



Minimally Invasive Tissue Sampling (MITS) Sample Collection Standard Operating Procedure (SOP)

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1. Purpose

The purpose of this Standard Operating Procedure (SOP) is to describe the procedures for specimen collection during the MITS procedure.

Objectives

- Describe the standard operation procedure of the MITS according to the study protocol
- List the materials and reagents not included in the MITS sample collection kit
- Articulate the steps to adequately prepare and disinfect the body for the MITS
- Identify the components of external inspection, the anthropometric measurements and the pictures
- Obtain MITS Samples for microbiology and pathology
- Identify the presence of ascites fluid and pleural effusions and how to obtain samples for analysis
- Describe the basis of the examination and sampling of the placenta
- State the steps to be done at the end of the procedure

2. Scope

This document is relevant to all tissue and non-tissue specimens collected from the following populations. Age-specific case instructions will be noted in the text.

- **Perinatal**
 - **Stillbirth** - baby born with no signs of life at or after 28 weeks' gestation
 - **Neonatal** - live born babies who die before 28 days of age
- **Infant** - baby who dies between 29 days and 1 year of age
 - **Infant, open fontanelle**
 - **Infant, closed fontanelle**
- **Child** - bodies of children > 1 year of age - 19 years of age
- **Adult** - bodies > 19 years of age

3. Roles and Responsibilities

The table below provides teams with an understanding of the individuals involved with the MITS SOP

Role	Responsibilities
MITS Specialist	Executes and ensures specimens are collected and assures compliance with the procedure. Specially trained to perform MITS. Referred to as "Specialist" throughout the SOP.
MITS Assistant	Assists the MITS specialist during the MITS procedure. Manages the specimen collection containers and the MITS Specimen Collection Form. Moves the body and helps to maintain the adequate positions of the body. Referred to as the "Assistant" throughout the SOP.

Note: Projects may prefer to have a third team member to assist during the procedure.

4. Related Documents

To be completed by study staff, if applicable

Title	Version Number

5. Useful Terms and Definitions

Review table and add additional project specific terms, as needed

Term	Definition
CNS	Central Nervous System
CSF	Cerebrospinal fluid
ID	Identification number
MIT S	Minimally Invasive Tissue Sampling
PPE	Personal protective equipment
Filter Paper	<i>Blood Spot Card</i>

6. Equipment / Reagents

6.1. MITS Sample Collection Kit with the Specimen Kit ID number

See Appendix A for all components

6.2. MITS backup box

See Appendix B for all components

6.3. Material to be obtained by participating site

Materials NOT included in MITS Kits
Disposable scrub, lab coat, gloves, boots, shoe covers, hat
Paper towels
Safety goggles
Forceps – Sterilized
Gauze rolls 36' x 100 yards, and 4x4
Cotton swabs for cleaning the body with reagents
Camera for pre-procedure pictures (high resolution, >300 ppi preferred)
Body weight scale <ul style="list-style-type: none">• for stillbirths and neonates calibrated in grams• for children / adults calibrated in kilograms
Rolling cart or auxiliary table
MITS cool box/cool packs to maintain temperature control of samples for microbiological analysis
MITS cryogenic vial rack
MITS test tube rack
MITS tray on which to place formalin jars
Biowaste container
Headrest
Scissors – Sterilized
Clock

6.4. Reagents to be obtained by participating site

Reagents NOT included in MITS Kits

Alcohol 70%

- **Conservation at room temperature | Long stability | Inflammable**

Iodine solution

- **Conservation at room temperature | Long stability**

Any reagent required for microbiological sample collection, processing, and analysis, if specified by the project protocol

- **For example, RNA^{later} or other stabilization solution**

Monsel's solution – Hemostatic agent

- **Ferric subsulfate 20%, used to stop bleeding after biopsy**
- **Conservation at room temperature in an airtight container protected from light.**
- **Crystallization may occur at temperatures below 22 degrees. Warming the solution may dissolve the crystals.**

Bleach 10%

- **Or project-specific standard for cleaning surfaces**

Project-specific supplies for blood culture, if applicable

7. Safety

Maintain the following safety procedures

Ensure appropriate personal protective equipment (PPE) is worn by all MITS personnel



Dispose of needles and all waste generated during procedure in appropriate container as per biosafety laboratory protocols

Please see Section 10.5 for a guide for donning and removing PPE.

8. Procedure

8.1. Preparation of the body and materials

Prior to starting the MITS specimen collection

The Specialist and Assistant don the appropriate PPE to perform the MITS (not included in kit).

The Assistant picks up the body from the morgue and checks the surname (or name of mother for stillbirths and early neonates) in the autopsy request document.

The Assistant takes the body to the MITS room and places it on the table in a supine position.

MITS Specimen Form Instructions:

- Use a new Specimen Collection Form for each MITS Case.
- Mark only one response unless otherwise noted.
- Mark only observations present. (i.e. do not put “x”s in boxes
- If additional space is needed for a description write in the “other notes” in Section 6.

Assistant and Specialist confirm MITS kit contains all items on the list of contents. Assistant arranges items from MITS kit on disposable placemat.

The Assistant and Specialist check once again the name, surname, official information related to autopsy report (if available), and informed consent

The Assistant prepares additional materials and equipment (not included in kit) required for MITS procedure. Apply labels, as needed

The Assistant completes the MITS Specimen Collection form Overview Section

8.2. Photography

Prior to starting the MITS specimen collection

The Assistant places body on a non-patterned light or dark blue background alongside the ID photo card with scale provided with the kit.

The Assistant takes high resolution (≥ 300 ppi, if possible) photographs and documents on MITS Specimen Collection Form.

The Specialist checks the quality of the photos for clarity and completeness. Assistant retakes, if needed.

8.3. Anthropometric Measurements

Prior to starting the MITS specimen collection

Note that different scales are used for weight

- Scale for stillbirths and neonates (Perinatal)
- Scale for children
- Scale for adults (in cases where a scale is not available document weight from clinical record.)

The Specialist places the body on the appropriate type of scale and measures the weight.

Note the body should be weighed alone, or the tare discounted if an adult or cart is used to hold the body.

The Assistant records the weight on the MITS Specimen Collection Form.

- **Grams** – perinatal (stillbirth/neonates)
- **Kilograms** –child, adult

The Specialist measures the basic anthropometric data using the tape measure (included in MITS kit). The Assistant records on the MITS Specimen Collection Form

Height/length (all):

The specialist places the body lying on side with legs extended and, using the flexible tape, measures the distance from vertex (top of head) to the heel of the right foot.



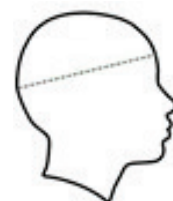
MUAC (6 month-5 years):

Specialist locates the acromial process on the RIGHT shoulder and the olecranon process at the elbow. At the midpoint between these two points place the tape measure perpendicular to the long axis and measure and record the circumference.



