Frequently-Asked Questions: Clinical Pathology

What does the Clinical Pathology Section do?
The Clinical Pathology Laboratory of the Diagnostic Center for Population and Animal Health (DCPAH), offers a full range of veterinary diagnostic testing in the areas of clinical biochemistry, hematology, hemostasis, urinalysis, and cytology. The laboratory is staffed by four board-certified veterinary clinical pathologists. Our highly qualified technical staff consists of certified Medical Technologists and Medical Laboratory Technicians with extensive experience in veterinary testing and knowledge of species differences. Careful attention to quality assurance ensures the accuracy of results.

How do I contact the Clinical Pathology Laboratory?
Regular business hours are:

Monday though Friday 8 AM to 10 PM
Saturday and Sunday 8 AM to 11 AM

Please call 517-355-1774 if you have any questions.

A clinical pathologist is available for service duty Monday - Friday from 8 AM - 5 PM.

When contacting the Clinical Pathology Laboratory with questions about a test result on a patient, please have as much of the following information available as possible:

- Owner name
- Animal name
- DCPAH account number
- DCPAH encounter number

Please do not hesitate to contact the Clinical Pathology Laboratory with any questions or concerns. We would like to help you with sample management before the sample is collected.

How should samples be submitted to the clinical pathology laboratory?
Submission Form

Complete a DCPAH Clinical Pathology submission form for each patient. Submission forms are available on the DCPAH CD, the DCPAH website, and upon request from the lab. Be sure to complete all sections of the submission form including

- Clinic Information
- Patient Demographics
- Specimen Type
- History and Clinical Signs
- Tentative Diagnosis
- Test Selection
- Specimen Source (for cytology)
- Sample Collection

**Hematology**

Perform a venipuncture using the proper technique. Blood should be collected into a lavender top (EDTA) tube.

- Specimen container must be at least half full.
- Mix at least 10 times using gentle inversion immediately after collection.
- For extremely small animals use an EDTA microtainer tube.
- Make two air dried blood smears as soon as possible following collection (particularly if the blood will not arrive at the laboratory the same day it is collected).
- Keep the slides at room temperature and protected in a rigid (e.g., plastic) slide holder.
- Label tube and slides with the animal’s name, the owner’s name, and collection date.
- Keep the whole blood refrigerated until shipping.
- Ship whole blood on cold pack. (Do Not Freeze)
- Do not include blood smear in the refrigerated portion of containers.

Fresh smears are particularly important when evaluating blood smears for:

- morphologic interpretation of cells
- Mycoplasma organisms

High quality slides should have:

- a feathered edge and extend 1/2 to 2/3 the length of the slide.
- a monolayer region where erythrocytes and leukocytes can be adequately evaluated.
Blood smears submitted without whole blood are considered submissions for pathologist review and are processed as a cytology submission. If a CBC was performed on an in-clinic analyzer, please send results from the analyzer along with the blood smears to facilitate interpretation by the pathologist.

**NOTE:** Submission of blood and slides for a CBC is less costly and provides more information than submission of blood smears only. If blood submitted for a routine CBC is found to have an abnormality, unusual cell type or other sources of concern, the smear will be sent to a clinical pathologist for further evaluation at no additional cost.

**Chemistry**

Perform venipuncture using the proper technique. For chemistry profiles, collect blood into a red top Vacutainer tube—a serum-separator tube (SST) is recommended.

- Mix SST tube by gentle inversion to distribute the clot activator throughout the sample immediately following collection. Red top tubes without an activator do not require mixing.
- Allow specimen to clot.
- Centrifuge at 3,000 x g for 10 minutes.
- Separate serum from cells as soon as possible following collection. It is also helpful to separate serum when using SST tubes because the barrier can break down in transit.
- Label tube with the animal’s name, the owner’s name, and collection date.
- Keep the serum refrigerated until shipping.
- Ship on cold pack.

When submitting samples for bile acid, collect both the fasting and 2 hr post prandial specimens into a red top Vacutainer tube—serum separator tubes (SST) are recommended.

