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| <b>Laboratory Services</b> | Title: Specimen Identification, collection and processing | Effective Date: |
|                            | Doc #: DRAFT  |                 |
| Author: Mathabo Lebina     | Version #: 0  | Approved by:    |

### **PURPOSE:**

The purpose of this procedure is to define the proper identification, collection and processing of patient specimens to ensure accurate test results. The accuracy of any test procedure is dependent on the quality of the specimen. The quality of the specimen is dependent on the specimen being collected and preserved in the appropriate manner.

## **SPECIMEN IDENTIFICATION**

### **General Requirements for Specimen Identification**

1. Each request must be accompanied by the appropriate test requisition form and matching specimen label.
2. The form must contain all of the patient's identification details
  - a. Patients Full Name / Unique Identifier
  - b. Name of the referring clinic/clinician
  - c. Name of test to be performed
  - d. Date and time of sample collection
  - e. Type of specimen
  - f. Clinical Details
  - g. Medication currently given

## **SPECIMEN COLLECTION**

### **1. Venous Blood Collection Requirements**

#### Collection Tubes:

- Specimens for testing should be collected directly into the appropriate colour top silicon-glass or plastic tube using standardized venepuncture / vacutainer methods.
- Avoid performing venepuncture using a syringe. This method is poor practice due to the increased risk of haemolysis and/or clotting of the sample.
- If a syringe must be used (21-23 gauge needles for paediatric patients), call laboratory for required volumes prior to collection as this may require special coagulation tubes. Blood must be transferred to the vacutainer tube within one minute of completion of draw
- Air bubbles or foaming in the tubes or syringes should be avoided.

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### Tube and Volume Requirements per Test

| Laboratory Tests  | Tube Colour  | Volume Needed  | Additive  | Purpose   |
|---|--|--|---|---|
| Chemistry<br>- LFTS<br>- Creatinine/Urea & Electrolytes<br>- Thyroid Function Tests<br>- Calcium<br>- RF -ASO<br>-Proteins -CK<br>-Lipase -Cholesterol<br>-Triglycerides<br>-HDL/LDL<br>-Endocrinology<br>-Tumour Markers | Red Top with clot activator or Yellow Top with Gel | Minimum 3ml in 5ml tube,                                 | None  | To allow blood sample to clot. This permits separation of serum when the serum needs to be tested |
| -Glucose  | Grey Top   | Min. 2ml   | Sodium Flouride Oxalate                                 | To prevent glycolysis<br>Fasting/Random   |
| HBA <sub>1</sub> C  | Purple Top   | 3-5ml  | EDTA  | To prevent Blood from clotting  |
| Karyotyping   | Green Stopper                                      | 4-5ml  | Lithium-Heparin   | To prevent glycosis   |
| Hematology<br>- Full Blood and Differential Count<br>- CD4 Count  | Purple Top   | Minimum 3ml in 5ml tube, Fill the microtube for children | Ethylenediamine Tetraacetic acid (EDTA) (anticoagulant) | To prevent Blood from clotting  |
| Malaria   | Purple Top   | Min.2ml  | EDTA  | NB: For Malaria collect sample when a patient is feverish   |
| PT & APPT   | Light Blue Top                                     | Fill a 5ml Tube  | Sodium citrate  | To prevent blood from clotting  |
| ESR   | Black top  | Fill up a Long Black tube                                | Sodium Citrate  | To prevent blood from clotting  |
| Osmotic Frigidity Test  | Green Top  | 5ml  | Heparin (anticoagulant)                                 | To prevent Blood from Clotting when Plasma needs to be Tested                                     |
| Microbiology<br>VDRL<br>Widal<br>Measles  | Red top with clot activator<br>Yellow top with gel | Minimum 3ml  | None  | To allow blood to clot  |

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Blood collection:

