THE NECROPSY IN VETERINARY MEDICINE:

A MANUAL FOR ALBERTA PRACTITIONERS AND RVTS

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"The cursory autopsy, with lack of attention to detail and without an understanding before the dissection begins of what one might seek, of what one must consider, and how tissues should be sought and prepared, can only yield missed opportunities." (1)

INTRODUCTION

This manual is intended for veterinary clinicians and Registered Veterinary Technologists in the Province of Alberta and will cover the place, procedure, and ancillary tests for postmortem examination in veterinary medicine.

The last two decades have seen two general trends in veterinary diagnostics in Canada that have pressured veterinary practitioners to perform a greater number of gross postmortems in the course of their duties. These are the retreat of governments from providing publicly funded diagnostic laboratories at both the provincial and the federal levels, and the takeover of small private laboratories by multinational laboratory companies. These trends have reduced access and increased the costs of postmortem diagnostic services to animal owners to the point that far fewer postmortem examinations are being performed, particularly in the livestock industry.

Veterinary clinicians, while trained in postmortem techniques as part of their education, are by nature of their interest more oriented towards treating and managing live animals, and are often uncertain in working with postmortem material. This series of articles is an attempt to provide clinicians with reference material to allow them to feel more comfortable in extending their practices to include regular postmortem examinations.

A necropsy, also known as a post mortem examination, is the examination of the body of an animal after its death. The word "necropsy" should not be interchanged or confused with the term "autopsy", which strictly means a human examining the body of another human following death of the latter, and as such does not apply to an animal postmortem. The goal of necropsy examination in veterinary medicine is to provide an analysis of dysfunction at the level of the entire animal or even the herd. Analysis of dysfunction only at the cell, organ or system level is inadequate (2).

Disease control requires information, and information is obtained by surveillance. Surveillance has two arms, commonly referred to as active and passive. Passive surveillance consists of examination of clinically affected cases of diseases in a population. Necropsy examination is one of the two main tools of passive surveillance in veterinary medicine[1]. The term “passive surveillance” is misleading and unfortunate because it implies a less robust approach than its

[1] The second is clinical examination of unhealthy animals

counterpart. Passive surveillance is actually “finger on the pulse” surveillance.

REASONS FOR CONDUCTING A NECROPSY

The information obtained from a complete necropsy is used primarily by veterinary clinicians and animal owners but in some instances more generally in society. There are many reasons to conduct a necropsy in veterinary medicine:

1. to determine the cause of death of an animal including the provision of a source of primary or corroborative information in cases of sudden, suspicious, or unexplained death, and to establish an etiology of disease.
2. to confirm, clarify or correct a clinical diagnosis and to rule out other disease processes. In this way the necropsy can serve as quality control for the clinician, monitoring the accuracy of interpretation of clinical signs and antemortem diagnostic tests. Clarification of clinical signs, especially those that were unexpected or atypical of the disease condition and correlation of clinical signs and pathologic findings is thereby provided.
3. to increase the accuracy of diagnosis in any of a number of conditions which are very difficult to accurately diagnose clinically.
4. to search for and to assess concurrent disease and management problems in order to establish causes of production loss.
5. as an information gathering device in research to assess the effectiveness of medical or surgical therapy, new medical and surgical techniques, and to determine the efficacy and toxicity of therapeutic agents.
6. to provide accurate information which can be used to compile provincial or national records of animal disease, to identify disease trends, to recognize, document and investigate diseases that are new to an area, emerging diseases, or novel disease processes. This is particularly important in veterinary medicine, because changing animal management systems may create entirely new diseases, or new opportunities for existing pathogens.
7. as a method for education of veterinary students, animal health technicians and those who may need to deal with animal disease such as wildlife officers.
8. to obtain forensic or legal information.
9. to identify emerging diseases.
10. to monitor the influence of environmental factors on physiologic processes.
11. to serve as an indicator of the presence of zoonotic diseases.

Very similar reasons are given for conducting autopsies in human medicine (3)

PRINCIPLES OF THE NECROPSY EXAMINATION

There are several principles that underlie necropsy technique:

1. to proceed every time with a standard, consistent, repeatable approach.
2. to expose the internal organs with minimal disturbance and contamination.
3. to open and examine organs in such a way that they may be reasonably reassembled into the shape and position that they occupied before they were cut.
4. to yield the best specimens possible for ancillary tests.

The necropsy is conducted in a routine manner every time. This is necessary to avoid chasing lesions as they are encountered and thereby being distracted from conducting a thorough examination. An orderly approach also has the benefit of making the prosector observe normal structures, and more importantly, variations of normal. This builds a basis of experience that will allow the abnormal to be recognized when it is encountered. To achieve this, the necropsy proceeds in a strict routine, progressing through a standard series of steps each time it is performed.

Necropsy procedure is designed to reveal organs as close to possible as they were in the animal at the point of death. This implies that there should be the minimum possible amount of handling of the carcass as it is moved into position for examination. This is relatively straightforward when the animal is to be examined in the field, but rather more difficult when it is to be transported to a clinic or laboratory. However, animals can be loaded and unloaded with relatively little disturbance of internal organs if care is taken. Rolling and twisting movements are particularly to be avoided as they potentially affect the relationship of abdominal organs, and may reduce torsions and volvulae prior to opening the carcass. In addition, there should be an economy of cutting: the most information should be revealed about the carcass and organ systems with the fewest incisions. This is one way of reducing the amount of damage. Contamination of the carcass and each individual organ by organisms and toxic compounds must be avoided so that when microbiologic or toxicologic techniques are required, there is no difficulty interpreting the significance of findings.

The necropsy procedure is designed to cause a minimal degree of damage to tissues, organs or organ systems and allow their reconstruction by reassembly if necessary following the necropsy. This capability is required as it may be necessary to verify the relationships of organs one to another or the presence or absence of an anatomic structure based upon some finding that has been made after the removal of that particular organ or part. This is true most commonly of the relationships between the great vessels and the heart and lungs which very often need to be reconstructed based upon finding anomalies or vascular pathology. The technique should proceed in such a manner that the organs can be reasonably reconstructed and identified as to location (left or right) if necessary.

Aimless cutting and hacking has no place in proper necropsy technique.

The basic necropsy examination is organized in such a manner that all organ systems are first evaluated grossly. Once this basic examination has been completed, the pathologist may then proceed to focus on the specific abnormal organs or tissues, but this should only occur after a thorough stepwise initial examination. A truism of veterinary practice is that you are more often wrong because you didn't look than because you didn't know. A standard procedure properly conducted requires the prosector to continuously search, look and be aware of what is seen.

Necropsy technique should also be designed to yield the best specimens possible for any ancillary tests that may be necessary following gross examination. The same principles apply: specimens should represent cell morphology and function as closely as possible to that which was present at the time of death. The necropsy must be conducted to avoid any exposure to water, contamination with foreign compounds, organisms or other material that could affect the findings and interpretations of further tests.

EUTHANASIA

Euthanasia is defined as "the deliberate ending of life of an animal suffering from an incurable disease" (4). Words that are used synonymously include euthenics and sacrifice. The verb for euthanasia is euthanatize, not euthanize. For a lively discussion of the correct English form, see references 5-7.

The various acceptable methods of euthanasia are reviewed and detailed elsewhere. For this discussion a few comments related to the circumstances and actions around euthanasia are relevant. The greatest paradox of veterinary medicine is that the first concern of the veterinarian should be the best possible care in the interest of the animal, yet often circumstances dictate that the best care of the herd is euthanasia of selected individuals. On the surface, this paradox appears to have its root in the greatest bane of the profession, economics. In fact, the situation is more complex. Usually there are several conflicting circumstances affecting the unwell animal or herd. The importance of each circumstance carries different weight according to the situation. The economic and sentinel value of the animal, economic situation of both owner and veterinarian, degree of suffering of the animal, emotional makeup and outlook of both owner and veterinarian, attachment of owner or some family member to the animal, quality of service offered by the practice, emotional symbolism of the animal, and many other factors bear upon the decision to euthanatize an animal. Veterinarians must be aware of these factors when assisting a client in this decision. This is more difficult than it appears, because the individuals involved may not be aware of all the relevant factors, or may turn a psychological blind eye to them. The relative demand and importance of each

factor must be judged carefully when advising an owner in order to render the best advice.

The word euthanasia is commonly used to sanitize the taking of animal life for the owner and the owner's family as well as for the veterinarian and his or her staff. The verb "to kill" is very often closer to the truth. This is very much the case in diagnostic veterinary practice when the disease problems encountered are often curable. Often the need for a high quality sample of a tissue or organ, or an untreated animal showing typical signs of a disease is a major factor in the decision to euthanatize a specific animal. In such situations, euthanasia is seldom in the interest of the animal, but is very often in the interest of the surviving members of the herd or flock.

There are many indications for killing or euthanatizing animals. Discussion here will be restricted to those most commonly encountered in diagnostic practice. These are:

1. As the best alternative to needless pain or other suffering.
2. When there is incurable illness or injury.
3. To obtain a particular type of sample.

a. a fresh tissue from a specific organ that cannot be obtained by other means.
b. an animal or bird showing typical signs of the disease process.

Once the decision to kill an animal has been made, the most important factor should be to minimize the discomfort and stress to which the animal will be subjected during the procedure. The methods of killing animals that cause the most rapid loss of consciousness with the least discomfort are considered the most humane. Safety of personnel involved in killing the animal is an important factor, especially if the animal is large, deranged, or vicious, if the area where the animal is to be killed has solid barriers to movement, an irregular floor surface or other dangerous features, or if there is some inherent danger in the method of euthanasia selected. The emotional effect of the procedure on the owner and other observers is an important, but secondary consideration to the two already mentioned, but must always be kept in mind. As a general policy, the author is of the opinion that owners should not be present when an animal is killed. If they wish assurance that the animal has been killed, they should be asked to sit in a waiting room during the procedure, and then invited to view the body when the animal is dead. This allows the veterinarian and staff to concentrate on the task, removes unnecessary pressure should the procedure not proceed smoothly, and allows time for the animal to be placed in a natural position following death prior to the owner viewing the body.

Agonal reflex respiratory movements made by large animals following barbiturate injection are often misinterpreted by owners. If an owner does wish to witness the euthanasia of an animal, a brief explanation of what is going to occur and the reactions to be expected from the animal will help to reassure the owner and pre-empt any

misinterpretation of events. Equally important is that new staff or students that are assisting or observing have the steps and events they see explained to them, especially if death of the animal is not as smooth and painless as anticipated. The veterinarian should never assume that paraveterinary staff know everything that is occurring, nor that they know how to respond in every situation. For new staff members, explanation can be given as the procedure is being carried out. This has a reassuring effect and involves them more deeply in their role. At all times, the veterinarian has to work in a calm and methodical manner, and the worse the situation becomes the calmer and more methodical he/she has to be.

Massive trauma to the central nervous system is an acceptable method of euthanasia, but carries some caveats. Captive bolt pistols produce instant unconsciousness, but can be both inhumane and dangerous in the hands of an inexperienced operator. They are of no use in older swine and bulls, both classes of animals having large frontal sinuses which prevent penetration of the brain by the bolt. These pistols cannot be used if it is necessary to properly examine the brain. A prerequisite for their use is complete physical restraint of the animal. Captive bolt pistols can be noisy when used inside concrete or steel structures. There is a role for firearms in killing animals in certain field or emergency situations. A single, well aimed shot into the brain administered by an experienced individual to a well restrained animal causes sudden death and is humane. Alternatively, a shotgun blast from short range laterally to the neck aimed such that the carotid arteries, trachea, cervical nerves and other structures are simultaneously destroyed can be an effective, humane, and safe method of killing a restrained large animal or wildlife under field or emergency conditions. However, use of firearms can become both dangerous and a nightmare of cruelty if the operator does not know the landmarks used to target the brain, misses the shot, or if the animal moves prior to discharge of the firearm. As with captive bolt pistols and for the same reasons, firearms should not be used on animals with heavy frontal bones or large frontal sinuses. Firearm use is precluded inside enclosed structures because of the possibility of ricochets.

POSTMORTEM CHANGES

Prior to examining a carcass, it is important to be familiar with the changes that may occur in the body of an animal in the periods immediately prior to, during, and following death. Such changes may give clues to the nature of the underlying disease process, but just as often may result in alterations that potentially confuse the diagnosis and interpretation of post-mortem findings and must be understood in order to properly interpret observations.