Head circumference (up to 12 months):

Specialist places the measure tape around the head so that the tape lies across the frontal bones of the skull, slightly above the eyebrows, perpendicular to the long axis of the face, above the ears, and over the occipital prominence at the back of the head.



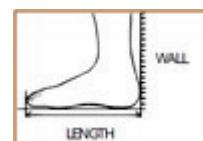
Lower leg length (Stillbirth/Neonate only):

Specialist measures the right leg from the medial malleolus to the medial condyle of the tibia.



Foot length (Stillbirth/Neonate only):

Specialist measures the distance from the heel to the longest toe of the right foot parallel to the long axis of the foot.



8.4. Body Inspection

Prior to starting the MITS specimen collection

Note: In perinatal cases, the body can be placed on top of the second disposable placemat

The Specialist performs an inspection of the external genitals. The Assistant records sex on the MITS Specimen Collection Form

Stillbirth only: The specialist determines whether the body is fresh or macerated (skin and soft-tissue changes such as skin discoloration or darkening, redness, peeling and breakdown), and in case of maceration evaluates the grade. Assistant records on the MITS Specimen Collection Form (Note: this is recorded under Section 6 of the MITS Sample Collection Form)

The Specialist performs a detailed external inspection of the whole body looking for visible congenital physical anomalies or malformations, evidence of trauma, external tumors, skin rashes and lesions or changes of the color of the skin (e.g. depigmentation, areas of darkness). The Assistant indicates presence or absence of findings on the MITS Specimen Collection Form.

8.5. Body Palpation

Prior to starting the MITS specimen collection

Note: NOT to be done on stillbirths

The Specialist performs a detailed palpation of the **abdomen** in order to detect hepatomegaly, other visceromegalies or abdominal masses.

The Specialist performs a detailed palpation of the **superficial lymph node areas** (anterior and posterior cervical, supraclavicular, axillary and inguinal areas).

The Assistant indicates presence or absence of findings on the MITS Specimen Collection Form.

8.6. Body Cleaning and Sterilization

Clean the areas of the body to be punctured before beginning the MITS procedure, as follows:

The Specialist cleans with water and then dries with gauze the areas of the body to be punctured. Areas vary by patient age and are indicated for each on the MITS Specimen Collection Form.

Specific areas to be cleaned are indicated below for each category of MITS patient on Specimen collection form.

The Specialist cleans with abundant alcohol the **project-specific areas** of the body to be punctured. Cleaning is performed with circular movements from the center to the periphery.



Allow alcohol to air dry for at least 5 minutes.

The Specialist cleans with abundant iodine solution the **project-specific areas** of the body to be punctured. Cleaning is performed with circular movements from the center to the periphery.



Allow iodine to air dry for at least 5 minutes.

9. Specimen Collection

9.1. Cerebrospinal Fluid (CSF) Occipital Approach for Microbiology

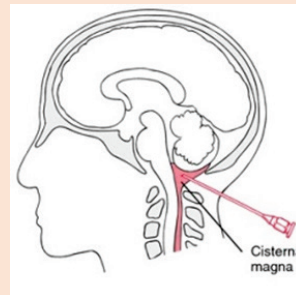
Materials Needed	MITS KIT Location
20G spinal puncture needle - Perinatal cases (Yellow)	Kit Bag 2
18G spinal puncture needle - Infants with closed fontanelle, child, adults (Pink)	Kit Bag 2
20 mL syringe	Kit Bag 2
Note: Projects working with perinatal cases may prefer a smaller 10 mL syringe	
10 mL sterile tube (pre-labeled)	Kit Bag 2

Note that the specific bag where materials will be located is project specific and the locations here are specific to the MITS Training Kits.

MITS specimen collection

a. The Specialist rotates and maintains the head in a lateral position, while the body is in a supine position

- **The assistant helps to maintain the head in the correct position**



b. The Assistant prepares a new and sterile 20 G (neonates/infants) or 18 G (adults and children) spinal puncture needle, a 20 mL syringe, and a 10 mL empty sterile tube for CSF fluid included in the MITS kit.



c. The Specialist selects and opens the spinal puncture needle (of the correct gauge for the type of case).



d. The Specialist stabilizes the spinal needle in the middle line below the occipital bone between the squama and the atlas bone

- Insert needle through the skin.
 - Advance the needle leaning it towards the orbital cavities. **The angle with the skin of the back should be approximately 75°. Advance the needle slowly but smoothly.**
- Occasionally, a characteristic “pop” is felt when the needle penetrates the dura.
- Otherwise, the guide should be withdrawn after approximately 1 cm and observed for fluid return.
- If no fluid is obtained, replace the guide, advance or withdraw the needle a few millimeters, and recheck for fluid return
- Continue this process until drops of fluid are successfully obtained



e. The Assistant opens the 20 mL new sterile syringe package

f. The Specialist attaches the syringe to the needle and draws off CSF.

- The CSF should be clear and colorless. If there is trauma or other that may not be the case. The CSF should still be collected if it is bloody or other colors.



g. The Assistant opens and labels the 10 mL sterile tube and the specialist transfers the CSF from the syringe to the tube, up to 10 mL.

- **Additional CSF collected can be placed into a backup tube with additional label**

h. The Assistant caps the tube.

i. The Assistant stores the 10mL sterile tube in the MITS test tube rack and fills the required information in the MITS specimen collection form

9.2. Brain/CNS MITS for Microbiology (Occipital Approach Only)

Materials Needed	MITS KIT Location
Materials Needed	MITS KIT Location
Bard Monopty 16G 160mm (labeled “BRAIN”)	Kit Bag 1
BRAIN/CNS cryogenic vial (pre-labeled)	Kit Bag 2

Posterior/Occipital approach-All cases

MITS Specimen Collection

a. The Assistant selects the CNS cryogenic vial and the BARD 16G, 160mm needle labeled “BRAIN” from the MITS kit

b. The Specialist charges the needle then starts the occipital puncture, using an entry point 2 cm below the approach used for the CSF puncture.

