IDEXX REFERENCE LABORATORIES

Specimen Collection Guidelines

CREATING CLARITY

Contents

IMPORTANT: Specimen quality directly impacts results

We take pride in the quality and accuracy of our reference laboratory services. In order to ensure the most accurate results and the best possible service, proper collection and handling of specimens are critical.

There are many variables during the 3 phases of testing that can impact results, including:

- Preanalytical phase: Patient preparation (fasting) and identification, tube filling, specimen handling and storage
- Analytical phase: Analyzer functioning and performance
- Postanalytical phase: Data logging and reporting

The pre- and postanalytical phases of the total testing process are more error-prone than the analytical phase.[1] Mindful practice and protocols will lead to a reduction in errors during the preanalytical phase of testing.

This guide provides a variety of preanalytical resources to aid you and your team in specimen collection and transport. Visit the **IDEXX Learning Center** at **idexxlearningcenter.com** for other useful specimen collection information, educational videos, and more.

Reference

1. Plebani M. Exploring the iceberg of errors in laboratory medicine. *Clin Chim Acta.* 209;404(1):16–23.

How to collect and submit quality specimens

Quality results begin with proper specimen collection and handling. Please refer to these guidelines for sample collection and shipment to our reference laboratories. For specific test details, please consult the Online Test Directory on **vetconnectplus.ca** for specimen collection protocols, interferences, storage, and stability information.

1. Choose proper collection tubes and containers.

The wrong tube or container can affect results. See the "**Specimen collection tube and swab guide**" for more information. For example, the gel in a serum separator tube (SST) can interfere with results of some specialized tests, including many endocrinology and drug tests. Consult the Online Test Directory on **vetconnectplus.ca** to determine the proper collection tube or container for specific tests.

2. Use proper collection techniques.

Blood specimens

The jugular vein is always preferred unless contraindicated (i.e., coagulation problems or a fractious patient).

Filling syringes

- Choose the largest accessible vein possible to ensure the continuous flow of blood when filling the syringe.
- Slow and difficult draws can rupture red blood cells, adversely affecting complete blood count (CBC) results and certain chemistries.

Transferring specimens

After collection, transfer blood as soon as possible to the appropriate blood collection tube to avoid risk of clumped platelets or clots, which may affect results.

- For vacuum tubes (e.g., Greiner Vacuette® tubes), pierce the stopper with the needle and allow the specimen to draw naturally into the tube by vacuum. Avoid forcing clotted blood into a vacuum tube as cells will lyse, causing hemolysis and inaccurate results.
- For nonvacuum tubes (e.g., a screw-top or flip-top plastic tube), remove the top from the blood collection tube. Carefully remove the needle from the syringe and slowly depress the plunger of the syringe to allow the blood to trickle down the inside of the collection tube.

Specimens clotted during blood draw may result in:

- Platelet clumps
- Falsely decreased cell counts (platelets, red blood cells [RBC], and white blood cells [WBC] on CBC)
- Hemolysis (when forcing blood into tube)

EDTA contamination may cause:

- Falsely decreased calcium
- Falsely increased potassium
- Interference with many specialized tests

Excess anticoagulant (underfilled tube) may result in:

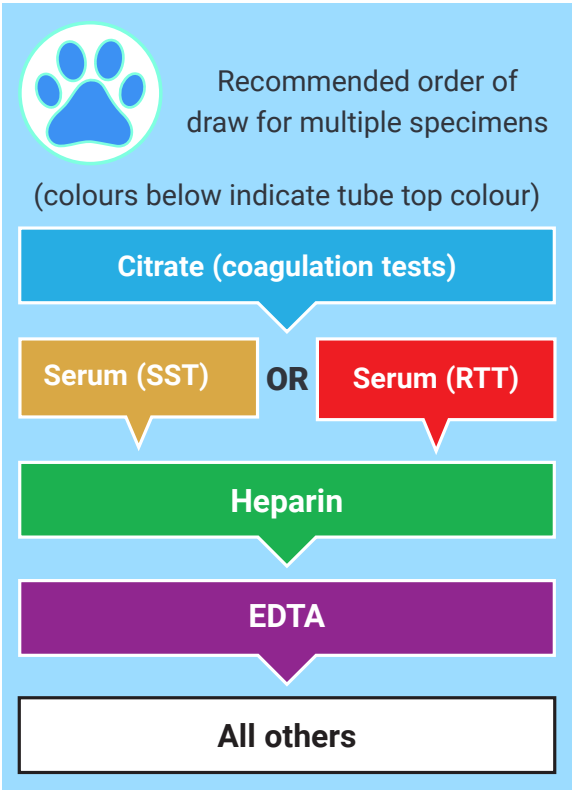
- Decreased RBC count and HCT due to dilution
- Altered cell morphology
- Inaccurate MCV, MCH, MCHC, and Hgb
- Falsely prolonged clotting times

Filling tubes containing additives

- With the exception of blue-top tubes for coagulation testing, which should be filled first, always fill tubes **without** additives first to prevent carryover of tube additives. For example, if you are filling an EDTA tube and an SST, always fill the SST first. Even a small amount of EDTA can interfere with many chemistry results.
- Overfilling or underfilling tubes produces an incorrect ratio of additives to sample and will generate erroneous results; therefore, fill lavender-top (EDTA) or blue-top (citrate) tubes as much as vacuum will allow.
- To prepare a sample in a tube containing additives and avoid clotting, always mix lavender-top (EDTA), blue-top (citrate), or green-top (heparin) tubes well, immediately after filling.

Centrifuging

Proper centrifugation is just as important as proper collection technique. Make sure collected samples are fully clotted before tubes are centrifuged; otherwise, the specimen sent to the laboratory will be plasma rather than serum. Note that some blood samples may take longer to clot than others.



Checking for hemolysis and lipemia

Before sending in your specimen, check for excessive hemolysis or lipemia. Hemolysis and lipemia can interfere with many tests. The reference laboratory will attempt to improve lipemic specimens by ultracentrifugation. However, lipemia can lead to hemolysis, which cannot be removed by ultracentrifugation. In addition, whole blood specimens cannot be ultracentrifuged, and CBC results can be altered by marked hemolysis or lipemia. If excessive lipemia is present upon specimen collection, consider redrawing the sample or delaying elective testing until after an overnight fast.

Consult the Online Test Directory at **vetconnectplus.ca** for detailed information on interferences.