- Collect a 12 hour fasting sample.
- Collect a 2 hour post prandial sample (we cannot provide reference intervals if the post-prandial sample is collected at some time other than 2 hours after feeding).
- Allow specimens to clot.
- Centrifuge at 3,000 x g for 10 minutes.
- Separate serum from cells as soon as possible following collection.
- Label all tubes with the animal’s name, the owner’s name, and collection date. Also include fasting or 2 hour post prandial on the appropriate tube.
- Ship on cold pack.
For other chemistry assays performed by the DCPAH Clinical Pathology laboratory please refer to the Available Tests section of the DCPAH CD or website (www.dcpah.msu.edu), or contact the laboratory.

Hemostasis

Atraumatic venipuncture is critical to minimize activation of coagulation factors and platelets. Collect blood into a light blue top (Na citrate) Vacutainer tube.

- Fill light blue top (Na citrate) to fill mark on tube (this is critical for accurate results).
- Do not over or under fill tube - this can falsely shorten or prolong clotting times.
- Mix at least 10 times using gentle inversion immediately after collection.
- Centrifuge specimen at 3,000 x g for 10 minutes and transfer plasma to a leakproof plastic tube.
- For species other than dog, cat and horse, please submit a citrated plasma sample from a healthy patient of the same species to serve as a control.
- Label tube with the animal’s name, the owner’s name, and collection date. If a control is included, label with animal name and “control”.
- Ship frozen plasma overnight on dry ice. (If the sample can not be delivered to the laboratory within 8 hours.)

NOTE: If blood collection is difficult, collection from a different vein using a fresh needle is recommended. Collecting blood directly into vacutainer tubes can be problematic, particularly in small dogs or cats because of collapsing veins. Blood can be collected using a needle and syringe (non-heparinized) then quickly transferred into a light blue top (Na citrate) tube.

Cytology

- Fluid Cytology

Typically, fluid should be collected aseptically into an appropriately-sized tube containing EDTA. If fluid volume permits, fluid may be submitted in an EDTA tube and a red top clot tube. Because cells deteriorate rapidly in many fluid samples, please prepare both direct smears and concentrated smears to be submitted with the fluid cytology. To prepare concentrated smears:
  - Centrifuge an aliquot of the fluid sample.
  - Remove a portion of the supernatant.
  - Resuspend the cellular material.
  - Prepare the concentrated smears.

For cerebrospinal fluid (CSF)

- Always collect CSF into a tube without anticoagulant.
- CSF requires special handling because of low cell and protein concentrations.
- Please contact the laboratory for hints about handling of CSF samples.

Remember:

- Smears should be allowed to air dry; fixation is not required and should be avoided.
- Label tube with the animal’s name, the owner’s name, and collection date.
- Label slides with the animal’s name, the owner’s name, specimen source, and indication of whether the smear is from direct or concentrated fluid.
- Slides should be kept at room temperature and protected from dust, scratches, or breakage.
- Rigid plastic slide holders work best. Avoid using cardboard slide holders.
- Fluid should be kept refrigerated until shipping.
- Ship fluid on cold pack. (Do Not Freeze).
- Do not include smears in the refrigerated portion of containers.

For synovial fluid submissions, each joint should be submitted as a separate cytology—each with a separate submittal form.

NOTE: Place a small amount of fluid into certain large EDTA-containing tubes will falsely increase specific gravity and total protein concentration, and may cause enough osmotic cell shrinkage to interfere with cytologic evaluation. A tube without anticoagulant may be used for samples that are not expected to clot (e.g., those without blood and without marked protein exudation).

- Fine needle biopsy

Most samples are collected by fine needle biopsy using a fine gauge needle (21-guage or smaller). Larger needles may be helpful for poorly-exfoliating tissue, but may cause more hemorrhage. The mass should be stabilized and the needle inserted into the tissue—with or without an attached syringe. The needle is redirected into the tissue several times (if the mass is large enough) using crisp forward motions to cut cores of tissue. If a syringe is used, gentle negative pressure may be applied help hold tissue in the needle. Use of suction should not replace forward cutting motions, and excess suction often yields samples that are more difficult to evaluate because of increased blood contamination. When used, negative pressure is released prior to removing the needle from the tissue. Material does not have to be visible in the syringe to have an adequate sample.

