MITS SURVEILLANCE ALLIANCE

MINIMALLY INVASIVE TISSUE SAMPLING (MITS) TRAINING

PARTICIPANT HANDBOOK

Version 3.2 January 01, 2023

Version History
1.0 - September 11, 2019
2.0 – September 25, 2019
3.0 – April 01, 2020
3.1 – October 01, 2020

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COURSE OBJECTIVES

This course is intended to provide pathologists and technicians with the skills necessary to conduct Minimally Invasive Tissue Sampling (MITS) according to the MITS Alliance Standard Operating Procedure (SOP). The MITS Alliance SOP was deliberately designed to optimize the MITS sample collection process by integrating the knowledge and experience regarding MITS sampling that exists across the MITS Surveillance Alliance. It is understood that course participants will learn to sample all tissues in the SOP but that they will also adapt the SOP to meet their own specific study objectives.

The course has been developed using principles of adult learning and thus the focus is on skills acquisition and practice. By the end of this training participants should be able to:

- Name each component of the MITS Kit and state its intended use
- Demonstrate correct cerebrospinal fluid (CSF), central nervous system (CNS)/brain, nasal pharyngeal (NP), blood, lung, liver, skin, and rectal MITS sampling technique
- State age-specific variations in the MITS Alliance SOP
- Demonstrate correct placenta sampling technique
- Demonstrate and maintain proper biosafety precautions
- Create a draft schematic for MITS operations in their own projects
- Identify the presence of external malformations and congenital anomalies

PARTICIPANT HANDBOOK

This handbook is intended to support your learning as part of the MITS Training. You are encouraged to keep it with you throughout the training in both didactic and practical sessions. There are designated spaces for taking notes, and you are encouraged to make notations anywhere that best facilitates your learning. This handbook includes important appendices including the MITS Alliance SOP for collecting MITS samples, Specimen Collection Forms, MITS Alliance Placenta SOP, and MITS Placenta Collection Form. You are encouraged to reference the appendices during the training, following along with the SOPs during demonstrations and practice.

AGENDA

The training agenda presented below is designed to progressively build on previous content and maximize skills practice. However, course facilitators may modify the agenda depending on the training environment including access to the morgue, availability of cadavers, etc.

TIME	LESSON	DAY 1
9:00-10:00	1.1	Course Welcome, Opening Remarks and Introductions
10:00-11:00	1.2	MITS Kits, SOP and Specimen Collection Form Review
11:00-11:15		Tea Break
11:15-12:15	1.2	MITS Kits and SOP Review (continued)
12:15-13:15		**Lunch**
13:15-13:45	1.3	Biosafety
13:45-14:00	1.3	Facility Walk Through
14:00-15:30	1.4	MITS Demonstration
15:30-16:00	1.5	Daily Debrief

TIME	LESSON	DAY 2
9:00-9:30	2.0	Arrive and Don PPE
9:30-12:30	2.1	MITS Practice
12:30-13:30		**Lunch**
13:30-14:00	2.2	Planning Your MITS Setup
14:00-15:00	2.3	Initial Handling of MITS Specimens
15:00-15:30	2.4	Daily Debrief

TIME	LESSON	DAY 3
9:00-9:30	3.0	Arrive and Don PPE
9:30-12:30	3.1	MITS Practice
12:30-13:30		**Lunch**
13:30-14:15	3.2	External Malformations
14:15-15:00	3.3	Placenta Examination and Sampling
15:00-15:30	3.4	Daily Debrief

TIME	LESSON	DAY 4
9:00-9:30	4.0	Arrive and Don PPE
9:30-11:30	4.1	MITS Practice
11:30-12:30	4.2	Demonstration of Placental Exam and Sampling
12:30-13:30		**Lunch**
13:30-15:00	4.3	Practice Placental External Exam and Sampling
15:00-15:45	4.4	Review of MITS Project Setup
15:45-16:15	4.5	Daily Debrief

TIME	LESSON	DAY 5
9:00-9:30	5.0	Arrive and Don PPE
9:30-12:30	5.1	MITS Practice
12:30-13:30		**Lunch**
13:30-14:00	5.2	MITS Implementation Moving Forward
14:00-15:00	5.3	Course Debrief, Closing Remarks, Certificates, and Evaluations

LESSON 1
MITS Training

LESSON 1.1
Welcome and Introductions

LESSON OBJECTIVES

- Describe background and experience of fellow participants and trainers
- Establish a safe learning environment
- Describe course learning objectives
- Resolve outstanding questions for Self-Study modules

LESSON LENGTH: 60 MINUTES

LOCATION

- Classroom

OBJECTIVES OF THE COURSE:

By the end of this training participants should be able to:

- Name each component of the MITS Kit and state its intended use
- Demonstrate correct CSF, CNS/brain, NP, blood, lung, liver, skin, and rectal MITS sampling technique
- State age-specific variations in the MITS Alliance SOP
- Demonstrate correct placenta sampling technique
- Demonstrate and maintain proper biosafety precautions
- Create a draft schematic for MITS operations in their own projects
- Identify the presence of external malformations and congenital anomalies

Participant responsibilities for a successful learning experience:

- Acknowledge that each person in this training brings unique and rich previous experiences.
- Participants are encouraged to use that experience to drive their individual contributions and inquiry.
- Seek the input and experience of your fellow participants.
- Active engagement will not only enhance your learning experience but will also benefit your fellow participants.

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Introduction to the MITS Alliance and RTI

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The MITS Surveillance Alliance

facilitates the use of MITS by bringing together interested institutions and researchers and expanding the network of partners using pathology-based surveillance in various populations, geographies, and contexts.

We bring together experts to

- **Standardize procedures and training** on the MITS technique
- **Create forums to share best practices** and lessons learned
- **Provide access to tools, materials, and expertise** to facilitate pathology-based surveillance

Our Members:
implement or are interested in postmortem research using MITS

19 countries represented by:

- **Institutions**
- **Investigators**
- **Researchers**

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Phase II Objectives of the MITS Surveillance Alliance

Surveillance Sites

- Identify sites with capacity and interest in integrating MITS into surveillance programs to provide high-quality mortality data for routine decision-making.
- Leverage resources from governmental agencies, bilateral and multilateral organizations, and foundations to incorporate MITS into existing mortality surveillance programs.

Toolkit Development

- Develop a package of guidelines, procedures, and related products to facilitate the initiation and implementation of MITS in new sites.
- Provide MITS surveillance sites with toolkits to guide planning and incorporate MITS-informed CoD within mortality surveillance platforms that inform Ministries of Health and other public health efforts.

Data Standardization and Harmonization

- Collaborate with the MITS Community of Practice and Technical Working Group platforms, core partners, and other key contributors to standardize data definitions for collecting and reporting MITS information.
- Contribute the MITS-informed CoD data to a future symptom-cause information (SCI) repository that will aid global and regional analyses.

Training and Capacity Building

- Build regional and local capacity for training and technical assistance in MITS.
- Utilize a diverse set of tools and methods, including expanding existing capacity to conduct and provide technical assistance for MITS sample collection and other related technical areas.

Communications

- Develop and execute a plan to improve communications about the value of MITS-informed CoD data and mortality surveillance, improve acceptability of the technique, and increase visibility.

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Support and Partners

RTI International was awarded a four-year grant by the Bill & Melinda Gates Foundation to serve as the MITS Surveillance Alliance Secretariat.

Core MITS Surveillance Alliance partners include

- Barcelona Institute for Global Health (ISGlobal)
- University of Nairobi School of Medicine/Kenyatta National Hospital
- The Ohio State University
- Child Health and Mortality Prevention Surveillance (CHAMPS)
- Centers for Disease Control and Prevention (CDC) Infectious Disease Pathology Branch
- CDC National Center for Health Statistics (NCHS)

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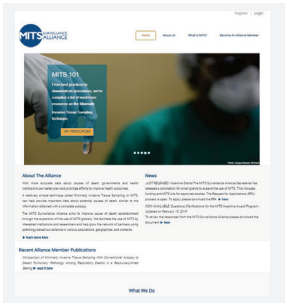
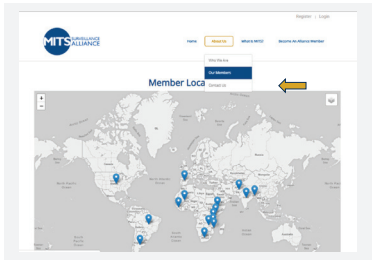
Surveillance Alliance Website





https://mitsalliance.org/

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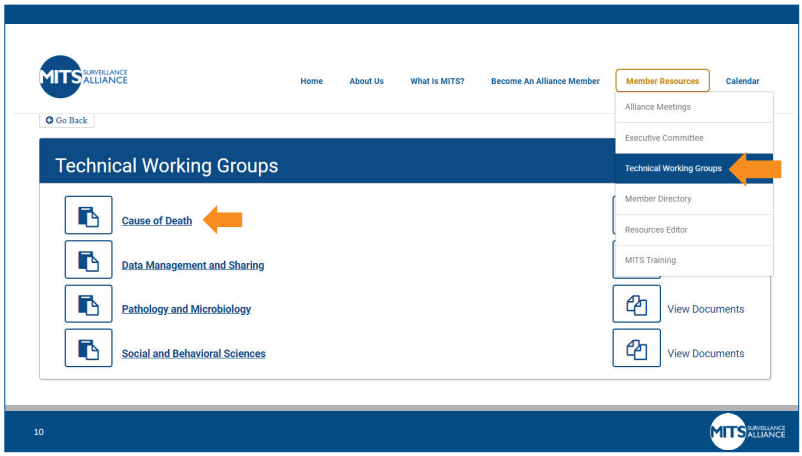

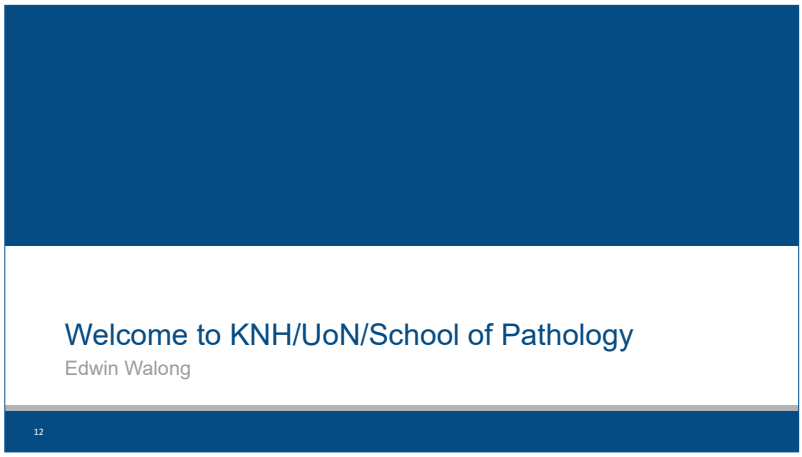

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Meet the Facilitators

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MITS Training Objectives and Course Materials

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Objectives

This course is intended to provide pathologists and technicians with the skills necessary to conduct MITS sampling according to the MITS Alliance Standard Operating Procedure (SOP).

