



## Clinical Pathology – Hematology & Clinical Chemistry Sample Guidelines

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### What sample type, tube type, and volumes should I use for CBC (complete blood count)?

Samples for CBC MUST be whole blood and MUST be collected in purple top K<sup>+</sup> EDTA tubes. Any other anticoagulant can cause swelling or shrinking of cells which will skew results. See chart below for recommended tubes & volumes. Please make sure you use an appropriate size tube for the species and fill tubes to the minimum volume line specified for the tube size. This ensures an accurate EDTA: whole blood ratio. Underfilling or overfilling results in artifact or uninterpretable results.

- The absolute minimum of whole blood required for CBC is 50 µl but minimum collection volume must be appropriate for the tube size. See chart below.
- We cannot accept tubes > 4 ml due to our centrifuge bucket sizes. If you need larger volumes, please fill multiple tubes at collection. Samples tubes >4 ml will be returned for aliquotting.
- Please use plastic (polypropylene) tubes, not glass. Samples submitted in glass will be returned for resubmission in plastic.

| Item                                     | Species        | Volumes    | Vendor | Catalog #  | Mfr      | Mfr #   |
|------------------------------------------|----------------|------------|--------|------------|----------|---------|
| <u>For CBC (whole blood) collection:</u> |                |            |        |            |          |         |
| Microvette 100 K3 EDTA                   | Small mice     | 50-100 µL  | Fisher | NC1675228  | Sarstedt | 20.1288 |
| Microvette 200 K3 EDTA                   | Small mice     | 100-200 µL | Fisher | NC9976871  | Sarstedt | 20.1278 |
| Microtainer, K <sup>2+</sup> EDTA        | Mice, rats     | 250-500 µL | Fisher | 02-669-33  | BD       | 365974  |
| Vacutainer K <sup>2+</sup> EDTA          | Larger animals | Up to 3 mL | Fisher | 02-683-99B | BD       | 367856  |

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**What sample type, tube types and volume should I use for serum chemistries?**

The ideal sample for clinical chemistries is serum and the ideal collection tube is a gold/yellow top commercially available serum separator tube that has a gel clot activator. Red top (no additive) or standard microcentrifuge tubes can be used but may result in decreased yield and increased hemolysis (creates artifacts). Plasma (green or purple top) may be acceptable for some parameters – but chelating agents (like EDTA) may interfere with calcium, ALKP, phosphorus and potassium. See chart below for some recommended tube types.

To obtain serum, blood should be collected into serum separator tubes and allowed to clot for ~30 minutes, then centrifuged for 10 minutes at 1800-3000g. If separation is inadequate or lipemia is present, longer or faster centrifugation times may be necessary. Separated serum can be transferred to new tubes (no additive tubes or standard microcentrifuge tubes) and submitted or stored. See question on “temperature/storage conditions below”.

Minimum serum volumes for chemistry test are listed after each test in our MiCORES site. In general, a minimum serum volume of 30 µL is required for one chemistry analyte, 50 µL for two chemistry analytes, 120 µL for a mini chemistry panel (9 analytes), and 270 µL for a full chemistry panel (13 analytes plus electrolytes). Remember that these are SERUM volumes - a standard estimate is that centrifugation will yield approximately half of the whole blood sample volume as serum. If volume is limiting, there is a question on our submission form in MiCORES that will allow you to prioritize your requested chemistry tests. Please DO NOT dilute your sample for reasons of insufficient volume – see question on “dilution” below.

| Item                                       | Species        | Volumes    | Vendor | Catalog #  | Mfr      | Mfr #   |
|--------------------------------------------|----------------|------------|--------|------------|----------|---------|
| <u>For chemistries or serology (serum)</u> |                |            |        |            |          |         |
| Microvette 100, no additive                | Small mice     | 50-100 µl  | Fisher | NC9255927  | Sarstedt | 20.1280 |
| Microvette 200 , Ser-gel                   | Mice           | 100-200 µL | Fisher | NC9315741  | Sarstedt | 20.1291 |
| BD Microtainer, Serum Separator            | Mice, rats     | 250-500 µL | Fisher | 02-675-185 | BD       | 365967  |
| Vacutainer Serum separator                 | Larger animals | Up to 3 mL | Fisher | 02-657-27  | BD       | 366668  |

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## At what temperature should samples be stored/submitted and how long can I wait before submitting?

