a serologic test is ever available for *Parelaphostrongylus tenuis* infection, using CSF for confirmatory testing becomes an even more important consideration than it is now for suspect ruminant infections. In addition, because CSF bathes the entire CNS bacterial and viral detection, testing on CSF may allow pathogen detection when areas of the CNS are not examined otherwise. It is difficult to collect spinal cord specimens when performing a field necropsy, but CSF can be aseptically collected fairly readily.

Testing not specified or samples to be tested not indicated. Failure to specify the testing needed when submitting samples results in errors, assumptions, disappointments or delays. Bacteriology, virology, and toxicology are not tests, they are disciplines. Bacteriological culture testing requires the submitter to indicate the exact tissue/specimen that should be individually cultured, and the types of cultures, for example aerobic culture, anaerobic culture, Salmonella culture, Campylobacter culture or Listeria culture. Viral or bacterial PCR (polymerase chain reaction) or FA (fluorescent antibody) testing generally requires the submitter to indicate which sample(s) they want tested. If multiple tissues should be tested, a definitive list should be provided. For suspect toxicoses, name the toxin(s) considered in the differential diagnosis or indicate "unknown toxin." Name the test requested, such as mass spectroscopy screen, toxic heavy metal panel, anticoagulant rodenticide screen. If you are unsure which tests are appropriate, contact the lab or the toxicologist.

Insufficient numbers of samples. Failure to test enough animals may not allow any patterns to be recognized. Not all definitive diagnostic samples are available at all stages of disease. High morbidity or mortality events should trigger testing multiple animals. Some state-funded subsidized testing may not cover a sufficient number of animals. In those instances when additional testing is indicated, it should be performed at unsubsidized cost. The goal should be to increase the odds of achieving a definitive diagnosis or defining a pattern that can be further investigated. Consider testing normal-appearing animals, too. Even when dead animals are available for necropsy, test live affected animals, including CBCs and chemistry profiles, blood cultures, virus isolations (nasal swabs, EDTA blood). Consider banking samples or sampling animals daily.

What is the magic number of necropsies to perform in a high mortality situation? Many laboratories or pathologists suggest a minimum of 3, but where does that number come from? It is all about recognizing patterns. One animal does not make a pattern. And a single animal could be an outlier to the problem. Likewise, 2 animals with different lesions will still not establish a pattern. Three animals, 2 of which have lesions that can be linked to a problem, start to establish a pattern and if all 3 are the same, the investigator can be more confident that they are investigating the correct problem. Remember that the physical exams and necropsies are really about defining the problem. Sometimes you get lucky and get the definitive diagnosis, too. Obviously, it may require more than 3 animals to detect lesions depending on the stage of illness, and it may take more than 3 to convince an investigator that a complete lack of gross, or even histologic, lesions is consistent with the problem at hand.

Limited investigation. Failure to be comprehensive in the overall work-up, probably as a result of cognitive biases in decision-making, often leads to a lack of definitive diagnostic results.[5] Assuming that the most likely scenario is the actual scenario often leads to omissions in testing and sample collection. After the top 2 or 3 differentials have not been confirmed by testing, it is often not possible to expand the testing to in-

clude additional differential concerns. For cost-containment concerns, comprehensive sampling should occur and testing may be staged to address highest priority rule-outs first. For most production animal health concerns, if it appears that a health concern is financially important enough to investigate at all, it is generally financially appropriate to be comprehensive. The time of the attending clinician is valuable and generally billed out to the client. Collecting additional samples from a single necropsy may not add considerably to the time or billable hours, whereas sampling a large number of animals or performing additional necropsies can result in significant changes in labor and potentially veterinary billable hours. Veterinarians are expanding the use of veterinary technicians and lay personnel in the collection of diagnostic specimens and also as necropsy prosectors for their own efficiency and affordability of services provided.

Communicating with the Laboratory

Diagnostic laboratories encourage submitters to call for assistance with test selection, sample handling, and other questions. Not every lab has veterinarians available to consult and veterinarians are communicating with individuals who have technical expertise in diagnostic disciplines such as bacteriology, toxicology, or parasitology. Laboratory personnel may not have familiarity with all drug or product names. When complicated instructions may be necessary, make sure the individual designated to make the phone call to the lab is well versed in diagnostics and the conditions being considered. Oversimplification of instructions or mistakes in understanding what has been discussed frequently contribute to submission errors, even when office staff call the lab in advance of submission. Staff communicating with the lab should understand the difference between antibody detection and antigen detection, should know the difference between serum and plasma, and should know the condition of the animal to be tested (is it alive or dead, sick or healthy screening test, neurologic signs vs respiratory signs). For complicated problems it is best if the veterinarian communicates with a diagnostician. Provide email addresses to receive written instructions or phone numbers for text messages.

Many laboratories, such as the Animal Health Diagnostic Center at Cornell University, offer written guidance for sample submission and testing for bovine disease presentations, either in laboratory manuals intended for the use of their veterinary clients, or in online formats.[1] In addition, textbooks and online manuals often provide similar guidance, for example *Rebhun's Diseases of Dairy Cattle*, 3rd edition, includes comprehensive diagnostic sampling and testing tables in Chapters 17 and 18 for a variety of clinical presentations.[6]

Proper diagnostic sampling, sample handling, and test selection are an important part of veterinary medicine's standard of care. Getting it right is just as important as giving the correct medication or performing the correct surgical procedure. Resolving simple or complicated problems in highly complex production systems is a rewarding part of veterinary medicine. Diagnostic medicine routinely finds answers that can lead to treatment success, disease control and prevention, market assurance, and better public health.

As a member of the laboratory diagnostician community, I can say that we pledge to continue to contact our submitters for information when forms are not completed, samples are not identified, test requests are not appropriate for the specimen provided, or there are quality concerns with the samples we receive. And we pledge to note on the result report each and every time the specimen provided is in a condition that is either unsuitable for testing or for which the results may be adversely affected. But we would rather just expedite your samples and always provide you with the highest quality results possible.

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