

This document offers some basic guidelines to follow when collecting and handling human samples for submission to Rules-Based Medicine (RBM), a Q² Solutions Company. Here we discuss the procedures for the most common sample types: plasma, serum, urine, and tissue homogenate. For more information on choosing your sample type, please refer to the FAQ <https://rbm.q2labsolutions.com/order/faq/> on our website or speak with your sales representative.

Blood, urine, and tissue samples have long been used as sources for measuring health and well-being and they remain important tools for biomarker research and clinical utility. However, the clinical information obtained from these specimens is influenced by the collection method, timing and handling. Following these guidelines will help ensure that submitted samples are uniformly handled in an optimum fashion to minimize the influence of environmental variables.

RECOMMENDED MATERIALS

It is necessary to have the proper materials ready when collecting, storing and shipping the specimens for your study. The materials and supplies listed here are recommendations only.

- BD Venous Blood Collection Tubes (for example, EDTA, Cat. # 367861) or equivalent [plasma only]
- BD Vacutainer® SST™ collection tubes (for example, Cat. # 367988) containing clot activator and gel for serum separation or equivalent [serum only]
- Temperature-controlled centrifuge [plasma or serum]
- Plastic microcentrifuge tubes- VWR Cat. # 87003-294 [plasma or serum]
- .5 mL screw-capped polypropylene (not polyethylene) tube [urine only- optional]- VWR Cat. # 10025-748
- 2 mL screw-capped polypropylene tube [urine only] VWR Cat. # 16466-032
- Potter-Eivehjem homogenizer (Teflon pestle and glass mortar) attached to a variable-speed drill a polytron or a tissuemizer [tissue homogenate only]
- -80°C freezer
- If a -80°C freezer is not available, then the samples should be stored at as low a temperature as possible. If stored at -20°C, the freezer should be of a NON frost-free type. Stability of samples has not been assessed at temperatures higher than -70°C.
- Dry ice
- Various packing supplies as required Styrofoam-insulated corrugated fiberboard outer packaging containers

AT A GLANCE:

Below are some basic guidelines to follow when submitting samples for analysis. Following these guidelines will help ensure streamlined reconciliation of samples. A study level RBM Sample Submission Form (SSF) should be provided within the first shipment email notification or prior to the first shipment.

Sample Tube and Manifest Guidelines:

- Only one tube per sample with sufficient volume for testing should be sent for analysis. Exceptions may apply depending on testing requirements and should be discussed with the project manager.
- Samples may be sent in a variety of tubes including screw and snap top microcentrifuge tubes, Matrix® tubes, or Microtiter® plates.
- Store samples in a 5"L x 5"W x 2"H cardboard cryobox with 81 count (9x9) grid.
- Samples with different testing requirements should be sent in separate clearly labeled boxes.
- Barcode labeling (preferred and must follow these guidelines):
 - 1D or 2D format
 - Must correspond to a unique ID
 - Must be applied in an orientation that can be scanned
 - Must be readable at -80°C.
- Non-barcode labeling:
 - A printed label should be used that is readable at -80°C.
 - Provide an electronic sample manifest in Microsoft Excel® format.
 - List the samples in the same sequence as the tubes.
 - Sample ID on the manifest must match the ID found on the sample tubes
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COLLECTION PROCEDURE

The collection procedures required vary according to the type of sample being collected. Refer to your sample type below for specific instructions.

Plasma

Whole blood samples should be collected by properly trained staff. Samples should be collected in BD Venous Blood Collection Tubes (Cat. # 367861 or equivalent). Immediately following collection, the samples should be mixed gently by inverting the tube four to five times to distribute the anticoagulant. To separate the plasma from the cells, centrifuge the collection tube for 10 minutes at 1,300 xg (or according to the manufacturer's instructions) using a temperature setting of 25°C, within a maximum of 2 hours after collection. We recommend centrifugation within 30 minutes of collection. The plasma should be aspirated by pipette and stored in plastic microcentrifuge tubes. Do not collect the buffy coat which is at the interface between the plasma and the red cells.

Serum

Samples should be collected in plastic BD Vacutainer® SST™ collection tubes (Cat. # 367988 or equivalent). Following collection, the samples should be allowed to clot in the collection tubes for a minimum of 30 minutes at room temperature. Serum should be separated from the clot by centrifuging the collection tube for 10 minutes at 1,300 xg (or according to the manufacturer's instructions) using a temperature setting of 25°C, within two hours of collection. If a fixed angle centrifuge is used the time should be increased to 15 minutes. The serum should be aspirated by pipette and stored in plastic microcentrifuge tubes.