By the end of this training participants should be able to:

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- Demonstrate correct cerebral spinal fluid, central nervous system/brain, nasal pharyngeal, blood, lung, liver, skin and rectal MITS sampling technique
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Participant Handbook Overview

The Participant Handbook is a tool to support participant learning in both the didactic and practical sessions.

Key documents included in the Appendices:

- MITS Alliance SOP (Appendix A)
- MITS Alliance Specimen Collection Form (Appendix B)
- MITS Alliance Placenta Examination and Sampling SOP (Appendix C)
- MITS Alliance Placenta Collection Form (Appendix D)
- MITS Sample Transport and Guidance (Appendix E)
- MITS Histological, Microbiological, (and other) Sample Analysis Guidance (Appendix F)

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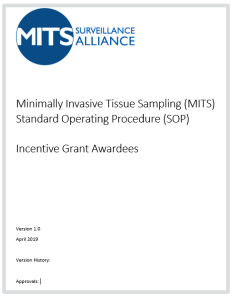
MITS Standard Operating Procedures

- Adapted from ISGlobal and CDC SOPs for current studies (children, neonates, stillbirths)
- Designed to be inclusive of all tissues and body fluids
- Will be adapted to each study's protocol
- Will dictate the MITS Kit contents for each study


MITS SURVEILLANCE ALLIANCE

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

Incentive Grant Awardees

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Participant Introductions

Please state the following:

- Name
- Title
- Project Location
- Study Objective and Population
- Role in Study
- Something Personal

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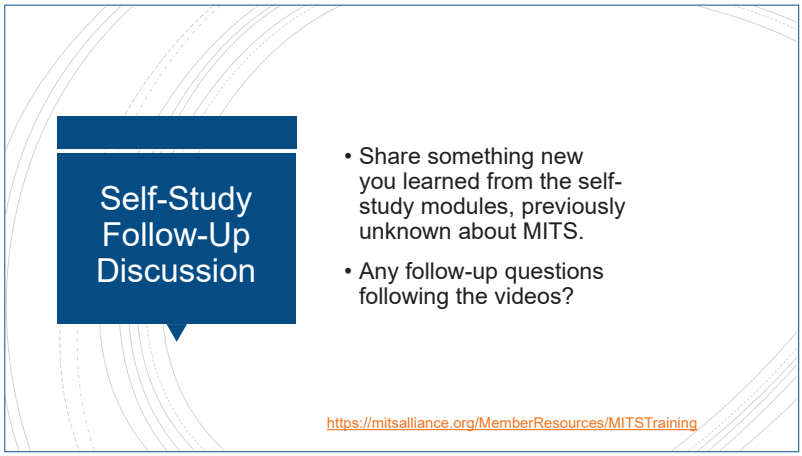

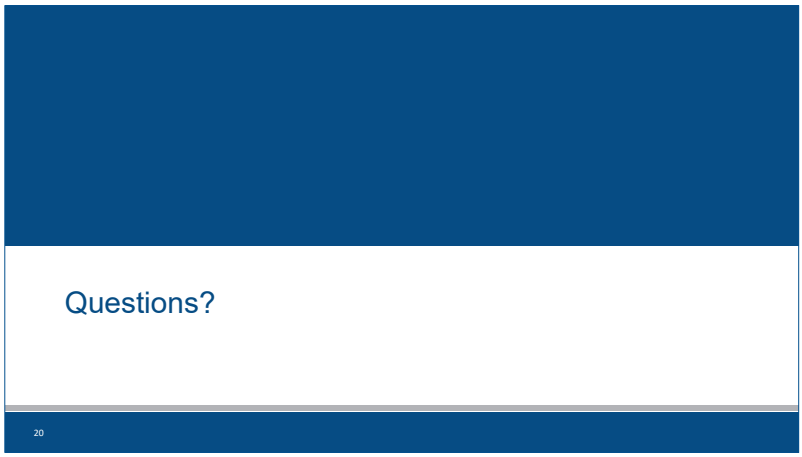

LESSON 1.2
MITS KIT Contents and Standard Operating Procedure Overview

LESSON OBJECTIVES

- Develop a broad understanding of overall MITS SOP
- Establish familiarity with MITS Kit contents and their use
- Explain the roles of the MITS Specialist and the MITS Assistant in conducting the MITS procedure
- Practice and review sequence of SOP using Specimen Collection Form, SOP, and MITS Kit materials

LESSON LENGTH: 120 MINUTES (WITH 15-MINUTE TEA BREAK)

LOCATION

- Classroom

NOTES FOR PARTICIPANTS

- Please recall that the MITS Alliance SOP is intentionally broad and includes sampling organs, tissues, and fluids that have been demonstrated to yield the most information based on previous studies.
- We acknowledge that not all projects will be collecting all samples addressed in this SOP but for the sake of the training all participants will learn how to take all samples included in the SOP.
- Note that your MITS Kit will be customized to your individual project SOP and that some materials used for the MITS Alliance SOP may not be included in the individual kits.
    - Incentive Grantee projects will coordinate directly with the MITS Alliance Secretariat (RTI) for kit contents and shipping.

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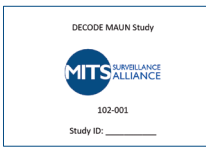
Lesson 1.2 MITS Kit Contents and Labeling

1

MITS Kit Collection Box

- Each kit: ~4" x 12" x 24", 3-4 lb. 10 kits shipped in one outer box: ~40" x 12" x 24" 30-40 lb
- Envelope: kit contents list, SOP, forms, labels, photo card, blood spot card
- Loose: measuring tape, paper mat

- Bagged:
  - Needles, syringes, swabs, bone marrow trephine
  - Labeled vials, vacutainer *(microbiology)*
  - Labeled formalin jars, tissue cassettes *(histology)*



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MITS Kit Backup Box

- One back-up box provided per 10 kits, same size as kit
- Extra sample collection supplies *(e.g., biopsy needles)*
- Supplies for rare samples *(e.g., skin biopsy punch)*
- Unlabeled extra containers and jars

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MITS Surveillance Alliance Kit Labels

- All kits created by the MITS Alliance will have the same labeling system
- The label identifies:
  - That the kit was developed by the MITS Surveillance Alliance
  - The study
  - The sample type (e.g., blood, liver)
  - The container type (e.g., formalin jar, cryogenic vial)
  - Whether duplicates should be expected for a given sample (e.g., 1 and 2 for left and right lung)

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What Kit Contents Have Labels?

Labels provided for:

- MITS Kit itself (top, side of box)
- Blood spot card
- Photo card
- Kraft envelope
- Paper forms (specimen collection, placenta collection)
- EDTA tube, rectal swab tube, NP swab tube
- Formalin jars
- Cryovials
- Slides
- Large jar for storing cassettes in ethanol

Unlabeled materials:

- Cassettes
- Materials in the backup box

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Some Labels Are Pre-applied to Containers, Some Are Not

Labels that are applied

- MITS Kit itself *(top, side of box)*
- Blood spot card
- Photo card
- Kraft envelope
- EDTA tube, rectal swab tube
- Formalin jars *(brain, lung, liver)*
- Cryovials *(brain, lung, liver, CSF)*
- Large jar for storing cassettes in ethanol

Labels that are not applied *(provided in a zip bag)*

- Paper forms *(specimen collection, placenta collection)*
- NP swab tube *(because swab tube comes individually wrapped and sealed)*
- Formalin jars *(placenta, cord, placental membrane, extra samples)*
- Cryovials *(placenta, cord, placental membrane, extra samples)*
- Slides

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MITS Surveillance Alliance Sample Identification Code:

| 1 | 00 | - | 000 | - | 00 | - | Z | - | 0 |

| MITS S.A. | Site ID | - | MITS Kit ID 001+ | - | Sample Code | - | Container Code | – | Duplicate # (optional) |

Sample Code		Container Code		Duplicate Number
01	CSF	P	Blood Spot Paper	1, 2, 3
02	NP Swab	E	EDTA	(if multiple samples are collected for one sample type)
03	Brain/CNS	H	Blood Culture	
04	Blood	T	NP Swab Tube	
05	Lung/Thorax	J	Formalin Jar	1= Left Lung, 2 = Right Lung
06	Liver	V	Cryogenic Vial	
07	Rectal Swab	C	Cassette	
08	Placental Membrane	B	Block	
09	Umbilical Cord	S	Slide	
10	Placenta	X	Extra	
11	Extra 1			
12	Extra 2			

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Examples:

- 100-008-01-V = MITS SA Training – Kit 8 – Cerebral Spinal Fluid – Cryogenic Vial
- 108-151-04-P = MITS SA MAHAN – Kit 151 – Blood – Blood Spot Paper
- 103-095-05-J1 = MITS SA Zambia – Kit 95 – Lung – Formalin Jar 1 (left lung)
- 112-038-09-S = MITS SA Argentina – Kit 38 – Umbilical Cord – Slide
- 114-123-11-J = MITS SA ZPRIME – Kit 123 – Extra Sample* – Formalin Jars

* Skin biopsy, placental lesion

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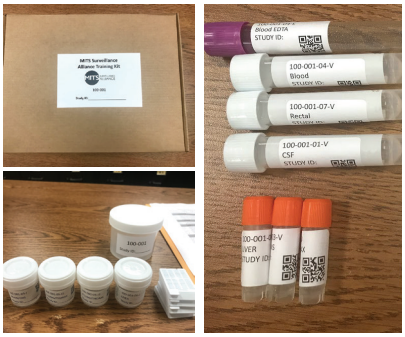
Example Labels



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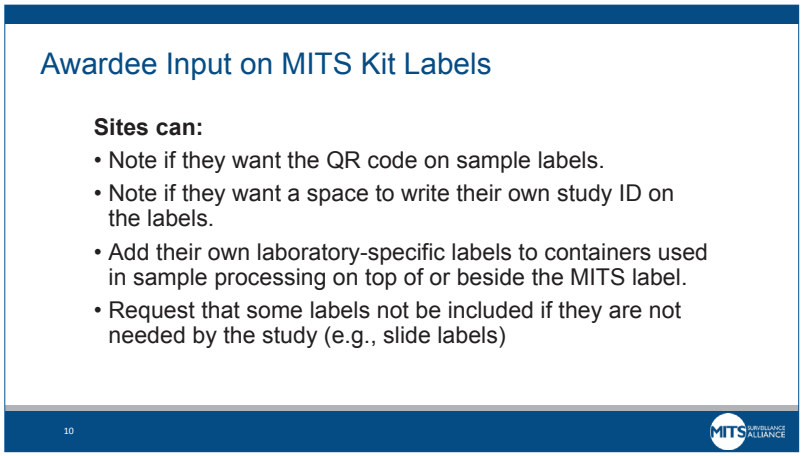

See proper setup of the MITS table below:

MITS roles:

- The MITS Specialist is responsible for performing anthropomorphic measurements, body inspection and palpation, cleaning and sterilization, and sample collection. NOTE that once cleaning and sterilization have been completed the MITS Specialist must not touch materials other than those in the collection kit or the body.
- The MITS Assistant is responsible for arranging and providing MITS materials to the Specialist, documenting findings on the Specimen Collection Form, and proper handling of samples following MITS procedure.
- NOTE that in projects with sufficient human resources sometimes the MITS Assistant role is split into two, one person to hand specimen collection materials to the MITS Specialist and another to document on the MITS Specimen Collection Form.

