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Veterinary Diagnostic Testing

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1.1 Introduction

Most veterinary diagnostic laboratories have websites or booklets describing requirements for diagnostic sampling. These resources have descriptions of the sample needed, volume, temperature requirements for shipping, and other valuable information to assist the referring veterinarian.

Obtaining diagnostic samples from animals may present zoonotic disease exposure to the veterinarian. The veterinarian should always be aware of zoonotic diseases, transboundary diseases and even potential bioterrorism acts when collecting diagnostic samples. One of the most recognized potential zoonotic exposures for veterinarians is rabies and this should be on the differential in any neurological case. Any neurological case should be carefully handled when obtaining brain or any samples from the horses.

Additionally, foreign animal diseases (FAD)/transboundary diseases should be on the differential when clinical signs suggest such. International movement of horses legally and illegally may introduce FADs into the United States and consultation with the USDA and state veterinarians should be done prior to any sampling should veterinarians have any concerns about these possibilities.

Veterinary diagnostic testing utilizes many of the rapidly developing testing platforms including PCR, sequencing, multi-array, and MALDI-TOF to assist in diagnosis. Testing procedures are changing frequently and veterinarians must familiarize themselves with their referral laboratories' website or contact the lab to stay abreast of new sampling requirements, and tests.

Many large state veterinary diagnostic laboratories are full-service laboratories and provide assistance to veterinarians in diagnostic plans, choosing tests and samples for suspected illnesses. State veterinary laboratories may be accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD), which is an organization that promotes the improvement of veterinary diagnostics and standards for testing (see www.aavld.org/mission-vision-core-values). Veterinarians should work closely with their laboratory to be assured that they are familiar with the most current and correct sample collection and handling required by the laboratory.

Most laboratories have specialized sections for testing which include: clinical pathology, anatomical pathology, endocrinology, coagulation, bacteriology, virology, molecular diagnostics, and toxicology. Referral to other laboratories is routinely done by large laboratories due to the extensive testing requirements and recognized expertise of other laboratories.

1.2 Diagnostic Sampling

1.2.1 Whole Blood

One of the most frequently tested body fluids in the equine is blood.

- Most veterinary blood tests are done on whole blood, plasma or serum.
- A number of different blood tubes, transport vials, and so on, should be available to veterinarians at all times to obtain diagnostic samples such as CBCs and blood chemistries.
- Some blood tests require specialized collection tubes or containers that are not routinely stocked at veterinary practices and may be purchased from the laboratory.
- Consultation with your laboratory or review of their website should be done prior to blood sample collections to ensure quality and diagnostic samples.
- Special attention should be made to the specimen, the manner of collection, appropriate transport container, temperature requirements, correct test requests, and

Interpretation of Equine Laboratory Diagnostics, First Edition. Edited by Nicola Pusterla and Jill Higgins.

complete paperwork. Most laboratories welcome assisting veterinarians to help ensure the correct samples are collected.

1.2.2 Order of Draw

The order in which blood samples are drawn when multiple blood collection tubes are being collected from the animal is called "order of draw." Although this is not routinely practiced in veterinary medicine, it is suggested to follow the order of draw. Advanced techniques and the improved detection levels in diagnostic tests may cause inaccurate results from carry over between tubes with additives. It has been determined which additives affects test results and drawing the blood in the correct order is necessary, but some researchers feel the difference is minimum. The order of draw for most veterinary applications is: sterile tubes (blood cultures), light blue, red top, or SST, dark green, and purple (Box 1.1). If additional tubes are going to be drawn consultation with the lab should be done.

1.3 Collection, Preparation, and Handling

1.3.1 Blood Collection Tubes

Various types of evacuated blood-drawing supplies should be kept on hand in a clinic or in an ambulatory vehicle for equine diagnostic testing. Additional blood collecting supplies may include specialized blood-drawing needles, needle holders, and butterfly collection device needles.

There are numerous specialized blood collection tubes that are used in human medicine that can be used in veterinary diagnostic testing for special and routine tests (Figure 1.1). These tubes include: (1) trace element tube (royal blue cap), (2) thrombin based clot tube with activator gel for serum separation (orange cap), (3) glucose determinations (gray cap), (4) lead determination (tan caps), purple/lavender caps, and (5) blood culture collection tubes and DNA testing tubes (yellow capped with sodium polyanethol sulfonate (SPS) and others for specified tests.

Important facts about evacuated blood collection tubes:

- Expiration date
  - Blood collection tubes expiration dates are stamped on the tubes.
  - Out of date tubes may lose vacuum because of dried out stoppers and cause incomplete seals, incomplete filling of tube, and additives may become inactive over time.
  - Plastic collection tubes may not maintain the same shelf life as glass.
- Tube size and complete fill
  - Evacuated tubes are designed to auto-fill to a designated amount and should be allowed to fill until blood stops flowing automatically.
  - Under-filling tubes with additives will adversely affect results.
  - If there is a likelihood that a tube will not be filled to the correct volume, smaller tube sizes should be used to ensure the correct dilution of blood to the additive. Blood collection tubes/containers come in various sizes.

Box 1.1 Key points of blood sampling.