For CBCs (complete blood counts):

- CBCs should be submitted at room temperature on the day of collection. Do NOT put samples on ice. If samples must be run the next day, they can be stored overnight at 4°C (refrigerator, NOT frozen). Because of the timing, a minimum of 48 hours advance notice is requested for CBCs, with longer notice preferred. Data can be entered into MiCORES <https://umich.corefacilities.org> and a submission date selected well in advance of your collection date. If you must submit CBCs on a Friday, this must be done early in the day so that our staff have time to run your samples. We do not process samples on weekends.

For serum chemistries:

- Samples should be collected into serum separator tubes, allowed to clot for 30 minutes at room temperature and centrifuged at 1800-3000g, followed by transfer of serum to new tubes. Samples should be separated on the day of collection and submitted at room temperature on the day of collection or frozen indefinitely at -70 to -80°C. Please separate serum before freezing - unseparated serum or serum frozen over the clot can result in hemolysis or other artifacts. If you do not have access to a centrifuge, submit your samples the same day as collected and we can separate it for you – additional charges for technician time may apply.

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## How can I get help or training in collecting blood samples from my animals?

- For training in blood drawing technique, contact the ULAM Training Core at [ulam-trainingcore@umich.edu](mailto:ulam-trainingcore@umich.edu)
- For fee-for-service assistance with sample collection, contact ULAM Technical Services at [ulam-techservices@umich.edu](mailto:ulam-techservices@umich.edu)

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## My results indicate artifact due to “hemolysis” or “lipemia”. What does this mean?

Ideally, separated serum will be clear. Red serum may indicate hemolysis, or lysing of red blood cells, which can occur in the animal in certain condition or during/after collection as an artifact. Yellow-white, semi-opaque serum may indicate lipemia, or high serum lipids. Many parameters can be artifactually elevated or lowered by these conditions and in some cases they will preclude obtaining a result. If your animals have a condition that you know will produce *in vivo* hemolysis or lipemia, please indicate this in sample submission notes – we may need to modify techniques to obtain a result. To avoid artifactual hemolysis after collection, practice gentle technique at blood collection, avoid expressing blood through a fine diameter needle too quickly, use a serum separator tube and separate serum promptly after clotting, and keep CBC samples at room temperature (no ice!).

For further information on hemolysis and other types of preanalytical variables impacting results, see Everds (2017) Toxicologic Pathology [PMC28178898](#).

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### **Can I/should I dilute my samples if there is low volume?**

We do NOT recommend dilution for low volume samples. For many parameters, the normal value range distribution is already skewed towards zero. Dilution of samples may put the results below the limit of detection. For tests that are measured as enzymatic activity, dilution also tends to generate non-linear results after dilutions of 1:1 or 1:2. See John JL et al (2018) Front. Vet Sci. [PMC5818404](#).

The only appropriate uses of dilution for a clinical chemistry sample are:

- Where extremely high values for a particular parameter are expected: here the values may be ABOVE the linearity range for the instrument. Examples may include liver enzymes (AST, ALT, AlkP) with severe hepatotoxicity and total serum cholesterol or triglycerides with high fat diets.
- Where lipemia is present: In this case, centrifugation and/or dilution may be used to reduce interference of lipemia.

If you anticipate dilution will be needed for your samples, please do not dilute your samples yourself. Indicate the anticipated condition (high values for particular parameters, presence of lipemia) in your submission and indicate which samples you think will be affected. Our staff will assess and perform dilution or centrifugation as necessary just prior to analysis. We typically use a 1:1 or 1:2 dilution in physiologic saline.