- **Midline 3-4 cm below the occipital bone**

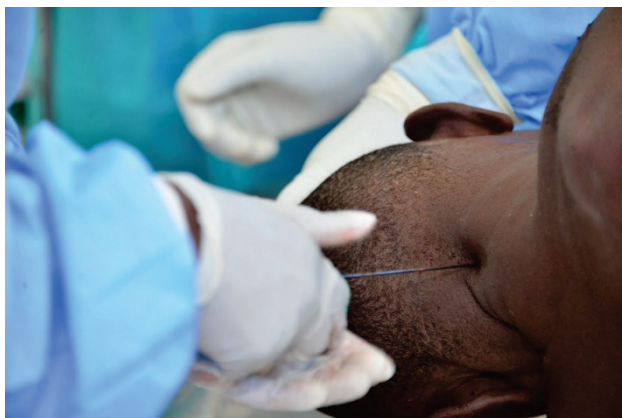


c. The Specialist advances the needle through the skin, with a 30° angle with the skin of the back, leaning it towards the orbital cavities, advancing the needle slowly but smoothly into the brain

Note: The needle should freely advance into the cranial cavity.



d. The Specialist obtains the biopsy



e. Withdraw the needle.

f. The Specialist removes the samples by rotating the needle handle clockwise and then depositing the sample into the BRAIN/CNS cryogenic vial. The specimen should appear white to tan or light grey in color.

g. The Specialist repeats steps b. through step g. Advancing the needle in different directions/depths to obtain samples from different brain regions. At least 3 samples are obtained.

h. The Assistant stores the cryogenic vial in the MITS collection rack or tray and fills the required information on the MITS Specimen Collection form



9.3. Brain/CNS MITS for Histology (Occipital and Fontanelle Approaches)

Materials Needed	MITS KIT Location
BRAIN/CNS formalin jar (pre-labeled)	Kit Bag 3
Same needle from Section 9.2 (BRAIN/CNS Microbiology)	re-use from 9.2

Posterior/Occipital approach - All Cases

MITS Specimen Collection

a. The Assistant selects the BRAIN/CNS formalin jar from the MITS kit

b. Using the same needle used for microbiology punctures.

- **The Specialist repeats the occipital sampling using the same entry points and following the same procedure and puts 6 samples in the BRAIN/CNS,POSTERIOR formalin jar**



c. The Assistant stores the BRAIN/CNS, POSTERIOR formalin jar in the MITS tray



d. The Assistant fills the required information on the MITS Specimen Collection form

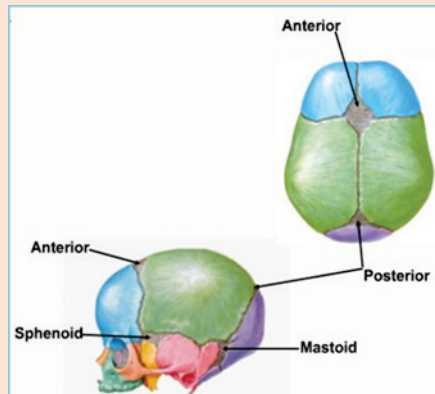
Fontanelle approach – Stillbirth, Neonates, and Infants with OPEN fontanelle Only

MITS Specimen Collection

a. The Specialist locates by palpation the anterior fontanelle and checks whether it is open

- **The anterior fontanelle remains opened the first 12-18 months of life.**
- **In older infants and in children, the anterior fontanelle is already closed and cannot be punctured.**

The approximate location of the fontanelle is shown in the figures.



b. Using the same needle that was used for the occipital puncture. The Specialist obtains 6 samples by puncturing the anterior fontanelle and puts them into the same BRAIN/CNS, formalin jar. The Specialist aims the needle in different directions/depths to obtain samples from different regions of the brain.



c. The Assistant fills the required information on the MITS Specimen Collection form

9.4. Nasopharyngeal (NP) Swab Sample

Materials Needed	MITS KIT Location
Nasopharyngeal (NP) swab tube with 1 mL viral transport media (individually wrapped, NOT pre-labeled)	Kit Bag 1
NP label	Kit Envelope

MITS Specimen Collection

Note: This procedure is NOT to be done on Stillbirths.

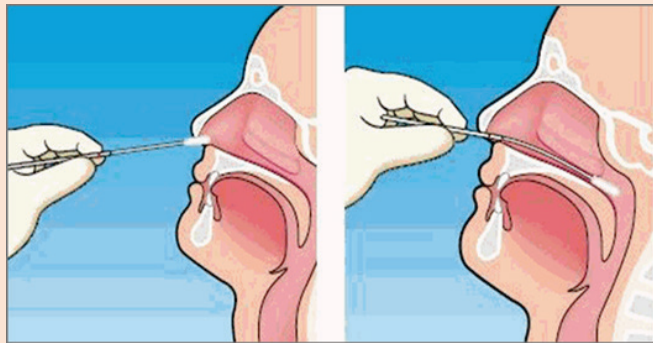
Note: This sample should be collected BEFORE Brain/CNS sampling through Trans-nasal approach.

a. The Assistant takes the NP swab tube 12 X 80mm with 1 mL viral transport media from the MITS kit and labels it.

b. The Assistant opens the NP swab tube.

c. The Specialist tilts the head back 70° degrees and inserts the NP swab into the nostril (until resistance is met at turbinates).

- **Note: the swab should reach a depth equal to the distance from nostrils to outer opening of the ear.**



d. The Specialist leaves the swab in place for 10 seconds to absorb secretions.



e. The Specialist slowly removes swab while rotating it.



f. The Specialist swabs the other nostril with same swab and procedure.

g. The Specialist places the swab into NP tube with viral transport media

Note: The swab should be deep enough that the medium covers the cotton tip portion.

h. The Specialist breaks off the top portion of the stick and discards it.

i. The Assistant caps the tube and fills the information on the MITS Specimen Collection form

j. The Assistant places the NP tube with viral transport media in the MITS test tube rack.

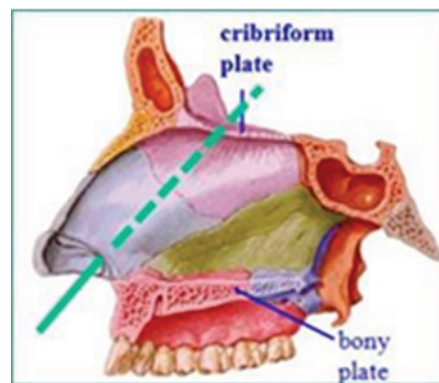
9.5. BRAIN/CNS MITS for Histology Trans-nasal approach – Infant with closed fontanelle, Child, Adult Cases

Materials Needed	MITS KIT Location
Bone marrow biopsy kit	Backup Box Bag 1
BRAIN/CNS formalin jar (pre-labeled)	Kit Bag 3
Same needle from Section 9.2 (BRAIN/CNS Microbiology)	re-use from 9.2

MITS Specimen Collection

- Trans-nasal brain specimens should NOT be collected for microbiology testing, due to contamination with nasal flora.
- To reach the brain parenchyma, the cribriform plate must be perforated. Bone marrow trephine kit is used for this.

a. The Assistant takes the bone puncture trephine and opens the container of a bone marrow biopsy needle.



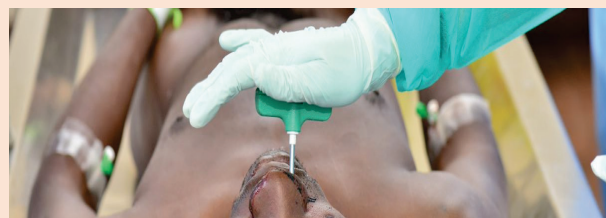
b. The Specialist inserts the stylet into trephine.

c. The Specialist introduces the needle with the internal guide into the nasal cavity with an angle of 45°, until reaching the roof of the nasal cavity (cribriform plate of the ethmoid bone).