- Review the referral laboratory website or contact the lab to obtain information.
- Required sample type: plasma, serum, whole blood, etc.
- Animal preparation: fasting, at rest, after exercise, after medications, etc.
- Volume of required sample. The minimum volume allows one single analysis including instrument dead volume.
- Collection tube type and size: EDTA, heparin, citrate, glass, plastic tube, microtube, etc.
- Sample handling after collection: clotting time, centrifugation, temperature requirements.
- Shipping and handling requirements: receipt at the laboratory within stated time, chilled, frozen, room temperature, and so on.
- Do not freeze sera in glass tubes.
- Storage temperature is specified as room temperature (15–30 °C), refrigerated (2–10 °C), or frozen (−20 °C or colder).
- Samples after collection should immediately be placed in appropriate temperature holding areas until testing is begun or until prepared for shipping to referral lab.
- An air-dried blood smear should accompany EDTA samples for hemogram if testing not performed within 3–5 h post collection.
- Slides should be labeled with a pencil or diamond point pen.
- Cells in collection tubes with anticoagulants/additives may develop artifactual changes; therefore, air-dried slides should be made to prevent these changes.
- Slides should be placed in slide mailers away from moisture and formalized tissues/samples. Formalin fumes affect air dried slides and may render cytology smear nondiagnostic.

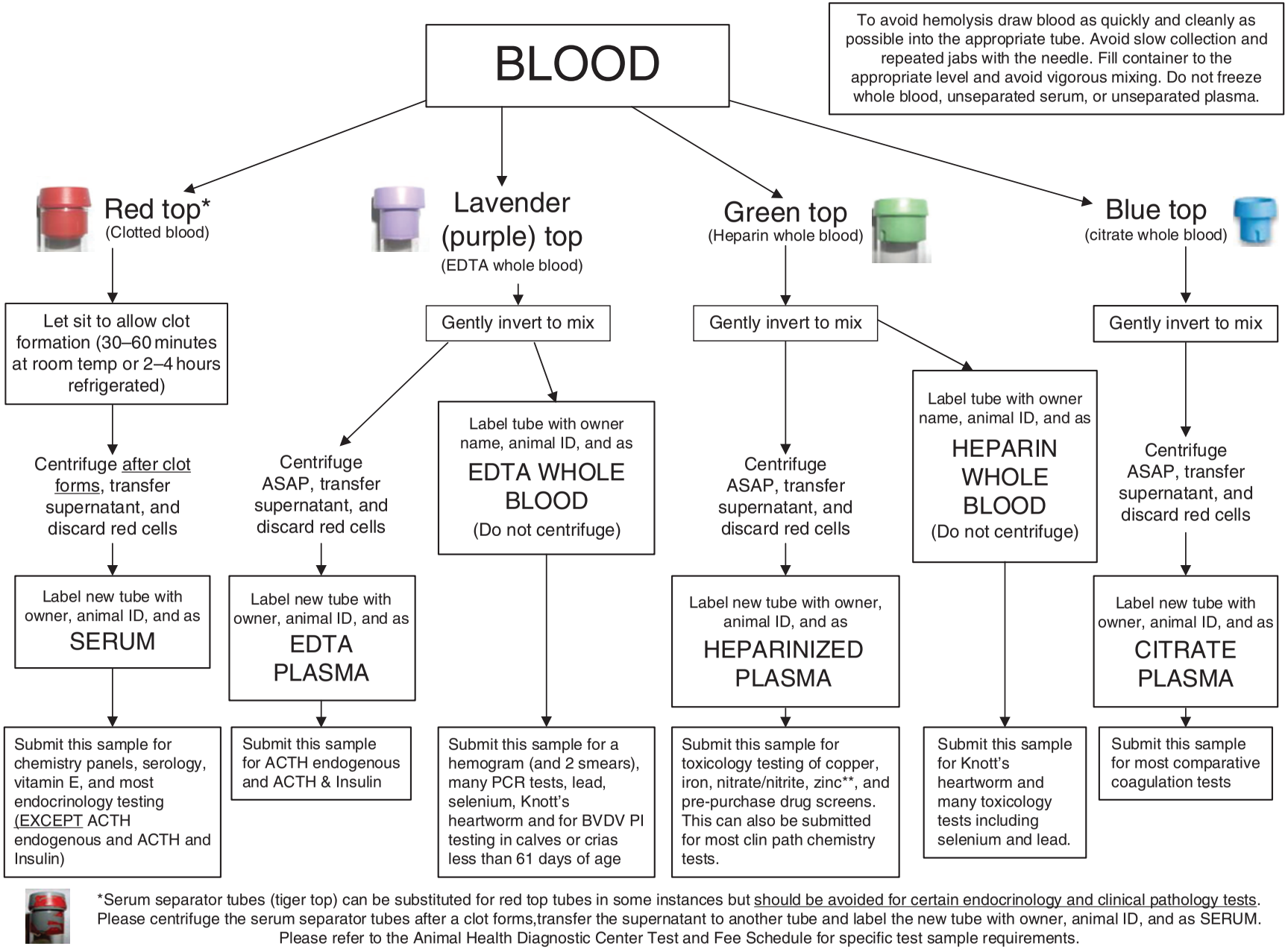

Figure 1.1 Blood flow chart. *Source:* Courtesy of Linda Mittel.