Hemolysis can result in:*

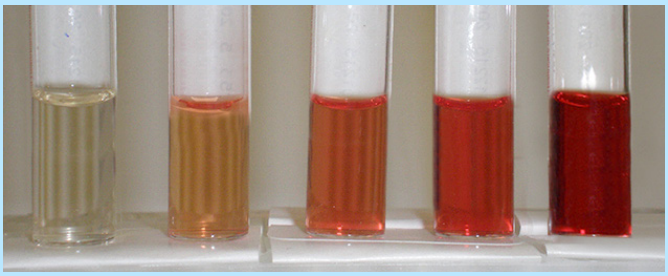
- Increased RETIC-HGB, MCH, and MCHC.
- Inability to read CBC parameters in severe cases.
- Altered bilirubin values.
- Decreased RBC count, ALP, and GGT.
- Increased albumin, fructosamine, total protein, AST, ALT, CK, phosphorus, and potassium.
- Interference with specialized testing.

*Chemistry values affected vary depending on species, methodology, and degree of hemolysis.

Lipemia may result in:

- Secondary hemolysis.
- Decreased AST.
- Interference with specialized testing.
- Altered RBC morphology.
- Falsely increased Hgb, MCH, and MCHC.

Hemolysis



N 1+ 2+ 3+ >4+

Lipemia

N 1+ 2+ 3+ >4+

Urine specimens

A urinalysis is an important component of a minimum database for both healthy and sick animals. Depending on the findings of the urinalysis, additional tests such as urine culture and sensitivity or urine protein:creatinine ratio may be indicated. The accuracy of urine tests is impacted by several preanalytic factors, including correct tube type, time to analysis, and collection method.

Obtaining a quality specimen

- **Sample collection:** Cystocentesis is preferred; however, free catch and catheterized specimens are also acceptable (if cystocentesis is not possible or when contraindicated [e.g., bleeding disorder, transitional cell carcinoma]). **Don't forget to indicate the collection method on the requisition form.**
- **Sample preparation and storage:** Transfer 5 mL of urine to a white-top tube (WTT) or a plain sterile container (without clot activator, serum separator gel, or other additives). Ensure sample is tightly closed to prevent leaking. Refrigerate the urine sample at 2˚C–8˚C until it is submitted to IDEXX Reference Laboratories.

- **DO NOT** submit syringes with needles attached as they cannot be accepted by IDEXX Reference Laboratories.
- Clot activator in serum red-top tubes can interfere with urine sediment examination.
- A lavender-top tube is not recommended for urine cultures (may inhibit growth).

A WTT (sterile plastic tube with no additives) is the recommended tube for urine or fluid cultures.

Fecal specimens

Fecal testing is an important part of preventive care that can ensure pets are free of intestinal parasites. Proper specimen submission—such as sufficient sample size, appropriate packaging, and specimens with limited debris—contributes to the overall accuracy and reliability of results.

Compared to microscopic examination methods, the parasite proteins detected by antigen testing maintain their integrity and do not degrade significantly in specimens held for several weeks refrigerated or at room temperature, as well as at freezing temperatures.

Obtaining a quality specimen

1. Request that the pet owner bring a fresh fecal sample to their appointment or use an IDEXX FecalChek® Home Collection Kit to allow the pet owner to collect the specimen at home after their visit.
   - Collect 3–5 g feces (equivalent to 2 grapes or 1–2 tablespoons; see images below).
   - Avoid samples that have had contact with soil for longer than a few minutes to reduce contamination with free-living nematodes.
   - Avoid samples that have been sitting in cat litter.
2. Transfer sample to a fecal collection container or a clean, leakproof plastic container.
3. If submitting for a Fecal Ova & Parasites centrifugation flotation test, refrigerate fecal specimen until it's shipped or picked up by the courier. Fecal specimens do not require shipment on ice.

For more information on IDEXX fecal testing,visit
idexx.ca/orderfecal

3. Label specimens properly.

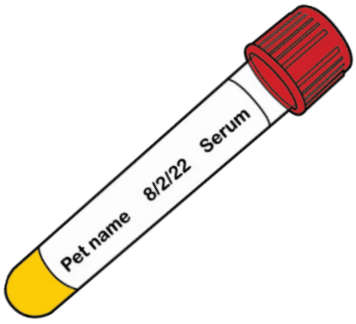
Each specimen should be identified with:

- Patient first and last name
- Date
- Sample type

If using a label:

- Do not apply label over cap/lid.
- Do not wrap label around tube.
- Do not leave excess label hanging off/sticking out from tube.
- Do not cover contents of tube with label.
- Do not use tape to seal the tube shut.

Use clear, legible writing—the clearer the handwriting, the less chance of error.



4. Submit a slide with the tube.

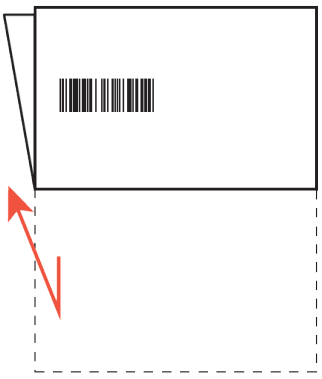
Air-dried slides (1–2 slides per site/lesion) should be included with specimens submitted for fluid analysis or urine cytology for accurate cytologic interpretation. This will preserve cell morphology and help with accurate interpretation.

Note: Submission of a blood film is no longer required when submitting for a CBC.
For your convenience, a hematology technician will now prepare a blood film for all IDEXX CBC-Select™ runs upon receipt of the specimen at the reference laboratory. However, if you suspect blood-borne parasites, especially *Babesia*, submission of a blood film prepared from an ear-prick collection is recommended. Submission of an air-dried blood film is also still recommended for avian and reptilian CBCs.

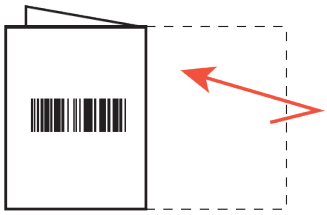
5. Include a test requisition form.

Complete and include an IDEXX Reference Laboratories test requisition form with all specimens. Fold the form in quarters as illustrated below. **IMPORTANT:** Place the form in the rear pocket of the specimen bag with the bar code facing out.