1. Collect all of the patient information required by the laboratory test requisition Form, and fill out the requisition form.
2. Collect other relevant information (drug dosage information, other relevant clinical information)
3. Ensure use of appropriate colour tubes for each requested laboratory test
4. Patient should be comfortably seated
5. Always collect blood from opposite arm from the site of intravenous infusion
6. Clean the skin on the site of venipuncture with 70% alcohol and allow to dry.
7. Apply a tourniquet 10 – 15cm above the puncture site. Do not leave the tourniquet for more than 3 minutes in place, as plasma proteins and protein bound constituents will be falsely elevated.
8. Collect blood using vacutainer needles and vacutainer tubes. For infants and neonates, use butterfly winged needles. **DO NOT USE A SYRINGE:** Using a syringe for blood collection causes haemolysis, which will greatly impede the accuracy of some laboratory investigations.
9. Release the tourniquet before the needle is withdrawn
10. For tubes with anticoagulant (Purple, Green, Blue), immediately and gently invert the tube 8 - 10 times to ensure complete mixture of blood and anticoagulant. **DO NOT SHAKE THE TUBE** as this will cause haemolysis.
11. Always put a dry pad (cotton wool or gauze) over the puncture site after needle removal.
12. Label Tube with patient name –date and time of blood draw
13. Arrange for prompt delivery of blood specimen to laboratory

**NOTES:**

- *When collecting venous blood in multiple tubes, always start with plain red top tube, then collect in tubes with anticoagulant.*
- *Multiple sticks and patting at the venepuncture site can affect the quality of the sample*
- *If only coagulation specimen is drawn; a pilot tube (red top) should be collected first and discarded*
- *If multiple specimens are collected, the anticoagulant specimen should be collected into the second or third tube.*

Blood Specimens after Collection

- *Blood specimens must be sent to the laboratory within 6 hours of collection.*
- *Whole blood specimens (FOR CD4 Count ) MUST NOT be stored in the refrigerator AND should NOT be transported in a box containing ice packs.*

*Please Note:*

*Several changes occur in blood specimen following collection including:*

- *Glucose is converted to lactate due to glycolysis by Red Blood Cells*

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- *Potassium (  $K^+$  ) and LDH pass through the RBC membrane into plasma leading to falsely elevated results.*
- *Phosphate increases due to hydrolysis of organic ester phosphate in RBC*
- *Decrease in activity of labile plasma enzymes*
- *Karyotyping samples should be sent to the lab/Central lab within 6hrs*

#### *Cytology:*

- *Fix sample with cytofixative to prevent cell morphology disintegration*
- *Transportation of fluids: Send to local lab and record the volume fluid tapped*

#### *Histology:*

- *All histology samples should be fixed with 10% Formalin – fully immerse the sample.*

## **2. Paediatric Dried Blood Spot (DBS) Collection Requirements**

Blood can be collected any day, any time, even on Friday afternoons, and stored at the hospital laboratory until it can be delivered to the testing laboratory.

### Blood collection through Heel Prick

1. Before the DBS is collected, the following information for the client should be written on the filter paper card:

- Patient name
- Clinic number
- Date of birth
- Hospital / Clinic

Do not touch the circles with anything other than the infant's blood. Your hands, glove powder, ink, or dirt may affect the result.

2. Put on gloves
3. Position baby with foot down
4. Select one of the newborn's feet. Warm the area before puncture
5. Thoroughly cleanse the back of the heel with 70% alcohol and allow to air dry for 30 seconds.
6. Use a sterile, disposable lancet to puncture the back of heel.
7. Wipe away the first small blood drop with a gauze pad.
8. Place the card close to the lanced area but do not touch it. Apply gentle pressure to the base of the foot and allow the second LARGE blood drop to fall from the heel onto surface of the filter paper. DO NOT TOUCH AND SMEAR THE BLOOD SPOT.
9. Fill entire circle with drop
10. Fill at least 3 circles

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11. When all circles are filled (or client no longer bleeds) apply cotton to the puncture site until blood flow stops.

### **3. Adult Dried Blood Spot (DBS) Collection Requirements**

Blood can be collected any day, any time, even on Friday afternoons, and stored at the hospital laboratory until it can be delivered to the testing laboratory.

#### **Blood collection through finger Prick**

1. Before the DBS is collected, the following information for the client should be written on the filter paper card:

- Patient name
- Clinic number
- Date of birth
- Hospital / Clinic

Do not touch the circles with anything other than the patient's blood. Your hands, glove powder, ink, or dirt may affect the result.