A number of changes occur with the process of dying. These include hypoxia/anoxia, responses to metabolic changes and aspiration of blood/rumen content into the lungs. Hypoxia is a common antemortem condition that is

reflected in the blood of an animal postmortem. While all blood can be thought of as hypoxic when the carcass of a dead animal is opened, close observation and experience will reveal differences in degree that allow differentiation of significant antemortem hypoxia occurring as a cause of death from hypoxia due to the endstage of other more important processes. Characteristically, when an animal is hypoxic for any length of time prior to death, the blood is dark and unclotted, even in the major vessels and heart. Blood that is adequately oxygenated at the time of death will be well clotted, especially in the heart and major vessels. In addition, in the left ventricle and aorta, it will be a distinctly brighter red than elsewhere.

Animals that have died slowly with gradually failing circulatory or respiratory systems will have associated metabolic changes that are grossly visible as varying degrees of paleness and swelling of the liver. The microscopic correlate of this change is parenchymatous degeneration of hepatocytes, a change that may be mentioned in histopathology reports received from reference laboratories.

The actual event of death is often accompanied by either aspiration or passive flow of gastric/rumen content into the larynx, trachea, or lung. Postmortem shifting of a carcass such as occurs when positioning it prior to examination, may also result in gastric/rumen content being introduced into the respiratory tract. It is important, and fairly easy, to differentiate between material entering the respiratory tract at this time from material entering the respiratory tract that is responsible for pneumonia and ultimately death. There is no tissue reaction to gastric/rumen contents entering the respiratory tract immediately at or following death. Material that enters shortly prior to death will elicit some hyperemia and fibrin exudation from mucous membranes. A much more severe inflammatory response is seen if true, active aspiration has occurred as part of the disease process that led to death, either due to accidental inhalation of ingesta, or more commonly, due to inhalation occurring as a consequence of some other process affecting laryngeal reflexes such as nervous disease or toxicosis. This is an important differentiation to make, because appreciation of gastric/rumen contents cannot occur in the presence of normal laryngeal reflexes. Aspiration is therefore an indication of either nervous disease affecting reflexes, or some systemic condition that has altered nerve function.

The process of decomposition begins at the ultrastructural level immediately following death. As circulation stops active arterial bleeding ceases. The blood that flows from cuts made at post-mortem examination is due to passive collapse of the walls of veins and arteries as their walls are incised. Blood gravitates to dependent parts of the carcass. Postmortem decomposition occurs at all times of the year in animals that die outdoors, but the rate varies with

the environmental temperature and is generally more rapid during the summer and fall. Decomposition is influenced by various factors that modify the accumulation and dissipation of heat such as thickness and type of haircoat, physiological condition of the animal at the time of death, rates of rumen fermentation and amount of body fat. The rate at which various animals decompose varies from species to species under the same environmental conditions. Insects will invade the carcasses of animals that die outdoors, and sequential changes in the populations of these occur. Such changes can be used to determine when the animal died in forensic cases (8). Some specific aspects of the post-mortem decomposition of ruminants are presented elsewhere (9)

Rigor mortis is the postmortem change that is most familiar to the veterinarian. Rigor is usually gone by 36 hours, but its speed of onset, progression and termination depend on a variety of conditions. These include physiological condition of the animal at death, amount and distribution of body fat, type and thickness of the haircoat and ambient temperature, among many others. Livor mortis is a second alteration that follows death. This is a purplish discolouration of the skin. In veterinary medicine livor mortis is seen most commonly in thin haired animals such as pigs, and will be present but not visible in animals with heavy haircoats. Dessication is a much longer term postmortem change which leads to mummification: drying and shrinking of non-durable parts.

At the cellular level, death does not necessarily occur at one defined point in time as it does with the organism as a whole. Cell death is a process which progresses through a series of stages. Different cell types commence and progress through these stages at different rates. The absolute requirement of a particular cell type for oxygen and other nutrients largely determines when the processes that ultimately lead to cell death commence, and how fast each cell will progress through these various stages. The various stages of cell death have been correlated with various microscopically observable anatomic changes. The stages and processes of cell death are excellently reviewed elsewhere (10).

IDENTIFICATION

Proper identification of an animal is required prior to commencing examination. Some species have legally required forms of identification. For example, in Canada, all domestic cattle must have a federally registered ear tag. When that animal dies, the ear tag must be reported as retired. The prosector is responsible for ensuring that this is done prior to commencing the postmortem examination.

Each species tends to have a different generally accepted method of identification. Species, breed and sex are the basic requirements for identification. Horses may have a lip tattoo, and are usually also described by

colour. Many companion species are microchipped, and microchip numbers need to be verified and recorded. In other species, specific colour patterns and marks or tags that will identify the animal should be noted. Tattoos, ear notches, wattles and other man made markings must be recorded. This latter point is very important both for identification and potential forensic purposes.

TOOLS FOR POSTMORTEM EXAMINATION

Tools needed for veterinary post-mortem examination are not expensive and are readily available, but some principles must be followed for best results. There is a common tendency to use old dull surgical instruments for postmortems. The subconscious thought is that the animal is dead and so the old, worn out equipment can be used for necropsies. This is a fallacy, as good quality, sharp instruments make the job easier and yield the best results whether in surgery or in the postmortem suite. Additional to the difficulties of working with dull and/or broken instruments there is the tissue damage and artefact caused by crushing, shearing and tearing that results from poor instruments. (11)

THE ENVIRONMENT

When a necropsy is conducted in the field, the environment in which the animal was found dead is the first item for examination. For farm animals, the type of operation, the method of feeding and the place in which the animal died are all important information. The way in which the carcass in lying and any signs of struggle or convulsions should be noted. Ruminant animals that become trapped underneath feeders or fences, or are heavily pregnant and are lying in hollows in the ground and are unable to move can bloat rapidly, and these features should be noted. Any damage to fences, possible toxic substances in the vicinity, old batteries, junk piles, etc., should be noted. When an animal has been moved from its environment and taken to a clinic or laboratory for examination, the owner should be questioned about the environment and the farm operation and this information should be recorded as part of the history prior to the necropsy examination.

EXAMINATION OF THE ANIMAL

The examination progresses from the environment to the animal itself. Animals that are presented alive should be given a clinical examination prior to euthanasia. Before any incisions are made in the carcass of an animal presented dead, an examination should be conducted from a short distance and then up close. The way in which the carcass is lying should be observed, as well as abnormalities such as blood or froth coming from the oral or nasal cavity, exudates or discharges from any body orifice, external damage such as scavenging of the carcass and any other information that may be useful such as bird droppings on the top line of the carcass. Time of death should be noted, and approximated if not definitely known. The time of necropsy should also be recorded. It is important to know

these two times for proper interpretation of autolytic changes.

From close up, the actual physical manipulation and examination of the various organ systems commences. Where practicable, the weight should be measured and recorded. The integumentary system is examined first. This is the largest organ of the body and the one which is often the least frequently examined at necropsy. The hair coat should be closely examined for general condition, matting, unusual substances, degree of roughness, unusual markings, singeing of the ends of hairs, and for the presence of ectoparasites. The feet should also be examined closely and in ruminants, particular attention should be paid to the coronary band and horn of the claws. In canines, the claws, foot pads and interdigital spaces should be closely looked at. The ends of claws of both dogs and cats should be examined particularly for evidence of splitting, entrapment of hairs, or other abnormalities suggestive of either fighting or trauma. The various body orifices should be closely examined with particular attention being paid to mucocutaneous junctions. Colours of mucous membranes should be noted. Ulcers, erosions, vesicles, and other abnormalities should be recorded. The eyes, conjunctiva, nictitating membrane, and both internal and external aspects of the ears should be closely examined. The skin, feet and nails/claws are examined, as are all body orifices.

After the initial examination of the integumentary system, the carcass can be positioned for incision. Certain mammals such as cats and rabbits have fine hair that will cling to necropsy instruments, stick to incisions and internal organs and generally obstruct examination. Therefore, prior to commencing necropsy of these species, the haircoat can be made more manageable by wetting with a mixture of liquid soap and water which serves as a wetting agent and reduces this tendency.

Ruminant animals are positioned with the left side down, while monogastrics are examined with the right side down. Ruminants are positioned this way so that the large and heavy rumen is out of the way when the abdomen is opened and the centre of balance of the animal is kept low. In contrast, monogastrics are examined right side down because more organs are visible in their natural orientation from the left body wall than from the right. Furthermore, in this orientation the spleen is directly accessible for microbiologic examination without further handling. Laboratory species and primates may be examined on their backs with their limbs pinned or tied laterally. Avians are also placed on their backs, as are most amphibians and reptiles. Fish should be examined in right lateral recumbency except those which are dorsoventrally flattened which should be examined on their backs.

The initial incision in a postmortem examination should be by made by lifting the uppermost front limb, placing the point of the knife in the axillary space, and cutting in an arc

anteriorly[2] underneath the scapula. A similar arc should be made posteriorly towards the back of the scapula. For very large animals, an assistant may be required to hold the limb while the prosector makes the cuts. The underlying connective tissue, fascia, pectoral and serratus muscles are severed, allowing the limb to be reflected dorsally at right angles to the carcass. A similar incision then should be made medial to the uppermost hind limb. The adductor and the other medial muscles are severed, followed by the capsule of the coxofemoral joint and the teres ligament. The hind leg is reflected dorsally at right angles to lie over the back of the carcass. A third incision follows, extending from the mandibular symphysis caudally along the ventral midline to the pubic symphysis. This incision should pass just lateral to the external genitalia in males, and to the mammary gland in those species in which it is large (cow, horse). The uppermost side of the carcass and the entire intermandibular space is then skinned. The skin is reflected on the dorsal side to the midline along the entire thorax and abdomen and from the ventral side of the incision to the surface on which the carcass is lying. The skin must be reflected far enough that the underlying musculature and connective tissue on each side of the incision can be clearly seen.

[2] In this discussion the terms dorsal, ventral, anterior and posterior are used with respect to the animal's normal position in life, and not its position on the postmortem table.

At this point, with three incisions, the prosector is able to continue examination of the integumentary system and commence examination of the musculoskeletal and reproductive systems. The suppleness of the skin and subcutaneous tissues give an estimate of the degree of hydration. The subcutaneous muscles are available for an appreciation of the general condition of the musculature and the amount and quality of subcutaneous fat reserves. If penetrating wounds are anticipated, then the entire carcass should be skinned and the skin examined from the subcutaneous side. Penetrating wounds are usually more easily seen from the subcutaneous side as they are not hidden by the hair coat and attention may be drawn to them by subcutaneous hemorrhage or trauma.

The coxofemoral joint of the uppermost hind limb has been completely opened for examination and any abnormalities in joint fluid, ligaments, capsule, articular surfaces and underlying bone can be immediately assessed. The mammary glands of female animals and the prepuce, penis, scrotum and testes of males may also be examined at this point.

The examination continues with removal of the abdominal and thoracic wall of the uppermost side. An incision is made immediately behind the xiphoid cartilage. With the cutting edge of the knife directed caudally along the linea alba and the point of the knife inside the abdominal cavity protected by the fingers of the prosector, the linea alba is incised back to the pubic symphysis. The incision is then completed by incising the

full thickness of the abdominal wall along the costal arch to the vertebral column, and carrying the incision from that point caudally along the dorsolateral side of the abdominal cavity immediately ventral to the transverse processes of the lumbar vertebral bodies. The incision is then curved ventrally to join with the incision on the midline at the pubic symphysis. In this way the abdominal wall is removed in a roughly triangular piece, and the organs of the abdominal cavity should be completely visible.

The diaphragm is punctured with the point of the knife immediately underneath the costal arch and observed to ensure that it collapses with an inrush of air to the thoracic cavity. This indicates a normal negative intrathoracic pressure at the time of death. If this does not occur, it is an indication that there may have been increased intrathoracic pressure. The diaphragm is then incised immediately along the costal arch from the midline to its dorsal attachments. The ribs are cut at or near their dorsal articulations with the vertebral column, and the sternum is cut along the ventral midline. In large or heavily muscled animals, a preliminary incision should be made with a knife through the muscles overlying the ribs. In this way, the direction of the incision can be marked for the rib cutters and muscle and connective tissue which might obstruct the rib cutter jaws can be cleared away. The knife is then used to remove any remaining attachments and the thoracic wall removed. In order to make removal of the thoracic wall easier, a handhold can be cut by making a 20 cm incision between any two ribs of the caudal region of the thoracic wall. The prosector can grasp the ribs through this incision with one hand while either cutting soft tissues with the other, or holding the rib cage for an assistant to cut ribs with the cutters.

The contents of the thorax and abdomen are now available for examination, yet have not been disturbed and should be in the position which they occupied at the time of death. At this point, serosal surfaces are examined, and organs evaluated for their correct anatomic position and for any grossly visible abnormalities in structure and/or pathologic processes.