- Adhere to volume requested by laboratory because requested volume is used for verification of results, add-on tests, and parallel (acute and convalescent serology) testing.
- Necessary volume should be calculated prior to collecting samples.
- Mature normal sized horses should yield 4 ml of serum from each 10 cc blood drawn: 5 ml of plasma should be obtained from 10 ml of whole blood.
- These volumes may vary with hydration, health status (anemia) and other conditions.
- Foals or seriously anemic animals may require that smaller volumes of blood be taken.
- Microtubes ranging from 200 to 600 microliters and other blood collection tubes are readily available ranging in sizes from 2 to 10 ml.
- Butterfly collection lines/winged infusion sets may be used to obtain blood samples in the case of inaccessibility to the jugular veins, small vessel size, fractious animals, or difficult approaches.
- Butterfly collection lines/winged infusion sets can be placed directly into the blood collection tube, but a syringe should be attached to butterfly lines to obtain the blood to prevent vessels collapse from undue pressures. Note that butterfly collection needles/winged infusion sets have been recognized to be one cause of a large number of needle sticks to technicians and staff. Appropriate care should be done to prevent this.
- Blood may be drawn directly into syringes and transferred to appropriate tubes. Special transfer devices are available to transfer blood from syringe to collection device/tube.
- Special handling of these samples drawn by syringes must be done to prevent hemolysis, damage to the cells, and under/over filling of the tubes. The needle should be removed from syringe carefully (do not recap) and push the plunger steadily, but gently to prevent hemolysis and run the blood down the side of the opened tube. The correct volume should be placed in the tube and immediately stoppered and inverted as required.

1.4 Blood Sample Handling after Collection

All blood samples should be collected and gently mixed by inverting the tubes immediately after collection. The inside of certain tubes is sprayed with additives and sample must be inverted multiple times to allow contact with the additive and mixing of the blood.

- EDTA, heparin and other additive invert 8–10 ×
- SST, red top and plastic serum tubes invert 5 ×
- Sodium citrate tubes invert 3–4 ×
- Blood culture vials invert 8–10 ×

Plasma and serum are obtained from different types of tubes. Plasma is obtained from whole blood with an additive/anticoagulant. Serum is obtained from clotted blood.

1.5 Centrifugation of Blood Samples

Blood samples and other diagnostic samples may need to be centrifuged to separate components.

- The normal waiting time for blood to clot is ~30 min.
- Centrifugation of clotted blood to obtain serum or anticoagulated blood to obtain plasma is typically done at 1000–1200 g for 10–15 min. Some blood collection tube manufacturers have specific centrifuge speed requirements and review of these requirements may be necessary prior to sample handling.
- Temperatures during centrifugation should be between 20–22 °C. If analytes are temperature-labile, centrifugation should be done at 4 °C or refrigerated.
- Serum collection
  - Serum collection tubes should be handled as suggested by tube manufacturer and as required by the laboratory.
  - Blood collected in a plain red top tube, serum separator tube (SST), or a tube to obtain serum should be allowed to clot at room temperature for a minimum of 30 min and no longer than 2 h before centrifugation and removal of the clot.
  - Special serum collection tubes are available to expedite clotting within 30 min. Orange capped SST tube with thrombin manufactured by Becton Dickson allows for clotting in 5 min and is commonly used in emergency situations.
  - The premature spinning of samples prior to full clotting will cause difficulty in separating the clot from the sera and may cause hemolysis, change in electrolytes, and analytes that may adversely affect results.
  - Refrigeration prior to the clot formation may affect results and cause spurious values particularly potassium levels.
  - Hemolyzed blood can adversely affect blood chemistry analytes.
  - Blood potassium, and total bilirubin can be affected by hemolysis.
  - Tubes should be spun in a centrifuge after clotting and serum should be promptly removed with a disposable pipette and placed into another plain red top tube or transport vial and stored at designated temperature.

  - Vitamin E and bilirubin are light sensitive and should be wrapped in aluminum foil or stored in dark container as should all light sensitive samples.
  - Ammonia, certain blood coagulation testing, and ACTH are temperature sensitive. These analytes must be collected and chilled/frozen immediately.
  - Some serum samples for serological testing may be kept at room temperature, but it is best to refrigerate or freeze sera after collection and during shipment.
  - Sera should be frozen to prevent protein breakdown and bacterial contamination,
  - particularly when samples will be held longer periods of time (2–3 weeks) for parallel testing of acute and convalescent serum samples.
  - Serum separator tubes (SST) or "tiger tops" (ref) have a special gel that allows for easier separation of the sera from the clot after centrifugation. This gel does not make a complete seal between the cells and serum (or plasma) and the tube should be centrifuged and serum removed from the cells to prevent changes in analyte values.
  - SST tubes should not be re-centrifuged because potassium values will be spuriously elevated.
- Plasma collection
  - Plasma is obtained from whole blood tubes with additives or anticoagulants such as EDTA, heparin, and citrate.
  - Whole blood is spun in a centrifuge and the supernatant, plasma, is removed with a disposable pipette. There are plasma collection tubes with gel to aid in the separation of the plasma from the cells. Plasma is collected and placed in a transport vial or plain red top tube. It is imperative to identify the plasma source (EDTA plasma, citrated plasma, etc.) because the additives/anticoagulant may affect the test and some tests are validated with a specific plasma type and it required for testing.