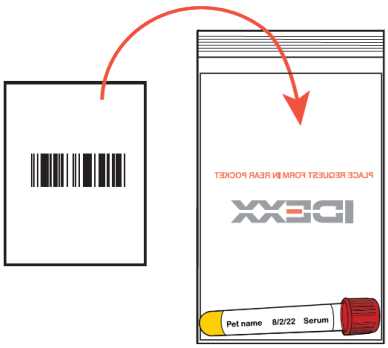
Save time and eliminate errors by using your practice information management system or VetConnect® PLUS to create an electronic requisition. For more information about VetConnect PLUS, call **1-800-667-3411** or visit idexxlearningcenter.com/vetconnectplus.



Fold in half horizontally, so bar code is visible.



Fold in half again vertically, so bar code is visible.



Place form in specimen bag rear pocket, **bar code facing out**.

6. Prepare specimens for pickup.

Specimens may require refrigeration, freezing, or room-temperature storage. Consult the individual test guidelines for instruction on proper storage.

Make sure that specimens are not exposed to heat or direct sunlight in warm weather or allowed to freeze in cold weather. Do not place specimens directly on or near air vents. All specimens must be well insulated during transportation to the laboratory.

When packaging samples, include one patient per bag.

Packaging for transport

- Secure all caps and lids.
- Use one bag for each patient.
- Do not place cold or hot packs directly on LTTs. Direct exposure to extreme cold or heat can cause cell damage and make it difficult or impossible to accurately perform a CBC.
- Protect slides, blood smears, and cytologies from condensation by avoiding direct contact with cold packs. Use plastic slide containers for blood smears and cytology slides.
- Always package nonhistology specimens separately from histology as items may be compromised if they come into contact with moisture or formalin.
- If shipping via IDEXX-Direct®: Absorbent material (e.g., paper towels) may be placed in specimen bag to protect specimens from breaking and to absorb spills. Package specimen(s) in an IDEXX-Direct shipping box. More than one patient specimen can be packaged in the box if tubes/jars are properly labeled, bagged, and sealed. To order more specimen bags or boxes, order online at **idexx.ca/order** or call **1-800-667-3411**.

Packaging for cold weather

- Keep specimens inside as long as possible.
- In the lockbox only: Place a bag of hot saline solution or a plastic bottle filled with hot water at the bottom of the lockbox (avoid direct contact with specimens).

Packaging for warm weather

- Keep specimens inside as long as possible.
- Place a wrapped cold pack in a separate sealed bag at the bottom of the lockbox or IDEXX-Direct shipping box (the cold pack should not be in direct contact with specimens).

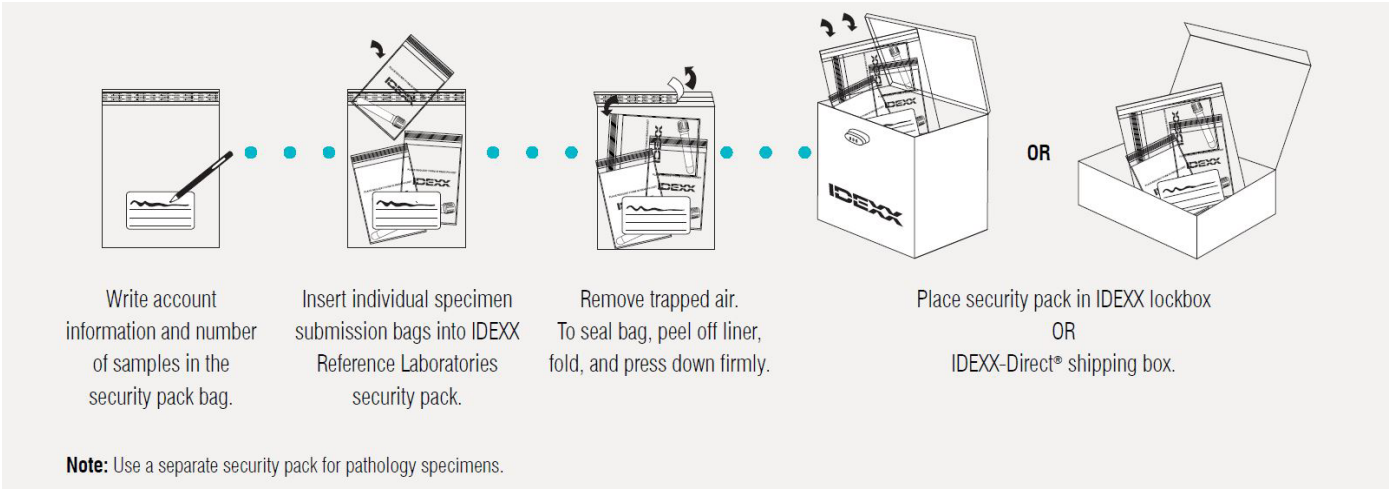
When shipping via IDEXX-Direct, including a cold pack in the box is strongly recommended.

7. Using security packs

Want extra protection for your sample submissions? Optional security packs add convenience and protect your sample submissions.

Security packs are large, tamper-proof bags that hold individual sample bags. These packs have an adhesive strip that provides a permanent seal, allowing for all the samples to travel as one to IDEXX Reference Laboratories. Security packs can be ordered directly from IDEXX Online Orders at **idexx.ca/order**.

Here is how you use security packs:



Note: Each specimen must be placed in a specimen bag before being placed in a security pack. If you have frozen samples, place those samples in their own security pack along with a frozen cold pack. If you have pathology samples, place those in their own security pack.

8. Ship your specimen (for IDEXX-Direct customers).

1. Package specimen.

- Place the IDEXX-Direct® box containing the patient specimens and requisition form(s) into a Purolator Express® Pack and seal.

2. Address.

- Attach a preprinted Purolator waybill to your package.
- **Important:** if shipping on a Friday for Saturday delivery to the IDEXX reference laboratory (available in select areas only), please ensure to use a preprinted "Saturday" waybill and "Saturday" Purolator sticker.
- Preprinted Purolator waybills and "Saturday" stickers are available to order from IDEXX. Order online at idexx.ca/order or call 1-800-667-3411.

3. Arrange pick up.

- Go to purolator.com or call 1-888-SHIP-123 (1-888-744-7123).

4. Track your package.

- Go to **purolator.com** and enter your tracking number in the Track a shipment field, or call 1-888-SHIP-123 (1-888-744-7123).

