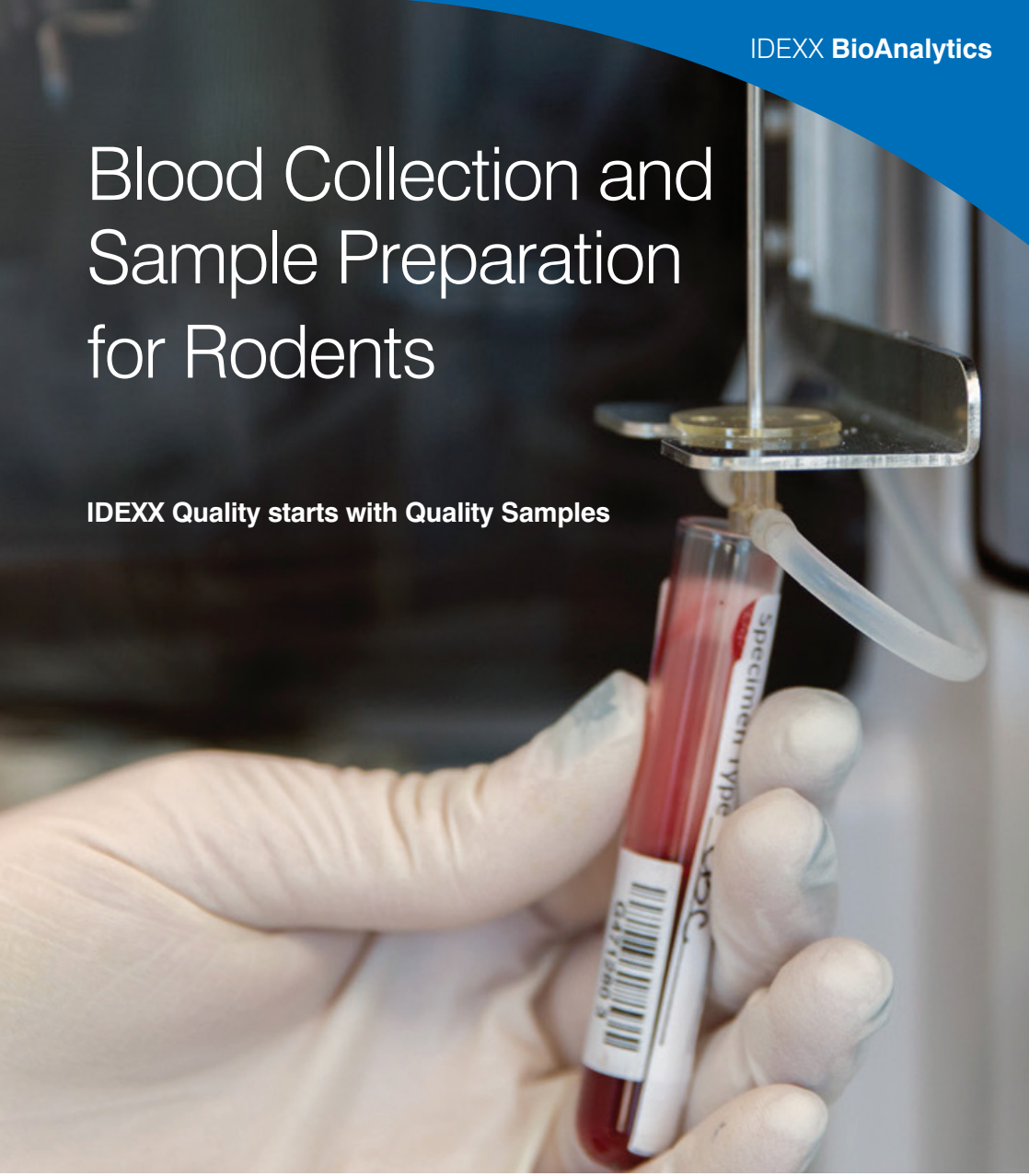
IDEXX **BioAnalytics**

# Blood Collection and Sample Preparation for Rodents

**IDEXX Quality starts with Quality Samples**




**Preclinical Services**

IDEXX

# Blood Collection and Sample Preparation

Definitions and sample preparation

**Whole blood** Blood drawn directly from the animal without any components such as fibrin, plasma, platelets, or white blood cells removed. It is not recommended to submit this type of sample without additives such as anticoagulants as hemolysis, clotting, and glucose breakdown can occur which can interfere with testing parameters.