1.6 Blood Culture Sampling

- Blood cultures are used in veterinary medicine in cases of sepsis, fevers of unknown origins, and other potential bacteremia/fungemic conditions. Use of blood cultures will assist in identification of the infectious agent associated with the illness and decrease the overuse of antibiotics.
- Specialized blood culture vials/tubes are required.
- A set of both anaerobic and aerobic blood culture samples should be drawn at the same time.
- Three sets (aerobic and anaerobic) should be drawn over a 24-h period. Sampling should occur prior to initiating therapy. In critically ill animals in need of antimicrobial therapy, two sets of blood cultures can be drawn within 15 min of each other and antimicrobials administered afterward.
- Samples should be drawn as a fever is rising to optimize isolating of bacterial organisms.
- Aseptic collection techniques are critical to prevent sample contamination and subsequent confusion on the interpretation and validity of results.
- Blood culture vials are available with resins to remove antimicrobials from blood for culture.
- Inoculated blood culture vials should be protected from temperature extremes, bright light and never be chilled.
- Blood culture vials should be taken to the laboratory as soon as possible (within 3 h) after collection, but if shipped to a referral lab, vials should be maintained at room temperature prior to and during shipping. Samples must be shipped overnight.
- Ship the blood culture vials in an insulated container to prevent temperature extremes.
- Patient identification should be noted on vial, time, and location of draw (which vein used, etc.) to prevent future resampling blood cultures in the same area.

1.7 Laboratory Validation of Blood Samples

Blood tests are validated on specific types of blood samples or products (plasma or serum) and reference values are established using these validated samples. Certain tests are required to be done on specific specimens; that is, CBCs must be done on whole blood from EDTA tubes or capillary tubes with EDTA anticoagulant in the tube.

- Heparinized whole blood causes distortion of the RBCs and is not acceptable for a hemogram.
- The specific type of plasma should be identified, that is, heparinized plasma, citrated plasma or EDTA plasma and noted on transport tube along with other animal identification, date, and initials of the person who drew the sample.
- Heparinized plasma is used for some toxicology testing.
- EDTA plasma is used for testing ACTH.
- Citrated plasma is used in coagulation studies.

Each laboratory may have their own specific requirements and this should be reviewed prior to sampling. Every veterinary laboratory does not have the same requirements.

1.8 Specimens, Transport Containers, and Media for Various Disciplines

1.8.1 Clinical Pathology

Emergency testing and routine tests are the norm for this section and turnaround times are usually quick. There are many routine tests that are performed in this section, but sophisticated testing is also done such as flow cytometry, immunophenotyping, and body fluid analysis (synovial fluid, pulmonary fluid, abdominal fluid, cerebrospinal fluid). Clinical pathology testing compliments most other laboratory sections and is often one of the first tests requested in diagnostic workups.

The section on blood collection and testing in this manual applies to most of the blood sample submissions to clinical pathology. Other equine samples that are processed in clinical pathology require special handling/collection include:

- Tracheal wash fluid, joint/synovial fluid, cerebrospinal fluid, bronchoalveolar lavage fluid, bone marrow, urine, and various needle aspirates submitted for cytological evaluation and analysis require submission in a sterile red top/EDTA tube.
- If cytological evaluation is requested, air dried smears of the fluid from either the red top tube or the EDTA tube should be sent with the sample in order to preserve the cellular components from breakdown and facilitate interpretation. These slides should be sent in slide mailers and kept dry.
- If culture will be requested on body fluid samples, a sterile red top tube or vial should be sent in addition to the EDTA sample. EDTA is bactericidal and not acceptable for aerobic or anaerobic bacterial culture or fungal culture. A sterile red top tube or sterile transport vial is required for aerobic fluid culture.
- Anaerobic culture on fluid samples requires the use of an anaerobic transport media. Anaerobic vials, large mouth screw top lids, vials with septum for needle injections or bottles are available for fluids, and tissues. Saturated swabs are not the preferred sample.
- Synovial fluid should be placed in a sterile red top and EDTA tube. Air dried slides should be made and submitted. Samples with small number of cells may require that the sample be cytospinned and slides made from the pellet on arrival at the lab to obtain a good representation of the cellular components.
- Bronchoalveolar lavage (BAL) samples should be sent in an EDTA blood collection tube and a plain red top tube chilled for overnight delivery. Air dried smears made direct from the EDTA tube should be submitted with the fluids (cells in a low protein fluid such as the saline lavage fluid may breakdown and become difficult to identify).
- Tracheal wash samples should be placed in both, a sterile red top tube for culture and an EDTA tube for cytological evaluation. Air dried smears should be made from the EDTA sample tube.

1.8.2 Microbiology

This lab section is responsible for the growth, identification, and antibiogram of bacteria, yeasts, and fungal agents. The advent of new technology has allowed for quick and novel bacterial identification. The MALDI-TOF™ machine has revolutionized the identification time of bacteria to minutes versus days. PCR and sequencing are other testing platforms that are used for bacterial and fungal identification. Collaboration with the molecular section of the laboratory is done many times to assist in identifications.

