

Table 1. Pooling Study Schedule of Events

	Enrolment	
	Day1	Day2
History and clinical exam	X	
CXR dual read	X	
TST	X	
Adult source tracing	X	
HIV ELISA ^a	X	
Gastric Aspirate (GA) for Smear, Xpert, MGIT ^b	X	
Nasopharyngeal aspiration (NPA) for Smear, Xpert, MGIT	X	
Induced Sputum (IS) for Smear, Xpert, MGIT	X	
Respiratory samples pooled for Smear, Xpert, MGIT #1) GA #2) NPA #3) Sputum induction with suction		X

^aHIV ELISA to be done unless a recent HIV test on the laboratory system and no risk factors for subsequent HIV exposure since last test. HIV Rapid Test would be performed only if not enough blood can be collected for the HIV ELISA. If ELISA test is positive in a child <18 months of age, blood must be collected for HIV DNA PCR as soon as possible to confirm HIV infection definitively.

HIV PCR to be done as the initial test if the child: a) is <18 months old; b) has had documented HIV exposure pre- or post-natally and c) does not have a documented record of a previous negative HIV PCR test OR if there is ongoing exposure (e.g. breastfed)

^bMGIT=Mycobacteria Growth Indicator Tube Culture

Minimum time fasting before procedures

Table 2 Minimum time fasting before collection of respiratory samples

Day 1		
Order	Sample type	Minimum hours nil per os (NPO)
1	Gastric aspirate (GA)	4 hours (preferably overnight fast)
2	Nasopharyngeal aspirate (NPA)	2 hours
3	Induced sputum	2 hours
Day 2 (for pooling)		
Order	Sample type	Minimum hours NPO
1	GA	4 hours (preferably overnight fast)
2	NPA	As soon as GA collected
3	Induced sputum	As soon as NPA collected

Note that the procedures should be performed in the order listed in the above table

Specimen collection

1. **Gastric aspiration**

Equipment required

1. Gloves, particulate respirator masks (N95 or equivalent), disposable aprons
2. Sputum specimen container (screw-top)- preferably Falcon tube
3. Laboratory forms
4. Laboratory bags
5. Nasogastric tube-NGT (6-10 French): preferred are Ryles (longer) or Levin tubes
6. 5, 10 and 20 cc syringe
7. Litmus paper/ pH strips
8. Sodium Bicarbonate (4%) solution for bedside neutralization
9. 3 bed sheets or surgical drapes: one for the bed, one for wrapping child into and one for putting over the child
10. Dropper
11. Normal saline (0.9% NaCl) or sterile water in single use vials

Performing gastric aspiration

This procedure is routinely performed by the study nurse or ward nursing staff.

2. The parent/ legal guardian will be instructed regarding overnight fasting of at least 4 hours before early morning gastric aspirate (GA). The procedure is preferably performed early in the morning when the child comes for a study visit or in the ward if child is an in-patient. The procedure may also be performed during the daytime, as long as the child has been kept nil per os (NPO) for minimum 4 hours.
3. Use an assistant (counselor) to help as this procedure requires 2-3 people.
4. Prepare all equipment for the procedure.
5. Disinfect all working surfaces including bed. Place a drape over the bed. Use one drape to immobilize the child and one to cover the child leaving head exposed.
6. Position the child in decubitus supine position with the help of an assistant.
7. Optional: instill 2 drops of oxymetazoline into each nostril to induce vasoconstriction and prevent epistaxis.
8. Measure the distance of the nasogastric tube to the stomach (from tragus of the ear, to nose, to xiphisternum): this estimates the distance that will be required to insert the tube.
9. Placing child's face in the "sniffing air" position, a nasogastric tube is passed from the nose into the stomach to aspirate gastric contents.
10. Attach a syringe (10 if using Levin or 20 ml if using Ryles tubes) to the nasogastric tube (size 6-10 French, depending on the size of the child).
11. Withdraw (aspirate) gastric contents using the syringe attached to the nasogastric tube.

12. Check NGT position by pushing some air from the syringe into the stomach (3-5ml), and listening with a stethoscope over the stomach.
13. Aspirate stomach contents gently and steadily, with the child in each of 3 positions: head central, left lateral and right lateral. Allow a few seconds before aspirating after changing position. If no fluid is aspirated, push tube 1-2cm deeper or pull out 1-2cm shallower, and aspirate. ANY VOLUME ≥ 1 ml is adequate for bacteriological examination. However, attempt to obtain as high volume as possible, aiming for a minimum 5mL, especially from a sick child (where a diagnosis is more important).
14. If < 1 ml is aspirated, a gastric lavage can be performed:
 - a. Sterile water (alternative: preservative free normal saline) 10 ml will be inserted down the tube, left for three minutes, and then aspirated until a minimum of 5-10ml aspirate is obtained.
 - b. If no fluid is aspirated, instill additional 10 milliliters of sterile water and aspirate again. If still unsuccessful, repeat this up to 3 times.
15. Transfer full volume of gastric fluid from syringe into a sterile container (Falcon tube).
16. Titrate volume of 4% sodium bicarbonate using a pipette or syringe and pH strips, adding to the specimen until pH 6-7 is reached (in order to neutralize the acidic gastric contents and so prevent destruction of tubercle bacilli)
17. Clean Falcon tube with alcohol swabs
18. Label sample: sample type and number, date, time, time of neutralization volume of bicarb added, total sample volume.
19. Place in sample bag, seal and put into a cold box for transport to lab.