Immediately after removal from the tissue, the material in the needle should be expressed onto clean microscope slides by placing the tip of the needle against the slide (not spraying droplets from above the slide).

- Place material close to the frosted end of the slide.
- Spread the material toward the center of the slide.
- All samples must be quickly spread before they clot or dry.
- Label slides with the animal’s name, the owner’s name and specimen source.
- Slides should be kept at room temperature and protected from dust, scratches, or breakage.
- Rigid plastic slide holders work best. Avoid using cardboard slide holders.
- Do not allow smears to come in contact with formalin fumes.

NOTE: Spreading is critical to provide an optimal zone that is thin enough to allow evaluation of cells. If material is not spread well, the smears are often too thick to allow adequate evaluation of the cells. If the drop of material is large, such that a smear will
be thick or extend off the slide, part of the drop should be picked up on the edge of another slide and spread onto a third slide with a gentle squash technique. This can be repeated to make several good smears rather than one thick smear.

**NOTE:** Order one cytologic evaluation per organ or tissue except for lymph nodes.
- You can include samples from multiple lymph nodes on one submission form, but clearly label slides with collection site.
- For all other samples, each organ or tissue requires a separate submission form and must be ordered separately.

**Sample Shipping**
- DCPAH shipping supplies and solutions can be found at in a document *(How Do I Submit Samples to DCPAH)* in the Shipping Options section of the DCPAH CD or website.
- Please ship DCPAH Clinical Pathology specimens or joint submissions for Clinical Pathology plus another DCPAH laboratory to:
  
  Diagnostic Center for Population and Animal Health  
  Clinical Pathology Section  
  A-215 Veterinary Medical Center  
  Michigan State University  
  East Lansing, MI 48824-1314

- There is no Saturday delivery at this address.
- Federal regulations govern packaging and labeling of diagnostic specimens. Packaging instructions and packaging solutions *(Product Order Form)* can be found in the Shipping Options section of the DCPAH CD or website.

**How quickly can I expect to get my results?**
The majority of testing is performed with results reported on the same day samples are received. Due to the labor, time and cost involved, a few specialty, low-volume tests are set up once a week. These include:

- ANAs (performed on Tuesdays with results available Wednesday)
- Protein electrophoresis (performed on Wednesdays with results available on Thursday)
- Bovine immunoglobulin assays by RID (set up Wednesdays with results available on Thursday).

If data are critical to your patient, you may request that a test be performed STAT for an additional fee. All test requests are considered important and samples are analyzed as rapidly as possible in the order in which they are received in the laboratory. A designation of STAT means that testing on that sample will be performed prior to testing of all pending routine samples.

Cytology samples that arrive at the Clinical Pathology Laboratory before mid-afternoon will be reported the same day if at all possible. A clinical pathologist will complete cytology and hematology review as expeditiously as possible each day.
What analyzers are used in the clinical pathology laboratory?

- Hematology – Advia 120
- Chemistry – Olympus AU640e
- Coagulation - STA Compact
- Blood gas - Nova CCX

What results are included in a mammalian CBC?

With the exception of llamas, camels and deer, the mammalian CBC includes:

- WBC concentration
- WBC differential
- RBC concentration
- Hemoglobin (Hgb) concentration
- Hematocrit (Hct)
- Mean cell volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean cell hemoglobin concentration (MCHC)
- Cell hemoglobin concentration mean (CHCM)
- Red cell distribution width (RDW)
- Hemoglobin distribution width (HDW)
- Platelet concentration
- Mean platelet volume (MPV)
- Plasma total protein (TP) by refractometer
- Spun Hct (packed cell volume, PCV)
- Fibrinogen concentration determined by heat precipitation (Equine and Food animal only)
- Cytograms and histograms from the automated hematology analyzer, Advia 120
- Microscopic examination of a blood smear