- Bacterial sampling and transport media
  - Sampling for isolation of bacteria and fungi may require specialized transport media (TM) to allow shipping/transfer to a referral laboratory.
  - Anaerobic and aerobic blood culture has specialized collection media. Amies transport media with or without charcoal and modified Stuart's medium are three of the commonly used aerobic bacterial TM. Amies TM with charcoal is used in veterinary medicine for the isolation of fastidious organisms such as *Taylorella* sp. and is required in contagious equine metritis regulatory testing.
  - Specialized enteric TM are available for assisting in the recovery of enteric organisms such as Para Pak™ transport media. This TM does not need refrigeration after inoculation for shipment.
  - Anaerobic vials, jars with large mouth lids, and tubes are available for fluids, tissue samples, and swabs, respectively. Some manufacturers sell anaerobic culture tubes with screw top tubes with special injection septum for liquid sample introduction or for swab introduction. Anaerobic transport media is required for swabs, body fluids, small pieces of tissue for anaerobic bacterial isolation. Anaerobic culture can be performed on fresh tissue that is > 2–3 cm in diameter (where the center of the tissue has maintained anaerobic conditions). The samples should arrive to the laboratory within 24 h of collection.
  - Tied off loops of bowel can be submitted for anaerobic enteric culture where laboratory will culture contents/tissue for anaerobes.
  - Fresh tissues samples must arrive chilled or frozen within 24 h after animal's death whereas inoculated

anaerobic transport media must be kept at room temperature for shipping and handling and arrive within 24 h.
  - *Clostridium* toxin tests can be done on fresh feces, but toxin proteins are extremely heat-labile and samples should be frozen as soon as obtained and shipped frozen within 24 h of collection.
  - Proper inoculation and handling of the anaerobic TM before inoculation and during is required to maintain anaerobic conditions. Tubes should be stored upright and when inoculating so to prevent loss of gas cap (see https://ahdc.vet.cornell.edu/docs/Anaerobic_Culture-Inoculation_of_Anaerobic_Transport_Media.pdf).
  - Inoculated anaerobic transport media must be maintained at room temperature.
  - Botulism PCR testing is done for the presence of *Clostridium botulinum* genes in feed, intestinal tissue and feces. This testing is done at the National Botulism Reference laboratory at the University of Pennsylvania, School of Veterinary medicine (www.vet.upenn.edu/veterinary-hospitals/NBC-hospital/diagnostic-laboratories/national-botulism-reference-laboratory).
- Fungal sampling and transport media
  - Transport media used for suspect systemic fungal infections is the same as for bacterial cultures. Consultation with the lab prior to suspect fungal submission is suggested. The use of molecular testing (PCR) for fungal identification directly from the clinical sample requires special handling and bacterial transport media cannot be used.
  - Dermatophytes do not require specialized TM.
  - Skin scrapings, hair, and horn/hoof samples should be sent in dry containers/paper envelopes to prevent moisture condensation and overgrowth with contaminants.
  - Skin, corneal fluid, tissue samples/biopsies should be placed into sterile screw top transport vials with a drop of sterile saline, chilled, and shipped for arrival to lab within 24 h.
  - Systemic fungal infection swab samples can be transported in aerobic and anaerobic bacterial media (Port a cul™) or the previously discussed anaerobic containers.
  - Inoculated bacterial transport media with fungal samples should be shipped and handled as discussed in the bacterial section.
  - Swabs obtained from the cornea, uterus/endometrium, and other locations should be inoculated into aerobic or anaerobic transport media and shipped chilled or room temperature, respectively. Actual tissue sample is preferred for culture.
  - All samples must be shipped overnight and arrive chilled to the laboratory to prevent overgrowth by contaminants.
  - If both fungal and bacterial testing is to be done, two swabs should be obtained to assure adequate sample volume.

1.8.3 Molecular Testing

The development of molecular assays has increased the breadth of testing for infectious pathogens. Molecular diagnostic laboratories utilize various molecular diagnostic modalities, including nucleic acid amplification techniques, and sequencing technologies.

- Universal viral transport medium (liquid) is available from various manufacturers and is room temperature stable for viral transport, maintenance, and long-term freeze storage.
- All body fluids including whole blood, serum, CSF, respiratory fluid samples, urine, and feces are acceptable samples for viral testing.
- Viral isolation is still very important even with the advent of PCR. Isolation allows for vaccine development, anti-viral treatments, and identification of novel agents. However, viral isolation requires that the sample contains at least a moderate viral load in order to successfully grow virus.
- Bacterial transport media cannot be used for viral PCR testing.
- Dacron- or rayon-tipped swabs are preferred for PCR and viral testing.
- Freezing tissues and samples can preserve samples for later viral testing, however, repeated freeze thaw is not recommended.

1.8.4 Parasitology

The parasitology section provides identification of parasites by various methods. These include direct fecal smear examinations, fecal flotations, fresh and fixed tissue samples for parasite identification, whole parasite identification, serological, and molecular testing.

- Fecal floatation testing requires 1–2 normally formed fecal balls (approximately 10 g of feces) from an average horse for quantification. Samples should be sent in a clean anaerobic leak-proof containers/plastic bag. Samples should not be submitted in an exam glove or rectal sleeve.
- Fresh feces submitted for fecal floatation must not be exposed to temperature extremes. Eggs may rupture/hatch in sample and the sample may become nondiagnostic.