- The Assistant helps to maintain the head in the correct position, when required

d. The Specialist performs a constant pressure as well as a rotation back and forth to penetrate into the cranial cavity. Forceful tapping of the trephine may be required to penetrate the ossified bone.



e. Specialist removes the trephine and stylet.

f. The Specialist introduces the CNS biopsy needle into the nasal cavity at an angle of 45°, until reaching the roof of the nasal cavity



g. Once the roof of the nasal cavity has been reached, the Specialist moves the needle around until the hole created with the trephine is identified

h. Once the hole is identified, the Specialist advances the needle into the cranial cavity and presses the button to obtain the sample.



i. The Specialist withdraws the needle and deposits the sample into the CNS, TRANSNASAL formalin jar.

j. Specialist repeats steps f through i, advancing the needle in different directions/depths to obtain specimens from different regions of the brain. At least 6 specimens should be obtained.



k. The Assistant stores the BRAIN/CNS formalin jar.

l. The Assistant fills the required information on the MITS Specimen Collection form

9.6. Blood Collection

Materials Needed	MITS KIT Location
21G spinal puncture needle – perinatal (stillbirth/neonate) and infant cases (Green)	Kit Bag 2
20 mL syringe Note: Projects working with perinatal cases may prefer a smaller 10 mL syringe	Kit Bag 2
EDTA vacutainer (pre-labeled)	Kit Bag 3
Blood spot card (pre-labeled)	Kit envelope

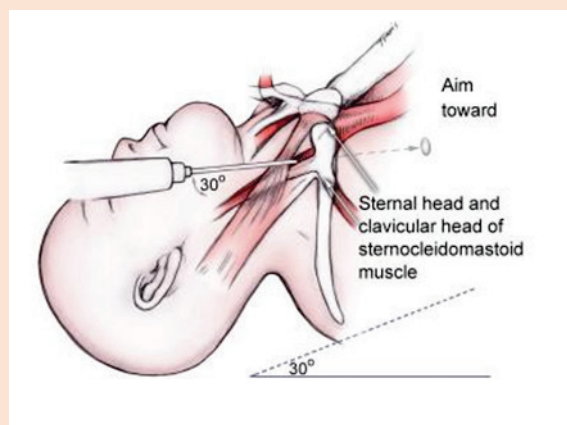
MITS Specimen Collection

a. The Assistant prepares a new and sterile needle (of the correct gauge for the type of case), a new 20 mL syringe, the EDTA vacutainer, and the blood spot card from the MITS kit box.

b. The Assistant opens the plastic wrap of a new sterile 20 mL syringe and a new 21G (green) spinal puncture needle (**perinatal/infant cases**) or 18G (pink) intramuscular needle (**child/adult cases**) and gives them to the Specialist.

Note: The guide of the needle should be withdrawn, and needle connected to the syringe prior to puncture

c. The Specialist locates the midpoint of the clavicle (mid-way between the sternal notch and acromioclavicular joint) and inserts the needle 1 cm lateral and superior or inferior to the clavicle.



d. For supraclavicular approach, use an angle of 30° above the clavicle and aim inferior, the specialist attempts to first aim for the clavicle. Once the clavicle is passed, pull the plunger to make negative pressure.



e. Maintaining the negative pressure with the plunger, the specialist continues advancing the needle in a plane almost parallel to the skin approximately 2-3 cm. Change the angle of penetration of the needle until venous blood is freely aspirated into the syringe.

Note: In case no blood is obtained with supraclavicular approach, use an infraclavicular puncture aiming to reach the subclavian behind the clavicle



In the case that no or very little blood is obtained following this procedure, perform a heart puncture, using the same needle.

f. The Specialist palpates the ribs to identify the left central, fifth intercostal space, and punctures there in a parasternal location.

g. The needle should penetrate approximately 2-4 cm (children) or 5-6 cm (adults) in a sagittal direction.



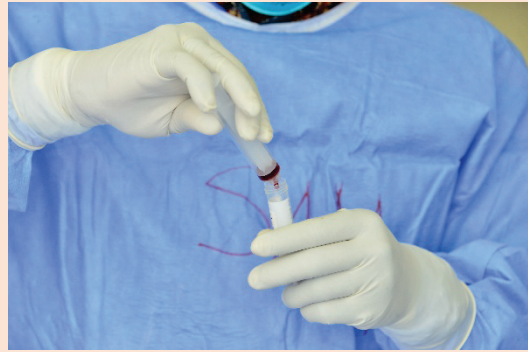
h. Aspirate and redirect the needle as needed until obtaining blood.

i. Fill the syringe with as much blood as possible.

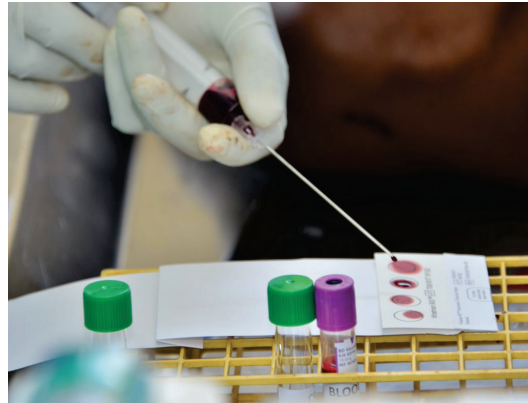
In case serum is obtained instead of blood, try again. In the case that no blood is obtained the second time, put the serum in the EDTA container only.

Note: Projects may have their own sample prioritization and should be addressed accordingly

j. The Assistant opens the EDTA tube and the specialist transfers up to 9 ml blood from the syringe to the tube.



k. The Assistant retrieves the blood spot card and the specialist deposits 4 large drops of blood onto each circle of the blood spot card, filling each circle.



l. The Assistant fills the required information on the MITS Specimen Collection form.

**ensure that entire circle on blood spot card is filled with blood*
**invert EDTA tube after filling*

m. The Assistant stores the EDTA tube in the MITS test tube rack and the blood spot card on the MITS tray.

9.7. Lung/Thorax for MITS for Microbiology

Materials Needed	MITS KIT Location
Bard Monopty 16G 100mm (unlabeled)	Kit Bag 1
1 LUNG 2mL cryogenic vial (pre-labeled)	Kit Bag 2

MITS Specimen Collection

Note: That if pleural effusion is suspected the fluid should be collected as extra sample.

a. The Assistant prepares the second, new, disposable Bard Monopty 16 G biopsy needle, and the LUNG cryogenic vials from the MITS kit.

b. The Specialist performs the puncture with the automatic needle in the mid-axillary line, upper region of the right thorax, trying to obtain a lung sample from the upper lobe. The needle should be oriented towards the head. The needle should penetrate as much as possible, for adults only, and the movement of penetration should be quick in order to avoid lung collapse. After reaching the limit, the needle should be retracted 2-3 cm, varies by body size.



c. The Specialist engages the biopsy needle by pressing with the thumb the button at the back end of the handle.