2. Put on gloves or wash hands
3. Warm the area before puncture
4. Thoroughly cleanse the finger with 70% alcohol and allow to air dry for 30 seconds.
5. Use a sterile, disposable lancet to puncture the skin.
6. Wipe away the first small blood drop with a gauze pad.
7. Place the card close to the lanced area but do not touch it. Apply gentle pressure to the finger and allow the second LARGE blood drop to fall from the finger onto surface of the filter paper. DO NOT TOUCH OR SMEAR THE BLOOD SPOT.
8. Fill entire circle with drop
9. Fill at least 3 circles
10. When all circles are filled (or client no longer bleeds) apply cotton to the puncture site until blood flow stops.

#### **Dried Blood Spot Drying**

- Allow the specimen to air dry horizontally for at least 3 hours
- Keep away from direct sunlight, dust, and bugs
- Do not heat, stack or allow DBS to touch anything during the drying process (including other DBS cards)

#### **Packaging DBS Card for storage**

1. At the end of each day, stack the dry DBS (leave for next day if not dry after 3 hrs) between sheets of glassine paper

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2. Insert into sealable plastic bag
3. Press air out of bag and seal
4. Add desiccant packets & 1 humidity card
5. Reseal bag
6. Store in refrigerator if not going to Lab that day

#### Storing DBS

Keep packaged DBS in sealed plastic bags in cool place until transported to Central Laboratory. Refrigerate if storing for 1+ week.

#### How to Package DBS for Shipping

1. Insert DBS bag into envelope
2. Include lab requisitions
3. Include specimen delivery checklist
4. Seal envelope
5. Label envelope clearly ("Infant DBS specimens")
6. Send to Central Lab on scheduled sending times

#### Rejection of an Invalid DBS Specimen can occur if:

- Specimen appears scratched or abraded. This may be due to the specimen not dried properly before shipping.
- Specimen appears super-saturated. This may be because the specimen may have been soaked on both side or blood may have been applied with syringe
- Specimen appears diluted, discolored or contaminated. The collection site may have not been allowed to air dry before sticking.
- Specimen appears clotted or layered. Multiple drops of blood may have been on top of each other.
- Name does not appear on card etc.

### **4. Urine Collection Requirements**

#### **Procedure for 24 Hour Urine Collection:**

- Obtain a dark urine collection bottle from the lab.
- Empty the bladder and discard this urine at the beginning of the collection period (e.g. 06.00 hrs of day 1)
- Collect all urine passed and keep in one container up to the end of collection time (e.g. 06:00 hrs of day 2)

URINARY PROTEIN: Use 24 hr urine collection as specified above.

URINARY CORTISOL: Use 24hr Urine collection as specified above.

URINARY FREE CATECHOLAMINES: Use 24 hr urine collection as specified above in 10ml concentrated hydrochloric acid (HCL).

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#### SPOT URINE COLLECTION:

- Early morning specimen preferably midstream urine.

### CLEAN CATCH URINE COLLECTION

**Wash hands with soap and water; Open the container; be careful not to touch the inside edges of the container and its lid**

#### Urine Collection Technique for Women

- Spread labia with one hand and hold apart
- Clean the area with a swab soaked in clean water
- Dry the area with sterile swab or gauze
- Void into the toilet for a few seconds and then stop
- Restart urine stream and collect into a sterile container
- Cap and avoid touching inside or edges of container

#### Urine Collection Technique for Men

- Retract foreskin if present
- Clean the tip of the penis with a swab soaked in clean water
- Dry the area with sterile swab or gauze
- Void into the toilet for a few seconds and then stop
- Restart urine stream and collect in sterile container
- Cap and avoid touching inside or edges of the container

#### Urine Collection Technique for Infants (Urine bag)

- Spread the labia and clean with a swab soaked in clean water **OR** Retract the foreskin and clean the tip of the penis with a swab soaked in clean water
- Place sterile urine bag over penis or labia
- Empty bag contents into a sterile urine container
- Re-clean and replace new urine bag if no urine in 30 minutes
- Recap the lid and avoid touching the inside or edges of the container

***Note:** Label the container with patients first name, last name, date and time of collection and the ward/clinic/hospital Refrigerate container after collecting the specimen, and deliver to laboratory the same day*

### 5. Semen Analysis Collection

Semen sample collected in the following manner

- Sample should be collected after a minimum of 48 hours and no longer than 7 days of sexual abstinence

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- Patients should abstain from sexual intercourse for 3 – 7 days (Ideally 4 days) before the test.
- Sample should be collected at MCH
- Sample should be obtained by masturbation and ejaculated into a clean wide mouthed glass or plastic container. Warm up the container to minimise the risk of cold shock.
- Semen must be taken, delivered and examined within 1 hour of collection.