**Plasma** Aqueous part of blood made noncoagulable by means of an anticoagulant such as EDTA, Heparin, or Citrate.

- Plasma can easily be obtained by placing whole blood into a tube containing an anticoagulant.
- When using a tube with an anticoagulant, do not shake the contents. Instead gently invert the tube end over end several times, watching the blood move through the tube, to ensure adequate mixing.
- When using an anticoagulant do not let blood sit without mixing. Instead place collected blood in the collection tube and immediately invert tube to mix.
- When using an anticoagulant coated collection tube such as the mini-LTT tube, be sure sample reaches the fill line and the tube is not overfilled. Overfilling the tube can alter the blood to anticoagulant ratio and result in clotting of the sample.
- Plasma collected using EDTA cannot be used to determine the following parameters: potassium, calcium, magnesium, iron, alkaline phosphatase, glucose, and lactate. For these tests, anticoagulants such as heparin or citrate may be used (for details on which anticoagulant to use for specific tests see the **Sample Handling and Testing Guidelines** table).

# Blood Collection and Sample Preparation

Definitions and sample preparation

- Blood samples containing an anticoagulant such as EDTA start to degrade as soon as the blood is outside of the animal. To help minimize this blood should be kept refrigerated and submitted for sampling as soon as possible. Extended storage can alter testing parameters such as increases in MCV and hematocrit values.

**Serum** Aqueous part of blood where fibrin and blood cells are removed by coagulation or with use of a serum separator tube.

- Serum can be obtained by placing whole blood in an empty tube and allowing the blood to clot or by placing in a specialized serum separator collection tube.
- When using an empty tube allow adequate time for the blood to form a clot (coagulation time may vary by species). Centrifuge the sample and transfer serum into a new, empty tube (for specific sample handling by test type see Sample Handling and Testing Guidelines table).
- It is important to allow an adequate time for coagulation and to separate the serum from the clot completely.
- Serum samples can be refrigerated if sampling will occur the following day or can be frozen for longer term storage.
- The gel from serum separator tubes can interfere with some specialized tests such as endocrinology and drug tests. Please refer to the **Sample Handling and Testing Guidelines** for information on specific tests.

# Blood Collection and Sample Preparation

## Did you know?

### Samples clotted during blood draw may result in...

- Platelet clumps
- Falsely decreased cell counts (platelets, red blood cells and white blood cells.
- Haemolysis (when forcing blood in to tube)

### Excess anticoagulant (under-filled 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

### EDTA contamination may cause...

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

# Submitting Samples

Sample collection and submission tips

## Submitting Samples

- Plasma samples should be kept refrigerated and submitted for testing as soon as possible.
- Plasma samples submitted in a shipping container containing freezer packs should be protected by an inner container to prevent samples from freezing.
- Serum samples can be submitted for testing as refrigerated or frozen samples.

## General Blood Sampling Tips to Improve Sample Quality

- To avoid hemolysis, blood samples should be taken immediately after the vein has been raised.
- Avoid “pumping or milking” the blood from the vein as this can induce coagulation and erythrocyte lysis.
- Avoid high negative pressure in the syringe, as this can cause erythrocytes to rupture.
- Do not squirt blood forcefully through the syringe needle into the tube. Instead apply gentle pressure and allow the blood to run down the side of the tube wall.
- Prevent plasma samples from freezing during shipping.