Using the MITS Specimen Collection Form and MITS SOP as references, simulate the procedure twice, once while serving as the MITS Specialist and a second time as the MITS Assistant.

LESSON 1.3
Biosafety and MITS

LESSON OBJECTIVES

- Describe the rationale behind the use of biosafety precautions in MITS
- Demonstrate how to apply and remove personal protective equipment (PPE) correctly and safely
- Identify clean and dirty spaces during the MITS procedure
- Demonstrate the correct use of PPE to protect from exposure to infectious diseases while collecting and handling MITS samples
- Reduce to an acceptable level the risk of worker exposure or environmental contamination
- Walk through the morgue identifying:
    - Where to enter the morgue area
    - The MITS changing room
    - MITS Room 1 and MITS Room 2
    - Handwashing locations
    - Dirty utility
    - Body storage

LESSON LENGTH: 45 MINUTES (30 MINUTES DIDACTIC/15 MINUTES WALKTHROUGH)

LOCATION

- Classroom

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Lesson 1.3 Biosafety

1

Lesson Objectives

- Describe the rationale behind the use of biosafety precautions in MITS
- Demonstrate how to apply and remove personal protective equipment (PPE) correctly and safely
- Identify clean and dirty spaces during the MITS procedure
- Demonstrate the correct use of PPE to protect from exposure to infectious diseases while collecting and handling MITS samples
- Reduce to an acceptable level the risk of worker exposure or environmental contamination

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NOTE:

- If there have been any suspected cases of viral hemorrhagic fever in the area, MITS should not be requested or performed.
- MITS is a minimally invasive technique; however, **universal precautions should be used** in the performance of all MITS procedures, because any patient coming to MITS may have an undiagnosed or known high-risk condition.
- Perform a risk assessment of the MITS procedure room to **reduce the risk to staff, the environment, and the facility** where the project will be performed.

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Key Considerations in the Location of the MITS Room

The MITS Room should be located:

- Away from public spaces
- Away from kitchen/dining areas
- In a space with adequate ventilation and light

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Clean, Transit, and Dirty Areas

Plan workflow to minimize movement between dirty and clean areas

Clean	Transit	Dirty
• Reception area • Waiting room • Interview/counseling room • Viewing room • Offices • Linen and supply storage • Observation area • Staff changing areas • Specimen store	• Body handling area • Disposal room • Postmortem room transit area	• Postmortem room • Dirty utility room • Body store

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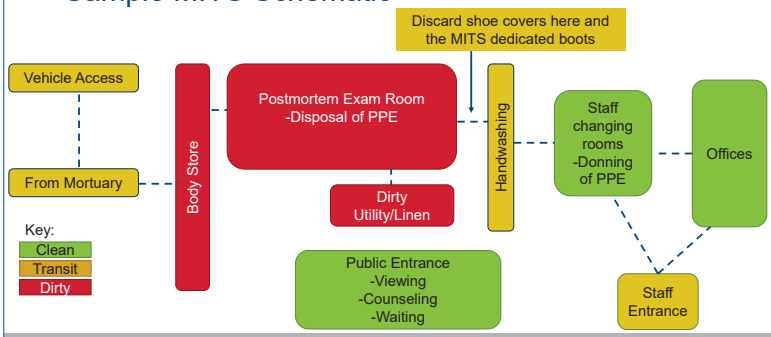
Sample MITS Schematic



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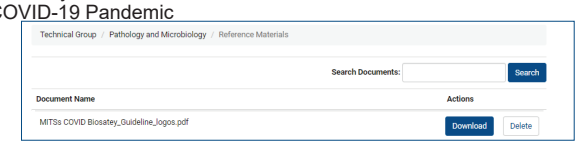
Infection Precautions in MITS

- When standard biosafety precautions are used properly, the risk of infection while performing MITS is very low
- Standard immunizations recommended for all health care workers also apply to those performing MITS
  - CDC recommendations can be found here: https://www.cdc.gov/vaccines/adults/rec-vac/hcw.html
- Biosafety Recommendations for MITS Practice in the Context of the COVID-19 Pandemic



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Required PPE

- Single-use, fluid-resistant, long-sleeved gown
- N-95 mask
- Disposable surgical hat or cap
- Goggles or facial shield
- Gloves (2 pairs “double gloving”) or autopsy gloves
- Closed shoes and shoe covers or boots

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Donning PPE

- Remove and secure all personal belongings (jewelry, watches, cell phones, pens, etc.) in changing room.
- PPE must be put on before leaving changing room and entering postmortem examination room.
  - MITS dedicated shoes remain in (or just outside) the postmortem examination room.
- Perform any PPE adjustment prior to the procedure. During the procedure, avoid touching or adjusting PPE.
- Put gloves on once inside postmortem examination room.

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Before the Procedure

- Maintain clean/dirty areas in MITS workspace.
- No materials outside of the MITS box (phones, cameras, etc.) should be handled by anyone also involved in specimen collection.
- Everyone in the MITS room who may come into contact with blood, body fluid(s), or tissues should wear disposable protective gloves.
- A "clean" circulating assistant should be available to obtain additional instruments, take notes, and use the camera during the MITS procedure.

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Material for Specimen Collection/Environmental Control

- Leak-proof infectious waste bag
- Ethyl alcohol, 70% (at least 100mL /MITS)
- Iodine solution (at least 100mL /MITS)
- 0.5% chlorine solution for environmental disinfection
- Gauze roll or 4X4
- Paper towels
- Cloths (2+)
- Rubber gloves for environmental cleaning
- Sharps container

Medical Waste Collection

- Contain all contaminated material in leak-proof storage bags or double bagged with biohazard warning label.
- Place all sharps (needles and syringes, scalpels, etc.) in puncture-proof sharps container for disposal
  - Do not fill containers beyond 75% of capacity
  - Don't recap needles
  - Close containers securely and tape if necessary

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After the Procedure: The Room

- Work surfaces should be cleaned and disinfected at the end of the day.
- The **postmortem room should be disinfected after each procedure**.
- Clean environmental surfaces can be decontaminated using 1,000 ppm (0.1%) sodium hypochlorite.
- If blood or body fluids remain on surfaces or if bloodborne illness is suspected disinfect using 10,000 ppm (1%) available **sodium hypochlorite**.

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After the Procedure: The Materials

- Soak reusable instruments in 10% sodium hypochlorite.
- Sterilization can be accomplished by using by steam or dry heat.
  - Boil for 20 minutes or soak in glutaral (Glutaraldehyde) 2% and hydrogen peroxide 6%.

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Accidental Exposure to Blood or Body Fluids

In the event of a percutaneous injury or mucocutaneous exposure to blood or bodily fluids:

1. Immediately wash the site with water
2. Report to supervisor or infection control officer for post-exposure management

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Appendix

1. Steps for donning gloves
2. Hand hygiene guidance
3. CDC Step-by-Step Guidance for donning and removing PPE

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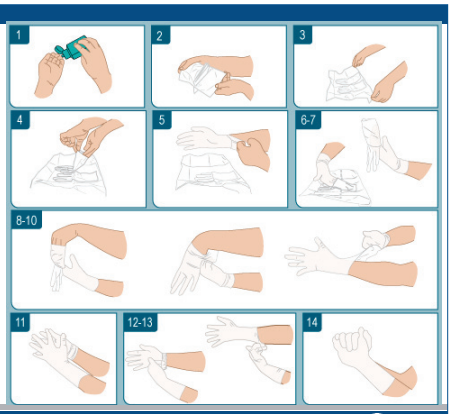
Donning Gloves

- Change gloves between patients
- Remove gloves if they become torn or damaged
- Perform hand hygiene before putting on new gloves



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Hand Hygiene Material

- Alcohol-based hand-rub solution, OR
- Soap and clean water

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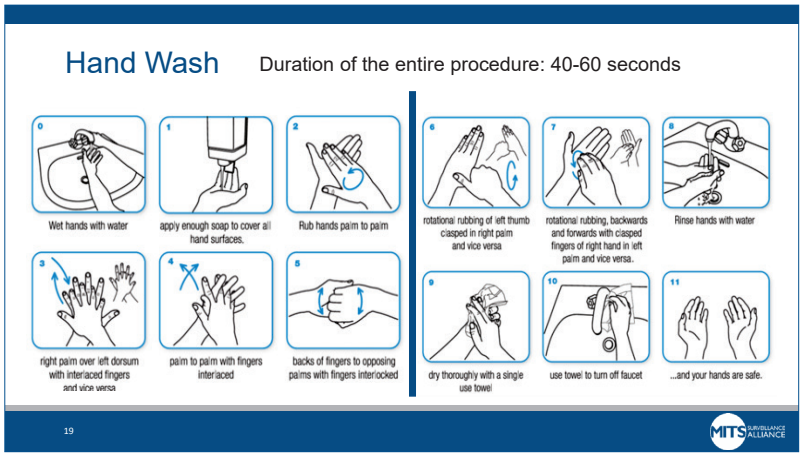
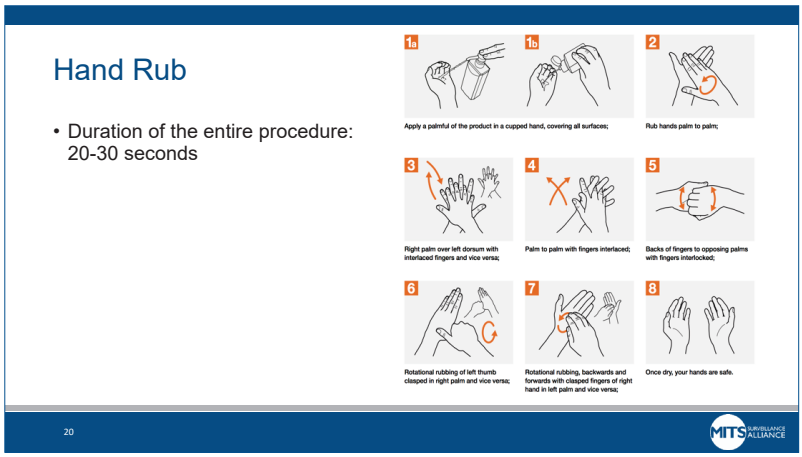


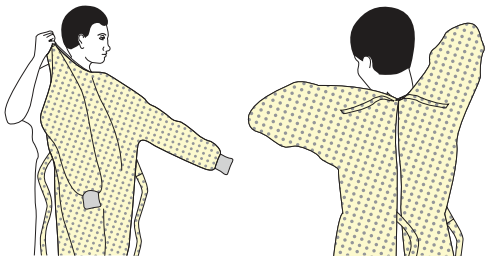


SEQUENCE FOR PUTTING ON PERSONAL PROTECTIVE EQUIPMENT (PPE)

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.