EXAMINATION OF INTERNAL ORGANS

Following opening of the carcass and exposure of all the internal organs, the examination enters a phase in which individual organs and organ systems will be removed and subjected to detailed examination. To commence this phase, the tongue, larynx, trachea and esophagus are removed back to the thoracic inlet. The tongue is loosened by placing the blade of the knife in the intermandibular space immediately medial to the mandible with the blade of the knife pointing caudally and the back of the blade against the mandibular symphysis. The knife is then pushed dorsally through the musculature and connective tissue into the oral cavity and an incision made caudally towards the larynx. A similar incision is made on the other side of the tongue. At this point, the prosector

should reach through the uppermost incision, grasp the tip of the tongue, turn it around, and pull it ventrally through the incision. This manipulation places the tongue in a position to provide a useful handhold for removal of the trachea, lungs and heart.

That part of the tongue which is ventrally protruding is grasped with one hand and the glossal frenulum and remaining attachments of the tongue are severed with the knife in the other. The larynx is removed by grasping the tongue firmly and inserting the knife blade medial to the stylohyoid bone on one side of the larynx with the cutting edge aligned ventrally. A slight ventral movement, turning the blade laterally at the same time will result in the cutting edge sliding along the medial aspect of the stylohyoid bone and out through the cartilaginous joint between the stylohyoid and the basihyoid bones. This procedure is then repeated on the other side and the larynx comes free of the neck. This latter movement is probably the most difficult of the entire necropsy procedure to learn, but once mastered, can be done very quickly and easily. The trachea and esophagus are dissected loose from the connective tissues of the neck back to the thoracic inlet.

Up to this point, no organ systems have been removed. The prosector should pause and examine the carcass with the thoracic and abdominal organs in place. The pleural surfaces, not only of the lungs but also of the reflected thoracic wall, should be examined for hemorrhage, adhesion, fibrin or other pathologic changes. The pericardium is examined from its external surface, and then incised and its contents and the epicardium are examined. The pericardium is examined at this point in the procedure because in some animals it is difficult to remove the pericardium without damage. The colour and pattern of vascular congestion of visible organs should be noted as well as the location, colour, turbidity and consistency of any fluids (i.e. pericardial sac, abdominal cavity, etc). The anatomic relationship of all organs and especially those of the abdomen should be noted, the degree of distention, congestion, enlargement or contraction from normal size, etc. should be noted. This is especially important in interpreting whether displacement has occurred, if it occurred pre or postmortem, and if a possibly previously displaced organ has returned to its normal location following death.

If abnormal fluids or exudates are present, samples should be collected at this time if required for microbiology or cytology. In addition, if there is any suspicion of disease of the cystic organs (gall bladder and urinary bladder) or if pathologic cysts are present samples can be taken by centesis before there is any further manipulation of the carcass with the attendant risk of contamination.

Removal of individual organ systems now commences. The loosened trachea and esophagus can be grasped and the knife used to cut the dorsal pleural reflections. The ventral attachments of the pericardium to the sternum and caudal diaphragmatic

aspects of the mediastinum, the vena cava and esophagus where they penetrate the diaphragm may be severed. The structures thus moved are set aside for more detailed examination at a later time.

Removal of the abdominal organs commences with the spleen of monogastric animals where it is present on the uppermost side. Ideally the gastrointestinal tract is divided into segments with dual ligatures at each intended point of incision and each section removed in turn. Ligation prevents spillage of gastrointestinal contents and bacterial contamination of other organs. The segments are divided between the ligatures by cutting. In practice, this is a time consuming procedure and sections of the gastrointestinal tract are usually removed without being tied. However, if ligatures are not used, care should be taken to hold the severed ends in such a way that spillage of contents is minimized. Ligation of the gut prior to cross sectioning and removal is essential when contamination must be avoided, and advisable when gut samples are required for virology, bacteriology or other procedures.

The esophagus of smaller monogastric animals can be tied off where it enters the stomach, the lower bowel tied off at the rectum, and the entire gastrointestinal tract removed as a unit and set aside for detailed examination later. For larger monogastric and ruminant animals, the tract can be divided into sections as follows: esophagus to duodenum; duodenum to ileocolic valve; ileocolic valve to rectum. Each segment is tied and removed. Working from posterior to anterior is easier with most species as this sequence removes the colon first in order to better expose the intestines and stomach. The intestine should be cut from the mesentery along the mesenteric border as it is removed. In this way, the intestine becomes straightened and the mesentery still attached to the animal serves as an anchor for one end of the intestine, making removal easier. In ruminant animals, because of the way they are positioned, the small intestine will disappear under the large intestine at the duodenojejunal flexure. At this point, the colon should be reflected dorsally to expose the duodenum. The ascending duodenum is then cut as far as its caudal flexure where it again disappears under the large intestine. The colon should be reflected to its normal position and the descending duodenum cut from the mesentery to its junction with the abomasum (a ligation point).

The pancreas is located in the mesentery between the descending and the ascending duodenum, and can be either removed by itself at this point in the procedure, or can be left attached to the duodenum. The latter approach permits sections to be trimmed for histopathology with duodenum attached, allowing clear identification of duodenum on microscope slides.

The abomasum and fore stomachs of ruminants are removed by pulling the exposed side ventrally and cutting the dorsal attachments. As this unit rolls out

towards the prosector, the spleen will become exposed and should be immediately separated and removed from the gastrointestinal organs to prevent contamination. The ruminant forestomachs and abomasum are removed with blunt dissection of ligaments between the abomasum, omasum and rumen. A point of technique is important here. While a knife is indispensible for proper postmortem examination, it can at times cut across normal anatomic boundaries, damaging tissues, and compromising the principles of technique elucidated above. Certain organs tend to separate more easily along normal anatomic boundaries when dissected bluntly than when cut. For those areas of the body in which careful application of blunt dissection can be used to good effect, the prosector should not hesitate to use it. Removal of the ruminant fore stomachs and abomasum is one procedure in which blunt dissection works extremely well. If the ruminant is a large one, and is on a postmortem table, then gravity may be used to good advantage to assist in removal. The dorsal ligaments of the rumen are torn bluntly. Application of traction to the rumen allows the the dorsal sac to be pulled out and towards the prosector. This allows the rumen to roll freely out of the abdominal cavity. The remaining attachments can be torn or cut with a knife as appropriate, and the forestomachs and abomasum will roll out with the rumen at the foot of the prosector. These are now placed aside for detailed examination a little later in the procedure.

The liver is easily removed at this point by incising the anterior connections with the diaphragm and the various lateral ligaments. The liver should be placed on a table and examined in detail. The vascular system can be examined by incising the vena cava and following its tributary veins back into each lobe. Similarly, the biliary system should be incised by following its tributaries backwards from the common bile duct. Finally, the parenchyma should be examined by placing the liver caudal side down on the table and incising it transversely, holding the knife blade parallel to the table. Such a cut allows complete reconstruction of the liver with examination of more parenchyma with a single cut than does the occasionally employed technique of dicing the parenchyma.

Prior to removal of the urogenital organs, the pelvic floor must be removed. This is most easily accomplished by cutting the musculature off the ventral side of the pelvis with a knife. The pubic symphysis in smaller animals can be split and the pelvis spread. In larger animals, a meat saw, Stryker saw, or bone cutters can be used to make cuts in the bone, the selection of tool depending upon the size of the animal being dissected. One cut is made on each side of the pelvis. Each cut extends from the pelvic brim to the obturator foramen of that side and from there through the ischium. These cuts should be kept as lateral as possible to allow the greatest exposure of pelvic

organs. After making these cuts the internal connective tissues of the ventral pelvis are incised, and the resulting plate of bone, which includes the complete pubic symphysis, is removed.

The urinary tract is removed as a unit by reflecting ventrally and dissecting the adrenals, kidneys and bladder free of their support ligaments, connective tissues and surrounding fat. The kidneys are often separated with less damage by blunt dissection than by using a knife. The organs are examined following removal by incising the ureters from the renal pelvices distally to the bladder. The uppermost adrenal gland and uppermost kidney should be incised longitudinally in situ and then each removed separately. The other adrenal and kidney should be incised transversely, examined and removed. The purpose of longitudinal and transverse incision is to indicate which came from the right side, and which from the left side once they are removed from the carcass. Further incisions are of course indicated to look at specific features or lesions but if the main incision, whether longitudinal or transverse, is maintained when trimming tissues for histology, the pathologist can rapidly tell from which side of the body each organ section originated.

In female animals, the genital tract is removed by dissecting the ovaries and uterus back towards the vagina, leaving the bladder attached and cutting the perineal skin in a circular fashion to allow removal of the vagina and the mucocutaneous junction with the other genital structures attached. The rectum is also removed with the urogenital organs in the same way. A circular incision in the perineal skin that encompasses the rectum and vagina allows the pelvic organs to be removed as a unit. For male animals, For male animals, the adrenals, urinary tract and rectum may be reflected and disected as in females. The perineal incision should be extended ventrally to include the scrotum, and then cranially to include the penis.

Following removal, the female reproductive tract is examined by sectioning the ovaries and then incising from the tip of each uterine horn distally into the body of the uterus and from there to the vagina. By incising these tracts in this direction, assuming proper cleanliness, the risk of introducing bacteria from lower in either the urinary or reproductive tract higher into the same tract with a knife or scissors is reduced.

EXAMINATION OF INDIVIDUAL ORGAN SYSTEMS

Removal of the main visceral organs from the carcass and examination of each is now complete. The next procedures to be performed are detailed examinations of the carcass as it remains and each of the organ systems that have been removed. First, the carcass is examined with particular attention being paid to the remaining musculature, now readily accessible. The bones are assessed for size, shape and strength. The femur is removed and split on a band saw, if available, allowing evaluation of bone marrow and the volume of medullary versus cortical bone and the

gross structure and strength of each. This examination can be facilitated by washing the bone marrow out of the medulla using water under pressure. Surfaces of several joints should be assessed following incision of the joint capsules. Costochondral junctions are also evaluated, with removal and sectioning of two or three representative ones on a band saw.

The head is removed from the carcass at this point. An incision is made into the vertebral canal at the atlanto-occipital joint. Dorsal flexion of the atlanto occipital joint will make it easier to cut the joint capsule and the skin on either side of the neck. As the joint is penetrated, the cerebrospinal fluid (CSF) which is released can be collected for examination. If CSF culture is required, the fluid should be collected with a needle prior to penetration of the joint capsule with the knife. Even if CSF is not collected, it should be evaluated for transparency, presence of fibrin, and whether it appears to be under pressure as it flows out upon incision of the meninges. The incision started prior to penetration of the atlanto-occipital joint is carried around the neck to include the skin and musculature on the dorsal side of the atlanto-occipital joint. The spinal cord is severed, the remaining attachments of the atlanto-occipital joint cut, and the head is then free and the brain may be removed. Removal of the brain of large species requires equipment that is not available in the field. Therefore, the intact head is transported back to the clinic or forwarded to a diagnostic lab for removal of the brain.

The brain can be taken out intact relatively easily. The dorsal head should be skinned, the skin reflected back to below the ears and the masseter muscles removed. The head is then secured, the method employed depending upon its size. The older the animal, the thicker the cranial bones that must be cut. If an animal has horns or antlers, it is best to cut these off below their base, i.e. remove the supportive bone into the frontal sinuses. The frontal sinuses of large animals enlarge with age, and therefore older animals will have two distinct plates of bone, separated by a sinus space, that must be cut through before the brain can be exposed. The brain is exposed by removing the dorsal cranial vault. There are three incisions required to do this, and some experience is needed to make the necessary cuts deep enough to allow the cap of the cranial vault to be removed without damaging the underlying brain tissue. The first cut is transverse, crossing the skull along a line drawn between the posterior commissure of each eye. The second incision is from the dorsal part of the right occipital condyle laterally around the poll region and anteriorly from this point to intersect with the first cut immediately medial to the eye. A similar cut is made on the other side of the head. (Figures 1 - 4). In very small species the head can be held in the prosector’s hand and scissors (mice) or orthopaedic bone cutters (rats, gerbils) will be adequate to make the necessary cuts. In medium sized species, the heads

may also be held in or steadied by one hand of the prosector while the cuts are made with the other hand using a hack saw or a Stryker saw. Stryker saws create an aerosol of bone particles, and so either a mask should be worn when using such a saw in pathologic work, or the work should be conducted in a biological safety cabinet. A Stryker saw should never be used to open the cranial vault of an animal that might have nervous disease transmissible to humans or on rabies suspect cases. The heads of large species should be secured in a head vise and a commercial butcher's hand saw used to make the cuts. Upon completion of the necessary cuts, the cap of the cranial vault may be pried dorsally with a mason's chisel or large screw driver and the brain revealed. In very large animals, a hammer may be needed as a persuader for the chisel.