## **6. Fecal Sample Collection**

- Label the specimen container properly with patient's name, time of collection and date of collection
- Ask the patient to pass the stool directly onto a cardboard or plastic container
- Collect about 20 – 40g of well formed stool or 5 – 6 spoonfuls of watery stool
- Specimens must reach the laboratory within 30 minutes of collection
- Do not keep specimens at warm temperatures, keep in cool shady places
- Do not contaminate with urine or dirt particles
- Stool **must not** be collected from bed pan containing disinfectant
- Close the lid properly to prevent drying

## **7. Blood Culture Collection**

Timing of blood collection

Whenever possible, blood should be taken **before** antibiotics are administered. The best time is when the patient is expected to have chills or a temperature spike. It is recommended that two blood cultures be obtained, separated by interval of approximately one hour.

The advantages of repeated cultures are as follows:

The chance of missing a transient bacteraemia is reduced

- ❑ The pathogenic role of saprophytic isolates (*Staphylococcus epidermidis*) is confirmed if they are recovered from multiple venepunctures.

Antibiotic therapy should be initiated after blood specimens for culture have been collected. If necessary, the choice of antibiotic can be adjusted when the results of susceptibility test become available.

### **QUANTITY OF BLOOD**

Because the number of bacteria per millilitre of blood is usually low, it is important to take a reasonable quantity of blood: 10 ml per venepuncture for adults: 2 - 5 ml may suffice for children: for infants and neonates 1 – 2 ml is often the most that can be obtained.



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## **BLOOD CULTURE MEDIA**

Choice of broth media

The broth should be able to support growth of all clinically significant bacteria.

### **Quantity of broth**

Ideally, the blood should be mixed with 10 times its volume of broth (5 ml of blood in 50 ml of broth) to dilute any antibiotic present and to reduce the bactericidal effect of human serum.

## **8. Sputum Collection Requirements**

### **To collect a proper sputum sample for the detection of AFB**

- Give the patient confidence by explaining to him/her the reason for sputum collection
- Instruct the patient to rinse his/her mouth with water before producing the specimen. This will help to remove food and any contaminating bacteria in the mouth
- Instruct the patient to take two deep breaths, holding the breath for a few seconds after each inhalation and then exhaling slowly. Ask him/her to breathe in again and then cough. This should produce a specimen from deep in the lungs. Ask the patient to hold the sputum container close to the lips and to spit into it gently after a productive cough. Sputum is frequently thick and mucoid, but may be fluid, with chunks of dead tissue from a lesion in the lung. The colour may be a dull white or a dull light green. Bloody specimens will be red or brown. **Thin, clear saliva or nasopharyngeal discharge is not sputum** and is of little diagnostic value for tuberculosis.
- If the sputum is insufficient encourage the patient to cough again until a satisfactory specimen is obtained. Remember that many patients cannot produce sputum from deep in the respiratory track in a few minutes. Give him/her sufficient time to produce an expectoration which she/he feels is produced by a deep cough.
- If there is no expectoration, consider the container used and dispose of it in the appropriate manner
- Check that the container is securely closed and labelled. Do not label the lid
- Wash hands with soap and water
- Give the patient a new sputum container and make sure that she/he understands that a specimen must be produced as soon as she/he wakes up in the morning
- Demonstrate to the patient how the container should be securely closed
- Instruct the patient to bring the specimen back the following day to the laboratory.

