


GenepowerRx[®] Sample Collection

White Paper | Version 1.0 | 17-March-2023

K. Kiran Kumar Y

Authored by: Kiran Kumar Y

Reviewed and supported by



Dr. Kalyan Ram Uppaluri
Managing Director



K. Sri Manjari
Scientific writer





Dr. Hima J Challa
Director


Dr. Kalyani P
CSO

Title: GenepowerRx® Sample Collection

Authors: Kalyan Ram Uppaluri, Hima J. Challa, Kalyani P, Kiran Kumar Yekula, K. Sri Manjari

The collection of appropriate and optimum samples is the responsibility of the healthcare provider collecting a sample in a clinical setting. The laboratory can help to ensure good samples by providing collection information to health care personnel at the collection site, making sure that appropriate containers and collection supplies are available, defining a good labeling system, and checking all samples carefully when they arrive in the laboratory.

Scope: This code of practice is intended to cover the practical work on human blood collection and FFPE from individuals for genetic testing

Assemble Supplies:

The following supplies should be available at any location where Venipuncture is performed routinely:

- Blood Collection tubes/blood culture bottles
- Needle
- Single-use tube/needle holder
- Syringe
- A tourniquet
- Alcohol prep pads
- 1 to 10% Povidone-iodine pads, tincture iodine, or chlorhexidine compounds if blood culture is to be drawn
- adhesive ANTISEPTIC bandages
- Gloves
- Sharps Container/ needle destroyer as per availability in the hospital
- Dustbin as per color code
- Blood Mixer (subject to availability)

Procedure:

- **Procedure for seating patient**
 - Ask the patient to be seated comfortably in a chair suitable for Venipuncture. The chairs should have arms to provide support and prevent falls if the patient loses consciousness.
 - Have the patient position his / her arm rested in a comfortable position and extend the arm to form a straight line from the shoulder to the wrist. Arm should not be significantly bent at the elbow.
- **Procedure for patient lying supine**
 - Ask the patient to lie down on his / her back in a comfortable position.

- If additional support is needed, place a pillow under the arm from which the specimen is being drawn.
- Have the patient position his / her arm extends to form a straight line from the shoulder to the wrist.

Apply Tourniquet

A tourniquet is used to increase venous filling. This makes the vein more prominent and easier to enter.

1. Precautions while using a tourniquet

Tourniquet application should not exceed one minute as localized stasis with haemoconcentration and infiltration of blood into the tissue can occur. If the patient has a skin problem, the tourniquet should be applied over the patient's gown or a piece of gauze pad or paper tissue should be used so that the skin is not pinched.

2. Tourniquet location

Wrap the tourniquet around the arm 3 to 4 inches above the Venipuncture site.

3. Blood Pressure cuff

If a blood pressure cuff is used as a tourniquet, inflate it to 40mm Hg.

4. Ensure the patient's hand is closed

The veins become more prominent and easier to enter when the patient forms a fist. There must not be vigorous hand exercise. Vigorous hand pumping can cause changes in the concentration of certain analytes in the blood.

5. Select Vein

Median cubital and cephalic veins are used most frequently. Veins on the back of the hand also can be used. Veins on the underside of the wrist must not be used.

Procedure for Vein selection

Palpate and trace the path of the vein with the index finger. Unlike veins, arteries pulsate, are more elastic, and have a thick wall. Thrombosed veins lack resilience, feel cord-like, roll easily, and should not be used. A tourniquet must be used to aid in the selection of a vein site unless specific tests do not require tourniquets (e.g. lactate, coagulation profile). The tourniquet should be released the moment blood starts flowing. If a tourniquet must be applied for the preliminary vein selection, it should be released and reapplied after two minutes.

Put on Gloves

The phlebotomist must wear gloves (sterile/unsterile as per the availability) before the Venipuncture is performed.