Notwithstanding the foregoing, the fastest and easiest way to remove the brain is to make a mid-saggital section of the head with a band saw. Each half of the brain may then be removed from the exposed cranial vault. This procedure should be used for only gross screening of the brain for unexpected lesions. It should never be used if the history or other evidence suggests nervous disease. Neither should it be used in young carnivores which have very friable brain tissue. Histology sections taken from areas adjacent to the cut will contain a slurry of bone chips, nervous tissue and other debris from the saw blade. Therefore, use of the bandsaw is also contraindicated if histology is to be performed on structures located on or near the longitudinal midline of the brain.

The meninges are examined and then removed by cutting with scissors. The falx cerebri and tentorium cerebellum must also be cut or the brain will be torn when removed. The underside cap of the cranial vault that is removed should be closely observed, as should the meningeal surfaces and the general shape of the brain and its adnexal structures. The head is then turned upside down and held so that the palm of one hand is located immediately under the brain. Removal of the brain is accomplished by cutting the cranial nerves and meninges, working anteriorly along the base of the brain from the foramen magnum. Once the brain has been loosened from its base, it will fall out into the palm of the prosector. The brain itself and structures of the skull may then be closely examined. The pituitary gland and eyes should be examined in situ and the eyes removed using the surgical procedure for eye enucleation (12). The remaining structures of the head including the teeth, mandibles, temporomandibular joints, pharynx, nasal cavity, nares and bones making up the skull should all be examined.

Attention is now returned to the heart and lungs. These should be placed on the table with the trachea and tongue facing the prosector. The tongue is examined and an incision made full

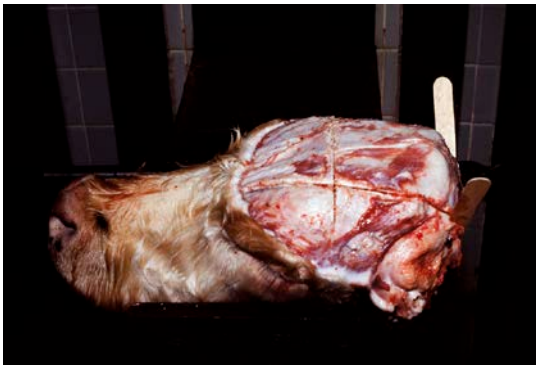


Figure 1: Lateral view of the head of a calf demonstrating the location of cuts needed to remove the brain. The location of these cuts is similar in all species with minor modification depending upon the shape of the cranium.

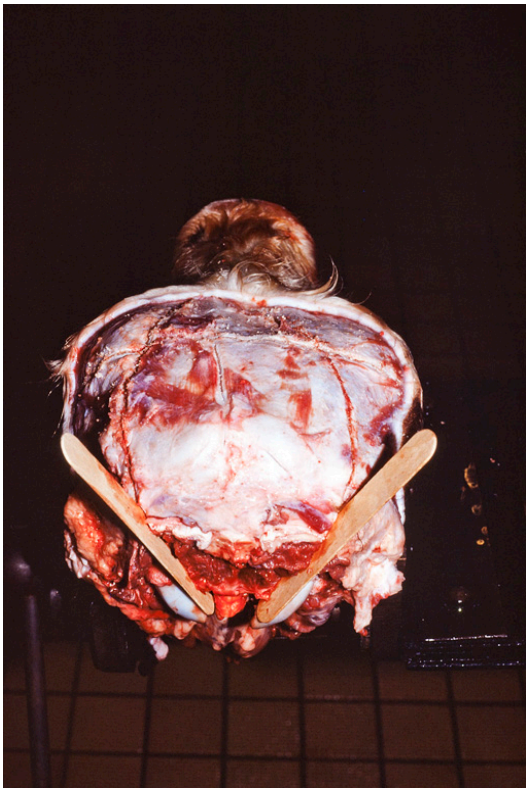


Figure 2: Caudal view of calf head in Figure 1

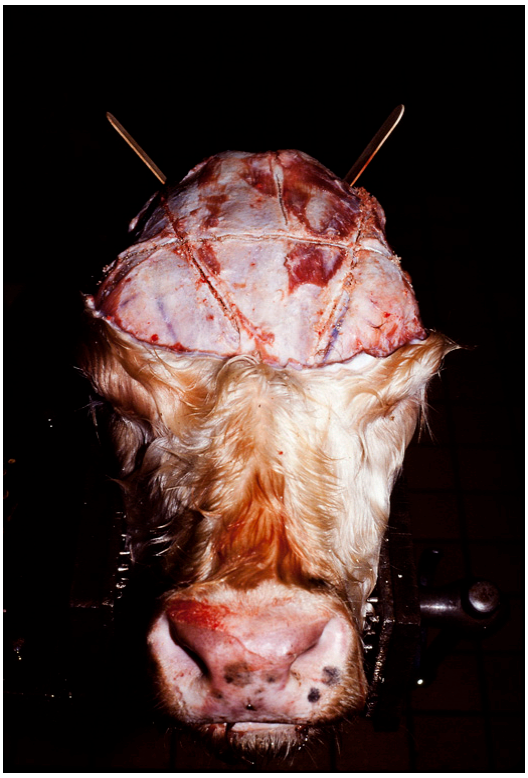


Figure 3: Frontal view of calf head in Figure 1

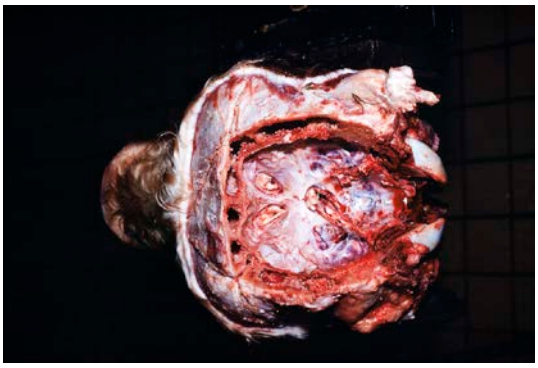


Figure 4: Caudal view of the calf head with cranial cap and brain removed.

thickness along the midline and another transversely across the thickest muscle at the base of the tongue. The mucosa and muscle should be closely examined. The lesions of selenium deficiency myopathy

in young ruminants are often seen at the base of the tongue and may not be observed elsewhere in the carcass. The structures of the ventral pharyngeal region including the tonsils should be examined and the esophagus incised and examined along its entire length. The trachea is incised from the dorsal larynx down to the bifurcation of the bronchi. The thyroid and parathyroid glands are examined. The laryngeal mucosa, cartilages, and content of these structures may all be examined. The incision of the trachea is then continued into each main bronchus and from each of these into secondary and tertiary bronchi in two or three areas of lung. Further incision of the lung can be made according to the discretion of the prosector. Following examination of the lung, attention is turned to the heart. (NOTE: Heart and lungs are kept as a unit, not separated). The pericardial and epicardial serosal surfaces are again examined as well as the veins and arteries visible underneath the epicardial surface. The heart is incised following the direction of blood flow through the organ. A cut is made into the right atrium adjacent to the septum and extended in two directions, into the vena cava and then by turning the knife 180^{o} through the atrioventricular valves into the right ventricle, still maintaining the knife adjacent to the septum. The ventricle is incised down to the apex and then from this point into the pulmonary artery up to its bifurcation. The atrioventricular and pulmonary valves, the interventricular and interatrial septae and the endocardial surfaces should be examined at this point. A cut is then made into the left atrium also adjacent to the septum and, continuing along the septum, extended through the left atrioventricular valves to the apex of the heart and then around to the base of the heart and along the entire length of the aorta. The structures of the left side are examined, as well as the papillary muscles and myocardium. The entire aorta and the brachiocephalic trunk are incised and the great vessels of the heart and their branches, including the coronary artery, examined in detail.

Examination proceeds to the alimentary system. In monogastrics, open the stomach following a line from the esophagus around the greater curvature to the pylorus. Gastric contents should be examined as well as the anatomic structures of the gastric wall. Is the content consistent with what is indicated on the history as being fed? Are there any unusual constituents present?

The ruminant forestomachs and abomasum, removed previously, are then laid out with the rumen on the left and the abomasum on the right. An incision is made through the esophageal opening of the rumen distally to the tip of the dorsal sac. A second incision is made from the end of the ventral sac to intersect the first incision immediately above the ruminal fold. A third incision is made from the first incision directly ventral into the reticulum. The omasum is incised in a plane transverse to the direction of the omasal folds and an incision made from this point into the abomasum around the greater curvature and out the pylorus. In

this way, the entire forestomachs and rumen have been opened in a manner which has resulted in complete exposure and yet that allows them to be reconstructed.

The contents should be closely examined and evaluated. In ruminants, pieces of toxic plants which are large enough to allow identification of the plant are often present. The smell of the contents of the forestomachs and abomasum should be noted and the pH of each area measured if the content appears in any way abnormal. A number of pH sticks are available which can be used to determine the pH of heavily contaminated materials. These can be rinsed in water without in any way compromising the reading of pH.

The anterior duodenum is opened longitudinally and the ampulla of Vater examined. In those species having a gall bladder, bile should be expressed from the gall bladder to determine patency of the common bile duct. Following this, the rest of the gastrointestinal tract from the duodenum to the rectum is separated from its mesentery allowing it to be laid out in long loops and incised. In smaller species, it can be incised from one end to the other quite readily. In animals such as cattle and horses with longer intestinal tracts and large cecae and/or colons, the entire tract can be removed as a unit, or it can be removed in sections: intestine to ileocecocolic junction, spiral colon, cecum and distal colon to rectum. Correspondingly, the entire gut can be opened, or representative sections of each area may be opened at the discretion of the prosector. Mesenteric lymph nodes, vessels and lymphatics should be evaluated and sectioned. The cecum, colon and rectum should also be incised following the normal flow of ingesta to the rectum.

The mammary gland should be examined at this point by opening each teat through the orifice and extending the incision through the teat canal into the glandular tissue proper.

Removal of the spinal cord is a difficult and time consuming procedure, especially in large species. In the absence of a history or other evidence of either central nervous or spinal cord disease it is not usually necessary to remove the entire spinal cord during postmortem examination. Sections of cervical, thoracic and lumbar cord can be easily obtained during routine postmortem by transecting a 2 cm width of the vertebral column with a meat saw or a band saw at each of these locations and removing the section of cord contained in the vertebral canal at this point. When detailed examination of the spinal cord is necessary, a band saw and/or other equipment and advanced techniques are required and must be employed by a skilled prosector. This is best done at a referral lab by a person skilled in removing spinal cords intact.

AVIAN POSTMORTEMS

The approach to an avian postmortem is similar to that for mammals. Flock diagnosis is very often the prime goal of an avian postmortem and often a specific diagnosis on an

individual bird is of little value if it is not representative of the flock. Therefore, in commercial poultry situations, several birds are often examined at one time, or sequentially to arrive at a flock diagnosis. Expected flock standards of performance are readily available for domestic poultry, and it is often necessary to compare individual birds to this standard to determine if there is a problem, and how serious it is. To perform and properly interpret results of a postmortem on birds from a commercial flock, the history is critical. Clinical signs are an important but often overlooked factor in diagnosis of disease in commercial poultry. As commercial poultry may often be submitted alive, an opportunity for a clinical examination is afforded in the clinic or diagnostic laboratory. The attitude and behaviour of the birds should be observed, and to do this they can be placed free on a table or the floor of the necropsy room. One of the biggest confounding factors in poultry diagnosis is that the birds submitted may not be representative of the disease process that is occurring. An owner may collect the easiest birds to catch, which may be the lame ones or culls rather than the sick ones. Another common mistake is to submit live birds when the problem is actually death losses. Therefore, the initial observations made on birds at postmortem should be compared to the complaint for which the birds have been submitted to assess whether they are representative of the problem.

The same principles given above for mammalian postmortems apply to avian postmortems. Blood samples should be collected and held prior to euthanizing a live bird in case the postmortem indicates that serology or a CBC is necessary. Feathers and down from avians will cling to necropsy instruments and stick to incisions and internal organs, interfering with examination. Therefore, prior to commencing an avian necropsy, the feathers should be washed with a mixture of water and liquid soap to dampen the feathers and reduce this tendency. As with mammals, the skin surface should be examined first, starting from the head. The beak, mouth, nares, eyes, comb, wattles, sear, ears, skin and feathers, wings, vent region, legs and feet should all be examined. Avians are placed on their backs and the body cavity is opened by making a midline incision from the beak to the vent and peeling back the skin. The coxofemoral joints are disarticulated and examined. This assists to stabilize the bird on its back for ease of examination. The abdominal wall is incised along the entire costal arch and then caudally to the ischium and medially across the midline so that the abdominal musculature is removed and the abdominal contents revealed. The pectoral muscles are incised down to the sternum on each side and examined. The ribs and pectoral bones are cut on each side in such a way as to allow complete removal of the entire sternum. At this point, the internal organs are

examined in situ with particular attention paid to the air sacs. The latter will be permanently damaged by manipulations to the carcass from this point on. The brachial plexi and vagus nerve are examined. One or both metatarsal bones are broken manually as a means of assessing bone strength and mineralization. An incision is made through the epiphysis of the proximal tibia into the tibiotarsal joint allowing examination of the bone, growth plate, and interior of the joint. The sciatic nerves are exposed and the mid-shaft of the femur cracked spirally to expose the bone marrow and medullary bone. The footpad is incised and examined.