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## **9. Cytology Collection Requirements**

### **TAKING CERVICAL SMEARS**

*Pre-counselling – Explain and give a patient confidence of why the sample is being collected*

- Put the patient's name and clinic number or date of birth on the ground glass end of the slide using lead pencil ( other markers are washed off by processing fluid). If smear is vaginal, mark 'V'. Please leave space for laboratory number.
- Clean cervix with 5% acetic acid. Position patient, adjust light and insert the warm speculum. Lubricate with minimal tap water. Use no antiseptics or greasy lubricants .
- Insert the specula into the cervical os using the bilobed end unless the cervix is very patulous or scarred when the spade end should be used. Firmly rotate the spatula through at least 360° ensuring that the scrape spans the squamocolumnar junction at all times. A brush sampler may be used where the squamocolumnar junction is high or where an endocervical abnormality is suspected.
- Spread material thinly on the glass slide. Use gentle longitudinal strokes rather than a circular motion. The aim is to get a single cell layer on as much of the slide as possible without damaging the cells.
- Place the slide on a horizontal surface and immediately dip in or apply the fixative generously, holding the spray 20cm away from the slide. Avoid exposure to direct sunlight. Allow to dry for a minimum of 20 minutes before inserting into the container
- Complete the request form with a ball point pen, pressing firmly on a hard backing surface. Ensure that all copies are legible.
  - Match the request form and the specimen to be transported
  - Tightly pack the specimen container by insuring that the lid is tightly closed
- Put the container in the box and label the names the requesting hospital/clinic
- Make sure the transportation box and the specimen are facing upwards direction
- Enter the specimen in the consignment book –(if the specimen is to be shipped and processed in a distant different center)
- Use the same format to record the consignment book with inclusive the name of the consigner and the consignee, and the column of the person receiving the sample in a distant center.

## **10. Histology Collection Requirements**

*This section advocates for caution in specimen handling to ensue safety of both the consigners, consignees, phlebotomists and or medical technologist at the reception.*

- After taking the biopsy, fully submerge a tissue in 10% formalin in a standard container.

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- Label the container with patient's name, date of collection and the requesting hospital
- Complete the request form with a pen
- Match the request form and the specimen to be transported
- Tightly pack the specimen container by ensuring that the lid is tightly closed
- Put the container in the box
- Make sure the transportation box and the specimen are facing upwards direction
- Enter the specimen in the consignment book

## Sample Rejection Criteria

### *A. Blood*

- **Incorrect Container:**  
Samples must be properly collected and placed in appropriate container. Samples that come in the incorrect container will be immediately discarded.
- **Inadequate request information:**  
If the request form and the container do not contain the proper specified information, samples will then be immediately discarded.
- **Under-filling:**  
Under-filled tubes will be rejected and a re-draw be requested. Inadequate filling of the collection tube decreases the required blood: Anticoagulant ratio (9:1), and may lead to false results.
- **Overfilling:**  
Overfilled tubes will be rejected. Overfilling increases blood: anticoagulant ratio, which may result in erroneous results or specimen clotting.
- **High Haematocrit:**  
Patients with elevated Haematocrits (polycythaemia  $\geq 55\%$ ) may require special coagulation tubes prepared by the laboratory. Contact the laboratory prior to drawing specimen on patients with known elevated haematocrits.
- **Clotted Samples:**  
Clotting leads to erroneous results. Clotted specimens are rejected. Clotting is caused by sample not collected in a correct tube or when the specimen is not well mixed with an anti-coagulant.
- **Haemolysis:**  
Results on haemolysed specimens may be inaccurate. Grossly haemolysed specimens will be rejected and a re-draw will be requested.
- **Soiled container or Form:**  
Any tubes, containers or forms with any trace of blood or any other sample will be immediately discarded.
- **Long standing specimens:** Specimens no longer produce reliable results after: Clinical chemistry tests > 6hrs

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FBC

4 days

#### B. Cytology

- HAEMOLYSED EFFUSIONS
- SOILED FORM AND SPECIMEN
- BROKEN SLIDES
- SLIDES WITHOUT A REQUEST FORM
- UNFIXED SLIDE-MATERIAL
- INADEQUATE CLINICAL INFORMATION

#### C. Urine

- Unsterile container for urine culture
- Long standing specimen > 30min
- Soiled sample and specimen form
- Overfilled specimen bottle
- Inadequate specimen

#### D. Stool

- Dry specimen
- Overfilled specimen
- Contaminated specimen (e.g. with urine)
- Soiled Container and a form

#### E. Sputum

- Soiled form and container
- Overfilled specimen

#### References

Lab Handbook,

National Medical Laboratory Quality Manua

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