If sample volume is low – indicate the priority order for your tests in your MiCORES submission.

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### **I don't see the test I am looking for, do you run it?**

We do offer send-out services to Charles River, Michigan State, and others, please contact us at [ULAM-PathologyCore@umich.edu](mailto:ULAM-PathologyCore@umich.edu) with the specific test you are looking for.

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### **How do I know my results are accurate?**

We run daily quality control reactions on our instruments using manufacturer supplied reagents. Additionally, we participate in the [Veterinary Laboratory Association's Quality Assurance Program](#), an independent organization providing quarterly quality assurance assessment relative to peer laboratories and instrumentation.

While we make every effort through our QAQC program to generate quality results, if you have results that raise questions or are inconsistent with other data from the same sample set, please reach out to us. Our veterinary pathologists are available for troubleshooting and to assist with interpretation – please submit a request for Pathology consultation through MiCORES. <https://umich.corefacilities.org>

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### What instruments do you use for analysis and how should I describe these in my methods?

Suggested methods wording is below. Minor changes will be necessary if you have non-standard anticoagulants or tubes. Please remember to include the name of our core in your acknowledgments or methods. This metric is important to our continued ability to provide services. For chemistry assays, more specific methods for any particular analytes are available upon request.

- For CBCs: Whole blood was collected into K<sup>2+</sup> EDTA anticoagulant tubes and complete blood counts (CBCs) were run on a [Heska Element HT5](#) (Heska Corporation, Loveland, CO) automated veterinary hematology analyzer.
- For chemistries: Whole blood was collected into serum separator tubes, allowed to clot, and separated into serum by centrifugation. Serum chemistries were run on an AU480 chemistry analyzer (Beckman Coulter Inc, Brea, CA).
- For both: Assays were performed within the ULAM Pathology Core laboratory at the University of Michigan. Quality control was performed daily using manufacturer-provided reagents and the laboratory is a participant in an external independent quarterly quality assurance program ([Veterinary Laboratory Association Quality Assurance Program](#)).

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**How do I interpret my results – should I use the reference ranges on my results print-out? Should I use statistical comparison? Where can I find help?**

In most cases, we do NOT recommend that you use these reference ranges or the indications of High or Low values. The reference ranges are a general guide and represent a broad range of sexes, ages, strains. This can be useful in clinically assessing a single animal but research studies should have a far tighter range of values. For more information about reference intervals in preclinical studies, see Hall (1997) Toxicol Pathol. [PMID9437812](#)

For an experimental study, the best comparison is between an experimental group and an appropriate age, sex, strain-matched control group from the same study. For larger animals, comparison of an individual animal to its own baseline is also sometimes used. For rodents, we do NOT generally recommend baseline assessment or repeated sampling for hematology or clinical chemistry parameters – repeated blood draws can result in artifacts due to tissue damage or stress and can generate a hematopoietic response even when volumes collected remain within regulatory parameters. Repeated hematology or chemistry sampling in rodents generally requires different groups for each timepoint.

Results should be compared to control animals both statistically and in light of biological importance. Consideration should be made for outliers and false positives. In rodents, individual outliers due to artifact are very common due to low volumes, clotting at collection, or artifactual hemolysis. Based on the number of parameters evaluated, it is also common to see statistically significant differences in one or more parameters arising by chance. This does not necessarily equate to biological significance. Values in a CBC or chemistry panel are not all independent of one another – if certain parameters are altered, other related parameters should also be affected. For this reason, assessment should consider BOTH biological and statistical significance. Related parameters that shift together in a logical way are more likely to indicate biologically important changes than single parameter changes. Our veterinary pathologists are available to assist with results interpretation. Please submit a request for pathology consultation through MiCORES <https://umich.corefacilities.org>

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