Nasopharyngeal aspiration:

Equipment required:

1. Suction apparatus
2. Disposable gloves and P2 respirator masks
3. Paper towels
4. Normal saline (0.9%)
5. Oxymetazoline (optional)
6. Sterile 6/7/8 G mucus extractor or nasogastric catheter
7. Cotton wool
8. Kidney dish
9. Laboratory forms
10. Laboratory bags

Procedure

This procedure for respiratory sampling in young children will be conducted before sputum induction.

1. The child's nose is cleaned with saline drops and cotton wool. If old enough, the child can be asked to blow the nose into a tissue. If the nasal mucus is too thick to be removed with the measures above, it can be suctioned prior to nasopharyngeal aspiration. A soft catheter size F6/7 is used for suctioning and is discarded immediately afterwards.
2. One drop of oxymetazoline may be instilled into each nostril to prevent nose bleeds.
3. Two drops of sterile saline are instilled into each nostril
4. The length of the cannula used for aspirating the NPA sample is measured as the distance from nostril to tragus of the ear; then the posterior nasopharynx is suctioned using a soft plastic cannula connected to a mucus trap.
5. Suctioning is activated only when the tip of the cannula is in the posterior nasopharynx. When the cannula is passed through the nostrils (during introduction and extraction), the suction is de-activated.

Sputum Induction

Sputum induction is typically used in patients who are unable to produce sputum spontaneously. The patient inhales nebulised hypertonic saline solution, which liquefies airway secretions, promotes coughing and allows expectoration of respiratory secretions. In young children, nasopharyngeal aspiration is usually required for sputum collection.

Contra-indications/precautions:

1. As hypertonic saline causes bronchoconstriction, the procedure should only be performed after pre-medication with salbutamol and under medical supervision in patients with asthma or severely impaired lung function.
2. As the procedure induces coughing, it should not be performed in patients in whom severe coughing may be harmful, including patients with:
 - a) Unstable respiratory state: acute respiratory distress, pertussis-syndrome, hypoxia (sats <92% in room air), pneumothorax.
 - b) Unstable cardiovascular status (including untreated cyanotic heart disease)
 - c) Recent surgery: attending surgeon to assess
 - d) Any condition where the patient is unable to protect the airway e.g. depressed level of consciousness

Infection control

The minimum requirement for sputum induction is a single room with door closed and air exhausted to the outside of the building without recirculation. Ideally, the room should be fitted with an air extractor allowing for generation of negative pressure in the procedure room. A "no-entry" sign should be fitted outside the door for the duration of the procedure.

Staff performing procedure (including child carer if present) must wear the recommended TB respiratory protection (particulate respirators) and disposable gloves when handling sputum specimen.

Equipment required:

1. Spacer with mask
2. Salbutamol (100µg/puff)
3. Suction apparatus
4. Pulse oximeter
5. Nebuliser with tubing and face mask

6. Hypertonic (3-5%) saline solution
7. Disposable gloves and P2 respirator masks
8. Paper towels
9. Kidney dish
10. Sputum specimen container (screw-top)
11. Laboratory forms
12. Laboratory bags
13. 5ml and 10ml syringes
14. 19G needle
15. Sharps container
16. Sterile 6/7/8 G mucus extractor or nasogastric catheter

Procedure:

1. Sputum induction is performed by a research nurse trained in this technique, and is undertaken after a 2–3 h fast.
2. Clinical evaluation form is completed before procedure, documenting general observations and chest auscultation. (See section 7 below). Detection of severe respiratory distress or severe tachycardia is a contra-indication for the procedure.
3. Oxygen saturation and pulse rate must be monitored throughout the procedure. Stop the procedure in event of a fall in saturation <90% and a pulse rate >180 or <100 bpm
4. Child is pretreated with 200µg salbutamol via metered dose inhaler with attached spacer to prevent bronchoconstriction. This is done by placing the assembled metered dose inhaler/spacer/mask onto child's mouth and nose. Child is allowed to settle until breathing freely. One puff is activated, keeping the mask in the same position, and the child is allowed to breathe 4-5 times. Mask is removed.
5. The child's nose is cleaned with saline and cotton wool to remove nasal mucus prior to sputum induction. If nasal secretions are thick, a soft catheter size F6/7 is used for suctioning and is discarded immediately afterwards.
6. A jet nebuliser attached to oxygen at a flow rate of 5-7 L per minute delivers 5 mL of 5% sterile saline for 15 minutes **or until child starts to cough.**

7. In young children who cannot expectorate spontaneously:

- a. Once the child starts to cough, sputum is obtained by suctioning through the nasopharynx with a sterile mucus extractor or catheter size 6 or 7. Pass the catheter up to a length equal to the distance from nostril to tragus of the ear, without applying suction. Once in position, apply suction. Catheter can be moved a little and turned to facilitate cough reflex and aspiration of sputum.