For llamas, camels and deer, the CBC includes:

- WBC concentration
- WBC differential
- Hemoglobin (Hgb) concentration
- Mean cell hemoglobin concentration (MCHC)
- Plasma total protein (TP) by refractometer
- Spun Hct (packed cell volume, PCV)
- Microscopic examination of a blood smear
Advia cytogram and histogram examples from a normal canine

- **RBC VHC**: (RBC Cytogram: X axis = hemoglobin concentration and Y axis = cell volume),
- **PEROX**: (WBC Cytogram: X axis = peroxidase staining and Y axis = cell volume) J. noise and platelets, K. lymphocytes, L. monocytes, M. neutrophils, N. eosinophils, O. LUCs (Large Unstained Cells that may be lymphocytes, monocytes, or blasts).
- **BASO**: (WBC Cytogram depicting X axis = nuclear complexity and Y axis = platelet volume), P. basophils, Q. lymphocytes and monocytes, R. neutrophils and eosinophils.
- **PLT SCATTER**: (Platelet Cytogram: X axis = refractive index and Y axis = platelet volume) S. platelets, T. large platelets, U. RBCs, V. RBC fragments, W. debris and ghost erythrocytes, X. ghost erythrocytes.

**What is included in an “avian” CBC?**

Avian CBC is the DCPAH term for a CBC performed on blood from any animal with nucleated erythrocytes and thrombocytes (e.g., birds, reptiles, amphibians). These samples cannot be analyzed by the automated hematology analyzer. An avian CBC includes:

- WBC concentration (calculated from microscopic cell count and WBC differential)
- WBC differential
- Plasma total protein (TP) by refractometer
- Spun Hct (packed cell volume, PCV)
- Microscopic examination of a blood smear
- Estimation of WBC adequacy from microscopic evaluation of the slide
**NOTE:** Some species of birds (particularly raptors) have lymphocytes that may lyse when they contact EDTA anticoagulant. Please prepare slides from fresh blood (not containing anticoagulant) immediately following collection for differential counting for these species.

### What can interfere with CBC results?

- Clotted samples will be rejected because CBC findings would be inaccurate.
- Underfilling EDTA tubes can alter erythrocyte parameters. Fill tubes to at least half the intended volume.
- Lipemia may falsely increase Hgb concentration, plasma TP, MCH, and MCHC. These results may be reported, but a comment will be included stating that they may be erroneous.
- Hemolysis may falsely increase MCH, MCHC and plasma TP. If the hemolysis occurred in vitro, RBC concentration, Hct and spun Hct may be falsely decreased. These results may be reported, but it will be noted that they may be erroneous.
- Erythrocyte agglutination interferes with RBC parameters.
- Platelet clumping may falsely decrease platelet concentration and increase MPV. When clumping is detected on a blood smear, the automated platelet concentration will be reported, but should be considered a minimum value.
- Ghost erythrocytes may falsely increase the automated platelet concentration. If ghost erythrocytes are detected and determined to be interfering with results, the platelet concentration will not be reported and the blood smear will be examined to estimate whether platelet concentration is adequate.

Blood smears are made from all samples and examined microscopically to:

- Verify that data from the automated analyzer are correct.
- Assess for morphologic changes or abnormal cells.
- Look for organisms or other non-cellular abnormalities.

**NOTE:** A manual WBC differential is provided if review of the blood smear appears to differ from the automated WBC differential. Smears with significant abnormalities are sent to a clinical pathologist for review.

### Which chemistry tests are calculated?

**Olympus AU640e**

- Globulin = Total Protein - Albumin
- Indirect Bilirubin = Total Bilirubin - Direct Bilirubin
- Serum Osmolality (mOsm/L) = [(2.0 x Na+) + (Glucose/18.0)] + (Urea/2.8)
- Anion Gap = (Na+ + K+) - (Cl- + HCO3-)
- Urine Total Protein:Creatinine ratio = Micro Total Protein/Urine Creatinine
- GGT:Creatinine ratio = GGT activity/Urine Creatinine
- % Fractional Clearance = (analyte urine/analyte serum) x (creatinine serum/creatinine urine) x 100
- Na:K ratio = Na/K

Nova CCX
- Osmolality (mOsm/kg) = 1.86 [Na+] + Gluc/18 + BUN/2.8 + 9
- Anion Gap = (Na+) - (Cl- + HCO3-)

What can interfere with chemistry results?