Specimen collection tube and swab guide

Type			 OR 
	BTT **Blue-top tube** Available in 2 mL; light blue cap.	SST **Serum separator tube** Available in 4 mL gold cap with gel in the bottom of tube.	RTT **Red-top tube**, serum tube, RTT with clot activator. Available in 4 mL and 9 mL; red cap.
Description	Contains sodium citrate anticoagulant.	Contains a gel medium that forms a barrier between the red blood cells and serum after centrifugation.	Plastic, silicone-coated plain red-top tube with clot activator.
Purpose	Citrated plasma for coagulation testing.	Serum for chemistries, immunology, serology, endocrinology. Do not use for transport of fluid specimens or urine.	Serum for chemistries, immunology, serology, endocrinology, therapeutic drug monitoring; also used for transport of separated serum or plasma.
Comments	Correct ratio of blood-to-anticoagulant very important. Specimen should be immediately centrifuged and plasma separated into a WTT (plain plastic tube without clot activator) and frozen.	Gel interferes with certain therapeutic drug monitoring tests (phenobarbital, digoxin, theophylline) and progesterone (use RTT with clot activator instead).	Centrifugation and separation of serum into a second RTT or a WTT is recommended. Clot activator will interfere with microscopic evaluation of urine or fluids submitted for cytology.

Type	 OR 	 OR 	
	LTT **Lavender-top tube** Available in 2 mL; lavender cap.	WTT **White-top tube;** plain plastic tube without clot activator. Available in 6 mL; white cap.	Royal BTT **Dark blue-top tube**, trace element tube. Available in 6 mL; royal blue cap.
Description	Plastic tube with EDTA anticoagulant.	Plain, no-additive, silicone-coated plastic tube without clot activator.	Plastic tube with clot activator; special stopper contains lower levels of trace elements.
Purpose	Whole blood for hematology; may also be used for submission of fluids for cytology.	Transport of serum or plasma for chemistries, endocrinology, immunology, serology, therapeutic drug monitoring, coagulation testing; urine for urinalysis, culture, or cytology; fluids for culture or cytology.	Use for collection of serum for trace elements, e.g., zinc.
Comments	Proper blood-to-anticoagulant ratio is important for accurate results. EDTA plasma is not acceptable for most chemistries.	After centrifugation, serum or plasma collected in other tube types can be transferred into a WTT for transport. Preferred tube type for submission of urine for urinalysis or urine culture. **IMPORTANT: Be sure to label the tube with the specimen type.**	

Type			
	Fecal collection container	Fecal culture transport media Enteric pathogen transport media	Oxoid Signal® blood culture bottle Available in 84 mL.
Description	Empty, sterile plastic tube with scoop.	Fecal culture media ideal for enteric pathogens.	Blood culture media ideal for both aerobic and anaerobic organisms.
Purpose	Feces for fecal ova and parasites, fecal antigen testing, or fecal PCR.	Feces for culture.	Whole blood for culture; use for patients >5 lb.
Comments	Collect fresh specimens only. Specimens allowed to sit on soil may be contaminated with free-living nematodes from the environment. Avoid specimens that have been sitting in kitty litter.	Avoid contamination with urine and soil. Refrigerate promptly to prevent overgrowth.	Keep at room temperature. Collection using sterile technique is critical for accurate results. See the "Microbiology specimen collection guidelines" section.

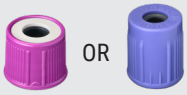
Type			
	Culture swab in transport media Aerobic/anaerobic culture swab, regular tip.	Culture swab with mini-tip Aerobic culture swab, mini-tip.	PCR swab
Description	Plastic-stemmed, sterile, flocked swab with liquid-based culture media.	Wire-stemmed, sterile, mini-tip, yellow-capped swab with gel-based culture media (Amies without charcoal).	Plastic-stemmed, sterile, polyester swab.
Purpose	Use for collection, transport, and preservation of specimens for aerobic or anaerobic culture.	Use for collection, transport, and preservation of specimens for aerobic or anaerobic culture of small sites, such as respiratory, choanal, or cloacal specimens from exotics.	Use for collection of respiratory specimens or tissue aspirates for RealPCR™ tests.
Comments	Refrigerate culture swabs promptly following specimen collection to prevent overgrowth. For instructions on sampling specific sites, see the "Microbiology specimen collection guidelines" section.	Refrigerate mini-tip culture swabs promptly following specimen collection to prevent overgrowth.	Submit swabs dry, without transport media, in an RTT or in an empty sterile tube. Keep refrigerated. For instructions on sampling specific sites, see the "RealPCR specimen collection guidelines," section.

Note: To increase supply availability, IDEXX has partnered with multiple manufacturers. There may be slight variations in appearance but no impact to performance. Tube and swab images are representative of those supplied by IDEXX. Tubes from other manufacturers may have different tube-top colours.

Specimen collection guidelines reference chart

For information on specimen collection guidelines for avian/exotics, microbiology, pathology, and RealPCR, see their sections on the following pages.

If sample is not fully clotted when spun, it will result in plasma rather than serum.

Type of testing	Specimen	Container	Description	Protocol	Storage
Chemistry, immunology, endocrinology	Serum	SST (serum separator tube) 	Gel to separate serum from clot and clot activator	Let specimen clot for 15–20 minutes or until a distinct clot has formed. Some specimens may take up to 30 minutes to fully clot. Centrifuge at 2,500 rpm for 10–15 minutes. Do **not** use SST for progesterone or therapeutic drug monitoring (phenobarbital, digoxin or theophylline).	Refrigerate
Chemistry, immunology, endocrinology, therapeutic drug monitoring	Serum	RTT (red-top tube, serum tube) 	Sterile, empty with clot activator	Let specimen clot for 20 minutes or until a distinct clot has formed. Some specimens may take up to 30 minutes to fully clot. Centrifuge at 2,500 rpm for 10–15 minutes, pipette serum from clot and transfer to a second RTT or a WTT (plain plastic tube).	Refrigerate
Coagulation (PT, PTT, D-dimer, VWD, and quantitative fibrinogen)	Citrated plasma	Collect in BTT (blue-top tube), then transfer plasma to a WTT (plain plastic tube without clot activator) 	Sodium citrate anticoagulant	Correct blood-to-anticoagulant ratio is very important. Fill tube as much as vacuum will allow to obtain proper ratio. Invert gently several times after filling. Centrifuge immediately at 1,500 rpm for 15 minutes. Pipette plasma from cells and transfer to a WTT (plain plastic tube without clot activator). Label as citrated plasma and freeze.	Keep frozen and ship with cold pack.
Hematology	Whole blood	LTT (lavender-top tube) and air-dried unstained blood smears 	EDTA anticoagulant	Fill tube as much as vacuum will allow to obtain proper blood-to-anticoagulant ratio. Invert gently several times. Air-dried blood smears preserve cell morphology.	Refrigerate; **Do not** freeze.
Parasitology (fecal ova and parasites and fecal antigen tests)	Feces	Fecal collection container or clean plastic container	No additive	Collect fresh fecal specimen from bowel movement or fecal loop. Do not allow specimen to sit on soil or in kitty litter.	Refrigerate or room temperature.
Stone analysis	Urolith	Dry sterile container	No additive	**Do not** place in formalin or other liquid.	Room temperature
Urinalysis, urine culture	Urine	WTT (plain plastic tube without clot activator) 	No additive	**Do not** submit a culture swab or syringe; transfer specimen to sterile tube and tightly seal. Indicate method of collection.	Refrigerate and prevent UV/sunlight exposure.