The beak is cut at the comissures and the lower jaw, tongue, larynx trachea and esophagus with crop are removed in the same way as for a mammal. The thymus, heart and lungs are removed with the trachea and esophagus, although it is much more difficult in an avian to remove the lungs intact as they are deeply inserted into the dorsal thoracic wall and ribs. The esophagus and crop, the trachea and lungs and the heart are all examined in turn exactly as described previously for mammals.

The intestinal tract is removed by severing the lower bowel at the cloaca and dissecting forward along the mesentery. Transect it just before the proventriculus. The spleen is removed and then the liver may be taken out, followed by the gastrointestinal tract. The intestine and cecae are then opened along their entire length with scissors. The Bursa of Fabricius and the cloaca are opened and examined. Attention is now turned to the reproductive tract which in females is opened along its length. The kidneys and adrenals are examined.

Returning to the head, cut the upper beak transversely behind the nares and examine the nasal cavity and sinuses. The head is then disarticulated at the atlanto-occipital joint and the brain removed using the same markers as for a mammal.

USE OF REFERRAL LABORATORIES

A number of changes in ownership and organization of veterinary diagnostic laboratories have occurred in Canada over the past two decades. These changes are similar to but perhaps more advanced than changes that have occurred elsewhere in the world over the same time period. The upshot of such change is that veterinary clinicians in private practice are sending more specimens of a wider variety to reference laboratories that are at greater distances from their clinics than previously.

This section of the manual discusses submission to and the use of a reference laboratory in veterinary practice. The object is to assist practitioners to obtain higher quality and more useful laboratory results. This discussion centres on “canned” animals, i.e. a combination of fresh and fixed tissues as well as blood, serum, and/or exudates for general investigation of a problem. Such samples can be intended for a wide variety of tests, usually at the discretion of the pathologist handling the case in consultation with the practitioner.

This is the type of material most commonly submitted by food production and equine practitioners. While such submissions are also made by companion animal and other practitioners, particularly from postmortem materials, the more common specimens from such practices are blood samples and biopsies.

There are several reasons for conducting tests additional to the gross postmortem:

1. to confirm or deny that the pathologic processes thought to be present at gross postmortem are indeed present.
2. to identify additional pathophysiologic processes that may be occurring.
3. to determine the etiologic agents present and to assess their relative significance both to the individual and to others when in a herd.

In some situations, particularly in large animal practice, the results of ancillary tests may lead to a need for additional testing, depending upon what is or is not revealed. The prosector must anticipate what tissues might eventually be required for further testing and preserve those that are not immediately needed into a freezer, refrigerator, or fixative as appropriate for possible future retrieval. It is far easier to obtain tissues at the time of postmortem and hold them than to regret not doing so after disposal of the carcass.

HOW TO USE A REFERRAL LABORATORY

A diagnostic laboratory is an ancillary arm of clinical practice. By analogy, it can be considered a tool to be employed in the practice of veterinary medicine in the same way as an anesthetic machine or computer. Like any tool, the laboratory has its strengths and weaknesses, can be frustrating, and gives better results to those who are experienced with it. A laboratory needs proper data input if the results that come out after processing are to have practical meaning or use. Therefore, if laboratory results are consistently frustrating or seem not to be helpful for the problem at hand, the first thing to check is whether the specimen or information input to the laboratory is appropriate. This is best accomplished by direct and frank discussion with the pathologists who are handling the daily case load in the referral laboratory (13). The job of the diagnostic laboratory is not to provide tests. It is to provide useful, relevant and applicable information based upon accurate test results, and to interpret that information as required. The laboratory test is one step in performing the job, not the job itself.

Most laboratories have hard copy and/or online specimen submission manuals. If more information is needed, a phone call or e-mail to the laboratory prior to submitting the animal or samples will save a lot of grief. A phone call will allow the clinician to express particular concern about the case which will increase its significance to the pathologist. This will also allow the laboratory time to ensure that any

necessary special resources will be available on arrival of the sample. A preliminary phone call will permit the pathologist to consult any relevant literature and if necessary, arrange additional tests with other laboratories prior to handling the case. This is especially true of particularly specialized tests which may require additional consultation.

THE HISTORY

The importance of the history in a referral case cannot be overestimated. The laboratory diagnostician, not having firsthand knowledge of the animal, is totally at the mercy of the person completing the history for information. Most referral laboratories provide history forms, and many have them available on line.

A good history has a number of characteristics. The history should provide direction to the necropsy. It provides the diagnostic pathologist with a verbal picture of both the environment and of the animal in question. It poses questions for which the practitioner and/or animal owner require answers. The history must identify the animal(s) in question and ties samples to that animal. Such basic information as age, breed, sex and the number of animals at risk as well as the number affected are all important as they imply certain types and groupings of disease differentials while ruling out others. For large animal submissions, information about the environment from which the animal came should be included.

The history should be completed legibly. A succinct description of the environment/ farm/residence and clinical signs of the animal in question should be provided. The animal should be clearly identified by microchips, ear tags, tattoos and/or markings. The reason for the submission as well as the number and type of samples submitted and whether they are fixed, frozen or fresh should be clearly stated. A list of possible differentials from the clinician's standpoint is very useful because it allows the pathologist to follow the clinician's reasoning about the case. Laboratory tests are selected and initiated based upon clues about the problem presented in the history. Some extremely sophisticated tests are available in the modern diagnostic laboratory, but sophistication applied in the wrong direction is useless.

Histories in cases for toxicology cases are particularly important. Toxicologic testing is expensive and can be time consuming. Therefore the history in such cases should be as precise as possible in describing the clinical signs, and as specific as possible about the compound(s) suspected. If it is not possible to suggest a specific compound, the clinical signs can be used to suggest a general class of compounds which might be responsible.

It is important to note whether and when an animal was euthanatised and by what method. Failure to include this latter information has on more than one occasion caused misdiagnoses in large animal cases. Nothing is more

annoying than for an owner to receive a report stating that an animal died of a gunshot wound when the animal was shot by the owner and submitted for toxicologic studies. The opposite has also occurred with an incomplete history: the wounds of animals that have been maliciously shot have been ignored because the pathologist assumed that the wounds were inflicted by the owner to euthanatize the animals (mea culpa). A clear statement about euthanasia and the reasons for submission of the animal will avoid unwarranted assumptions and misunderstanding on the part of client, clinician and pathologist.

As much information as possible should be given. It is better to provide too much information rather than too little. However, despite the foregoing, *the history does not have to be lengthy to be complete*. It should be concise yet comprehensive. Often two or three well composed lines are all that is needed, even in a complex case.

While a case can be made that in certain situations the history should not be read until after the necropsy is completed in order not to bias the pathologist, in most diagnostic situations the pathologist should be familiar with the history prior to commencing examination of the animal. It should be consulted at every stage in the process of postmortem examination and interpretation of results. Investigation of a disease is a dynamic event, and especially in herd investigations, the history may need to be added to as the case evolves.

As most practices use computerized record keeping systems, there is a temptation for a practice to simply run off the entire clinical record of an animal and send that with laboratory specimens as a history. Such temptations should be resisted, as the sheer mass of information in such records may obscure the issue. The diagnostician works much better when directed by a few short sentences that detail the specifics of the problem that necessitate laboratory input, concluded by one or more questions that the clinician would like answered by the laboratory.

Occasionally there are situations in which there is information that is relevant to the history but that the practitioner does not want to commit to writing. Such situations are difficult because ethical considerations require a complete written history. The medical record on any animal belongs to the owner and in legal cases, complaints made by owners against veterinarians and similar situations information that the clinician would not like the owner to see may come to light. It is prudent to discuss in person or via the telephone any case that is complicated or which might end up in some type of legal process or complaint with the pathologist handling the case. A note must be made in the clinical record of the call and its content. The best guideline is that all relevant information should be on the clinical record or laboratory report and anything extra which the clinician does not want known

to the client should remain unwritten and unspoken. It should be noted that there are some situations in which the veterinarian might wish to enter certain information into the clinical record precisely because it would come to light in a complaint or legal proceeding. For example, such items as accounts that are chronically unpaid or relevant notes about the client's apparent state of mind related to the case (14), although uncomplimentary to the client, might be important to enter into the record.

The history should be forwarded to the laboratory with the samples. Enclosing the history in a waterproof sleeve or sealable plastic bag physically attached to the specimen container is advisable. Waterproof protection for the history form is necessary because leakage of body fluids and fixatives can occur in transit causing damage and in extreme cases, destruction of the history form (Figure 5). Contaminated history forms that may be handled by several individuals at the receiving laboratory are fomites and can play a role in the spread of infectious and zoonotic disease as well as allergens. Samples forwarded with the history should be identified on the history form, and the containers in which they are held should be marked.

As a rule of thumb, the amount of extra time and effort put into a case by a laboratory will often be determined by the amount of effort that has been put into the history and preparation of the accompanying samples.

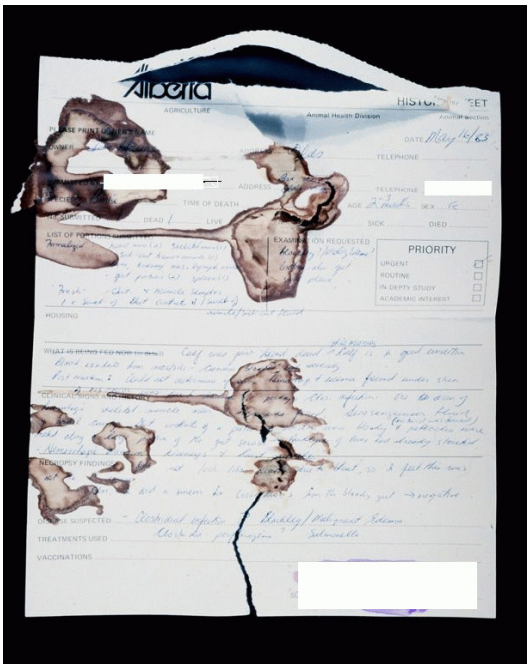


Figure 5: Protection of the history from damage by tissue fluids is necessary from the standpoint of public health, legibility and esthetics. Leakage of body fluids from the specimen to the history form during shipment resulted in damage in this instance. The history is best packaged in a plastic sleeve or bag to prevent damage should bodily fluids leak from it.

GENERAL ISSUES RELATED TO SPECIMENS

The better and more appropriate for testing the specimen that is received by the laboratory, the more useful will be the report the clinician receives. Therefore, when very specific types of samples are required for certain tests, visiting the laboratory website, consulting the laboratory manual, or placing a phone call to the laboratory to obtain information on the types of samples needed and how they should be preserved is time well invested. This is

particularly relevant when the clinician is performing an in clinic postmortem examination and the entire carcass is available from which to select samples. It should be noted that the types of samples, methods of preservation and protocols used for the same tests can differ between laboratories. A caution: samples required for various tests listed in standard reference textbooks should be used only as a general guideline since testing emphasis, priorities, expertise and technology vary between institutions and locations, and may differ significantly from textbook information. Textbook information often lags technological innovation, and newer, faster, more accurate tests become available more quickly than the cycle time of the average clinical textbook. Time spent collecting and preparing a good set of specimens will be rewarded in the usefulness of the report received.

In any but the most straightforward of cases, a preliminary telephone call to the laboratory is advisable. A call enables the clinician to express particular concern about the case, including potential legal aspects and client concerns and allows the laboratory to ensure that staff and necessary special resources are available when the specimen arrives.