- Leaving serum or plasma on cells causes artifacts in chemistry results. Glucose will be utilized by cells, resulting in an artifactual hypoglycemia. Cells will leak contents including electrolytes and enzymes. To avoid these problems, serum should be separated from cells as soon as possible following collection.

- ID (iditol dehydrogenase) is a relatively labile enzyme. Activity may decrease in samples during routine shipping. Results should be interpreted accordingly.

- Chemistry values can be affected by hemolysis, hyperbiliurinemia, and lipemia. The effect of these substances is indicated in the table below.

- Lipemic samples are cleared by ultracentrifugation for an additional charge if the lipemia is marked and likely to affect results. When possible, animals should be fasted for at least 12 hours prior to blood collection to help avoid lipemia.

- Hemolysis can occur in vivo or in vitro. In vitro hemolysis can be avoided by proper blood collection and handling. The laboratory will notify you if the submitted sample is too hemolyzed to analyze. The effect of hemolysis on chemistry tests depends upon the substance being measured. Hemolysis interferes with results by:
  - Color interference
  - Release of substances that are in higher concentration in cells than in serum or plasma (e.g., the psuedohyperkalemia that occurs in hemolyzed samples from animals, such as horses, with high K-containing erythrocytes).
  - Release of substances that interfere with the test method.

Sample Interferences on the Olympus AU640e

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<tr>
<th>Test</th>
<th>Hemolysis</th>
<th>Lipemia</th>
<th>Icterus</th>
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<td>++&lt;1%</td>
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<td>Ammonia</td>
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<tr>
<td>Amylase</td>
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<td>++&lt;5%</td>
<td>+&lt;10%</td>
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<tr>
<td>AST</td>
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<td>++&lt;5%</td>
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<tr>
<td>Bicarb</td>
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<td>++&lt;0%</td>
<td>+&lt;0%</td>
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<tr>
<td>Bilirubin D</td>
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What is included in a fluid cytology report?

All fluid cytology requests include:

- Additional slide preparation from any submitted fluid
- Microscopic evaluation of all direct and/or concentrated slide preparations by a clinical pathologist
- Cytology report
  - Sample
  - Microscopic Description
  - Interpretation

In addition to the above:

- Fluid from enclosed spaces (e.g., pleural cavity, peritoneal cavity, subcutaneous cyst) will include:
  - Nucleated cell concentration
  - Total protein (TP) by refractometer
  - Spun Hct (PCV) if result is greater than 2%

- Synovial Fluids will include:
  - Nucleated cell count
  - Spun Hct (PCV) if result is greater than 2%
  - Total protein (TP) by refractometer
  - Viscosity
  - Turbidity

- CSF will include:
  - Nucleated cell concentration
  - RBC concentration
  - Microprotein measured biochemically

**NOTE:** Whenever possible, cell concentrations are measured using an automated hematology analyzer. If that is not possible, cells are counted manually using a hemacytometer. If a sample contains a clot, cell concentrations will be measured whenever possible; however, the resulting nucleated cell concentration will be falsely low and should be considered a minimum value.
What is the clinical pathology policy about cancellation of tests?

A specimen that arrives at the Clinical Pathology Laboratory and has not been processed may be canceled. If the ordered tests have already been performed when the cancellation notice is received, the routine fee for the test performed as originally ordered will be billed.

What is the clinical pathology policy about repeating tests?

Clinical Pathology Laboratory tests will be repeated at no charge when the submitting veterinarian has good reason to question the validity of a test result. Please contact the laboratory and talk to the clinical pathologist on duty if you have questions about a possible erroneous test result.