Cleanse Venipuncture Site

The puncture site must be cleansed

1. Cleansing Method for Venipuncture

- Use a gauze pad/swab with 70% isopropyl alcohol solution.
- Cleanse the site with a circular motion from the center to the periphery.
- Allow the area to air dry to prevent hemolysis of the specimen and to prevent the patient from experiencing a burning sensation when the Venipuncture is performed.
- Chlorhexidine gluconate is recommended for infants two months and older and patients with iodine sensitivity.
- Cleanse the site with 70% alcohol, then swab concentrically, starting at the middle of the site with a 1 to 10% povidone-iodine solution (0.1 to 1% available iodine) / or chlorhexidine gluconate.
- Allow the site to air dry and then remove the iodine or chlorhexidine from the skin with alcohol.

Venipuncture Procedure When Venous Blood Collection Tubes Are Used

There are several different blood collection systems available that collect blood samples using different principles.

- Thread the appropriate needle into the holder until it is secure.
- When drawing blood for cultures, wipe the stopper with a suitable antiseptic solution. Make certain the stopper is dry before performing the Venipuncture.

Make sure the patient's arm or other Venipuncture site is in a downward position to prevent reflux of "backflow".

- Hold the patient's arm firmly distal to the intended puncture site. The phlebotomist's thumb should be used to draw the skin taut. The thumb should be 1 to 2 inches (2.5 to 5.0 cm) below the Venipuncture site.
- To prepare the patient, inform him or her that the Venipuncture is about to occur.
- With the bevel up, puncture the vein with the needle at an angle of insertion of 30 degrees or less. Keeping the needle as stable as possible in the vein, pushy/connect the first tube onto the needle. Maintain the tube below the site when the needle is in the vein.
- Release the tourniquet as soon as possible after the blood begins to flow. Do not change the position of the tube until it is removed from the needle. During the collection, do not allow the contents of the tube to contact the closure. Movement of the blood back and forth can cause reflux into the venous system and possible adverse patient reactions.
- Allow the tube to fill until the vacuum is exhausted and blood flow ceases. For tubes that contain additives, this will ensure there is a correct ratio of blood to additives.
- When the blood ceases to flow, remove/disconnect the tube from the needle/holder. The sleeve re-covers the needlepoint that pierces the tube closure, stopping blood flow until the next tube is inserted/connected to the needle/holder. To obtain additional specimens, insert/connect the next tube to the needle/holder prior to withdrawing the needle from the vein. If only one tube is collected this must be removed prior to withdrawing the needle from the vein.
- Immediately after drawing each tube that contains an additive, mix the blood gently and thoroughly by inverting the tube five to ten times. To avoid hemolysis, do not mix vigorously.

Venipuncture Procedure Using Needle and Syringe

In general, Venipuncture using a needle and syringe should be avoided for safety reasons. In case of difficult collections by needle/holder and vacuum blood collection tubes, the Venipuncture procedure can be performed using a syringe draw; the following procedure is recommended.

- Assemble the needle and syringe.
- Hold the patient's arm firmly distal to the intended puncture site. The technician's thumb should be used to draw the skin taut. This anchors the vein. The technician's thumb should be 1 or 2 inches below the Venipuncture site.
- Prepare the patient by informing him or her that the Venipuncture is about to occur.
- With the bevel up, puncture the vein with the needle at an angle of insertion of 30 degrees or less.
- Keeping the needle as stable as possible in the vein, slowly withdraw the desired amount of blood.
- Release the tourniquet as soon as possible, after the blood begins to flow.

Fill the Tubes If a Syringe and Needle Are Used

The syringe method of drawing venous blood is not recommended since it is much safer and easier to use a closed, venous blood collection tube system. If it is necessary to use a syringe, proceed with the following recommendations to transfer the blood from a syringe to a blood collection tube.

- Use the same “order of draw” as for a venous blood collection tube system.
- Blood Draw-2ML in EDTA vacutainers.
- To transfer the blood from the syringe to a venous blood collection tube by removing the stopper.
- Mix additive tubes by inversion.

Ensure the Patient’s Hand Is Open

Opening the patient’s hand reduces the amount of venous pressure as muscles relax. The patient must not be allowed to pump the hand.

Releases the Tourniquet

Release the tourniquet as soon as possible after the blood begins to flow.

Place the Gauze Pad/Swab

A clean gauze pad/swab should be placed lightly over the Venipuncture site. Cotton balls may be used but ideally, it is not preferred because of the possibility of dislodging the platelet plug at the Venipuncture site.