- The McMaster, Wisconsin, and other modified methods may be used to obtain approximate numbers of strongyle egg counts and are frequently performed in private veterinary clinics.
- Testing fecal samples for tapeworms, *Anaplocephala perfoliata*, by floatation is not a reliable test due to the intermittent shedding of eggs by the adult tapeworms. Serological testing has been developed but has not gained favor due to the inability to interpret positive results in horses that have been successfully treated for tapeworms, but still remain seropositive.
- Fecal sampling for floatation to assist in determining resistance patterns using fecal egg count reduction test (FECRT) should be obtained 10–14 days post administration of an anthelmintic.
- Fecal samples that cannot be tested soon after collection (within 7 days or less) may be placed into TM such as 10% formalin or polyvinyl alcohol to assist in preserving the ova and the delicate trophozoites forms seen with enteric protozoal infections.
- Fecal samples for larval parasite and identification (strongyle family) should be fresh, kept at room temperature, and contain large numbers of ova on fecal floatation (>100 epg) to insure adequate numbers of larval hatching for identification.
- Lungworms, *Dictyocaulus arnfieldi* can be diagnosed in fresh fecal samples, but requires active floatation techniques and special sugar solutions. Clinical signs or suspect disease should be provided to parasitology lab to allow proper techniques to be performed. Baermann testing is used for diagnosis of lungworms if eggs are not found in fecal samples that have been tested by active floatation methods.
- Pinworms are not routinely found in fecal floatations and the "cellophane tape test" can be used to assist with diagnosis of pinworms ova (cellophane tape is stuck to a clear glass side and examined microscopically).
- Enteric protozoal infections are not thought to be pathological in apparently normal equine adults and foals, but antigen (fecal) ELISA detection tests are readily available for *Giardia*, and cryptosporidium.
- EPM causative agents, *Neospora hughesi* and *Sarcocystis neurona* antibody levels can be detected in serum and CSF by IFAT and ELISA. IHC and PCR are available for detection of the organisms in neurological tissue, but may not be rewarding due to the focal localized areas of infection.
- Skin scrapings and entire/partial parasites submitted for identification must be submitted in a clean escape-proof container such as plain red top blood collection tube or transport vial with screw top lid. Isopropyl alcohol in a red top tube/leak-proof vial can be used to transport and preserve ticks, mites, and other parasites.
- Skin scrapings should be obtained after lightly scraping the affected area until small drops of fresh blood are seen.
- Tissue samples or fresh tissue biopsies for parasite evaluations such as *Oncochera* sp. can be submitted in clean leak-proof containers with a few drops of sterile saline to keep samples moist and prevent dessication of parasite.
- Skin parasites maybe "washed out" during histological sample processing; therefore, a fresh biopsy in addition to the fixed sample should be submitted in a transport vial that prevents desiccation for parasite evaluation.

1.8.5 Toxicology

Toxicology laboratories utilize various types of analytical equipment and instruments, techniques for the detection, identification, and quantification of organic, inorganic, and toxic compounds. Vitamins and mineral testing are often performed in these laboratories. The accurate diagnosis of a toxicosis like many other diseases is made by utilizing information made from criteria. Forensic and legal cases tested in toxicology have stringent requirements for sampling, identification, shipping, and handling. These should be reviewed prior to obtaining samples to prevent serious errors in sampling. Chain of custody may be necessary particularly in forensic cases and possible legal cases. This should be discussed with the laboratory and client that is requesting testing so that the samples are not compromised for use in legal cases.

Drug screens for regulatory, and pre-purchase drug screens have specific requirements such as (1) sample type (i.e., whole blood, urine), (2) blood tube collection types, including EDTA, heparin or serum, and (3) testing volumes. It is critical to follow the laboratory guidelines for testing and sampling since many of these drug screens are associated with legal repercussions and cannot be redrawn.

Ante-mortem samples may include whole blood (blood tube additives may vary on testing and should be discussed with toxicologist), serum, urine, hair, body fluid, reflux, and feces. If unable to contact toxicologist prior to testing whole blood, tubes with EDTA or heparin are generally acceptable. Certain drugs are protein-bound and necessary sampling tubes may vary with each compound; therefore, using both tubes would prevent errors on the part of the submitter.

Samples should be placed in individually identified containers such as plastic sealable bags, sterile urine sample cups or wrapped in aluminum foil for testing lipophilic toxins. Excess air should be removed from plastic bags. Samples should be frozen as soon as possible and kept frozen in a deep freezer (not frost free) until analyzed. Serum should be removed from the clot and frozen.

SST tubes are not appropriate for drug monitoring or toxicological analysis. The gel in SST extracts lipophilic substances which is most drugs; therefore, causing falsely low drug concentrations.

Testing plant materials and forage for possible toxicities should include part of the leaves, stems, flowers and roots. Forage samples should be kept cool and dry or even frozen. Photographs of suspect plants showing stems, roots, flowers, seeds, should be submitted along with plants if available.

Post mortem/necropsy cases should always include a complete "tox set" and be held frozen until needed for testing. This link describes the information and suggested samples for toxicological workups and drug screens (https://ahdc.vet.cornell.edu/docs/Toxicology_Submissions_and_Analytica_Screens.pdf). The "tox set" can be used if necessary after histopathology results are obtained or for use in ancillary testing. Tissue material from a necropsy should include brain, liver, kidney, fat, urine, aqueous humor or intact eyeball, skin (site of exposure), heart blood collected in lithium heparinized blood collection tubes, stomach, reflux, intestinal contents, and feces. Collect stomach, intestine, and feces last to prevent contamination of entire carcass. Each sample or tissue should be placed in individually identified container similar to the ante-mortem testing. Most toxicological samples should be frozen and stored in a non-frost proof freezer. Other samples to collect may include paint chips, soil, supplements, and feed, forages, water, and cohort blood and urine samples.