Urine

Urine samples, at least 25 mL, should be collected into a sterile, screw-capped polypropylene container. If a pour-off tube is used, we recommend using a 2 mL screw top polypropylene tube.

First Morning Specimen - This is the specimen of choice for biomarker analysis, since the urine is generally more concentrated (due to the length of time the urine is allowed to remain in the bladder) and, therefore, contains relatively higher levels of biomarkers. Also called an 8-hour specimen, the first morning specimen is collected when the patient first wakes up in the morning, having emptied the bladder before going to sleep.

Spot Urine - This is the specimen most commonly sent to the laboratory for analysis, primarily because it is the easiest to obtain and is readily available. This specimen can be submitted for biomarker analyses, although it is not the specimen of choice. Spot specimens can sometimes give an inaccurate view of a patient's biomarker levels if the specimen is too diluted and analyte values are artificially low.

24-hour collection – If 24-hour urine collection is required then the collection containers must be amber colored (to protect from light) and contain an antimicrobial agent. These preservatives may include: boric acid (preferred), hydrochloric acid, acetic acid, and toluene. It should be noted, however, that the stability of most biomarkers has not been established for this collection type.

Tissue Homogenate

Tissue samples should be collected, weighed, and added to lysis buffer (100 mg of tissue per 900 µL lysis buffer). Our recommended lysis buffer is 50mM Tris-HCl with 2mM EDTA, pH 7.4. If the samples are not homogenized immediately then the samples should be frozen in liquid nitrogen and stored at -80°C. While EDTA is a good inhibitor of divalent metal requiring proteases, you may want to minimize other protease activity by adding the following inhibitors: aprotinin, antipain, leupeptin, and pepstatin A (all at 1µg/mL) and 2mM PMSF(phenylmethylsulfonyl flouride).

Tissues may be homogenized using a Potter-Elvehjem homogenizer (Teflon pestle and glass mortar) attached to a variable-speed drill, a polytron or a tissuemizer. During the homogenization process, the tube should be submersed in an ice bath to maintain the sample at 2-8 °C. Following homogenization, the tissue preparation is centrifuged for 2 minutes in a microfuge at 13,000 xg. Making sure that the cell pellet is not disturbed; aspirate the supernatant.

RECOMMENDED STORAGE

All specimens must be frozen immediately after collection and processing (at -80°C or on dry ice if possible).

It is recommended that the samples be stored at -80°C. If samples are stored at higher temperatures, it should be for as short a period of time as possible. If the samples are stored at -20°C, the freezer must be of a NON frost-free type. Stability of samples at higher temperatures than -70°C have not been assessed.

DOCUMENTATION AND LABELING

Identify specimens with labels generated by your facility. These should be -80°C compliant so that they remain attached to the samples while frozen. Barcodes (1D or 2D) are acceptable and preferred. Text should be electronically printed or or hand-written in felt-tip permanent marker. RBM's Sample Submission Form should be filled out prior to shipping. The sample submission forms can be found on our website at <https://rbm.q2labsolutions.com/order/>

SAMPLE SHIPPING AND HANDLING

Samples are to be shipped to RBM for overnight delivery, if using FedEx, please select "Priority Overnight" service. Preferably, samples should be shipped Monday-Wednesday to arrive Tuesday-Thursday. Samples should not be received during holidays and weekends. Holiday schedule can be found at <https://rbm.q2labsolutions.com/order/faq> The sample tubes (when possible) should be placed in a 5"L x 5"W x 2"H cardboard cryobox with 81 count (9x9) grid, rather than bags, to minimize damage to the samples. If shipping a Matrix® tube rack or Microtiter® plate, it is recommended to secure the lid and place in a plastic bag. The shipment should be prepared according to biohazard regulations and shipped frozen, in a Styrofoam container with sufficient dry ice to maintain temperature (less than -80°C) for at least 48 hours for US domestic shipments; 72 hours for international shipments. Service that includes automatic replenishment of dry ice is recommended.

Please remember to include an itemized list of contents and any other documentation that is specifically required by the country of origin for the samples being shipped. Upon receipt, the samples will be stored at -80 °C until scheduled for testing. Samples may be rejected for analysis if the sample container is broken or leaking, or if the samples show evidence of thawing.

Shipment notification including project/study ID, sample manifest in excel format, courier name, and tracking number should be sent to RBM_Receiving@q2labsolutions.com at least one day in advance for standard processing timelines. RBM's Sample Submission Form (SFF) should be included in the first email notification if not already provided. The SFF can be downloaded here: <https://rbm.q2labsolutions.com/order/>

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