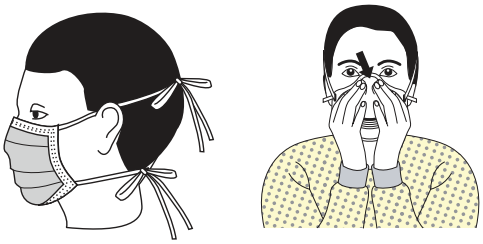
1. GOWN

- 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

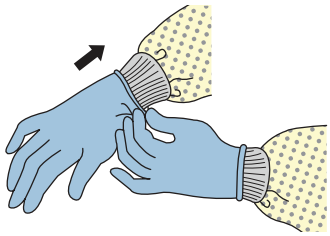


3. GOGGLES OR FACE SHIELD

- 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

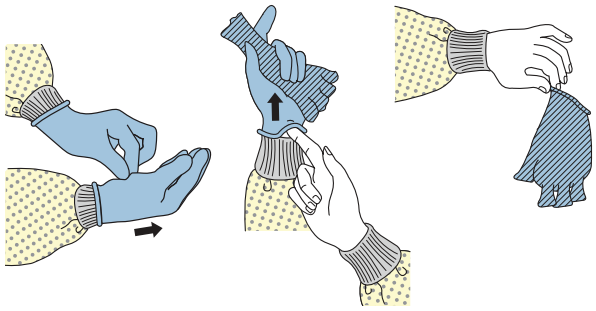
- Keep hands away from face
- Limit surfaces touched
- Change gloves when torn or heavily contaminated
- Perform hand hygiene

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

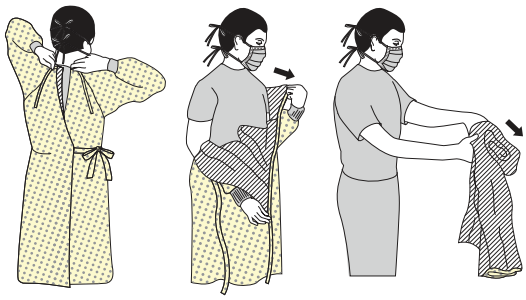


2. GOGGLES OR FACE SHIELD

- 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

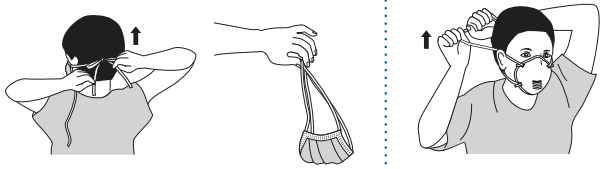
3. GOWN

- 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

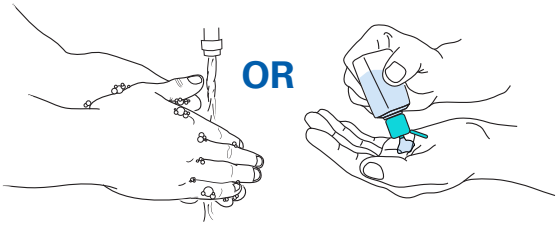


4. MASK OR RESPIRATOR

- 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



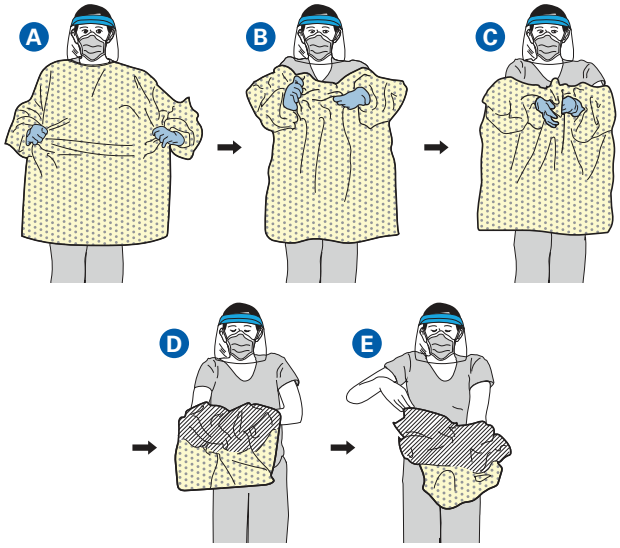
PERFORM HAND HYGIENE BETWEEN STEPS IF HANDS BECOME CONTAMINATED AND IMMEDIATELY AFTER REMOVING ALL PPE

HOW TO SAFELY REMOVE PERSONAL PROTECTIVE EQUIPMENT (PPE) EXAMPLE 2

Here is another way to safely remove PPE without contaminating your clothing, skin, or mucous membranes with potentially infectious materials. **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. GOWN AND GLOVES

- Gown front and sleeves and the outside of gloves are contaminated!
- If your hands get contaminated during gown or glove removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Grasp the gown in the front and pull away from your body so that the ties break, touching outside of gown only with gloved hands
- While removing the gown, fold or roll the gown inside-out into a bundle
- As you are removing the gown, peel off your gloves at the same time, only touching the inside of the gloves and gown with your bare hands. Place the gown and gloves into a waste container

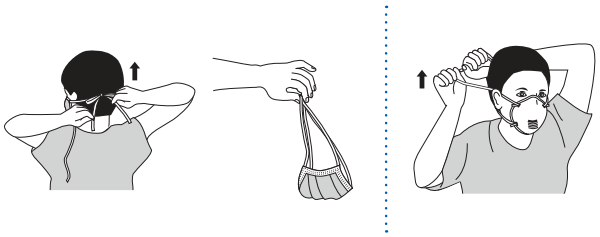


2. GOGGLES OR FACE SHIELD

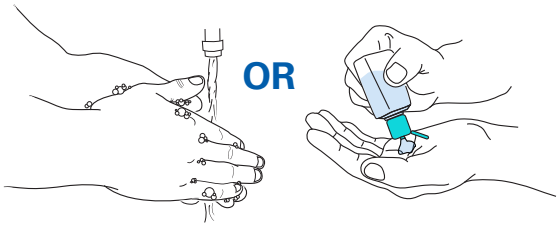
- Outside of goggles or face shield are contaminated!
- If your hands get contaminated during goggle or face shield removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Remove goggles or face shield from the back by lifting head band and without touching the front of the goggles or face shield
- If the item is reusable, place in designated receptacle for reprocessing. Otherwise, discard in a waste container

3. 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



4. 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

LESSON 1.4
MITS Demonstration

LESSON OBJECTIVES

- Observe correct CSF, CNS, NP, blood, lung, liver, skin, and rectal MITS sampling technique
- Observe correct establishment and maintenance of proper biosafety precautions

LESSON LENGTH: 90 MINUTES

LOCATION

- MITS Room

NOTES FOR PARTICIPANTS

Follow along with the MITS Specimen Collection Form and MITS SOP as the course facilitator conducts MITS.

NOTES FROM MITS PRACTICE

LESSON 1.5
Day 1 Debrief

LESSON OBJECTIVES

- Identify and describe the highlights of the day
- Identify the challenges or aspects that remain unclear
- Identify learning goals for Day 2

LESSON LENGTH: 30 MINUTES

LOCATION

- Classroom

NOTES FOR PARTICIPANTS

This is an opportunity to reflect on your learning, identify remaining questions, and set learning goals for the next training day.

1. What went well today?

2. What aspects or lessons remain unclear or challenging?

3. Identify one or two learning goals for tomorrow:

LESSON 2
MITS Training

LESSON 2.1
MITS Practice

LESSON OBJECTIVES

- Demonstrate correct CSF, CNS, NP, blood, lung, liver, skin, and rectal MITS sampling technique

LESSON LENGTH: 180 MINUTES

LOCATION

- MITS Room

NOTES FOR PARTICIPANTS

- Meet in the classroom and facilitators will escort the group to the MITS Room(s).
- The focus of today's MITS practice is to gain confidence and skill to correctly and successfully obtain MITS samples.
- Use the MITS Alliance SOP and MITS Specimen Collection Form as a guide.

NOTES FROM MITS PRACTICE

LESSON 2.2
Defining Your MITS Project Setup

LESSON OBJECTIVES

- Apply knowledge of the flow of MITS samples to identify and troubleshoot potential operational challenges in implementing MITS within projects

LESSON LENGTH: 30 MINUTES

LOCATION

- Classroom

NOTES FOR PARTICIPANTS

- This activity will be completed outside of class time and presented to the group on Day 4.
- The purpose of this session is to concretely consider how you can organize and arrange your space to operationalize MITS.
- Specifically, the goal is to begin to define the flow of materials, samples, and people that will take place.
- Making note of the key spaces below, use the following page to sketch the layout for your MITS project identifying clear areas, transit areas, and dirty areas:
    - Away from public spaces
    - Away from kitchen/dining areas
    - In a space with adequate ventilation and light
- In your sketch be sure to identify the following spaces:
    - MITS/postmortem exam room
    - Handwashing
    - Changing room
    - Body storage
    - Dirty utility/linen

Be prepared to present your sketch on Day 4.

Use this page to sketch.

LESSON 2.3
Initial Handling of MITS Specimens

LESSON OBJECTIVES

- Describe and demonstrate initial handling of MITS sample once collected

LESSON LENGTH: 60 MINUTES

LOCATION

- Classroom

NOTES FOR PARTICIPANTS

- Refer to Appendix E: MITS Sample Transport and Guidance and Appendix F: MITS Histological, Microbiological, (and other) Sample Analysis Guidance for further detail.

NOTES

SLIDES

Lesson 2.3 Initial MITS Sample Processing

1

Lesson Objectives

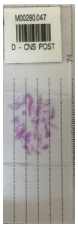
- Describe and demonstrate proper handling of MITS samples once collected
- List key components of gross examination of the MITS specimens

2

Fixed Tissue Specimen

Formalin Jar – Local Lab

- The labels for the slides should always be together with the samples



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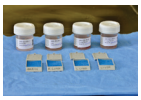
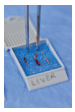
NOTES

SLIDES

Formalin Jars: Histological Processing First Day

1. **Formalin jars MITS specimens** (all except placenta)
   - **Time of fixation: 4-6 hours**
     - Limit fixation in formalin to no more than 6 hours
   - Put the samples into the cassettes
   - Put the cassettes into a large recipient and fill it with **distilled water/alcohol depending on logistics, infrastructure, and study requirements**



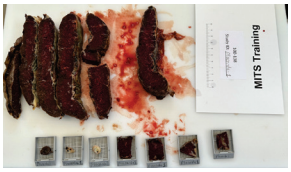


2. **Formalin jar placenta sample or any large sample (e.g., skin)**
   - Be sure formalin covers the whole sample. If required, refill with formalin.
   - Leave the tissue in formalin until fixation (should be day 2).