Avian/exotic specimen collection guidelines

Accurate results depend on quality specimens. Please follow the guidelines detailed below.

Specimen collection tubes

Type			
	Mini SST **Gold-top tube**	Mini GNTT **Green-top tube**	Mini LTT **Lavender-top tube**
Description	Contains a gel medium that forms a barrier between the red blood cells and serum after centrifugation.	Plastic tube with lithium heparin anticoagulant.	Plastic tube with EDTA anticoagulant.
Purpose	Serum for chemistries, immunology, serology, endocrinology.	Heparinized whole blood for avian/exotic hematology; heparinized plasma may be used for most chemistries.	Whole blood for avian/exotic hematology
Comments	Gel interferes with certain therapeutic drug monitoring tests (phenobarbital, digoxin, theophylline) and progesterone (use RTT with clot activator instead).	If plasma is desired, specimen must be spun and separated into a RTT or a WTT.	Proper blood-to-anticoagulant ratio is important for accurate results. EDTA plasma is not acceptable for most chemistries.

Chemistry

- Serum (collected in a mini SST) or heparinized plasma (collected in a mini GNTT) may be submitted for avian/exotic chemistries.
- When using a mini GNTT or a mini STT, it is important to separate the serum or plasma after spinning to avoid artifactual changes in chemistry values.
- Mini GNTT: invert the mini GNTT 6–8 times immediately after adding blood and then spin down.
- Mini SST: allow the mini SST to clot for 15–20 minutes before spinning.

Hematology

- For differentials, immediately make an air-dried, unstained blood film using glass slides. Do not use coverslips. Use an individual slide holder for each patient.
- Submit blood in an EDTA lavender-top tube (LTT) or GNTT (without plasma-separator gel). Invert tube 6–8 times immediately after adding blood. Minimum blood volume in GNTT/LTT should be ≥0.3 mL.

DO NOT use an LTT with reptile, kookaburra, crow, ostrich, or emu specimens.

Guidelines for limited sample sizes

- If specimen size is limited, indicate priority of testing in case the specimen size is inadequate.
- If submitting a single specimen for both chemistry and hematology, submit an unspun GNTT (without plasma-separator gel) with an unstained, air-dried blood smear. Some chemistry values may be affected.

Always prioritize tests on your requisition form in case the specimen size is inadequate.

Microbiology

For avian cultures, organisms are categorized as nonpathogenic (reported as normal gram-positive flora), including A-hemolytic streptococci (alpha-strep) and G-hemolytic streptococci (gamma-strep), *Enterococcus*, *Corynebacterium*, *Lactobacillus*, and nonhemolytic staphylococci, or are characterized as pathogenic, including *Enterobacteriaceae* and other gram-negative rods, fungi, *Mycoplasma*, hemolytic staphylococci, *Pasteurella*, and yeast on respiratory and fecal samples. Yeast are noted as present but are not identified on routine bacterial culture.

Susceptibility testing is routinely performed on all *Enterobacteriaceae*, gram-negative rods, and hemolytic staphylococci. Pasteurella is NOT tested because it is predictably susceptible to many antibiotics used in birds.

Note: Antibiotics tested for avians are dependent on the organism and may include amikacin, ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime, cephalexin, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, piperacillin, tetracycline, tobramycin, and trimethoprim/sulfa. Other antibiotics available upon request.

Clinical presentation	Source	Tube/storage	Protocol	Test to request
Upper respiratory signs	Respiratory discharge, aspirate or flush	Culture swab in transport media or fluid in a sterile tube (WTT or RTT)	Sinus aspirate or flush using nonbacteriostatic saline; swab of choanal slit in cases of visible discharge/ inflammation	Aerobic Culture or Aerobic Culture with Anaerobic and/or Fungal Cultures
Lower respiratory signs	Flush or swab of lower airways	Culture swab in transport media, fluid in a sterile tube, or tissue in a sterile tube (WTT or RTT)	Tracheal wash using nonbacteriostatic saline or swab taken through sterile ET tube; swab or biopsy of air sacs or lungs	Aerobic Culture or Aerobic and Fungal Cultures
Diarrhea	Fresh feces	Fecal culture media (preferred) or sterile tube (WTT or RTT)	Fresh feces	Fecal culture
Abscess	Abscessed material	Culture swab in transport media, fluid in a sterile tube, or tissue in a sterile tube (WTT or RTT)	**Note:** Avian abscesses often contain caseous material which may yield poor results. Submission of a combination of peripheral purulent material, as well as tissue from the margin or wall of the abscess will often provide the best results.	Aerobic Culture or Aerobic and Anaerobic Cultures
Wound	Infected tissue	Culture swab in transport media or tissue in a sterile tube	Flush wound with sterile nonbacteriostatic saline, then swab or collect biopsy of affected tissue.	Aerobic Culture or Aerobic Culture with Anaerobic and/or Fungal Cultures

Microbiology specimen collection recommendations

In birds, the major microbiology analyses are for respiratory infection, diarrhea, and abscesses.

Histology

Exotics species will be assigned to the IDEXX Reference Laboratories team of anatomic pathologists with special expertise in avian and exotic species.

Microbiology specimen collection guidelines

Special instructions

- Inform the laboratory if the animal is being treated with antimicrobial drugs.
- Indicate if the patient is immunocompromised.
- **DO NOT** submit syringes with needles.
- Clearly specify sites of all cultures, including method of collection for urine specimens.
- Urine, fluids, aspirates, and tissues may be submitted in a WTT (plain plastic tube) or an RTT (red-top tube). An LTT (lavender-top tube) is not recommended for cultures as it may inhibit bacterial growth. An SST (serum separator tube) is not appropriate for cultures.
- Culture specimens should be refrigerated promptly to prevent overgrowth, with the exception of blood cultures, cerebrospinal fluid (CSF), and joint fluid, which should be kept at room temperature.
- Recent antibiotic therapy (within the previous 2 weeks) may result in lower yield or negative cultures. Withdrawal from antibiotics for a minimum of 72 hours, ideally 7–10 days, is recommended when culturing a patient following antibiotic administration.
- When submitting two swabs from different sites, submit each swab with its own requisition form to be plated separately. If you would prefer these swabs to be cultured together on the same plate under a single test code, submit together and indicate this on the requisition form.