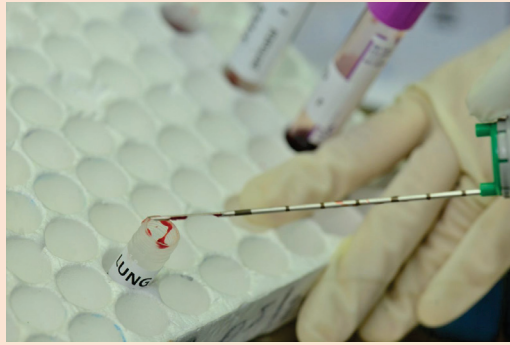
d. The Specialist removes the needle from the body.

e. The Specialist opens the needle to obtain the sample by rotating the handle in a clockwise direction.

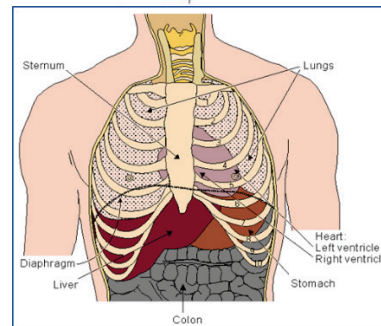
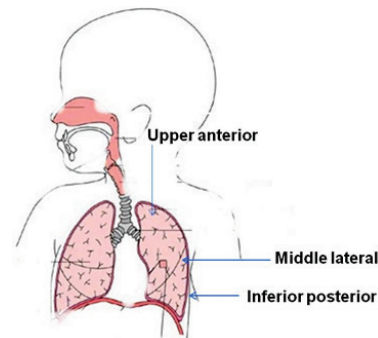
f. The Specialist checks that the tissue cylinder has been obtained from the correct organ (the cylinder should be about 20 x 1 mm and show a pink or reddish color and soft consistency)

g. If there is no sample or the sample has not an adequate appearance (yellow color indicates fatty tissue, brown color and increased consistency may indicate liver tissue), the puncture should be repeated using the same needle until an adequate sample is obtained

h. The Specialist puts the tissue sample in the LUNG cryogenic vial



i. The Specialist repeats the sequence of punctures (repeat steps Section 9.6 Step a-j), aiming the needle in different directions to try to obtain samples from the upper, middle, and lower lung regions/lobes. Remove needle after a sample from each lobe is taken. Have to reset the needle for every sample. One sample from each region should be obtained for the cryogenic vial. In the left thoracic region using the same needle and entry hole, and following the same procedure and placing the samples in the same LUNG cryogenic vial



j. The Specialist repeats the same procedures in the left axillary region to obtain left lung samples, which are collected into the same LUNG cryogenic vial.

k. The Assistant stores the LUNG cryogenic vial in the MITS cryogenic vial rack and fills the required information on the MITS Specimen Collection Form

Note: The punctures of the lung can also be obtained using a posterior approach (back of the body, below the inferior angle of the scapula). Only use if difficulty with axillary approach.

- For this approach, the assistant should help the specialist to turn the body in a lateral or prone position.
- In this case, the biopsy needle should also be directed to the upper, middle and lower areas.

9.8. Lung/Thorax for MITS for Histology

Materials Needed	MITS KIT Location
Same needle as Section 9.6 (Lung/Thorax Microbiology)	re-use from Section 9.6
RIGHT LUNG formalin jar (pre-labeled)	Kit Bag 3
LEFT LUNG formalin jar (pre-labeled)	Kit Bag 3

MITS Specimen Collection

- a. The Assistant takes the RIGHT LUNG and LEFT LUNG formalin jars from the MITS kit.
- b. The Specialist repeats the sequence of puncture approaches of the RIGHT and LEFT Lung using the same entry points and following the same procedure.
- c. The Specialist puts 2 samples of each right lung region (upper, middle, lower) in the RIGHT LUNG formalin jar and 2 samples of each left lung region (upper, middle, lower) area in the LEFT LUNG formalin jar (*a total of 12 samples, 6 from the right lung and 6 from the left lung*).
- d. The Assistant stores the RIGHT LUNG and LEFT LUNG formalin jars in the MITS tray and fills the required information on the MITS Specimen Collection Form.

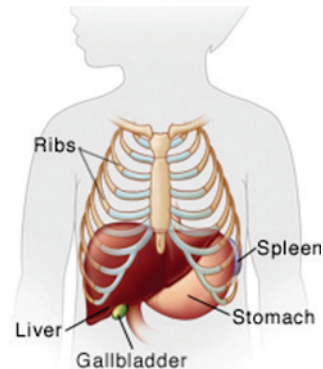
9.9. Liver MITS for Microbiology

Materials Needed	MITS KIT Location
Bard Monopty 16G 100mm (unlabeled)	Kit Bag 1
LIVER 2mL cryogenic vial (pre-labeled)	Kit Bag 2

MITS Specimen Collection

Note: If ascites is detected collect the fluid as an extra sample

a. The Assistant takes the biopsy needle Bard Monopty 16G, 100 mm and the 2mL cryogenic vial labeled as LIVER from the MITS kit



b. The Specialist performs a puncture with the needle in the mid-axillary line, in one of the three last intercostal spaces.

- The needle should be oriented 30° in a cranial and 15° in a posterior direction.
- The needle should penetrate 2-4 cm depending on the size of the body

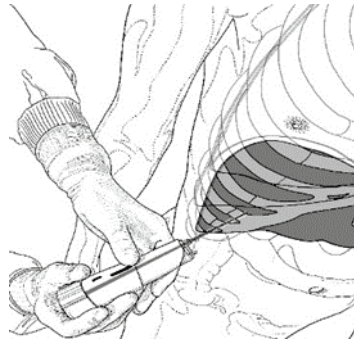


c. The Specialist engages the biopsy needle by pressing with the thumb the button at the back end of the handle



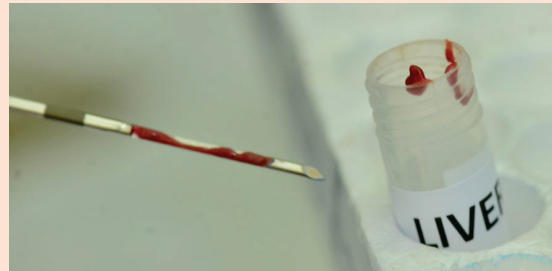
d. The Specialist removes the needle

e. The Specialist opens the needle to obtain the sample by rotating the handle in a clockwise direction



f. The Specialist checks that a correct tissue cylinder has been obtained

- The cylinder should be about 20 x 1 mm and show a brownish color



g. The Specialist puts the sample in the LIVER cryogenic vial

h. Repeat the same procedure (step c. to step g.). Using the same entry point used for the first puncture, in order to reduce the marks in the body. Change the direction of the needle in order to sample different parts of the liver. At least 3 samples from different areas should be put in the LIVER cryogenic vial.