4

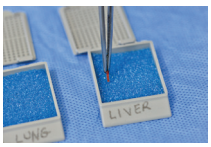
Formalin Jars: Histological Processing Second Day

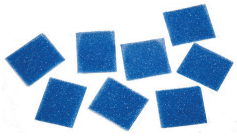
1. **Formalin jar placenta sample**
   - With a scalpel prepare a 15 x 15 x 3 mm section of the placenta (including the maternal side) and a membrane roll
   - Put the placental sample in the cassette
   - Put the cassette into the recipient containing all the other cassettes



5

Tissue Cassettes





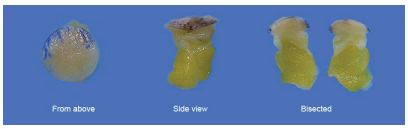
6

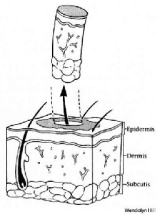
NOTES

SLIDES

Formalin Jars: Skin Processing Second Day

- Following fixation, with a scalpel bisect the skin specimen from the epidermis to the hypodermis (fatty tissue)
- Split skin into two samples, keeping layers intact
- Put the skin sample in the cassette





7

Histology Cassettes: Histological Processing Second Day

- Keep the cassettes in distilled water or alcohol 70% until time to put them into the tissue processor
- Put the cassettes **in the tissue processor**
- **Avoid the formalin step in the processor!**

8

Histology Cassettes: Histological Processing Third Day

- Prepare the paraffin blocks
- Prepare the histological sections
- Stain the sections with hematoxylin and eosin
- Mount the sections
- **Let the slides dry until day 4**
- Keep the specimen collection form and the pathology report with the samples during the process
- Once the histological diagnosis has been made, put all the slides and paraffin blocks in the archive

9

NOTES

SLIDES

Quality Assessment of Histological Samples

10

Quality Assessment of Histological Samples

Results can only be as good as the specimens and how they are processed

11

Quality Assessment of Histological Samples

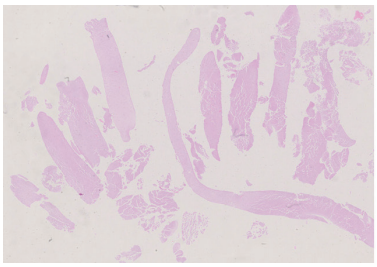
- Sample collection
  - Technical adequacy
    - Target tissue
      - Number and size of fragments
    - Other tissues present
  - Specimen quality
    - Autolysis
    - Postmortem bacterial overgrowth
    - Presence of key anatomical structures
      - Meninges in brain specimens
      - Bile ducts in liver specimens
    - Contamination
      - Postmortem bacterial overgrowth
      - Nasal content in transnasal brain specimens
      - GI tissue in liver specimens
- Sample processing
  - Stain color/intensity
  - Thickness
  - Artifacts
    - Folds
    - Knife marks
    - Pigments
    - Bubbles/drying
    - "Floaters"

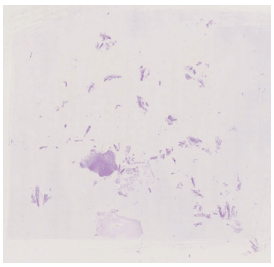
12

NOTES

SLIDES

Technical Adequacy





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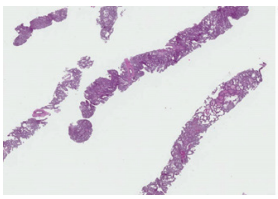
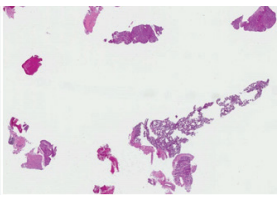
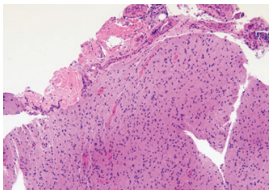
Technical Adequacy



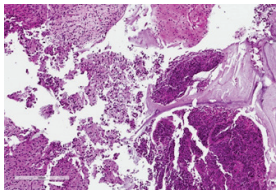


14

Specimen Quality



Meninges with brain



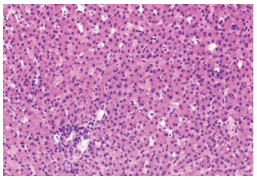
Nasal contamination of brain

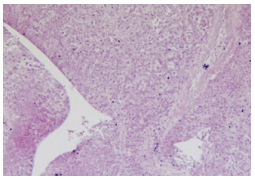
15

NOTES

SLIDES

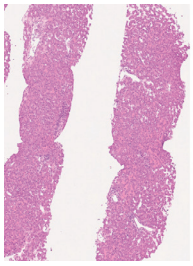
Specimen Quality - Autolysis

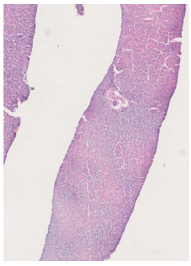




16

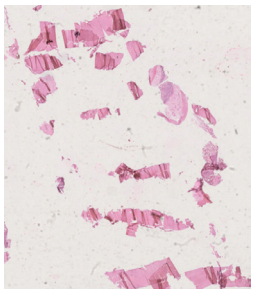
Processing - Staining



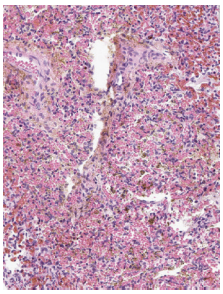


17

Processing - Artifacts



Tissue folds

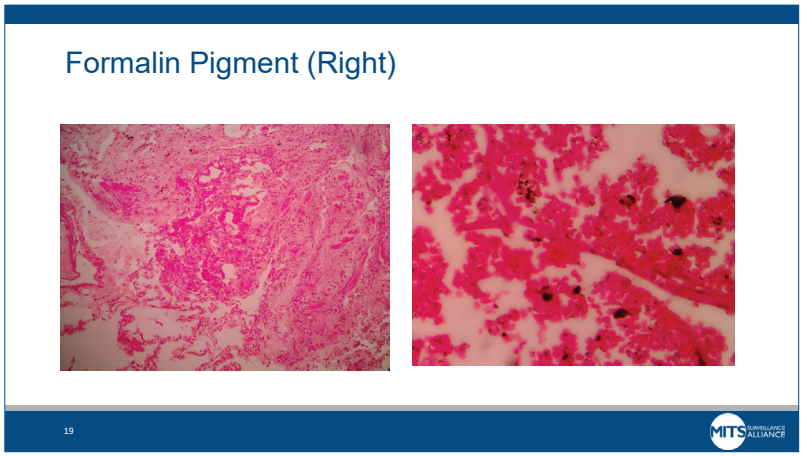


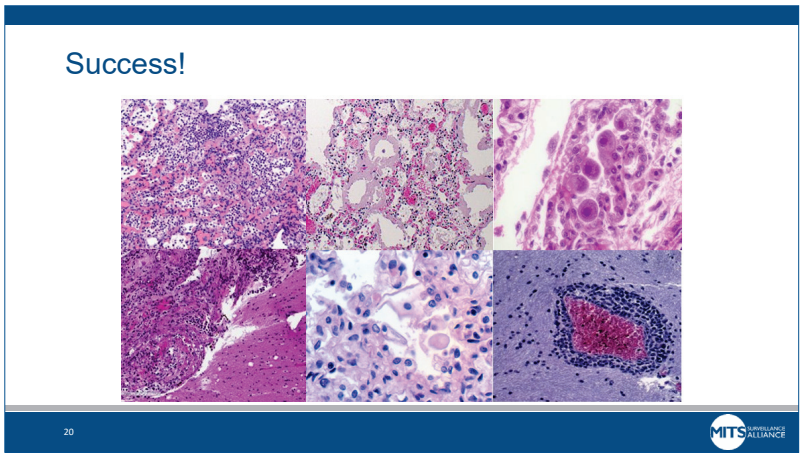
Formalin pigment

18

NOTES

SLIDES





LESSON 2.4
Day 2 Debrief

LESSON OBJECTIVES

- Identify and describe the highlights of the day
- Identify the challenges or aspects that remain unclear
- Identify learning goals for Day 3

LESSON LENGTH: 30 MINUTES

LOCATION

- Classroom

NOTES FOR PARTICIPANTS

This is an opportunity to reflect on your learning, identify remaining questions, and set learning goals for the next training day.

1. What went well today?

2. What aspects or lessons remain unclear or challenging?

3. Identify one or two learning goals for tomorrow:

LESSON 3
MITS Training

LESSON 3.1
MITS Practice

LESSON OBJECTIVES

- Demonstrate appropriate technique of the MITS SOP in the role of MITS Specialist
- Demonstrate appropriate technique of the MITS SOP in the role of MITS Assistant

LESSON LENGTH: 180 MINUTES

LOCATION

- MITS Room(s)

NOTES FOR PARTICIPANTS

- The goal of MITS practice today is to practice becoming more familiar with the sequence for collecting MITS samples.
- You will have the opportunity to go through the SOP twice, once as the MITS Specialist and once as the MITS Assistant.
- The MITS Sample Collection Form (Appendix B) should be used as a job aid to for sequencing and conducting the procedure.

NOTES FROM MITS PRACTICE

LESSON 3.2
External Malformations

LESSON OBJECTIVES

- Identify the major types of malformations
- List the four developmental mechanisms of congenital anomalies
- List the four major categories of clinical presentation of congenital anomalies

LESSON LENGTH: 45 MINUTES

LOCATION

- Classroom

NOTES FOR PARTICIPANTS

This presentation is not intended to be an exhaustive study of congenital anomalies and external malformations. The purpose is to provide a general overview. Those wishing a more comprehensive etiologic description and illustration of congenital anomalies should consult the relevant perinatal, pediatric, and genetic textbooks.