# Sample Submission Quick Reference

| Type of Testing | **Chemistry, Immunology Endocrinology** including progesterone and therapeutic drug-monitoring (digoxin, phenobarbital or theophylline) | **Chemistry, Immunology Endocrinology** | **Haematology** | **Coagulation** (PT, APTT and quantitative fibrinogen) |
|---|---|---|---|---|
| Sample | Serum | Serum | Whole blood | Citrated plasma |
| Container |  **RTT/R** (red-topped tube, Vacutainer®) Any glass, red-topped tube. Plastic Vacutainer® with clot activator |  **SST** (serum separator tube, yellow-topped Vacutainer®) |  **LTT/L** (Lavender-topped tube and air-dried unstained slides) |  **BTT** (blue-topped tube) to collect, then transfer to plastic tube |
| Contents | No additives (empty/sterile) | Gel to separate serum from clot (during centrifugation) and a clot activator | Anticoagulant EDTA | Anticoagulant sodium citrate |
| Protocol | Let specimen clot 15–20 minutes, centrifuge at 2,500 rpm for 10–15 minutes, remove serum from clot and transfer to a red-topped tube or a clear screw-topped tube. | Let specimen clot 15–20 minutes, centrifuge at 2,500 rpm for 10–15 minutes. **DO NOT** use SST for progesterone or therapeutic drug-monitoring (digoxin, phenobarbital or theophylline). | Fill tube as much as vacuum will allow to obtain proper blood-to-anticoagulant ratio. Invert gently several times after filling. | 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. Separate plasma from cells. Transfer plasma to plastic tube and label as citrated plasma for submission. Freeze sample and ship on ice. |
| Storage | Refrigerate | Refrigerate | Refrigerate **DO NOT** freeze | Keep frozen (refrigeration OK if received at laboratory within five hours) |

# Blood Sample Collection Parameters

When designing an experiment that will require blood collection from rodents, several basic parameters such as the following, should be carefully considered.

- Survival blood collection versus terminal blood collection
- Species of animal
- Size of the animal and estimated total blood volume
- Amount of blood sample and frequency of sampling
- Type of blood sample
- Experience of technical staff
- Effect of sampling site, restraint, and/or anesthesia on blood parameter measured.

For survival blood collection, the frequency and quantity of sample collection is dependent on the circulating blood volume and the rate of red blood cell turnover of the animal and varies by species. For mice and rats, the circulating blood volume is approximately 55-70 ml/kg of body weight (1,2).

Percentage of the blood volume and frequency of sampling that can be safely performed in mice and rats is listed in the **Blood Sample Volumes for a Range of Body Weights** table. More frequent collection or sample volumes greater than those listed in the table can be performed but require fluid or cellular replacement and additional supportive care of the animal.

# Approximate Blood Sample Volumes

for a range of body weights

| Body weight (g) | *CBV(ml) | 1% CBV every 24 hrs ** | 7.5% CBV every 7 days | 10% CBV every 2 - 4 wks ** |
|---|---|---|---|---|
| 20 | 1.10 - 1.40 | 11 - 14 µ | 90 - 105 µ | 110 - 140 µ |
| 25 | 1.37 - 1.75 | 14 - 18 µ | 102 - 131 µ | 140 - 180 µ |
| 30 | 1.65 - 2.10 | 17 - 21 µ | 124 - 158 µ | 170 - 210 µ |
| 35 | 1.93 - 2.45 | 19 - 25 µ | 145 - 184 µ | 190 - 250 µ |
| 40 | 2.20 - 2.80 | 22 - 28 µ | 165 - 210 µ | 220 - 280 µ |
| 125 | 6.88 - 8.75 | 69 - 88 µ | 516 - 656 µ | 690 - 880 µ |
| 150 | 8.25 -10.50 | 82 - 105 µ | 619 - 788 µ | 820 - 1000 µ |
| 200 | 11.00 -14.00 | 110 - 140 µ | 825 - 1050 µ | 1.1 - 1.4 ml |
| 250 | 13.75 -17.50 | 138 - 175 µ | 1.0 - 1.3 ml | 1.4 - 1.8 ml |
| 300 | 16.50 -21.00 | 165 - 210 µ | 1.2 - 1.6 ml | 1.7 - 2.1 ml |
| 350 | 19.25 -24.50 | 193 - 245 µ | 1.4 - 1.8 ml | 1.9 - 2.5 ml |

* Circulating blood volume (1ml = 1000 µ)