i. The Assistant stores the LIVER cryogenic vial in the MITS cryogenic vial rack and fills the required information on the MITS Specimen Collection Form

9.10. Liver MITS for Histology

Materials Needed	MITS KIT Location
Same needle from Section 9.8 (Live MITS for Microbiology)	re-use from Section 9.8
LIVER formalin jar (pre-labeled)	Kit Bag 3

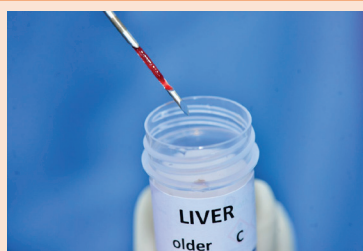
MITS Specimen Collection

a. The Assistant takes the LIVER formalin jar from the MITS kit

b. Using the same needle used for microbiology punctures, the Specialist repeats the sequence of liver puncture approaches using the same entry point and changing the direction of the needle, to obtain 6 liver biopsies.



c. The Specialist places the 6 biopsies in the LIVER formalin jar.



d. The Assistant stores the LIVER formalin jar in the MITS tray and fills the required information on the MITS Specimen Collection Form.

9.11. Rectal Stool Sample

Materials Needed	MITs KIT Location
Rectal brush	Kit Bag 1
10 mL sterile tube (pre-labeled)	Back up Box - Bag 2

Specimen Collection

Note: This procedure is NOT to be used on stillbirths

- The Assistant takes the rectal brush from the MITs kit and a 10mL sterile tube from the MITs box.
- The Assistant opens the protective wrap of the rectal brush and gives it to the Specialist.
- The Assistant positions the head and leg as needed.
- The Specialist inserts the rectal brush 3 to 4 cm into the rectum and gently rotates.



- The Specialist removes the brush.



- If sample is collected, the Assistant opens the protective wrap of the 10mL sterile tube, labels the tube and gives it to the Specialist.
- The Specialist places the brush into the vial and breaks off the top portion of the stick and discards it.
- The Assistant caps and places the 10mL sterile tube in the MITs test tube rack and fills the required information on the MITs Specimen Collection Form.

9.12. Skin Sample

Materials Needed	MITS KIT Location
Extra label	Kit Envelope
Punch biopsy	Backup Box Bag 1
Extra formalin jar	Backup Box Bag 3

Specimen Collection

Note: These samples are only collected if skin lesions (papules, nodules, vesicles, macules) are detected.

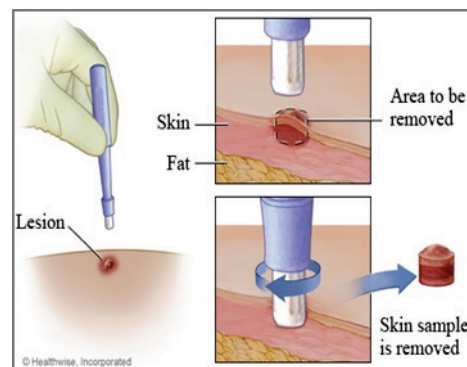
- a. The Assistant takes a skin biopsy punch and a formalin jar from the MITS backup box.



- b. The Assistant sticks one of the extra labels from the MITS kit in the formalin jar.

- c. The Specialist takes a punch biopsy of the border of the lesion, by pushing and rotating the punch in the skin, then removing the punch.

Note: The sample should contain epidermis, dermis, and subcutaneous fatty tissue.



- d. The sample is placed in the extra, labeled formalin jar and stored.

- e. The Assistant fills the required information on the MITS Specimen Collection Form.

Note: When possible umbilical samples should be taken if placenta sampling is not possible.

10. End of the procedure and completion of the MITS Specimen Collection Form

10.1. MITS Specimen Collection Form

After the MITS specimen collection procedure

- a. The Assistant writes the time (24 hour) in which the procedure has ended on the MITS Specimen Collection Form
- b. The Assistant writes on the MITS Specimen Collection Form, by indication of The Specialist, any additional samples collected and/or notes from the procedure

10.2. Excessive seepage or bleeding though the biopsy entry points

After the MITS specimen collection

Note: If body fluid leakage is a social concern in your community, you can use Monsel's solution (ferric subsulfate 20%) to stop excessive bleeding or seepage if it occurs during MITS collection. Monsel's solution is not included in the MITS kit.

- a. The Assistant and Specialist check for excessive seepage or bleeding.

- b. The Assistant prepares the container with the Monsel's solution and a swab for its application and opens the Mosel's solution jar.



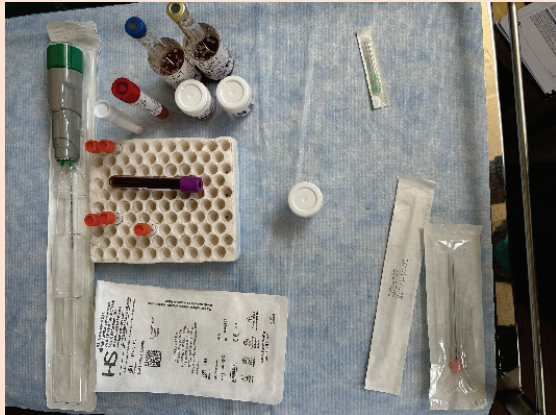
- c. The Assistant takes with the swab some Monsel's solution from the jar.
- d. The Assistant applies the Monsel's solution to the bleeding entry point.
- e. If necessary, take a gauze and roll it around the bleeding area to make pressure and reinforce the hemostatic effect of the Monsel's solution. Before delivering the body to the family REMOVE the gauze.

Optional step: Rinse body from head to toe with water following the procedure.

10.3. Containers and Tools

After the MITS specimen collection

- a. The Assistant makes sure that all the containers and jars are properly labeled and closed.



- b. The Specialist and Assistant make sure that any unused and unlabeled materials (*e.g. swabs*) are stored in a backup box and any unused materials labeled with an ID are disposed of in the biowaste container.

- c. The Specialist and Assistant dispose of any sharps in sharps container.

- d. The Assistant puts the MITS cryogenic vial rack with all the used cryogenic vials and the MITS test tube rack with the used tubes in the MITS cool box with cold packs.

- e. The Assistant puts the MITS tray with all the used formalin jars, the unused large screw-cap jar, and any MITS tissue cassettes in the MITS kit box.

Note: The MITS cool box (microbiology) and the MITS kit box (pathology) should be sent for further processing

- f. The Assistant washes the surfaces used during the process with the standard 10% bleach followed by 70% ethanol to dry.

- g. The Assistant puts away the digital camera and charging.

10.4. MITS Envelope

After the MITS specimen collection

- a. The Assistant puts the Specimen Collection Form back into the MITS envelope

- b. The Specialist makes sure that the project-specific gross examination form and pathology form, remaining labels, and the 5 cm ruler are in the MITS envelope.