NOTES

SLIDES

Lesson 3.2 External Malformations

1

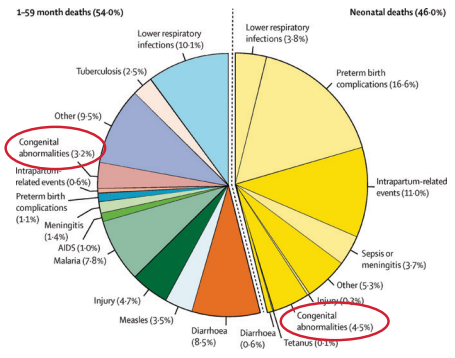
Lesson Objectives

- Identify the major types of malformations
- List the four developmental mechanisms of congenital anomalies
- List the four major categories of clinical presentation of congenital anomalies

2

Congenital Anomalies

- Structural or functional anomalies (e.g., metabolic disorders) that occur during intrauterine life and can be identified prenatally, at birth, or later in life
- The most common severe congenital anomalies are heart defects, neural tube defects, and Down syndrome



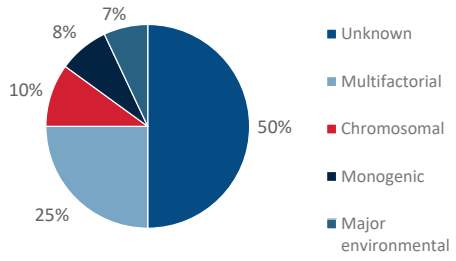
Source: Creative Commons Perin J, Mulick A, Yeung D, Villavicencio F, Lopez G, Strong KL, Prieto-Merino D, Cousens S, Black RE, Liu L. Global, regional, and national causes of under-5 mortality in 2000-19: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet Child Adolesc Health. 2022 Feb;6(2):106-115. doi: 10.1016/S2352-4642(21)00311-4. Epub 2021 Nov 17. Erratum in: Lancet Child Adolesc Health. 2022 Jan;6(1):e8. PMID: 34800370; PMCID: PMC8786667

3

NOTES

SLIDES

Causes of Congenital Anomalies



Adapted with permission from Keith L. Moore BA MSc PhD DSc FIAC FRSM FAAA, T. V. N. Persaud MD PhD DSc FRCPath (Lond.) FAAA MGTMsP-. Developing Human- Clinically Oriented Embryolog. In: The developing human. 2016. p. 458.

4

Congenital Anomalies and MITS

- The presence of a congenital malformation or anomaly does not in itself contraindicate MITS.
- The nature of the anomaly and study objectives must be considered when determining whether to proceed.
- If MITS is conducted all external anomalies should be noted on MITS Specimen Collection form.

5

Developmental Mechanisms of Congenital Anomalies

1. Malformation
2. Disruption
3. Deformation
4. Dysplasia

6

NOTES

SLIDES

1. Malformations

- Morphological defects of organs or organ parts resulting from abnormal development, which was pathologic from the start.
- Malformations can occur on a genetic or environmental basis.

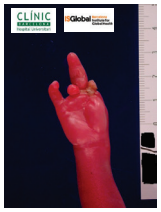
Examples:

7

2. Disruption

- Secondary defect in an initially normally developing organ or its part
- Etiological factors are either external or internal, but are not hereditary

Example:



8

3. Deformation

- Develop later in gestation because of mechanical powers (pressure) causing abnormal shape or position of a body part.
- Fetal development is diverted.
- There are many etiological factors, for example a small or malformed uterus, leiymyomatosis, oligoanhydramnion, abnormal positions of the fetus, or multiple gravidity.

9

NOTES

SLIDES

4. Dysplasia

- Abnormalities in histogenesis and tissue formation
- Tumors

10

Other Lesions

Agenesis: complete absence of an organ and its primordia

Aplasia: absence of an organ resulting from damaging its primordia

Atresia: absence of an opening of mostly visceral organs

Hypoplasia: a not-so-serious stage of aplasia, the organ is not completely developed and the number of cells in its structure is decreased.

11

Clinical Presentation of Congenital Anomalies

1. Isolated
2. Sequence
3. Association
4. Syndrome

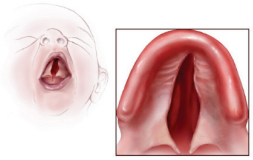
12

NOTES

SLIDES

1. Isolated

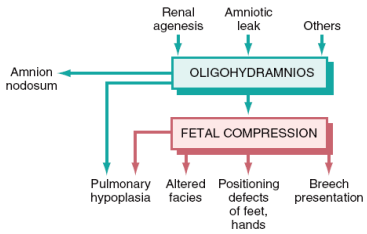
- Approximately 75% of congenital anomalies are isolated and no other anomalies are present.
- Isolated major anomalies can be associated with one or more minor anomalies.



13

2. Sequence

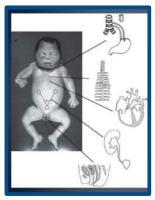
Secondary anomalies that develop as part of cascade of primary anomaly



14

3. Association

- Pattern of multiple anomalies that occur with a higher than random frequency
- VACTERL
  - **V**ertebral, **A**nal, **C**ardiac, **T**racheo–o**E**sophageal fistula, **R**enal, and **L**imb defects
- MURCS
  - **MU**llerian duct aplasia, **R**enal aplasia, **C**ervicothoracic **S**omite dysplasia



15

NOTES

SLIDES

5. Syndrome

- Symptoms and anomalies present together
- The collective occurrence is not random, but relates to the etiology of the process
- Not caused by any primary malformation
- Syndromes often originate from one etiologic agent, for example some specific chromosomal aberration

16

Down Syndrome

- Trisomy 21
- Highly associated with maternal age
  - 1:550 in women < 20 years
  - 1:25 in women > 45 years
- Characteristic facies:
  - Flattened face
  - Epicanthic folds

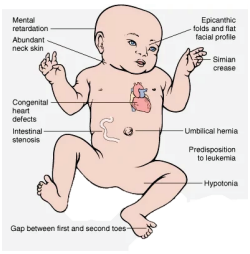


Image credit: USMLE Forums

17

Anomalies of Multi-gestation

Fetal-fetal transfusion

18

NOTES

SLIDES

Gastrointestinal System: Omphalocele

19

Gastrointestinal System: Gastroschisis

20

Appendix

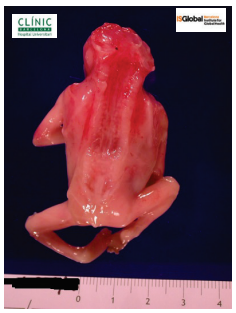
Other examples of congenital anomalies

21

NOTES

SLIDES

Malformations: Anencephaly and Rachischisis



22

Malformations: Mielomeningocele

23

Malformations: Polydactilia

24

NOTES

SLIDES

Edwards Syndrome

- Trisomy 18
- Microcephaly, micrognatia
- Finger superposition
- Clubfoot
- Congenital heart defects
- Renal malformations

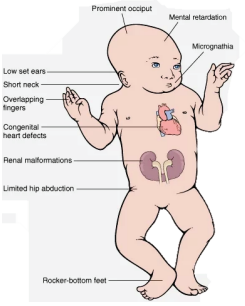


Image credit: USMLE Forums

25

Patau Syndrome

Musculoskeletal and cutaneous

- Polydactyly (extra digits)
- Cyclopia, proboscis
- Congenital trigger digits
- Low-set ears
- Prominent heel
- Deformed feet known as rocker-bottom feet
- Omphalocele (abdominal defect)
- Abnormal palm pattern
- Overlapping of fingers over thumb
- Cleft palate

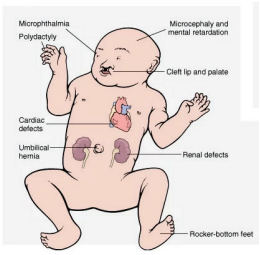


Image credit: USMLE Forums

26

Digeorge Syndrome

- Chromosome 22q11.2 deletion syndrome (Digeorge syndrome/velocardiofacial syndrome)
- Abnormalities:
  - Congenital heart disease
  - Defects in the palate
  - Abnormal facies
  - T-cell immunodeficiency

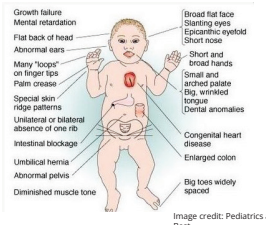


Image credit: Pediatrics at Best

27

NOTES

SLIDES

Fragile X Chromosome

- Mutation in chromosome X
- 1:1550 boys; 1:8000 girls
- Mental retardation
- Characteristic external phenotype:
  - Large, protruding ears (both)
  - Long face (vertical maxillary excess)

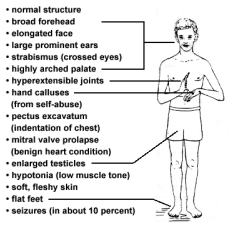


Image credit:
https://my.vanderbilt.edu/speds2430/topics/sample-page/

28

Hydrops Fetalis

- Abnormal accumulation of liquid in the interstitial space in the fetus
- Immune hydrops: maternal-fetal Rh incompatibility
- Non-immune hydrops:
  - Cardiovascular diseases
  - Chromosomal abnormalities: trisomy
  - Thoracic: diaphragmatic hernia
  - Fetal anemia
  - Multiple gestation
  - Other: infections, malformations, neoplasms

Image credit: Zineb B, Boutaina L, Ikram L, Driss MR, Mohammed D - The Pan African medical journal (2015)
https://openi.nlm.nih.gov/detailedresult?img=PMC4742050_PAMJ-22-137-g002&query=hydrops%20fetalis&it=xg&lic=by&req=4&npos=24

29

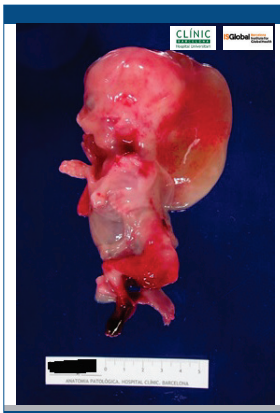
Cystic Hygroma

- Congenital multiloculated lymphatic lesion
- Classically found in the left posterior triangle of the neck and armpits
- Frequently associated with other malformations

30

NOTES

SLIDES



Cystic Hygroma

- Congenital multiloculated lymphatic lesion
  - Familial neck webbing (autosomal dominant)
  - Lymphedema distichiasis syndrome (autosomal dominant)
- Chromosome disorders
  - 45,X Turner syndrome
  - X-chromosome polysomy
  - Trisomy 18 syndrome
  - Trisomy 21 syndrome
  - Trisomy 22 mosaicism syndrome
  - Teratogenic disorders
  - Fetal alcohol syndrome

31

Tumors: Teratoma

32

Tumors: Angioma

33

NOTES

SLIDES

Achondrogenesis

34

LESSON 3.3
Placental Sampling

LESSON OBJECTIVES

- Describe the gross appearance of a normal placenta and the basic normal histology
- Describe gross examination of the placenta according to the MITS Alliance SOP
- Identify the macroscopic appearance of the most frequently appearing placental lesions
- Describe the steps in placental sampling
- Name the major categories of placental malformations