Remove and Dispose of the Needle

Remove the needle and activate the safety mechanism according to the device manufacturer’s instructions. Safely dispose of the unit into an easily accessible sharps container.

Techniques to Prevent Hemolysis (which can interfere with many tests):

- Mix all tubes with anticoagulant additives gently (vigorous shaking can cause hemolysis) 5-10 times.
- Avoid drawing blood from a hematoma; select another draw site.
- If using a needle and syringe, avoid drawing the plunger back too forcefully.
- Make sure the venipuncture site is dry before proceeding with a draw.
- Avoid a probing, traumatic venipuncture.
- Avoid prolonged tourniquet application (no more than 2 minutes; less than 1 minute is optimal).
- Avoid massaging, squeezing, or probing a site.
- Avoid excessive fist clenching.
- If blood flows into the tube slowly, adjust the needle position to remain in the center of the lumen.

Blood specimen collection and handling:

- Each request for a blood specimen / any type of sample collection should be accessioned to identify all paperwork and supplies associated with each patient. Assign the Barcode with the following information in the EMD
- Accession is in the form of entry in the Out Patient Sample Collection Register by the registration assistants and affixing a number on the request form.
- For specimens other than blood, appropriate containers should be labeled and given to the patients with proper instructions for collecting and handling specimens.
- For blood collection, the patients are directed to collection counters according to the accession numbers which is displayed.

Label Blood Collection Tubes and Record the Time of Collection

The patient and the patient’s blood specimen must be positively identified at the time of collection. Blood specimens must be obtained in tubes identified with a label bearing at least the following.

- The patient’s first and last name
- Age/sex

- An identification number
- The date
- The time (as required e.g. therapeutic monitoring)

The completed label must be attached to the tube before leaving the side of the patient, and there must be a mechanism to identify the person who drew the blood. Alternatively, the manufacturer's tube label can be inscribed with the patient's complete information.

The laboratory documents the time when the specimen was collected. A small signature or initials of the personnel responsible for collecting the specimen shall be recorded whenever possible.

Specimens that need transport at a cool temperature

- Certain tests require that blood specimens be cooled immediately following the Venipuncture and transported with ice packs:
- Transportation of primary sample with specifications about time frame, temperature, and carrier

Rejection of primary samples

- Mislabeled/Unlabeled specimens
- Improper container
- Quantity not sufficient for testing
- Without a test request
- Haemolysed
- Clotted (Where not indicated)

Samples not adhering to the Vacuum blood collection tubes specifications

Receipt, Labeling, Processing, and Reporting of primary sample

To receive a complete requisition form W.R.T

- a) Name of the physician or other person legally authorized to make a request for examination
- b) Type of primary sample and the anatomic site of origin.
- c) Examination requested.
- d) Clinical information of the patient for interpretation purposes
- e) Date and time of receipt of the sample in the laboratory.

Unique identification of the patient

TO BE GIVEN BY LAB PERSONNEL as per the individual sections.

SAFETY AND BIOMEDICAL WASTE DISPOSAL

- In general, all human biospecimens and materials contacting with biospecimens must be treated as bio-hazardous materials.
- Protective clothing, including a laboratory coat, gloves, and protective glasses, must be worn at all times when performing this procedure
- All equipment should be sterile.
- Ten categories of biomedical waste have been described in schedule I of BMW rules 1998. According to category 4, sharps and wastes must be disposed of as per Biohazard Waste and Sharps Management policy.
- Waste sharps (needles, syringes, scalpel, blades, glass, etc. that may cause punctures and cuts. This includes used and unused sharps). Disinfection (chemical treatment using at least 1% hypochlorite

solution or any other equivalent chemical reagent. It must be ensured that chemical treatment ensures disinfection/ autoclaving/ microwaving and mutilation/ shredding must be such to prevent unauthorized reuse.

- Soiled waste (items contaminated with blood, and body fluids including cotton, dressing, and soiled) require local autoclaving/ microwaving/ incineration. There will be no chemical pretreatment before incineration. Chlorinated plastics shall not be incinerated.