SPECIMENS FOR HISTOLOGY AND IMMUNOPEROXIDASE TECHNIQUES

The principle of preparation of tissues for histopathologic examination is to try to have the fixed, processed and stained specimen on the slide reflect as closely as possible the situation of that tissue at the time it was last in the living animal. Thus, tissues intended for histopathologic examination should be removed from the surgical site or carcass and handled with the same care as living tissues during surgery (15). A number of techniques can be used to reduce or avoid artifacts induced by tissue handling. While forceps may be used to grasp tissue and remove it from the carcass, the tissue underlying the points of contact of the forceps may be crushed. This may be avoided by using scissors to trim tissue distal to the forceps, allowing the undamaged portion to drop directly into a vial of fixative. Paradoxically, tooth forceps cause less tissue damage than those with a plain end because they require less force to grip a tissue and the crushing artifact they induce is applied to a much smaller area than for forceps without teeth. Alternatively, tissues that are surrounded by fat, connective tissue or prominent capsules can be held by these structures, avoiding artifactual damage to the areas of interest. Lung is a resilient tissue, and can be easily damaged by excessive force employed to overcome its resilience on trimming. Lung is more effectively cut by scissors than by a knife or scalpel, scissors producing less artifact than these other techniques.

It may be necessary to clean tissues that are being selected for histopathology. Rinsing with saline is preferable to water in that there is less chance of tissue artifact production with

isotonic fluid. Water is hypotonic and will cause cell damage (16).

A number of fixatives are suggested in the literature for use in veterinary practice. The most common of these are 10% neutral buffered formalin, 70% alcohol, Bouin's, Carnoy's, Zenker's and Mirsky's fixatives. In the author's opinion, the best all round fixative and only one of these needed in veterinary clinical practice is 10% neutral buffered formalin. Alcohol and alcohol containing fixatives (Bouin's, Carnoy's) make tissues extremely brittle and have a very short useful life due to evaporation of the alcohol. Zenker's fixative contains mercury and is a health and disposal problem. Carnoy's contains chloroform and must be made up fresh prior to use and Mirsky's seems to lose effectiveness after a week or so. Alcohol, Bouin's and Zenker's fixatives penetrate tissues slowly and are really only suitable for fixing thin structures such as mucosal surfaces or corneas. Bouin's solution is often recommended for use in fixing uterine biopsies from mares and specimens from eyes. The author's opinion from looking at both types of samples fixed with both types of fixatives is that there insufficient discernible difference to justify maintaining Bouin's solution in a clinic setting.

Solutions of formalin stronger than 10% are not satisfactory for fixation because they cause tissues to become hard and brittle, inducing artifacts and making the technical job of sectioning at five microns extremely difficult. Buffering of formalin fixative to a pH of 7.4 is necessary because formalin in solution is acidic, causing precipitation of the pigment acid hematin in tissues (15, 16). This pigment cannot be removed, obscures detail, and might be confused with pathologic pigments. Ten percent neutral buffered formalin is most easily obtained by either purchasing it in bulk, premixed from a scientific supply house, or purchasing pre-filled specimen jars from a veterinary supply company. Alternatively, it can be produced quite cost effectively by diluting commercial 37% or 40% formalin solution[3] to 10%. Once diluted, it must be buffered with sodium phosphate. In some rural locations, the local pharmacist will prepare 10% neutral buffered formalin for a veterinary clinic for a nominal fee[4].

Fixatives penetrate tissue at a rate depending upon their molecular weight and the resistance of the tissue to the particular fixative. The rate of penetration differs for each fixative and for each tissue. The rate of penetration sets a practical limit to the size of a piece of tissue that can be fixed in any particular solution. As a rule of thumb, fixation should be complete within 12 hours for adequate microscopic

[3] Note that commercial formalin that is referred to as 100% is actually only either 37% or 40%. Formalin solutions of 100% are not attainable.

[4] The formula for 1L 10% neutral buffered formalin is as follows:

- Distilled water 900ml
- NaH_2PO_4 (anhydrous) 4.0 gm
- Na_2HPO_4 (anhydrous) 6.5 gm
- Commercial 37 - 40% formaldehyde solution 100ml

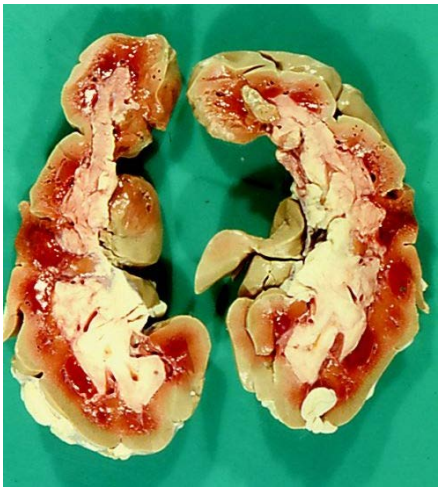
examination. Formalin penetrates tissue at the rate of approximately 3 to 4 mm in 24 hours (16). Therefore the maximum thickness of tissue sections placed in formalin should be 6 – 8 mm (penetration from each side at 3 to 4 mm per 12 hours equals complete fixation in 24 hours). Tissues can be longer than 8 mm in 2 other dimensions, but where practicable, an 8 mm cube of tissue is an ideal size for fixation (Figure 6).

Figure 6: Samples of lung of an appropriate size to allow formalin penetration and complete fixation within 12 hours.

Rate of penetration is not the only factor of importance. Many tissues from adult animals contain approximately 70% water while those of younger animals and certain diseased tissues (i.e. edematous lungs) contain a much greater percentage of water. Fixative displaces these tissue fluids as it moves through a tissue. The tissue fluid displaced causes dilution of the remaining fixative. Dilution of fixative as penetration progresses thus occurs. In addition, the fixative is also consumed during the process of fixation as it complexes with protein. Therefore, although the initial solution was 10% formalin, it very rapidly becomes considerably more dilute than this (Figures 7a and b). In order to overcome this dilution effect, a volume of formalin 10 times the volume of tissue to be fixed is required (16). A quick test of formalin fixative effectiveness is to observe the colour of blood and tissue in formalin. If fixation is progressing properly, the blood should turn brown and tissue develop a grey brown colour within 2 hours. Formalin does not fix fatty tissue well, and so excess fat around a structure of interest should be trimmed as much as possible.

The processes of decomposition of tissue, both autolysis and putrefaction begin at the time of death and continue until the fixative fully penetrates all the tissue. Thus, pieces of tissue which have as their smallest dimension more than 1 cm or those that have been placed in insufficient volumes of fixative will have autolytic and or putrefactive processes continuing beyond 12 hours. If fixative never penetrates to the middle of such samples, they will be rotten and unusable for examination. A related problem occurs when tissues, most notably lung, float in formalin. Fixation of floating tissues will occur more slowly than for those that are submersed. In this situation fixation may be accelerated by covering the surface of the formalin with absorbent cloth such as bandage material or pieces of old surgical towels.

A problem occasionally encountered during severe winter weather is freezing of formalin in transit from the clinic to the laboratory. Tissues that have been completely fixed in



Figures 7a and 7b: The ratio of the volume of tissue to volume of fixative and rate of penetration of tissue by fixative are important factors. This bovine kidney has continued to putrefy in fixative since the large volume of tissue in relation to the volume of formalin prevented proper fixative penetration. Note the clear line of demarcation between the fixed outer rim of tissue and the unfixed central region in Figure 7b. It was at this point that the concentration of formalin dropped below the threshold required for tissue fixation due to dilution by displaced tissue fluids

formalin prior to freezing of the formalin can often be processed with reduced freezing artifact if the formalin is changed (refreshed) prior to shipment. If there is any chance that a fixed sample may be frozen in transit, it is best to hold the tissues in the clinic for at least 24 hours and then replace the formalin solution prior to shipment.

Thin slices of tissue placed in formalin swell slightly and warp. For this reason, while the smallest dimension of a piece of tissue placed in formalin should not be more than 1 cm, it should not be much less. Fixed tissue samples are trimmed further in the histology laboratory to compensate for warping in fixation and optimize them for processing and fitting on a slide. Deformation of slices of tissue less than 1 cm thick during fixation makes it difficult for laboratory personnel to properly trim such tissues to the final optimum size for histopathological processing.

Large masses of tissue swell and harden considerably in formalin solution. This poses a problem when such tissues are placed in narrow necked containers for shipment. A large piece of fresh tissue is quite pliable and can easily be forced through the narrow neck of a bottle or jar into a fixative solution, but once fixed it can only be retrieved by either cutting into smaller pieces, pulling it out with tooth forceps or smashing the container (15). Smashed glass containers pose a risk of injury to those handling the specimens (Figures 8a and b) and at least one laboratory technician has

suffered a severed tendon from handling such specimens. Retrieving such tissues without smashing the container may induce artifacts in the sample, degrading its usefulness as a diagnostic specimen. This may all be avoided by sticking to the 1 cm thick guideline.

Figures 8a and b: a. Glass specimen jar containing a large hardened formalin fixed piece of lung that cannot be retrieved through the neck of the bottle. b. retrieval of the sample by smashing the bottle. This results in fragments of glass that pose a safety hazard. Small pieces of glass can become embedded in the specimen, inducing artifacts and causing a safety hazard.

The size of a piece of tissue that can be fixed is governed by the physical properties of the fixative, but the number of samples sent from that tissue is not. Selection of tissues for histopathologic examination must be based upon knowledge of anatomy and the disease processes that may be present. While a large animal such as a horse or a cow has large organs, it does not follow that a large piece of the organs from such an animal be cut for microscopic examination. Proportionately, a 1 cm cube of kidney from a horse is much less representative of that organ than the same volume of kidney from a rat, but there is no restriction on the total number of 1 cm cubes that can be collected. Therefore, numerous sections of an ideal size may be collected from a large structure such as an equine lung, and as many different areas should be sampled as needed to provide an adequate microscopic examination of the entire structure. There is no restriction to the number of pieces of tissue of the optimum sizes described above that may be taken from an organ provided a sufficient volume of fixative is available to allow complete fixation of all the specimens collected. If a large number of samples is taken from an animal at postmortem, it is advisable to regularly agitate the contained to ensure complete mixing of the fixative and tissues as displaced tissue fluid builds up around the specimens from the volume of tissues collected.

Selection of samples for histopathologic examination must be based upon knowledge of anatomy and the disease processes that either are evident or that may be present. Areas of an organ from which tissues are removed

for microscopic examination should be selected to include:

1. as many anatomic areas as possible and
2. represent the grossly visible disease processes accurately.

Organs collected at postmortem tend to fall into two categories, those that have distinct anatomic areas, eg. kidney, brain, adrenal gland, and those that are relatively anatomically homogeneous eg. liver, lung, spleen. The approach to collecting of each of these types of organs needs to be slightly different. For example, organs with distinctly different anatomic areas such as kidneys can usually be cut in such a way that single tissue sections can be taken that will encompass the capsule, cortex, medulla and pelvis. For many species, this anatomic requirement is readily accomplished by a 1 cm thick coronal section from cortex to pelvis. Such a section should be made through any grossly visible diseased area to satisfy both of the above anatomic requirements. If there are areas of pathologic change that vary from one area to another, then as many 1 cm thick transverse sections as are needed to accurately represent all such areas should be taken. At least one section of tissue should be taken from the advancing edge of a lesion. It is often at the advancing edge of a lesion that the most characteristic features of a disease process are found, not yet being obscured by the subacute or chronic reaction that may occur deeper in the lesion. To give an extreme example, a section from the edge of an inflammatory lesion is of more diagnostic use than a section from the centre of an abscess.

Organs that have a generally homogeneous structure do not have to be cut quite as exactly, since the basic organizational structures of the organ tend to be recurrent, and are likely to be encompassed in any block of tissue that is collected. However, some thought is still required in order to ensure that all the essential anatomic structures of the organ are present on the slide that ultimately is produced. Lung is an organ with a homogeneous structure, and can be cut so that a pleural surface is located along at least one edge, and a cross section of a secondary bronchus is situated in the middle of the block. This will ensure that all of the major airway, vascular and pleural structures are included on a single slide.

In the same way, all other organs of the body can be sampled in such a way that all anatomic areas as well as typical diseased regions are represented on the microscope slide that is produced.

It may be desirable to know the area of an organ from which a particular block of tissue is taken. This can be accomplished by shaping the block that is cut from a particular region. The lung is a good example of this identification technique. Sections from cranial lobes of lung can be trimmed as isosceles triangles, middle lobes as equilateral triangles, accessory lobes as rhombi and caudal lobes as cubes. Right and left can then be further identified by making a

shallow scalpel cut in sections from the left side but not the right. Any such scheme of marking tissues must be indicated for the receiving laboratory on the history sheet.

Intestine provides special problems in collection due to the resident population of bacteria. Autolytic changes progress very rapidly in the intestine. The intestine is best fixed by trimming short one to two cm pieces from different levels and gently flushing the lumen of debris using fixative in a syringe. The flushed intestine can then be placed into an appropriate volume of fresh fixative. This simple procedure will result in well fixed intestinal sections that can be interpreted in detail.