Normal flora, predictable susceptibility patterns, and nonpathogenic organisms

IDEXX follows guidelines set by the Clinical and Laboratory Standards Institute (CLSI) as well as our own interpretive standards gained from years of experience in performing susceptibility testing. We believe these are the "best practice" microbiology techniques, and we would be happy to discuss the following policies with you:

- Susceptibilities will not be performed on normal flora or nonpathogenic organisms.
- Pathogens with predictable susceptibility patterns or with no CLSI interpretive standards will be reported with a recommended list of antimicrobials. Examples include ß-hemolytic streptococci (beta-strep) and *Pasteurella* in nonsterile sites.
- Pathogens with atypical growth characteristics (i.e., slow-growing or anaerobic) render susceptibility testing inaccurate and misleading. Therefore, recommended antibiotics will be reported in an effort to guide therapy. Examples include *Corynebacterium pseudotuberculosis* and *Actinomyces*.

Gram stains

- Aerobic cultures on avians and exotic species automatically receive Gram stains.
- For other species, Gram stains must be requested and will incur an extra fee (see test code GRA [Gram Stain]).
- The best sources for valuable Gram stain information are wounds, abscesses, fluids, tracheal washes, and sterile sites.

Microbiology site-specific guidelines

Source	Tube/storage	Protocol	Test to request
Abscess or wound	Culture swab in transport media or tissue/fluid in sterile tube*	Aseptically prepare collection site. Aspirate fluid or pus from abscess, pustule or vesicular wounds.	Aerobic Culture or Aerobic and Anaerobic Cultures
Blood	One blood culture bottle per time point. Do not submit swabs, serum or whole blood in LTT or RTT. For animals ≥5 lb, use 84 mL Oxoid Signal® blood culture bottle. For animals <5 lb, (and for add-on fungal culture if desired)use yellow-top Wampole® Isolator™ blood culture tube. Keep at room temperature.	Collection using sterile technique is critical for accurate results. Aseptically prepare venipuncture site. Disinfect the top of culture bottle with alcohol and let dry. Ideally two samples drawn approximately one hour apart from different venous sites should be submitted. • >40 lb, place 10 mL blood in one 84 mL bottle. • 20–40 lb, place 7.5 mL blood in one 84 mL bottle. • <20 lb, place 5 mL blood in one 84 mL bottle.	Blood Culture
Bone marrow	Sterile tube (WTT or RTT)*	Aseptically prepare collection site.	Aerobic Culture or Aerobic and Anaerobic Cultures
Brucella canis	Specimens should not be submitted for culture if *Brucella canis* is suspected. See the "Submission of highly pathogenic zoonoses" section for additional details.		
Cerebrospinal fluid (CSF)	Sterile tube (WTT or RTT)* Keep at room temperature.	Collect CSF by aseptic subdural tap, ventricular aspiration or lumbar puncture.	Aerobic Culture or Aerobic and Anaerobic Cultures
Ears	Culture swab in transport media	Posterior pharyngeal cultures may also reveal organisms causing otitis media. **Note:** Topical treatments may inhibit bacterial growth.	Aerobic Culture
Eyes	Culture swab in transport media	Use swab to collect suppurative material from cul-de-sac or medial canthus. **Note:** Topical anesthetic may inhibit bacterial growth.	Aerobic Culture

For help interpreting your culture results, visit **idexx.ca/mic**.

Microbiology site-specific guidelines (continued)

Source	Tube/storage	Protocol	Test to request
Feces	Fecal culture transport media (preferred) or sterile tube (WTT or RTT)*	Avoid contamination with urine and soil. If *Clostridium perfringens* and *Clostridium difficile* enterotoxin testing will be performed, include 3–5 g fresh feces in sterile container.	Fecal Culture
Hair	Sterile tube (WTT or RTT)*	Hair should be plucked (not cut) in order to include follicles.	Fungal Culture
Joint fluid	Sterile tube (WTT or RTT)* or blood culture bottle. Keep at room temperature.	Aseptically inject fluid into sterile tube or blood culture bottle. Specimens >48 hours old are not suitable for culture.	Aerobic Culture or Aerobic and Anaerobic Cultures
MRSA	Culture swab in transport media	If screening for nonclinical carrier, collect swabs from nasal passages and rectum. For clinical patients, collect specimen from lesion and submit for Aerobic Culture. *Staphylococcus* spp. isolated in routine cultures are screened for methicillin resistance and further identified to species level when indicated.	If screening for nonclinical carriers: MRS Screening Culture If lesion is present: Aerobic Culture
Nail or Skin	Sterile tube (WTT or RTT)*	Use sterile blade or swab to collect material from infected nail. Scrape or swab active border of skin lesions.	Aerobic and/or Fungal Culture
Sinus	Culture swab in transport media or tissue/fluid in sterile tube (WTT or RTT)*	Aspirate from maxillary, frontal or other sinuses. **Note:** Chronic sinusitis often involves anaerobic bacteria.	Aerobic Culture or Aerobic and Anaerobic Cultures
Tissue	Sterile tube (WTT or RTT)*	Place tissue in sterile tube with small amount of sterile LRS (Lactated Ringer's solution) to keep specimen hydrated.	Aerobic Culture or Aerobic and Anaerobic Cultures
Tracheal wash/BAL	Sterile tube (WTT or RTT)*	Place wash fluid in sterile tube. Best results can be expected when buffered solution, such as LRS (Lactated Ringer's solution), is used rather than acidic isotonic saline solutions.	Aerobic Culture
Urine	Sterile container (WTT [plain plastic tube] preferred)*	Indicate collection method. Cystocentesis is strongly recommended (except in large animals). Avoid contamination with feces. Swabs are not acceptable specimens for semiquantitative urine culture.	Urine Culture

*LTT (lavender-top tubes) are not recommended for cultures as they may inhibit bacterial growth. SST (serum-separator tubes) are not appropriate for cultures.