LESSON LENGTH: 45 MINUTES

LOCATION

- Classroom

NOTES

SLIDES

Lesson 3.3. The Placenta

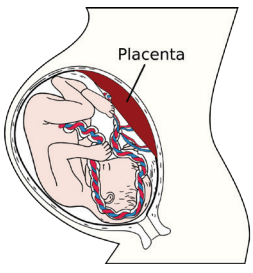
1

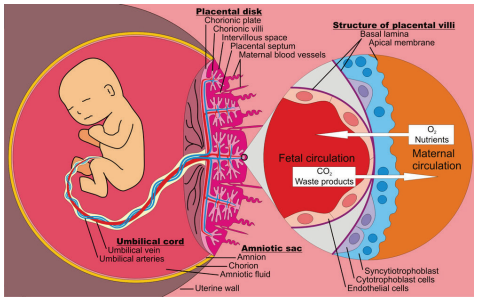
Lesson Objectives

- Describe the gross appearance of a normal placenta and the basic normal histology
- Describe gross examination of the placenta according to the MITS Alliance SOP
- Identify the macroscopic appearance of the most frequently appearing placental lesions
- Describe the steps in placental sampling
- Name the major categories of placental malformations

2

The Placenta





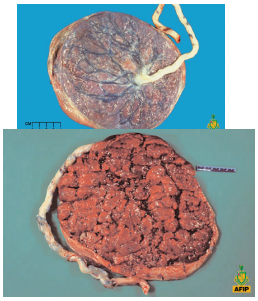
3

NOTES

SLIDES

The Placenta: Macroscopic Features

- Placental section
  - Spongy appearance
  - Soft to medium consistency
- Limited by
  - Chorionic plate on one side *(fetal surface)*
  - Basal plate on the other side *(maternal surface)*

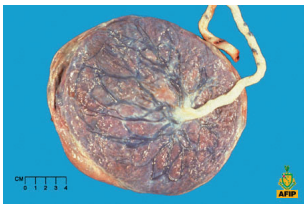


4

The Placenta: Macroscopic features

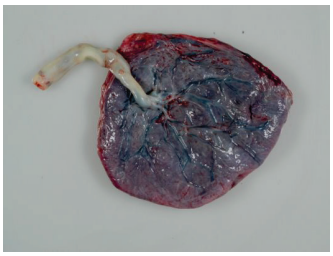
Disc-like, flat round to oval shape

- Average diameter: 22cm
- Average thickness at the center: 2.5 cm
- Average weight: 470 gr



5

Fetal (Chorionic or Amniotic) Surface



- Glossy/shiny appearance:
  - epithelial cover *(amnion)*
- Semitranslucent
- Vessels coursing over its surface
- Umbilical cord insertion

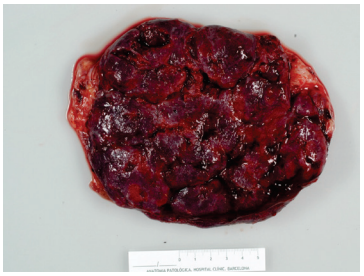
6

NOTES

SLIDES

Maternal (Uterine or Basal) Surface

- Opaque/reddish appearance
  - Histology: mixture of decidual, trophoblastic cells and fibrinoid
- Incomplete system of grooves subdivide the maternal surface in 10-40 slightly elevated areas *(lobules or cotyledons)*



7

Placental Examination and Sampling

8

Equipment

- Disposable lab coat, gloves and N-95 mask
- Safety goggles
- Gauze rolls 36 feet x 100 yards
- Camera for pre-procedure pictures (high resolution, >300 ppi preferred)
- Weight scale calibrated in grams
- Measuring tape
- Tweezers, knife, and scalpel
- Adequate container for placenta fixation

9

NOTES

SLIDES

Placenta Microbiology Sampling

- Microbiology sampling must occur as soon as possible after delivery and before any contact with formalin.
- Placental disc:
  - Use one sterile scalpel blade to cut two pieces of tissue 0.5 x 0.5 x 0.5 cm from the periphery of the placenta.
    - Take one sample from the periphery and another sample from the center.
    - As needed, sample gross lesions (e.g., abscess).
  - Place the two pieces of placenta in the cryogenic vial using clean forceps.

10

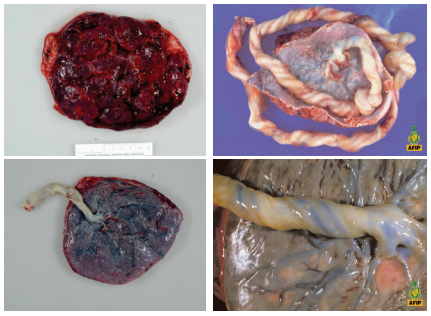
Placental Microbiology Sampling (con't)

- Cord: Using a new sterile scalpel blade, cut two thin transverse sections of the cord (one from the fetal end and one from the maternal end).
  - Place the cord samples in the cryogenic vial using clean forceps.
- Membrane: Using a sterile scalpel blade, cut two pieces of placenta membrane.
  - Place the two pieces of membrane in the cryogenic vial using clean forceps.

11

Photos: Mandatory

- Maternal surface
- Fetal surface
- Other grossly visible lesions *(hematomas, knots, etc.)*



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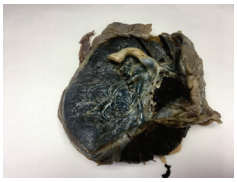
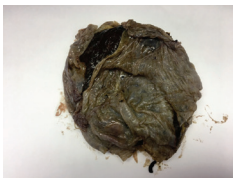
NOTES

SLIDES

Photos: Mandatory

13

Measuring Placental Disc

- Measure the maximal linear length and width (greatest dimension of the axis perpendicular to this linear measurement) of the placenta disc.

14

Weigh the Placenta

- Trim extraplacental membranes and umbilical cord prior to weighing.
- Record whether the placenta was fresh or fixed when measured.

15

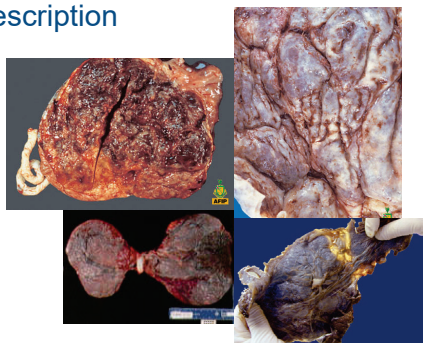
NOTES

SLIDES

Placental Disc and Description

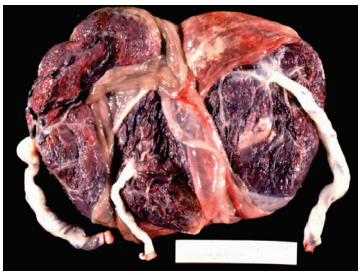
- **Color of fetal surface**
- Color of maternal surface
- Transparency of fetal side
- Any other gross abnormalities
  - **Look for hematomas**
  - Areas of atrophy
  - Unusual shape

  (Placenta bilobate or multilobate, accessory lobes)



16

Multiple Gestation: Describe What You See

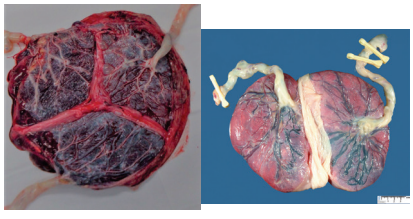


Indicate the number of discs

- Single
- Multiple *(number)*

17

Multiple Gestation: Describe What You See



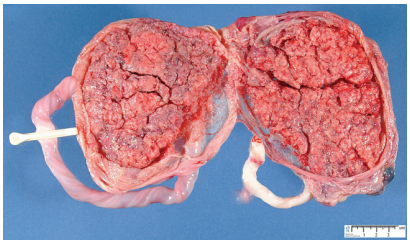
- **Features of dividing membranes:**
  - Thin translucent: monochorionic placenta
  - Thick and ridge: dichorionic placenta
- **Describe any gross abnormality:**
  - Hematomas
  - Areas of atrophy
  - Cord abnormalities

18

NOTES

SLIDES

Multiple Gestation
If Separate Discs: Describe as Separate Placentas

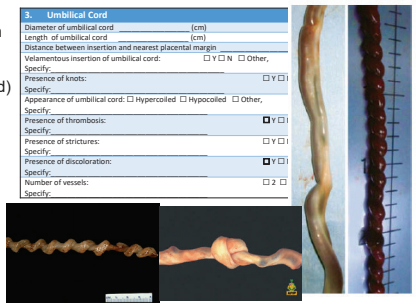


- Color of fetal surface
- Color of maternal surface
- Transparency of fetal side
- Any other gross abnormalities
  - **Hematomas**
  - Areas of atrophy
  - Cord abnormalities
  - Unusual shapes

19

Describe the Cord

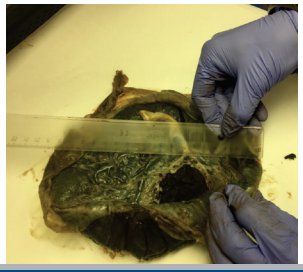
- Average diameter of the cord; length
- **Number of vessels**
  - in the middle or fetal third of the cord)
- Site of insertion in relation to the center/margin of the placenta
- Direction of coiling (handedness) if possible
- Any gross abnormalities
  - Abnormal color
  - Strictures, knots, thrombi
  - Areas of hypo- or hypercoiling



20

Describe the Cord

Distance between the insertion site and nearest placental margin



Length of the cord

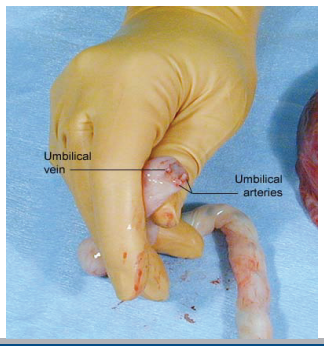
21

NOTES

SLIDES

Describe the Cord

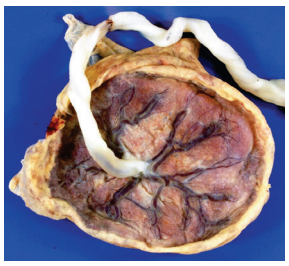
Number of umbilical cord vessels (2 arteries and 1 vein)



22

Central or Eccentric Insertion *(Variants of Normal Insertion)*

Central insertion



Eccentric (slightly away from the center) insertion

23

Marginal Insertion

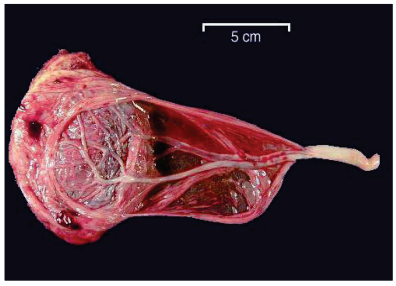
24

NOTES

SLIDES

Velamentous Insertion

Attachment to the membranes around the placenta



- Intramembranous vessels (unprotected by Wharton jelly) are at greater risk for compression and rupture.