At times, there may be compelling reasons to fix and submit an entire structure for anatomic examination. The brain is a complex organ which may need to be sent for referral as intact as possible in order that specific areas be sampled at the receiving laboratory. As the brain has no collagenous septae other than the meninges and vascular matrix, it is relatively easily penetrated by formalin. With care it can be fixed intact for submission to a laboratory. The brain should be removed from the cranium, the meninges removed and both brain and meninges placed in 10% neutral buffered formalin: 1L for animals up to the size of dogs and cats; 4L for animals the size of cattle and horses. A transverse section down to the lateral ventricles aids in formalin penetration. Formalin solution should be stirred every two hours or agitated gently and continuously on a laboratory shaker if one is available. After 24 hours, the formalin solution should be completely replaced with fresh formalin and stirred regularly as before. After another 24 hours the brain will be fixed, at which point both brain and meninges can be placed in a small volume of formalin in a container for shipment to a laboratory.

A similar approach will work for larger specimens such as a heart with an anomaly that the clinician may desire to submit intact so that the orientation of the parts is not lost. Such samples can be adequately fixed by incising the cardiac chambers sufficiently to allow free entry of formalin and then placing in a large volume of formalin under regular agitation and treated as indicated above. In the same way, a solid mass such as a tumor which includes the edges of the surgical incision may be fixed in entirety. A series of incomplete transverse cuts are made no further apart than 1 cm and the entire structure is placed into a suitable volume of formalin with regular agitation. Such a preparation allows examination of the edges of the entire mass for invasion of tumor cells.

Eyes for histology present a special challenge. They are best trimmed in the laboratory by specialist personnel. The globe can be removed intact from the skull at postmortem and then fixed entire for shipment to the histology laboratory. Prior to placing into fixative, a cut should be made

through the sclera halfway around the circumference of the globe with a sharp scalpel. This should penetrate the full thickness of the sclera but no further, the idea being to keep the globe intact but to allow formalin into the chambers of the eye.

The advent of immunoperoxidase staining that can be performed on paraffin embedded fixed tissues has revolutionized veterinary diagnostics, particularly with respect to identification of antigens of pathogens in tissue sections. In general, most immunoperoxidase stains for common animal pathogens can be performed on formalin fixed tissues processed by standard histological techniques. Although the polymerizing action of the aldehyde group of formalin will alter the reactivity of tissues to histochemical stains, such changes are usually reversible by washing with water, application of heat, microwaving, or a combination of these techniques. As a general rule, histochemical techniques are more successful if processing is occurs within 24 hours of fixation. Advance contact with the referral laboratory to discuss fixation is advisable if immunochemistry for non-routine pathogens or antigens is desired.

SPECIMENS FOR HEMATOLOGY AND CHEMISTRY

Blood and serum samples are by far the most common samples shipped from veterinary clinics to laboratories. Most laboratories provide specimen shipping packages for blood and serum, and so minimum detail will be provided here. Prior to shipping whole blood, two smears should be made and air dried, while serum should be spun down and separated. Cardboard or plastic slide mailers are readily available for slides, but should be strongly reinforced to prevent breakage of slides in transit (17). Blood vials can be wrapped in bubble wrap if a specifically designed styrofoam or other protective mailer is not available. Figure 9 demonstrates a simple way to prepare blood samples for shipment in the absence of a specific blood vial mailer.

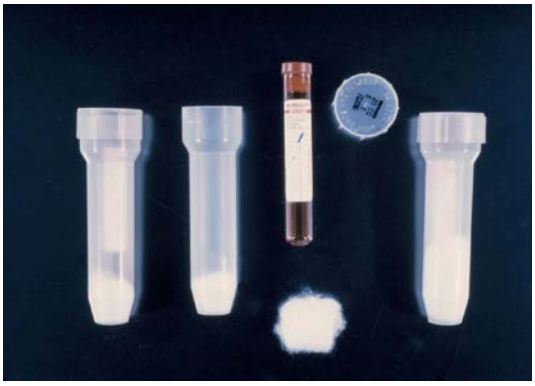


Figure 9: A well packaged submission of blood tubes. The tube is padded top and bottom inside a syringe case. Addition of absorbent material between the outside of the blood tube and the inner wall of the syringe case would make this shipment meet the packing regulations for dangerous goods.

BACTERIOLOGY SPECIMENS

Despite the advances in immunoperoxidase, *in vitro* hybridization, PCR and ELISA testing that have changed veterinary diagnostic microbiology, attention to good handling and shipping techniques is still important, and there are situations in

which recovery and identification of viable organisms is necessary. Among the most difficult specimens to collect and interpret are those for bacteriology. The microbiologic flora of animals and their environment is an ecosystem in its own right. Many organisms are present all the time in both animals and the environment, and very few of these are pathogens. Pathogens generally tend to be at a competitive disadvantage to nonpathogens in dead tissue, making recovery from necropsy specimens more difficult. Furthermore, many species have large populations of motile organisms in their gastrointestinal tracts which start to invade tissues within minutes of death. Therefore, care and attention in selecting samples for microbiologic culture is of the utmost importance.

Techniques employed in selecting samples for microbiology should be intended to minimize contamination and ideally, aseptic procedures should be used. This is very difficult, but not impossible in post-mortem examinations. For sites that are normally protected from contamination such as closed wounds, joints, abscesses etc, sterile needle aspiration is best. Sites that are not protected from contamination such as the surfaces of skin and mucosa and draining tracts should be collected by surgical biopsy. Fluids and tissues are at all times more desirable specimens than swabs when they can arrive in the laboratory within a few hours. Swabs are less desirable because they are easily contaminated, collect only small volumes of tissue, absorb bacteria into the swabbed material making recovery more difficult, and expose bacteria to both drying and oxygen. These reasons make swabs especially undesirable to obtain cultures of anaerobic organisms. Nevertheless, in many field situations the laboratory is at some distance from the place that samples are collected. Fresh tissues may become less than fresh in the time that it takes to transport them to a laboratory and overgrowth of normal flora or contaminants may occur. Under such circumstances, swabs placed in transport medium are a useful compromise, if collected in as clean a way as possible under the circumstances.

Parts of organs taken at necropsy for culture should be cut in such a way that contamination from the knife is reduced. The normal anatomic borders of the organ form a barrier that helps retard bacterial invasion and which may be used to assist in reducing contamination. If a sample representative of the disease process in an organ can be obtained by cutting off one end of the organ, for example, the lobe of a lung, this is ideal. Such a procedure results in only one cut being made across the lobe, and this provides only one surface from which contaminating bacteria from the knife blade may enter the organ. Organs for bacterial culture should be larger than those taken for histopathology since the concern is invasion by contaminants, an undesirable event, not rapid penetration of fixative. Removing both the organ and the regional lymph node and packaging them separately provides the best

selection of tissue for bacteriologic workup. Intestine should be tied off at both ends or in a loop to prevent spillage of contents.

Samples for bacteriology/ mycology may be collected and packaged for shipment to a laboratory in many ways. A variety of commonly available containers are quite satisfactory for such use. Commercially available plastic specimen bags are sterile until opened and can be used for a wide variety of samples, including fluids (Figures 10, 11).

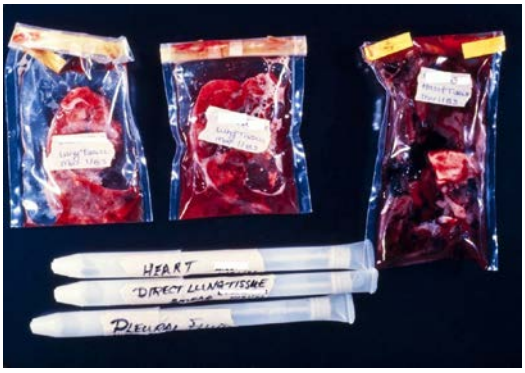


Figure 10: A well prepared and properly packaged submission which uses both swabs and commercially available plastic bags. Note the individual packaging and labeling of each sample.

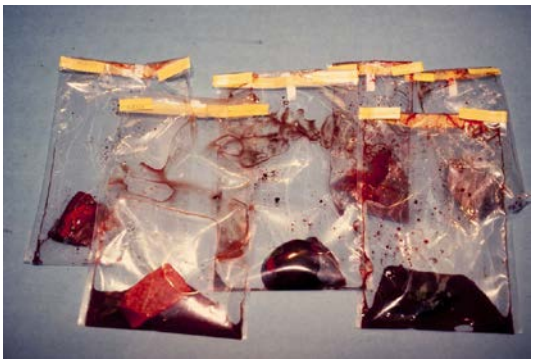
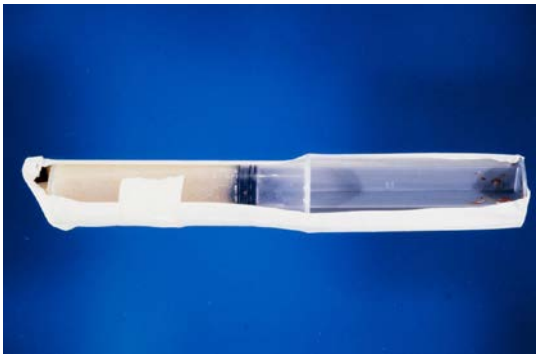
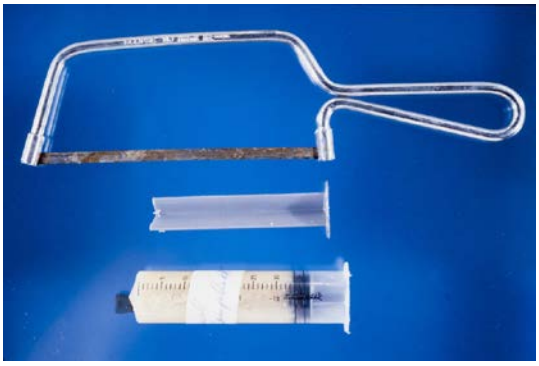


Figure 11: Samples from a neonatal foal well packaged for microbiology. Note the individual packaging and labeling of fresh tissues. Note also how much blood and tissue fluid may leak during transit from samples that appeared relatively dry upon collection.

Syringes are also sterile until their packaging is opened. After aspirating material to be cultured from abscesses, joints, the bladder, etc. into a syringe, the plunger can be cut off with a hacksaw at the point at which it protrudes from the barrel (Figures 12a and b).





Figures 12 a and b: A convenient method for collecting and containing aspirated fluids in as sterile a manner as possible for microbiology or cytology. The protruding part of the syringe plunger is cut off with a hacksaw after aspiration is complete. The barrel of the syringe is then replaced into a plastic syringe case which is taped shut. This forms a durable protective inner package for shipment, and requires a minimum of handling.

The syringe is placed back into the plastic syringe case from which it came. This can be taped closed and serves as an excellent inner container for shipment. In this way, a syringe provides a satisfactorily sterile sample for culture with minimal handling of the material to be cultured, reducing the chances for contamination. Alternatively, the liquid can be transferred from the syringe to transport media or blood culture media in those situations where this is necessary.

Transport medium assists in maintaining viability of bacteria during shipment and swabs should be forwarded in transport media. *It is important to realize that transport medium is not a growth medium nor does it favour one organism over another*. Swabs should not be submitted in a tube without transport medium as bacteria are exposed to drying and to oxygen, both of which are detrimental and may result in failure to recover a pathogen. Transport medium should be neither frozen nor subjected to excessive heat. Both storage and transport should be at either refrigerator temperature (4^{o}C) or at room temperature.

Occasionally the practitioner is faced with a situation in which the swabs being used do not have their own transport medium. The extended length guarded swabs that are used for reproductive cultures are an example. If transportation time to the laboratory will be greater than 20 minutes, the tip of the swab must be placed into a vial of transport medium and the shaft of the swab cut off with side cutters or heavy scissors to allow the vial to be closed. This must be done carefully as there is potential to introduce contamination to the swab or transport medium at this point.

Each fresh tissue or swab submitted for culture should be packaged separately. This is especially important when septicemic diseases are suspected. Many contaminant organisms are motile and if several tissues or swabs are placed together in the same container, these motile organisms will move into and overgrow all tissues. Pathogens may be impossible to recover in such situations, even though they may be present. A diagnosis of septicemic disease requires that the same organism be recovered from 3 separate organs. If organs have been packaged together, cross-transfer of bacteria from one organ to another could have occurred during transport, and accurate interpretation of findings will not be possible

Laboratory submissions of large numbers of microbiology samples at one time for herd survey work requires prior consultation with the lab. Since media is usually prepared or purchased to meet average daily demands, and special media may have to be prepared in advance for certain tests, it is best to contact the laboratory as far in advance of a large multiple submission as practical. This will permit production of sufficient media of appropriate types for the tests required and will allow the scheduling of workload and assignment of staff to permit processing of the samples upon arrival. Herd surveys for mastitis and

reproduction are the submissions that most commonly require such advanced discussion with the laboratory.