- c. The Assistant puts the MITS envelope back into the MITS kit box

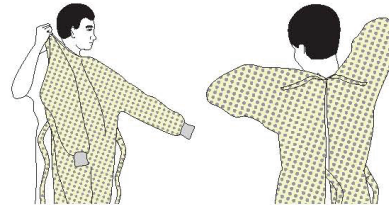
10.5. Donning and Removing Personal Protective Equipment

SEQUENCE FOR PUTTING ON PERSONAL PROTECTIVE EQUIPMENT (PPE)

The type of PPE used will vary based on the level of precautions required, such as standard and contact, droplet or airborne infection isolation precautions. The procedure for putting on and removing PPE should be tailored to the specific type of PPE.

1. GOWN

- Fully cover torso from neck to knees, arms to end of wrists, and wrap around the back
- Fasten in back of neck and waist



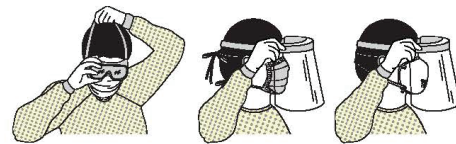
2. MASK OR RESPIRATOR

- Secure ties or elastic bands at middle of head and neck
- Fit flexible band to nose bridge
- Fit snug to face and below chin
- Fit-check respirator



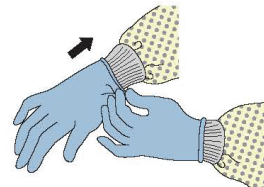
3. GOGGLES OR FACE SHIELD

- Place over face and eyes and adjust to fit



4. GLOVES

- Extend to cover wrist of isolation gown



USE SAFE WORK PRACTICES TO PROTECT YOURSELF AND LIMIT THE SPREAD OF CONTAMINATION

- Keep hands away from face
- Limit surfaces touched
- Change gloves when torn or heavily contaminated
- Perform hand hygiene



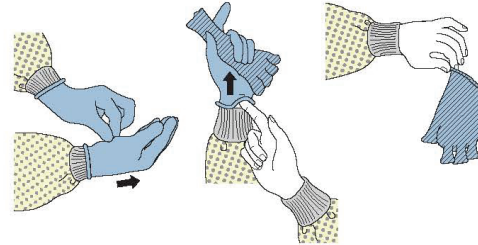
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HOW TO SAFELY REMOVE PERSONAL PROTECTIVE EQUIPMENT (PPE) EXAMPLE 1

There are a variety of ways to safely remove PPE without contaminating your clothing, skin, or mucous membranes with potentially infectious materials. Here is one example. **Remove all PPE before exiting the patient room** except a respirator, if worn. Remove the respirator **after** leaving the patient room and closing the door. Remove PPE in the following sequence:

1. GLOVES

- Outside of gloves are contaminated!
- If your hands get contaminated during glove removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Using a gloved hand, grasp the palm area of the other gloved hand and peel off first glove
- Hold removed glove in gloved hand
- Slide fingers of ungloved hand under remaining glove at wrist and peel off second glove over first glove
- Discard gloves in a waste container



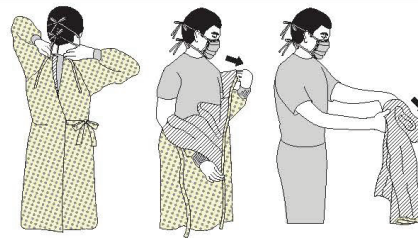
2. GOGGLES OR FACE SHIELD

- Outside of goggles or face shield are contaminated!
- If your hands get contaminated during goggle or face shield removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Remove goggles or face shield from the back by lifting head band or ear pieces
- If the item is reusable, place in designated receptacle for reprocessing. Otherwise, discard in a waste container



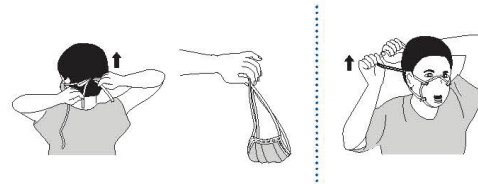
3. GOWN

- Gown front and sleeves are contaminated!
- If your hands get contaminated during gown removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Unfasten gown ties, taking care that sleeves don't contact your body when reaching for ties
- Pull gown away from neck and shoulders, touching inside of gown only
- Turn gown inside out
- Fold or roll into a bundle and discard in a waste container

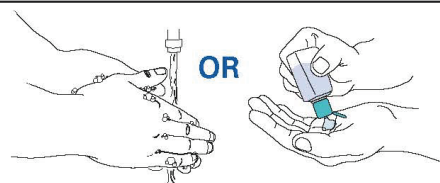


4. MASK OR RESPIRATOR

- Front of mask/respirator is contaminated — **DO NOT TOUCH!**
- If your hands get contaminated during mask/respirator removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Grasp bottom ties or elastics of the mask/respirator, then the ones at the top, and remove without touching the front
- Discard in a waste container



5. WASH HANDS OR USE AN ALCOHOL-BASED HAND SANITIZER IMMEDIATELY AFTER REMOVING ALL PPE



PERFORM HAND HYGIENE BETWEEN STEPS IF HANDS BECOME CONTAMINATED AND IMMEDIATELY AFTER REMOVING ALL PPE



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11. References

Castillo P, Ussene E, Ismail MR, Jordao D, Lovane L, Carrilho C, et al. Pathological Methods Applied to the Investigation of Causes of Death in Developing Countries: Minimally Invasive Autopsy Approach. PLoS One. 2015 Jun 30; 10(6):e0132057.

Martínez MJ, Massora S, Mandomando I, Ussene E, Jordao D, Lovane L, et al. Infectious cause of death determination using minimally invasive autopsies in developing countries. Diagn Microbiol Infect Dis. 2016; 84(1):80-6.

CDC. National health and nutrition examination survey (NHANES). Anthropometry procedures manual. January 2011

12. SOP Appendices

12.1. Appendix A: MITS Kit Components

Note: MITS kit contents project-specific. These are the contents of a kit consistent with this SOP.