** Maximum sample volume for that sampling frequency

# Sample Collection Technique Guide

Survival Procedures (Volumes refer to Mice)

| Collection Technique | Volume | Advantages | Disadvantages |
|---|---|---|---|
| **Submental Vein (mice)** | <0.2ml* | • No anesthesia required<br>• Ease of locating target vessels for sampling with training<br>• Moderate to large sample recovery<br>• Easy to stop post collection bleeding<br>• Can be performed rapidly decreasing risk of hemolysis and platelet clumping | |
| **Lateral saphenous** | <0.2ml* | • No anesthesia required<br>• Ease of locating target vessels for sampling with training<br>• Moderate to large sample recovery<br>• Can use the same site multiple times by alternating hind legs<br>• Can be performed rapidly decreasing risk of hemolysis and platelet clumping | • Harder to stop post collection bleeding<br>• Can cause temporary lameness of the limb if procedure is prolonged |
| **Submandibular/ Facial Vein (mice)** | <50µl* | • Can be done with or without anesthesia<br>• Ease of locating target vessels for sampling with training<br>• Moderate to large sample recovery<br>• Can use the same site multiple times by alternating sides of the face<br>• Can be performed rapidly decreasing risk of hemolysis and platelet clumping for sampling | • More challenging to visualize target veins for sampling<br>• More challenging to stop post collection bleeding |
| **Tail Tip Puncture** | <0.2ml* | • No anesthesia required<br>• Ease of locating target vessels with training<br>• Can use the same site multiple times<br>• Easy to stop post collection bleeding | • Small sample recovery<br>• Slower blood flow which can increase risk of platelet clumping and hemolysis of sample |
| Terminal Procedures (Volumes refer to Mice) | | | |
| **Intra-cardiac Puncture** | <1ml* | • Easy to perform with training<br>• Large sample recovery | • Can cause increased hemolysis if inappropriate size needle and syringe or vacutainer used. |
| **Caudal Vena Cava** | <1ml* | • Large sample recovery | • Requires more training than intra-cardiac puncture |

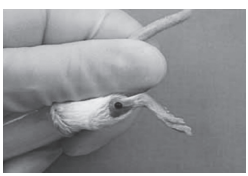
*Estimated whole blood volumes only based upon an adult mouse. Actual volumes should be determined by NIH phlebotomy guidelines by total blood volume and approved IACUC animal use protocols.

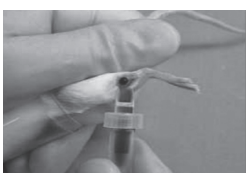
# Blood Collection Techniques (listed by species)

Guidelines for Survival Blood Collection of Mice and Rats

## Lateral Saphenous Vein

### Materials needed for collection:





- Restraint device (modified 50 ml conical tube, DecapiCone, or plexiglass rodent restrainer)
- Small rodent fur clippers
- Blood lancet or 25-gauge needle
- Blood collection tube

### Technique

- Label blood collection tubes, and place in tube rack.
- Remove the lid from the first sample tube.
- Encourage mouse or rat to enter the restraint device while gently holding the back leg outside of the restrain device.
- Hold the tail base with your index finger to help extend the limb.
- Shave the hair of the leg with mini fur clippers.
- Use your hand to apply gentle pressure below the knee to hold the leg against the side of the restraint device rim. This will occlude blood flow, allowing blood to pool and the lateral saphenous vein will become prominent.

# Blood Collection Techniques (listed by species)

Guidelines for Survival Blood Collection of Mice and Rats

- Use a blood lancet or 25-gauge needle to gently puncture the vein being careful to not push the needle all the way through the vein.
- Quickly remove the needle and drops of blood will form at the venipuncture site. Keep the leg extended with slight pressure below the knee for the best blood flow.
- Touch the rim of the blood collection tube to the venipuncture site to draw blood into the tube.
- Once blood collection is complete, use sterile gauze or cotton ball to apply gentle pressure to the venipuncture site. Allow the leg to bend, releasing any pressure on the back of the knee.
- Once bleeding has stopped at the venipuncture site, return the mouse to the cage.
- Handle blood samples according to sample type and testing parameters (please refer to **General Information and Advice for Blood Collection and Sample Preparation.)**