Blood agar or other plates cultured in clinic and growing organisms are not suitable to send to a laboratory for further identification of such organisms. Organisms will die out on such plates during transit due to desiccation and other adverse environmental conditions. Rather, the organisms of interest on the plate can be removed from the plate, placed in bacterial transport medium, and shipped.

VIRAL SPECIMENS

As with bacterial culture, advances in immunoperoxidase, in vitro hybridization and ELISA testing have changed veterinary diagnostic virology, and tests have been developed that have less rigid preservation and shipping precautions than are required for keeping viruses viable for culture. Nevertheless, there are occasions when there may be a need to ship samples for viral culture. Samples for virus isolation require the most delicate handling of any clinical specimens. Not only are such samples most subject to loss of viability following death of the animal, they are also most subject to damage in collection and shipment. Furthermore, viral isolation procedures are delicate, difficult, time consuming and expensive. In order to have the best chance of recovering viruses tissues must be taken from live or recently euthanatised animals. The sample must be appropriate for the agent in question, taken at a time in the disease process when viral particles are still present, and must be packaged and shipped such that viability will be maintained. Viruses are susceptible to light, heat, dehydration and pH change. Speed of transmission to the laboratory and sterility of the specimen container are of great importance for viral specimens. It is generally observed that the success of isolation decreases with increasing distance from the point of collection to the virology laboratory.

Preserving samples for viral isolation presents a dilemma. Ideally, such samples should be shipped frozen. Dry ice is the best compound for keeping samples frozen in transit. Freezer packs and wet ice will keep a sample cold, but not frozen. However, as it evaporates, dry ice will decrease specimen pH due to the formation of carbonic acid, and it is subject to Transport of Dangerous Goods (TDG) regulations. If the specimen can reach the laboratory within 24 hrs of collection, wet ice or ice packs will be adequate to maintain the viability of most common veterinary viral pathogens. Samples that require longer than 24 hours to reach their destination should be frozen[5] and shipped on dry ice, observing TDG regulations. As an added protection for the samples, if dry ice is used for shipping, the container

[5] Note that some viruses are susceptible to freezing (e.g. Malignant Catarrhal Fever Virus) and diagnostic specimens from which it is hoped to recover them should only be shipped refrigerated.

holding the sample (but not the dry ice) must be airtight.

Swabs for viral culture should be placed in viral transport medium or sterile normal saline solution, and refrigerated or frozen. Swabs in viral transport medium cannot be used for bacterial culture, and if bacteriology is required, separate swabs in bacterial transport medium should be submitted. Viral transport medium should be stored at refrigerator temperature prior to use, and most viral transport media have a storage life of approximately one month. Feces for virology should be sent frozen in 5 ml volumes rather than as swabs in transport medium.

Samples from vesicular diseases of the skin or mucous membranes are a particularly important sample for laboratory evaluation in veterinary practice. These may be collected into a capillary tube. The ends should then be sealed with plasticine or wax. Tubes can be protected during shipment by placing them into a stoppered serum tube and protecting with cotton wool.

PARASITOLOGY

Skin scrapings for parasitology are best forwarded in serum tubes. The scalpel blade and mineral oil which are used to obtain the sample should also be dropped into the serum tube along with the scraping. Often material present on the blade of the scalpel is as useful for parasitologic examination as the overlying skin and hair obtained by scraping. Samples of lung for Baermann testing and fecals for flotation and other techniques can be shipped in plastic specimen bags or commercially resealable plastic bags.

TOXICOLOGY

Toxicologic testing is expensive and can be time consuming. Therefore, the clinician needs to be as specific as possible about the compound for which he or she would like the toxicology service to search. If it is not possible to suggest a specific compound, the clinical signs should be used by the clinician to suggest a general class of compounds which could be involved. General directions such as "screen for toxins" on a history usually cannot be followed due to the time and cost involved in general screening procedures.

Samples for toxicology usually consist of fresh organs, gastrointestinal contents or body fluids. Such samples are usually shipped frozen, but this is not an absolute requirement. It is important to remember that residues of compounds inside shipping containers can contaminate specimens for toxicology and lead to false results. Containers that have previously held chemicals or drugs should not be used to package and transport toxicology samples. It is also important to be aware of possible environmental contamination of toxicology samples. Accidental contamination of abomasal contents from a calf by permethrin occurred in one instance when the prosector sprayed himself and the immediate area with insecticide to kill flies. This could have caused false interpretation of laboratory

findings if it had not been recognized as a potential source of contamination.

Occasionally a toxicologic problem may be suspected, but the clinical signs were not observed and the class of toxin is unknown. In such cases, a minimum of 10 cc of each of liver, lungs, kidney, stomach contents and fat, as well as all the bile and urine that is available should be forwarded for toxicology. Preservatives should never be added to samples intended for toxicologic analysis: preservation should be achieved by refrigeration or freezing. As with all other types of samples, clear identification of each specimen should be marked on its container.

Brain (both fixed and fresh frozen) should always be included with tissues examined in suspected poisoning situations. Many nervous diseases will produce signs that mimic those produced by various toxins, and nervous disease should always be ruled out as part of a toxicologic investigation.

ELECTRONMICROSCOPY

Electron microscopy (EM) is a specialized technique and only occasionally might a clinician require electron microscopic services. However, it will be covered briefly in this overview for the sake of completeness. The electron microscope is a good example of the sophisticated technology that can be applied in modern diagnostics but which must be carefully employed if it is to be of any benefit. Its high degree of specificity requires that it be applied to a problem in a directed way that will give the best results. One feature of the use of EM in veterinary practice is that the type and/or quality of specimen do not have to be as pristine as for research purposes to give useful diagnostic information. In select circumstances, such as direct examination for viral particles, even autolytic tissues may be examined to advantage. The same principles of fixation apply for EM as apply for light microscopy, with universal fixative being the best general fixative for EM in general veterinary practice[6,7].

Direct EM examination of feces for viral particles is occasionally a useful diagnostic technique, although it has been replaced in many situations by PCR. Unfortunately, concentrations of virus in clinical specimens may be too low for detection by this method, and viral

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[6] The formula to make 100 ml Universal Fixative is as follows:

- 1.16 gm Sodium phosphate monobasic (NaH2PO4.H2O)
- 0.27 gm Sodium hydroxide (NaOH)
- 4.0 ml 25% glutaraldahyde
- 20.0 ml 10% Formaldehyde
- 76.0 ml Distilled demineralized water

[7] 10% Formaldehyde (for universal fixative): Add 25 gm paraformaldehyde to 200 ml distilled, demineralized water. Heat slowly to 60^0C on a hot plate in the fume hood. When the temperature reaches 60^0C, add 1 NaOH dropwise until the solution clears. After the solution has cooled, make it up to 250 ml with distilled, demineralized water.

WARNING: Universal fixative is unstable and deteriorates rapidly (one month in the dark in the refrigerator or five days at room temperature). The glutaraldehyde must be of high quality and glutaric acid free. Universal fixative is not available commercially. The pH of universal fixative should be 7.2 and the osmolality approximately 940 mOsm.

concentration procedures may need to be employed by the laboratory. Interpretation of findings often needs to be carried out in conjunction with other test results. Interpretation of findings of such tests at the level of the individual animal is fraught with danger and interpretation is best made at the herd level.

Tissues submitted for standard ultrastructural examination (transmission EM) must be collected and fixed as soon after death as possible. They are diced into cubes of no more than 2 mm per side. This is best done on a tongue depressor with a new scalpel blade. The sample should be placed in a vial with a minimum 1 ml of cold universal fixative. Tissues submitted for surface structure examination (scanning EM) should be prepared in the same way except that larger 1cm x 1cm blocks may be used

SHIPPING CONSIDERATIONS

Samples leave veterinary clinics in pristine condition. Unfortunately, they do not necessarily arrive in such condition at the end of their journey. In preparing a sample for shipment it is important that one imagine not only what the sample looks like when it leaves the clinic but what it might look like upon receipt at the other end of a trip on a hot day after several hours of jostling, rough handling and delay in transit. The principle should be that the sample is packaged in order to arrive in the same condition in which it left the clinic.

The quality of packaging used for samples will have a significant bearing upon the condition of the specimen when it arrives at the laboratory. This will affect the quality of the work that can be achieved with the specimen, and therefore the usefulness of the laboratory report. Containers do not have to be elaborate or expensive to be effective. They must be strong enough not only to hold the specimen but must be capable of withstanding rough handling in transit. It needs to be of a material that is strong and durable. If very cold conditions might be encountered in transit, it needs to be of a material that will not fracture if bumped. It should be capable of being fastened securely and should provide some insulation capability, especially if frozen or chilled material is being sent or if samples are being shipped in very hot conditions. There should be a place on the container to readily display the address of the laboratory and the clinic of origin.

Adequate packaging consists of a leak-proof inner container, a layer of absorbent material capable of soaking up the liquid volume of the inner container, a waterproof outer container and a shipping box. A number of commonly available clinical items make good inner packages. The best containers for both fixed and fresh tissues are made of heavy plastic with screw caps. Heavy glass containers with screw caps are second best. Resealable plastic bags are also suitable if closed properly and contained in a leak proof outer container. Containers

with snap on lids and baby food containers are unsuitable for shipping samples. The latter are quite popular for shipping liquids, but they do not have a true screw lid but rather a screw type gasket that is not intended for reuse and is not watertight upon reuse. Styrofoam drug and vaccine boxes are useful for samples that need to be shipped frozen or chilled. Freezer packs tend to keep samples chilled but will only keep very small specimens frozen. Dry ice is useful to keep samples frozen but is subject to regulations under the Transport of Dangerous Goods Act (TDGA, see below).

Blood, serum and other samples in glass tubes are especially prone to damage in transit. These tubes are rigid, readily broken if compressed and the tops pop off easily. They are small enough to ship in standard envelopes tempting the shipper to mail them with inadequate padding or protection. When damaged, they have sharp jagged edges that cut surrounding packaging and pose a safety threat to anyone handling them. Therefore they must be packaged in such a way to prevent damage and to safely contain broken glass if they are damaged.

Containers that may be used for microbiology samples have been described previously in Part 6 of this series. Serum tubes are clean but not sterile, and are excellent shipping containers for samples for fungal culture, small parasites or skin scrapings for mange examination. Samples for toxicology can be placed in individual clean plastic or heavy glass containers or individually wrapped in aluminum foil. As with samples for microbiology, tissues should not be packaged together. Rather, they should always be placed in separate clean containers.

Once a specimen has left the clinic it will be transported by a carrier to the laboratory. Specimens should be sent by the most direct route since delays in transit will result in autolytic changes in unfixed tissues, contaminant overgrowth of microbiologic specimens and decreased viability of a variety of organisms. It is advisable to ship specimens for microbiology early in the week so that there is extra time available if delay occurs in transit.

Shipment of samples to laboratories within Canada is governed by the Federal Transportation of Goods Act (TDGA) implemented by Transport Canada in 1985. Over 3,000 items in 9 classes are considered dangerous goods, including common household substances such as bleach. These goods require special packaging and documentation and can only be shipped via carriers qualified to handle dangerous goods by people trained in dangerous goods shipment. Persons receiving shipments of dangerous goods also require dangerous goods training certificates.

The regulations under the TDGA contain two types of materials of concern to veterinarians sending samples to a diagnostic laboratory. The first of these is infectious substances, and the other is material used to preserve samples while in transit. When an

organism that is directly infectious to animals or humans is known to be present, it is classified as an infectious substance, is subject to the TDGA regulations and must be labeled and documented in accordance with TDGA regulations. A substance or tissue that is not known to be directly infectious to humans or animals, but is being sent for laboratory screening is not classified as an infectious substance. Rather, it is considered a diagnostic specimen and can be shipped without TDGA documentation. Nevertheless a diagnostic specimen should be packaged in the same way as a dangerous good.

The second area of concern in shipping samples centres on compounds used as preservatives. In practical terms for veterinarians, fixatives and dry ice are the two materials most likely to be subject to TDGA regulations. The small volumes of formalin that are used in preserving specimens for laboratory shipment are exempted from dangerous goods regulations[8]. However, any amount of dry ice is a regulated preservative and must be shipped as a dangerous good, adhering to all the regulations for labelling, documentation and training of personnel. Freezer packs are not subject to the TDG and are an alternative to dry ice. However, in hot weather, when shipment time may be lengthy, or for certain types of specimens, freezer packs may not be adequate and dry ice may be needed.

[8] TDGA regulations classify the liquid formaldehyde solutions mentioned in this manual as Class 9.2, UN#2209. This allows the unregulated shipment of any amount up to 50kg.

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