Suction pressures by ages, according to the American Association for Respiratory Care (AARC) Guidelines for appropriate sub-atmospheric nasotracheal suctioning pressures:

- Neonates: 60 - 80 mm Hg (0.079- 0.10 Bar)
- Infants: 80 - 100 mm Hg (0.079- 0.13 Bar)
- Children: 100 - 120 mm Hg (0.13- 0.15 Bar)
- Adults: 100 - 150 mm Hg (0.13- 0.19 Bar)

Negative pressures should not exceed 150 mm Hg as higher pressures have been shown to cause trauma, hypoxemia and atelectasis.

Once sputum is obtained, the catheter is withdrawn from the nose. DO NOT aspirate as the catheter passes through the nose. If mucus is to be obtained from the oral cavity as well, the mouth should be rinsed/wiped with anti-bacterial, non-alcohol-based mouth wash prior to the entire procedure, in order to avoid contamination.

- b. If the child does not cough after nebulisation, chest percussion is done over the anterior and posterior chest wall. Mucus is then extracted as (7) above. Nebulisation and chest percussion followed by nasopharyngeal aspiration can be repeated if an inadequate sample is obtained (volume <1mL).

8. In older children who can expectorate spontaneously:

- a. Once the child starts to cough, encourage the expectoration of sputum into a sputum container. The child should continue to expectorate until no more sputum can be produced. Nebulisation can be repeated if an inadequate sample is obtained (volume <1mL or watery sample indicative of saliva)
 - b. If the child does not cough after nebulisation, encourage the child to perform deep breathing. The child can also be made to jump or run on the spot if clinically stable and able to do so. Chest percussion is done over the anterior and posterior chest wall. Encourage the child to expectorate as 8a. above.
9. Specimens will be transported directly to the laboratory for processing.
10. Spacers and nebuliser equipment will be sterilised after use in every patient.
11. Complete clinical evaluation form after procedure. Any new signs of respiratory distress not settling after 5-10 minutes of supplemental oxygen via face mask must be reported immediately to attending clinician. SAE form must then be completed.

1.1. Quality Control (QC) of samples

- a) All forms must be checked and signed-off as correct before they depart from the clinical site. This is done by an appropriate responsible party which may be a study nurse, counsellor, doctor or the study coordinator.
 - a. If the form has not been filled out to satisfaction, it must be returned to the person who filled it in.
 - b. Once a person has signed as “QC,” the responsibility of the form’s accuracy and completeness lies with the “QC-er”.

1.2. Transport boxes

- a) At the beginning of every day, ice bricks must be collected in the freezer in room XXXX and packed into the appropriate transport boxes housed in room YYYY. These must be taken to the procedure room at the hospital at the start of the day on days when sampling is planned.
- b) At the end of each day the ice bricks must be returned to room XXXX.
- c) Transport boxes should be returned to room YYYY and wiped clean on the inside and outside at the end of each day using an alcohol based disinfectant. Leave open to dry. Also clean the ice bricks the same way.

- d) Transport boxes and ice bricks can also be kept in the procedure room, as long as they are clean at the end of each day. The boxes should not be left open when respiratory samples are collected and should be wiped after the procedure as well.

1.3. Sample transport

- a) Once the samples have been collected, begin the transport procedure by checking that all required forms have been completed and the barcodes match across forms as well as on the samples being transported. If there are any errors here, return the forms to the person who completed them, for correction.
- b) Place samples into a transport box that has the correct temperature.
- c) Forms:
 - a. Complete the Departure side of the Transport Log and re-check that all samples listed there are in the transport box.
 - b. Depart the site with all the appropriate forms (including Requisition Forms and Transport Log).
 - c. On arrival at the laboratory, complete the Transport Log by filling in the Arrival side of the form. Make sure that there is a person present to receive the sample, this person signs to verify sample reception.
- d) Leave the sample and the requisition form in this person's possession.

Table 3 Volumes, Storage and Transport Conditions for Sample Types

Sample	Minimum Volume	Temperature to keep until arrival in lab	Storage in Cough Room until transport to lab	Transport to lab	Max time between collection and arrival in lab ^A	Destination Lab
GA	Take time to collect 5-10 mL ideally (1mL min)	2 to 8°C	Fridge	with ice brick	Same day	NHLS TB Lab (9 th floor) ^D
All other respiratory samples	As much as possible, minimum 1 mL	2 to 8°C	Fridge	with ice brick	Same day	NHLS TB Lab (9 th floor)
HIV ELISA Test	Minimum 0.6 ml	18°C to 25°C	NHLS Tube rack	18°C to 25°C	n/a	NHLS Serology
HIV PCR Test	Minimum 0.5 ml (0.25 ml accepted)	18°C to 25°C	NHLS Tube rack	18°C to 25°C	n/a	NHLS Virology