Sample requirements:

FFPE tumor tissue sample or Fresh/frozen tumor tissue in normal saline (within 24hrs of biopsy)

Formalin-fixed, paraffin-embedded (FFPE) tissue samples are routinely prepared from human surgical tissue samples by fixation with formalin and embedding in paraffin. Thin sections of FFPE samples are commonly subjected to histopathological analysis, and remaining paraffin-tissue blocks are usually archived. Existing extensive archives of FFPE tissue samples represent a valuable source for retrospective studies of gene expression patterns and mutation analysis. However, the use of such samples for DNA analysis is limited due to chemical modification by formaldehyde and fragmentation of the DNA during tissue processing (sampling, fixing, embedding) and storage (humidity, time, temperature) of the samples.

The quality of laboratory results is highly dependent upon proper specimen collection and handling. Specimen storage conditions and stability are critical in isolating high-quality nucleic acids and insuring maximum sensitivity of detected significant variants.

- The tissue must contain sufficient tumor to be macro-dissected to a concentration of greater than 20% tumor cells in a normal cell background.
- Number of slides is dependent on size of the tissue and the tissue fixation and processing.
- Store samples at room temperature or refrigerated.

Transportation of Samples

Transport of specimens takes place at the following four temperatures:

- Frozen
- Refrigerated (2-8°C)
- 18-22°C
- Room Temperature

Use transport boxes supplied by GenepowRx. All gel packs should be frozen for at least 24 hours < 0°C prior to use. Use separate transport boxes for each of the temperature ranges e.g., all frozen samples in one box.

Frozen

- Empty transport (thermocool) box
- Place a layer of perforated sponge at the bottom
- Place a pre-frozen gel pack over the perforated sponge
- Place specimens sealed in a 'Zip lock bag' over the gel pack
- Place another pre-frozen gel pack over the samples
- Cover with a second layer of perforated sponge
- Place the un-perforated sponge and close the lid
- Seal the cardboard box and transport it to the laboratory immediately
- Indicate 'Frozen Samples' on the cardboard box.

Refrigerated (2-8°C)

- Empty transport box
- Place a pre-frozen gel pack at the bottom
- Place a layer of perforated sponge over the pre-frozen gel pack
- Place specimens sealed in a 'Zip lock bag' over the perforated sponge
- Cover specimens with a second layer of perforated sponge
- Place another pre-frozen gel pack over the samples
- Place the un-perforated sponge and close the thermacol lid.
- Seal the cardboard box and transport to the laboratory immediately
- Indicate 'Refrigerated Samples' on the cardboard box

18-22°C

- Empty transport box
- Place a pre-frozen gel pack at the bottom
- Place a layer of perforated sponge over the pre-frozen gel pack
- Place a second layer of perforated sponge
- Place another pre-frozen gel pack over the perforated sponge
- Place the un-perforated sponge and specimens sealed in a 'Zip lock bag' over the un-perforated sponge
- Seal the cardboard box and transport to the laboratory immediately
- Indicate '18-22°C Samples' on cardboard box

Room Temperature

- Empty transport box
- Place a layer of perforated sponge at the bottom
- Place specimens sealed in a 'Zip lock bag' over the perforated sponge
- Cover specimens with a second layer of perforated sponge
- Place un-perforated sponge and close the lid
- Seal the cardboard box and transport to the laboratory immediately
- Indicate 'Room Temperature Samples' on the cardboard box

Note: Patient Questionnaire, Consent & other medical reports are mandatory for the Transport of the samples.

References:

1. <https://extranet.who.int/lqsi/sites/default/files/attachedfiles/LQMS%205-3%205-6%20Sample%20collection%20transport.pdf>
2. <https://www.nhsfife.org/media/33755/sop25-sample-processing-and-storage-v3-final.pdf>
3. <https://dshm.delhi.gov.in/pdf/QAC/SoPs/Laboratory.pdf>
4. <https://ncdc.gov.in/index1.php?lang=1&level=1&sublinkid=691&lid=542>
5. <https://tmc.gov.in/research/pdf/TMC-HEC-SOP.pdf>