

This document offers some basic guidelines to follow when collecting and handling human samples for submission to Myriad RBM. 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 (<http://www.MyriadRBM.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 (EDTA ,Cat. # 367861) or equivalent [plasma only]
- BD Vacutainer® SST™ collection tubes (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 # 1415-2500
- Screw-capped polypropylene (not polyethylene) container [urine only] - VWR Cat # SCT-5mL
- 2 mL screw top polypropylene tube [urine only - optional] VWR Cat # 16466-032
- Potter-Elvehjem 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 not be a 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

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.

[See sample volume requirements »](#)

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. [See sample volume requirements »](#)

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 1 µg/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 submerged 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 13,000xg. Making sure that the cell pellet is not disturbed; aspirate the supernatant.

RECOMMENDED STORAGE REQUIREMENTS

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 that -70 C have not been assessed.

DOCUMENTATION AND LABELING

Label specimens with labels generated by your facility. These should be -80 °C compliant so that they remain attached to the samples while frozen. Text should be electronically printed or hand-written in felt-tip permanent marker. Barcodes (2D or 3D) are acceptable and preferred.

Myriad RBM's [standard shipping form](#) should be filled out prior to shipping and included in the package so that we may follow our standard operating procedures for receiving and handling. These sample submission forms can be found on our website at MyriadRBM.com/order/how-to-order/

SAMPLE SHIPPING AND HANDLING

Samples are to be shipped to Myriad RBM overnight, for receipt no later than 24 hours after shipment. Preferably samples are shipped Monday-Wednesday to arrive Tuesday-Thursday. The sample tubes (when possible) should be placed in 9"x9" cryoboxes, rather than bags, to minimize damage to the samples. 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. Urine specimens should be clearly labeled with smudge-free pens or bar codes and require triple packaging, consisting of a leak proof primary receptacle (the cup), a leak-proof secondary packaging that meets 49CFR173.199(b) requirements, and an outer packaging. Use Styrofoam-insulated corrugated fiberboard outer packaging containers.

Please remember to include a completed copy of Myriad RBM's standard shipping form along with 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 the samples show evidence of thawing.

Note: "Universal Precautions" for bloodborne pathogens should be employed when handling all samples.

SHIPPING SPECIMENS

Please forward tracking numbers to receiving@myriadrbm.com

Collected and packaged specimens should be sent to -

Kalyn Sowell

ksowell@myriadrbm.com

(512) 275-2644

Myriad RBM, Inc.

3300 Duval Road

Austin, TX 78759

QUESTIONS

Please direct questions regarding the packaging and/or shipping of samples to info@myriadrbm.com

Please send sample collection questions to clientservices@myriadrbm.com