1.8.6 Virology

The virology diagnostic section provides testing for viral agent detection and monitoring in multiple species using viral isolation and serology as the mainstay of testing. The development of PCR and molecular testing has increased the breadth of testing, and this section now utilizes various diagnostic modalities including, nucleic acid amplification techniques, and sequencing technologies.

Fresh tissue samples, and body fluids and products in viral transport media are acceptable samples. Some viruses are unable to be cultured easily or even at all and PCR techniques are being used successfully with these viruses. Viral transport media may optimize viral isolation and can be used in PCR techniques.

- Viral isolation requires that sample has a high viral load in to grow virus. Low numbers of viruses in sample may cause false negatives.
- Multiple species tissue cell lines may be necessary to isolate viruses from various animal species.
- Viral isolation is still very important even with the advent of PCR. Isolation allows for vaccine development, anti-viral treatments, and identification of novel agents.
- Turnaround time with viral isolation may range from 3–30 days.
- Bacterial transport media cannot be used for PCR testing.
- Dacron or rayon flocked swabs are preferred for PCR and viral isolation.
- Acceptable samples for viral isolation or PCR includes nasal swabs, body fluids/discharges, and target tissue samples.
- Universal Transport Medium, is a room temperature stable viral transport media for collection, transport, maintenance, and freezer storage.

1.8.7 Immunology/Serology

This laboratory section is responsible for testing areas that include allergies, autoimmune diseases and presence of antibodies in serum or other body fluids such as CSF, peritoneal fluid, and aqueous humor. Testing includes various platforms such as serum neutralization (SN), hemagglutination inhibition (HI), complement fixation (CF), Western Blot, ELISA, flow cytometry, multiplex, indirect fluorescent antibody (IFA), agar gel immunodiffusion (AGID), microscopic agglutination, serum hemagglutination inhibition (SHI), and cytokines. Most serology tests use an antigen as a reagent to capture antibodies.

- Serum is the most common sample tested, but other body fluids that are validated can also be used such as peritoneal fluid, CSF, and so on.
- Serum should be obtained in a clot tube (SST or red top) and allowed to clot at room temperature and centrifuged. Serum should be removed and placed in a transport tube.
- Acute samples and convalescent sera should be submitted together for parallel testing. Convalescent sera should be drawn 10–21 days after illness depending on agent to be tested for.
- Leptospirosis MAT serum samples should be drawn approximately 10 days post beginning of suspected illness. Further, antimicrobial treatment may blunt leptospirosis antibody response.
- *Anaplasma phagocytophilum* IFA titers develop 5–7 days after infection with agent.
- Titers associated with *Borrelia burgdorferi*, EHV-1/-4, *S. neurona*, *N. hughesi* may produce lifelong antibodies and positive titers are not always associated with active illness.
- Vaccine titers do not correspond to disease-protective levels in animals.
- IgM is the first isotype to elevate after infection followed by IgG.

1.8.8 Anatomical Pathology

Surgical biopsies, post mortem gross examinations, and histology are the most frequently submitted cases. Histology is the most frequently requested test and supporting stains and tests assist in diagnosis.

- Formalin preservation of tissues or biopsy samples should be done as soon as possible to prevent autolysis. The minimum dilution of formalin to tissue should be 10:1.
- Small pieces of tissue are required to allow for fixation. Large pieces of solid tissue should be cut into pieces that are 0.5 cm thick to allow fixation of tissues.
- Formalin preserved tissue should not be allowed to freeze.
- Bouin's solution may be used for fixation of delicate tissues such as with ophthalmic, intestinal tissues and reproductive histological evaluation.

1.8.9 Endocrinology

This laboratory section tests reproductive and metabolic hormones, and vitamins in the horse. This includes progesterone, PMSG, testosterone, granulosa cell tumor testing, metabolic testing including ACTH, leptin, and thyroid tests.

- Serum is the preferred sample for the majority of equine tests except for ACTH testing.
- Serum should be removed after centrifugation from the blood collection tube after clot formation and placed into a plain red top tube or transport vial.
- Hemolyzed samples may adversely affect results.
- Blood collection tubes with activators, SST tubes and activators, or any additives are not acceptable for serum collection.
- Sera should be chilled/frozen after removal from clot and shipped to laboratory to arrive chilled.
- EDTA whole blood testing for Cushing's disease should be chilled immediately after collection and prior to centrifugation. Equine ACTH testing requires EDTA plasma that has been collected after gravitational separation should not be frozen, but chilled. Proteolytic enzymes that may be still in plasma may affect results and cause ACTH values to be inaccurate. Do not place EDTA whole blood too close to ice packs prior to plasma separation for the same reason. EDTA plasma must be frozen as soon as possible after removal from cells and placed in a plain plastic red top tube or plastic transport vial. EDTA plasma should not be placed back into EDTA tubes for transport to laboratory. If liquid additives have been used as the tube additive sample dilution may occur.

1.8.10 Coagulation

Vascular injury is the most common cause of hemorrhage in the horse, but there are various conditions in the horse that may cause hemostatic failure. Diagnostic testing can aide in this determination, but careful sampling techniques, proper collection and handling are necessary to obtain accurate meaningful results. If the animal is excited splenic contraction may occur and cause elevated blood cell counts and increased platelet counts, alcohol from the skin preparation, sedatives, and analgesics may also affect the results.