A WTT (sterile plastic tube with no additives) is the recommended tube for urine or fluid cultures.

Submission of highly pathogenic zoonoses

There are no designated Biosafety level 3 (BSL-3) microbiology laboratories at IDEXX Reference Laboratories in Canada or the United States. Therefore, specimens suspected of containing organisms requiring BSL-3 containment should **NEVER** be sent for culture and will **NOT** be accepted for culture. These highly pathogenic zoonotic organisms include, but are not limited to, the following:

- *Bacillus anthracis*
- *Brucella abortus*
- *Brucella canis*
- *Brucella melitensis*
- *Brucella suis*
- *Burkholderia (*formerly *Pseudomonas) mallei*
- *Burkholderia (*formerly *Pseudomonas) pseudomallei*
- *Francisella tularensis*
- *Mycobacterium bovis*
- *Mycobacterium tuberculosis*
- Prions, such as bovine spongiform encephalopathy (BSE), etc.
- Rabies virus
- *Yersinia pestis*

If any of the previously listed organisms are suspected, contact your provincial or territorial veterinarian and/or provincial or territorial veterinary diagnostic laboratory. See the Public Health Agency of Canada (PHAC) website at **canada.ca/publichealth** for more information on pathogenic zoonotic organisms.

In addition, agar plates with fungal growth or environmental specimens suspected of containing the following organisms will **NOT** be accepted for culture testing:

- *Blastomyces dermatitidis*
- *Coccidioides immitis*
- *Coccidioides posadasii*
- *Histoplasma capsulatum*

Cytology or histology of affected sites or fungal serology tests may be more appropriate alternatives for the diagnosis of the above organisms.

Pathology specimen collection guidelines

Accurate results depend on quality specimens. Follow the guidelines detailed below:

- Provide patient signalment (including breed), relevant history, clinical signs, physical examination findings, therapies instituted, and the anatomic site sampled.

Patient history and clinical findings contribute to an accurate result.

- Include the following information, where applicable:
  - Gross lesion description, including size, shape, consistency, symmetry, and border definition (well demarcated vs. invasive)
  - Radiographic findings (especially for bony lesions or oral masses) and/or ultrasonographic findings (especially for samples from internal organs)
  - References to prior laboratory results (prior cytology/biopsy, recent CBC), including accession numbers.
  - Any specific questions you would like answered
  - Working clinical diagnosis

To learn more, view the *Important Considerations in Cytology Sample Collection and Submission* and *Important Considerations in Histology Sample Collection and Submission* snippets at **idexxlearningcenter.com**.

IMPORTANT! Only biopsy submissions submitted in a concentration of 10% buffered formalin are acceptable. The liquid contents in the biopsy jars must be indicated and clearly labeled on the jars.

Refer to: **go.idexx.com/submitbiopsy.**

Choose the correct test code

Fees are determined by the number of sites, lesions/masses, or organs submitted. Use the appropriate test code based on number of sites. For help determining the correct number of sites, visit **idexx.com/submitpathology.**

Biopsy submission guidelines

Learn more about shipping and packaging requirements for biopsy specimens at: **go.idexx.com/submitbiopsy.**

Quick tips:

- Use new jars, one jar per site, and label with the patient's first and last name, collection date, specimen ID, and veterinarian's name.
- 10:1 formalin to tissue ratio. Completely cover the mass. Microcassettes are available for small specimens.
- Ensure jars are labeled with "10% buffered formalin" or "saline" if using non-IDEXX issued jars.
- Submit using the standard shipping or courier service process.

Place specimen(s) in the appropriate-sized IDEXX provided formalin container(s).

- Do not reuse containers or use containers not approved for formalin.
- Use separate, individually labeled jars for each site in a multi-site submission.
- If the specimen is very small, place it in a microcassette before putting it in a formalin container (microcassettes are available through IDEXX Reference Laboratories).
- Make sure to use a compliant biopsy container that is large enough to completely cover the entire mass/lesion. Use a 10:1 formalin concentration to allow for adequate fixation.

Label each container with the patient's first and last name, collection date, specimen ID, and veterinarian's name.

Package submission(s) individually for each patient, along with their requisition form.

- To ensure all specimens for the same patient are processed together, package all jars with the requisition form into one bag.
- If there is not enough room to fit all jars into a single bag, use a larger resealable bag that will keep all sites submitted for the patient together, or wrap an elastic band around multiple bags for the same patient.
- Submit samples using your standard courier service process.

Large tissue specimen shipping instructions

- Samples that are too large to fit in IDEXX-provided formalin jars should be shipped fresh, wrapped in gauze wetted with saline (not soaked). IDEXX provides a Large Biopsy Sample Submission Kit (see next page) for convenience. Instructions are included.
- Triple bag the specimen, using 1- to 5-gallon freezer bags or 10-gallon red biohazard bags. Place absorbents and ice packs inside the middle layer bag.

Note: Larger specimens require additional time for fixation; the normal turnaround time may not apply.

			
Type	Biopsy Jar—60 mL with 30 mL Formalin	Biopsy Jar—120 mL with 60 mL Formalin	Large Biopsy Sample Submission Kit
Description	60 mL jar for histology specimen; contains 30 mL of formalin.	120 mL jar for histology specimen; contains 60 mL of formalin.	Heat-sealed kit contains: • (1) 4-mil double-zipper bag (12" × 15.7") • (1) 2-mil double-zipper bag (12" × 15.7") • (1) 2-mil IDEXX Specimen Bag (14" × 18") • Kit instruction sheet.
Purpose	Collection and fixation of samples submitted for pathologic testing and review	Collection and fixation of samples submitted for pathologic testing and review	Submission of fresh tissue samples for pathologic testing and review
Comments	Make sure you use a compliant biopsy container that holds enough to completely cover the entire mass/lesion. Use a 10:1 formalin concentration to allow for adequate fixation. Lids of biopsy jars have a double-click sealing closure. Please be sure the lids are secured appropriately. Do not place any labels on top of the label included on the jar.	Make sure you use a compliant biopsy container that holds enough to completely cover the entire mass/lesion. Use a 10:1 formalin concentration to allow for adequate fixation. Lids of biopsy jars have a double-click sealing closure. Please be sure the lids are secured appropriately. Do not place any labels on top of the label included on the jar.	Samples that are too large to fit in IDEXX-provided formalin jars should be shipped fresh, wrapped in gauze, wetted with saline (not soaked).