	Envelope	Units
E.1	MITS SOP	1
E.2	Blood spot card (pre-labeled)	1
E.3	Photo card (pre-labeled)	1
E.4	Labels for: extras samples, NP swab, slides	1
O.1	Tape Measure	1
O.2	Disposable placemat	2
	Bag 1	Units
1.1	Bard Monopty needle 16G, 100mm <i>Lung/thorax, liver</i>	2
1.2	Bard Monopty needle 16G, 160mm <i>Brain</i>	1
1.3	Bone Marrow Trepine <i>Brain transnasal approach if closed fontanelle</i>	1
1.4	Nasopharyngeal (NP) swab with viral transport media	1
1.5	Rectal brush	1
	Bag 2 - Microbiology	Units
2.1	20G 1.5" spinal puncture needle (yellow) <i>Perinatal/infant CSF collection</i>	1
2.2	21G 1.5" intramuscular needle (green) <i>Perinatal/infant blood collection</i>	1
2.3	18G 3.5" spinal puncture needle (pink) <i>Child/adult CSF and blood collection</i>	2
2.4	20mL syringes <i>CSF, blood collection</i>	2
2.5	10mL EDTA vacutainer (purple top, pre-labeled) <i>Blood</i>	1
2.6	10mL sterile tube (prelabeled) <i>CSF, rectal swab</i>	2
2.7	2mL cryogenic storage vials (pre-labeled) <i>Brain, lung/thorax, liver</i>	3
	BAG 3 - Histology	UNITS
3.1	Large (120 ml) screw cap jar (pre-labeled) <i>Storage of fixed tissue cassettes in ethanol</i>	1
3.2	Tissue cassettes (unlabeled) <i>Brain, right lung/thorax, left lung/thorax, liver</i>	4
3.3	20 mL jars (prefilled 10% formalin) (pre-labeled) <i>Brain, right lung/thorax, left lung/thorax, liver</i>	4

Supplies for MITS of: brain, lung, liver, cerebral spinal fluid (CSF), blood, nasopharyngeal swab, rectal swab (for histology and microbiology where appropriate).

Supplies for placenta sampling are included in the backup box

12.2. SOP Appendix B: Back-up box Components

Note: Back-up box contents are project-specific. These are the contents of a MITS back-up box consistent with this SOP. Supplies include those for MITS of two placentas, two skin biopsies, as well as 1-2 replacements per MITS kit item. Placenta samples for histology include placental membrane, placenta, cord fetal end, cord placental end, 2 placenta tissue, intraamniotic membrane, and up to 3 lesions. Placenta samples for microbiology include placental membrane, placenta, placental cord (both ends together)

Envelope		Units
E.1	MITS SOP	2
E.2	Placenta SOP	2
E.3	Blood spot card	1
E.4	Photo card <i>2 placenta sampling, 1 back-up</i>	3
E.5	Millimeter ruler (5 cm) <i>2 placenta sampling, 1 back-up</i>	3
E.6	Scalpel blade #24 <i>2 placenta sampling, 1 back-up</i>	3
O.1	Tape Measure <i>2 back-up</i>	2
O.2	Disposable placemat <i>2 placenta sampling, 2 back-up</i>	4
Bag 1		Units
1.1	Bard Monopty needle 16G, 100mm <i>Lung/thorax, liver</i>	2
1.2	Bard Monopty needle 16G, 160mm <i>Brain</i>	1
1.3	Biopsy punch needle 5mm <i>Skin</i>	2
1.4	Bone Marrow Trephine <i>Transnasal approach if closed fontanelle</i>	1
1.5	Nasopharyngeal (NP) swab with viral transport media	1
1.6	Rectal brush	1
2.1	20G 1.5" spinal puncture needle (yellow) <i>Perinatal/infant CSF collection</i>	1
2.2	21G 1.5" intramuscular needle (green) <i>Perinatal/infant blood collection</i>	1
2.3	18G 3.5" spinal puncture needle (pink) <i>Child/adult CSF and blood collection</i>	2
2.4	20mL syringes <i>CSF, blood collection</i>	2
2.5	10mL EDTA vacutainer (purple top, pre-labeled) <i>Blood</i>	1
2.6	10mL sterile tube (prelabeled) <i>CSF, rectal swab</i>	2
2.7	2mL cryogenic storage vials (pre-labeled) <i>3 back-up, 4 extra samples (e.g. skin), 2 placentas* 3 samples</i>	13
3.1	Forceps <i>2 placenta sampling</i>	2
3.2	Scalpel handle <i>Placenta sampling (reusable)</i>	1
3.3	Large (120 ml) screw cap jar <i>2 placenta sampling, 1 back-up</i>	3
3.4	Tissue cassettes <i>4 back-up, 2 extra samples (e.g. skin), 2 placentas* 4 cassettes</i>	14
3.5	20 mL jars (prefilled 10% formalin) <i>same as cassettes</i>	14

Supplies for MITS of two placentas, two skin biopsies, as well as 1-2 replacements per MITS kit item

* Placenta samples for histology include placental membrane, two samples of placenta tissue, placental cord (both ends together)

* Placenta samples for microbiology include placental membrane, placenta tissue, placental cord (both ends together)

12.3. Appendix C: SOP Labeling systems formalin jars, cryogenic vials, slides

I. Formalin jar labels (Histology)

Tissue Specimen Description	Label Sample ID	Label Text	Label location
Brain/CNS	1##-###-03-J	Brain/CNS	Pre-applied to formalin jar
Left lung/thorax	1##-###-05-J1	L Lung/Thorax	
Right lung/thorax	1##-###-05-J2	R Lung/Thorax	
Liver	1##-###-06-J	Liver	
Placental Disc membrane junction	1##-###-08-J	Placental Memb.	In Kit envelope
Umbilical Cord	1##-###-09-J	Cord	
Placenta 1 of 2	1##-###-10-J1	Placenta 1/2	
Placenta 2 of 2	1##-###-10-J2	Placenta 2/2	
Extra sample (e.g. skin, placental lesion)	1##-###-11-J	Extra 1	
Extra sample (e.g. skin, placental lesion)	1##-###-12-J	Extra 2	

II. Cryogenic Vial labels (Microbiology)

Tissue Specimen Description	Label Sample ID	Label Text	Label location
Brain/CNS	1##-###-03-V	Brain/CNS	Pre-applied to formalin jar
Left Lung/thorax	1##-###-05-V	Lung/Thorax	
Liver	1##-###-06-V	Liver	
Placental disc membrane junction	1##-###-08-V	Placental Memb.	In Kit envelope
Umbilical cord	1##-###-09-V	Cord	
Placenta	1##-###-10-V	Placenta	
Extra sample (e.g. skin, placental lesion)	1##-###-11-V	Extra 1	
Extra sample (e.g. skin, placental lesion)	1##-###-12-V	Extra 2	

III. Slide labels (Histology)

Tissue Specimen Description	Label Sample ID	Label Text	Label location
Brain/CNS	1##-###-03-S	Brain/CNS	In Kit envelope
Left lung/thorax	1##-###-05-S1	L Lung/Thorax	
Right lung/thorax	1##-###-05-S2	R Lung/Thorax	
Liver	1##-###-06-S	Liver	
Placental Disc membrane junction	1##-###-08-S	Placental Memb.	
Umbilical Cord	1##-###-09-S	Cord	
Placenta 1 of 2	1##-###-10-S1	Placenta 1/2	
Placenta 2 of 2	1##-###-10-S2	Placenta 2/2	
Extra sample (e.g. skin, placental lesion)	1##-###-11-S	Extra 1	
Extra sample (e.g. skin, placental lesion)	1##-###-12-S	Extra 2	