There are primary hemostatic (platelet plug tests) and secondary hemostatic and fibrinolysis assays (fibrin clot formation/coagulation) available to assist in the diagnosis.

- Primary hemostatic tests include platelet counts that can be obtained from a stained blood smear by examination of the feathered edge of a smear to detect platelet clumping. This can be done in a clinical pathology laboratory when a CBC is done.
- Routine EDTA tubes used for hemograms are acceptable for making blood smear for platelet evaluation. This requires a careful venipuncture (atraumatic and away from recent venipuncture sites) directly into an evacuated EDTA (purple cap tube), heparin (light green cap) or citrate (light blue cap) collection tube. Complete fill of the tube for the proper ratio for testing is required. After collection mix by inverting 8–10 times. The sample should remain at room temperature, and the smear prepared as soon as possible after collection.
- Secondary hemostatic and fibrinolytic assays are often done with POC units. Automated POC units are available in clinical settings for stall-side testing and require the same correct careful sampling handling as in primary testing. Sample must be collected into a citrate blood collection tube (light blue tube), allow complete autofill, and mix by inversion 8–10 times. Sample may be drawn through intravenous catheter, but sample must be obtained after the catheter has been flushed with 20 cc of sterile calcium free saline. Maintain citrate blood collection tube at room temperature until it is centrifuged. Following centrifugation, place the plasma into a plastic tube. Ship sample chilled or frozen to the laboratory. Hemolyzed samples are not acceptable.

Further Reading

AHDC. https://ahdc.vet.cornell.edu/docs/Blood_Tubes_and_Labeling_Guidelines.pdf. [Online] 2016. https://ahdc.vet.cornell.edu/docs/Blood_Tubes_and_Labeling_Guidelines.pdf.

Arronson DM. 1987. Bor. Annals of Internal Medicine. 106(2): 246–253.

Asirvatham JR, Moses V, and Bjoransen L. 4, April 2013. Errors in potassium measurement: A laboratory perspective for the clinician. North American Journal of Medical Sciences, 5: 255–259.

Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson RB Jr, et al. 2013. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)(a). Clin Infect Dis. 57(4): e22–e121.

BD Vacutainer Venous Blood Collection Tube Guide. 07 2010.

BD. BD Vacutainer Venous Blood Collection. [Online] 2010. [Cited: October 20, 2015.] https://www.bd.com/vacutainer/pdfs/plus_plastic_tubes_wallchart_tubeguide_VS5229.pdf.

Bowen Raffick AR and Remaley AT. 2014. Interferences from blood collection tube components on clinical chemistry. Biochemia Medica, 24: 31–44.

Brooks MB. 2008. Equine coagulopathies. In BW Parry (ed.), Veterinary Clinics of North America. Philadelphia: Elsevier Saunders, Vol. 24, p. 335–355.

Center or Phelbomtony. [Online] https://www.phlebotomy.com/pt-stat/stat0510.html.

Dow SW, Jones RL, and Rosychuk RAW. 1989. Bacteriologic specimens: Selection, collection, and transport for optimum results. The Compendium Small Animal, 11: 686–701.

Frank C, Madden DJ, and Duncan C. 2015. Field necropsy of the horse. Veterinary Clinics of North America Equine Practice 31: 233–245.

Guder WG, Narayanan S, Wisser H, and Zawta B. 2009. Diagnostic Samples: From the Patient to the Laboratory: The Impact of Preanalytical Variables on the Quality of Laboratory Results, 4th Updated Edn. John Wiley & Sons, Ltd, p. 124.

Henry JB and Kurec AS. 1996. The clinical laboratory: Organization, purposes and practices. In JB Henry (ed.), Clinical Diagnosis and Management by Laboratory Methods. Vol. 19. Philadelphia: WB Saunders,

LabCorp. Microbiology Specimen Collection and Transport Guide. [Online] 2015. www.LabCorp.com.

Lappin VS and Michael R. 2012. Enteric protozoal infections. In CE Greene (ed.) Greene's Infectious Disease of Dog and Cat, 4th Edn. Elsevier, pp. 785–792.

Marjani A. 2006. Effect of storage time and temperature on some serum analytes. The Internet Journal of Laboratory Medicine. 2(2): 1–6.

McPherson RA and Pincus MR. 2011. Henry's Clinical Diagnosis and Management. Philadelphia: Elsevier/Saunders.

Meridian Biosciences, Inc. Meridian Bioscience, Inc. [Online] www.meridianbioscience.com/.

Sanger. Sample Collection for Microbiological Samples. 2008.

Tuck MK, Chan DW, Chia D, Godwin AK, Grizzle WE, Krueger KE, et al., 2009, Standard Operating Procedures for Serum and Plasma Collection: Early Detection Research Network Consensus Statement Standard Operating Procedure Integration Working Group. Journal of Proteome Research, 8: 113–117.

Willard MD and Tvedten H. 2012. Small Animal Clinical Diagnosis by Laboratory Methods. St. Louis: Saunders, an imprint of Elsevier Inc.

Young A. 1990. Blood culture: Indications and technique. Equine Veterinary Education, 2: 38–40.