Cytology submission guidelines

Prepare slides

- Make slides using either a squash technique or a blood smear technique. Stain one slide to ensure adequate cellularity and quality.
  Note: The following handling conditions may result in nondiagnostic slides:
  – Material being expelled onto slides but not smeared
  – Excessive pressure being applied when smearing material
  – Material being too dense or thick
- Both prestained and unstained (preferred) slides are acceptable for cytology.
- Slides should be air-dried only. Do not fix slides, spray with hair spray, or apply a coverslip. Avoid contact with oil.
- Store slides at room temperature, and keep specimens away from formalin fumes.
- For fluid specimens, submit fluid in a lavender-top tube (LTT; EDTA), along with 1–4 slides prepared from the fluid.
  – Do not submit fluid in a serum separator tube, serum RTT with clot activator, or in a syringe for cytology.
  – Fluid in a WTT (plain plastic tube) may be included along with the LTT if a culture may be ordered based on cytology results.

Label specimens

- Using pencil, label the slide(s) and any tubes clearly with patient's first and last names and place into slide containers.
- Label slide holders and tubes with patient's first and last names, collection date, specimen ID, and veterinarian's name.
- If submitting for multiple sites, label both slides and slide boxes with site.

Slide submission

- Provide signalment, breed, relevant history, clinical signs, and specimen site location(s) to help our pathologists give you the most comprehensive interpretation. Include the following where applicable:
  – Gross lesion description, including size, shape, consistency, symmetry, and border definition (well-demarcated vs. invasive).
  – Radiographic findings (especially for bony lesions or oral masses) and/or ultrasonographic findings (especially for samples from internal organs).
  – References to prior laboratory results (prior cytology/biopsy, recent CBC etc.), including accession numbers.
  – Any specific questions you would like answered and working clinical diagnosis.
- For fine-needle aspirates (FNAs) or fluids, submit up to four slides per site. Submission of more than four slides per site may result in additional charges and a longer turnaround time.
- For lymph node(s), 3–10 slides can be submitted (1–4 slides per lymph node) using the specific test code for Lymph Node Cytology. This will ensure the most appropriate processing of your specimen and allow for the appropriate larger number of slides associated with these cases.
- For fecal cytology, submit 1–4 prepared slides. Do not submit fecal swabs or fresh feces.
- For hair, fur, or skin cytology, submit 1–4 prepared, stained slides. Do not submit specimens in a tube. Submission of tape preps is not recommended.

To learn more about submitting samples for cytology or biopsy, visit idexx.com/submitpathology.

RealPCR specimen collection guidelines

Polymerase chain reaction (PCR) testing offers a versatile testing modality for infectious diseases. However, accurate results depend on collecting the appropriate specimen type for the clinical manifestation. Provided below are detailed protocols for each specimen type.

All specimens should be stored and shipped refrigerated, unless otherwise indicated. Specimens should be collected prior to antibiotic, antifungal, or antiviral treatment directed against the organism of interest or collected at least 2 weeks after withdrawal of these medications. Specimens received within 72 hours of collection are preferred, but most specimens are stable for up to 10 days if stored refrigerated, unless otherwise indicated. Vaccination with an attenuated or modified live vaccine may result in positive PCR test results for up to a few weeks postvaccination.

Every RealPCR™ specimen submission is processed with up to 7 quality controls. If a specimen fails the preanalytical quality control (DNA and RNA), a repeat extraction is attempted. If quality control criteria are still not met for a particular diagnostic specimen, the submitting veterinarian will be contacted and encouraged to submit a fresh specimen at no additional charge.

Source	Tube/storage	Protocol
Whole blood	EDTA whole blood (LTT)	2 mL (0.1 mL minimum)
Feces	Sterile container (preferred) or empty, clean container	5 g (1 g minimum) fresh feces
Urine	Sterile container	2 mL (0.1 mL minimum) urine
Fluid (CSF, pleural effusion, respiratory wash specimens, uveal fluid, ascites, abscess aspirates, etc.)	LTT (preferred), RTT, or WTT (plain plastic tube)	0.5 mL (0.1 mL minimum) fluid
Swabs	RTT or WTT (plain plastic tube)	Submit dry, plastic-stemmed swabs, without transport media. Respiratory specimens may include a deep pharyngeal swab (with visible organic material on swab; please rub firmly), and a conjunctival swab (wipe eye clean; swab inside of eyelid), which can be submitted together in the same tube, or a nasal swab (equine respiratory panels or systemic mycoses). When acquiring a pharyngeal swab, precautions should be taken to prevent patient from biting and/or ingesting part of swab. Collect specimens prior to fluorescein staining.
Tissue aspirates	Submitting tissue aspirates on a swab improves stability of the specimen. 1–3 air-dried, unstained slides (keep refrigerated) are also acceptable specimens but may result in lower sensitivity.	1. Aspirate tissue into the hub of the needle using standard techniques. 2. Remove the syringe from the needle and fill syringe with air. 3. Reattach the syringe to the needle and squirt out aspirate onto the end of a dry, plastic-stemmed swab. 4. Submit the swab in an RTT or a WTT (plain plastic tube); keep refrigerated.
Tissue biopsies	PCR testing can be performed on previously submitted histopathology specimens (formalin-fixed, paraffin-embedded tissue). Fresh, refrigerated tissue specimens submitted within 24 hours of collection are also acceptable. Freezing is not recommended unless the specimen can be maintained frozen until arrival at the PCR laboratory.	

Serum is not a good specimen for PCR because the cells containing DNA have been removed from the sample.

Specimen tube quick guide

Recommended order of draw for multiple specimens

Cap color	Tube type	Sample type	Number of inversions	Clot or not	Spin time	Spin speed
Blue	Sodium citrate	Plasma	4	N/A	10 minutes	1,500 g
Red	Clot activator	Serum	5–10	15–30 minutes	10–15 minutes	2,500 g
Gold	Clot activator with gel	Serum	5–10	15–30 minutes	10–15 minutes	2,500 g
Green	Lithium heparin	Plasma	5–10	N/A	15 minutes	1,500 g
Lavender	K2 EDTA	Whole blood/ plasma	8–10	N/A	N/A	N/A
White	No additive	Urine, transfer for separated serum or plasma	5–10	N/A	N/A	N/A

One complete inversion

- Turn the filled tube upside down and return it to an upright position.
- Repeat required number of times for each tube type.

= 1 inversion

Coagulation draw volume guide

Ensure the correct blood-to-additive ratio is met by checking the draw volume against the nominal fill mark on the tube or by holding the tube up to this guide.