25

Describe the Membranes

- Note the color/opacity and completeness.
- Record, if possible, the shortest distance between the site of rupture and the placental edge.
- If circumvallate or circummarginate, the percentage of the circumference involved should be noted.

4. Membranes
Describe color: ☐ White ☐ Brown/Green ☐ Red/purpose ☐ Other, Specify:_____
Describe opacity: ☐ Clear ☐ Slightly opaque ☐ Opaque ☐ Other, Specify:_____
Are membranes complete: ☐ Y ☐ N ☐ Other (specify):_____
Are the membranes: ☐ Circumvallate: _____% ☐ Circummarginate _____% ☐ Neither ☐ Other, Specify:_____

26

Describe the Membranes

Greenish color: indicative of chorioamnionitis

27

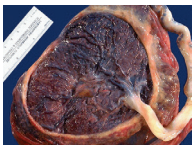
NOTES

SLIDES

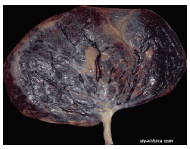
Placenta Extrachorialis

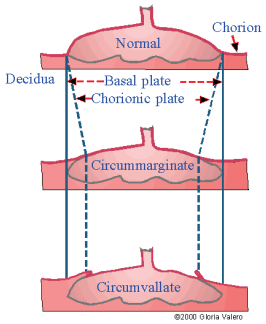
- The transition from membranous to villous chorion does not take place at the edge of the placenta.

Placenta circumvallate



Placenta circummarginate





28

First Day: Perform Serial Sections

- Using knife or scalpel, serially incise the disc from the maternal to the fetal surface at 1-2 cm intervals.
- Put placenta in formalin for 24 hours.

29

Second Day: Complete Sectioning

- Following fixation rinse with water to remove formalin.
- Using knife or scalpel complete the previous sections and carefully separate the slices.

30

NOTES

SLIDES

Second Day: Photograph Sections

- All the placental slices are put on a clean surface and a photo is taken.
- If placental lesions are identified take extra photos.

31

Second Day: Measure the Placenta

- Measure both mural minimal and maximal thickness.

32

Observe for Lesions

- **Any grossly identified lesions should be described.**
- In case of lesions:
  - Give the number and measure of lesions.
  - Describe appearance.
  - Describe location (central/paracentral or peripheral).
  - Estimate of the percentage of the total placental volume they affect.
- The location(s) of the lesions should be stated: central/paracentral or peripheral.
- Lesions that are microscopically different may appear similar grossly.
- **Do not try to diagnose the lesions based on gross examination: histology will come…**

33

NOTES

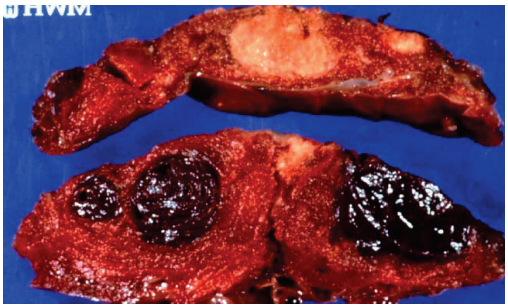
SLIDES

Observe for Infarcts

- Localized area of ischemic villous necrosis. Can be quite extensive.
- Macroscopic features: depend on the evolution.
  - Recent infarcts: Red
  - Old infarcts: White
- Look for placental floor infarction.
  - Abnormal whitish color of maternal surface

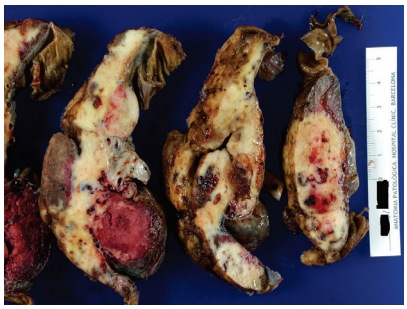
34

Recent and Old Infarcts: Involvement of 60% of the Placenta Volume



35

Recent and Old Infarcts: Involvement of 90% of the Placenta Volume



36

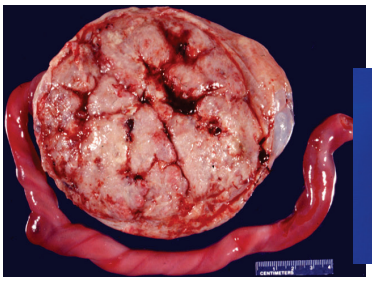
NOTES

SLIDES

Old, Central Infarct: Involvement of 10% of the Placenta Volume

37

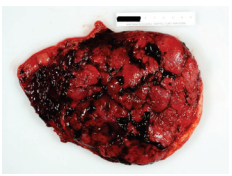
Placental Floor Infarction



38

Other Lesions

- Subchorionic fibrin
- Retroplacental hemorrhage/hematoma



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NOTES

SLIDES

Other Lesions

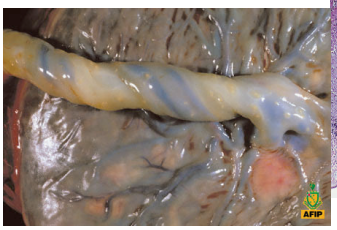
- Cysts
- Tumors

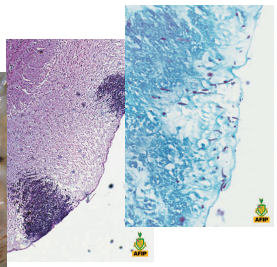
40

Other Lesions

Suggestive of infection

- *C. albicans*, other





41

Placental Sampling

Submit **five blocks** as a minimum:

- One block to include a **roll of the extraplacental membranes** from the rupture edge to the placental margin, including part of the marginal parenchyma.

42

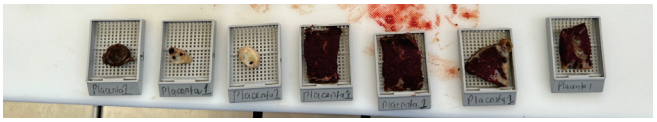
NOTES

SLIDES

Placental Sampling (con't)

Submit **five blocks** as a minimum:

- Two **cross-sections of the umbilical cord**, one from the fetal end and another approximately 5 cm from the placental insertion end.
- Two blocks each containing a full thickness section of **normal-appearing placenta parenchyma.**



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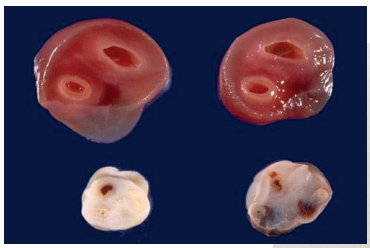


44

Placental Sampling (con't)

- Full-thickness samples should be taken from within the **central two-thirds of the disc.**
- **If the transmural thickness is greater than the length of the cassette, divide the gross slice and submit it in two cassettes: the upper third (chorionic plate and subjacent tissue) and lower third (basal aspect) of the parenchyma.**

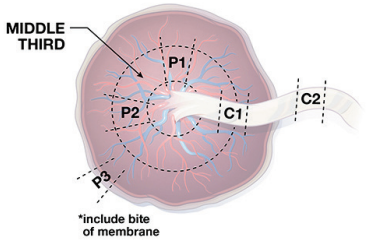


Photo credit: https://www.cdc.gov/zika/hc-providers/best-specimens-at-time-of-birth.html

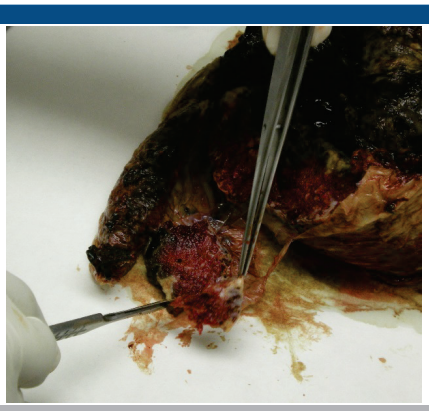
45

NOTES

SLIDES

Placental Sampling (con't)

- In case of lesions, additional blocks of the lesion(s) should be sampled, with adjacent normal parenchyma if possible.



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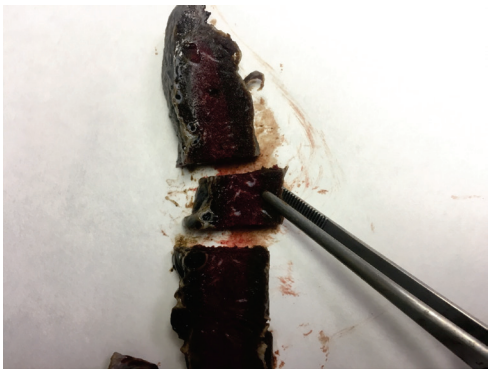

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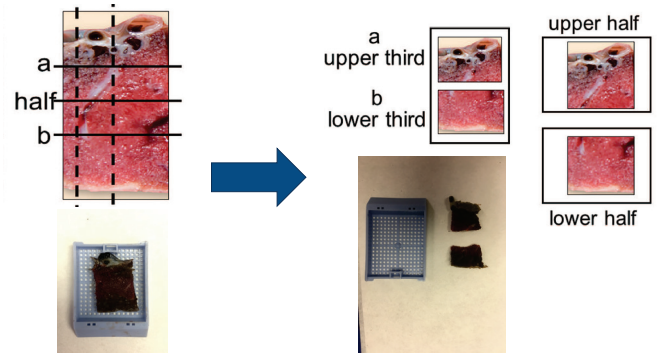

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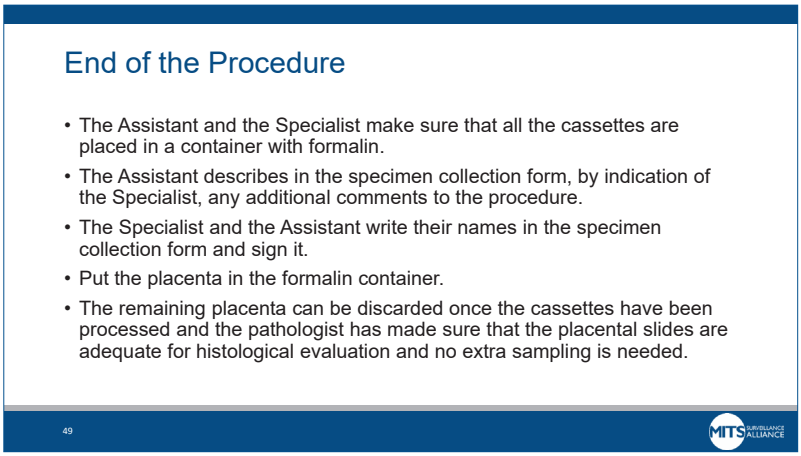
NOTES

SLIDES



LESSON 3.4
Day 3 Debrief

LESSON OBJECTIVES:

- Identify and describe the highlights of the day
- Identify the challenges or aspects that remain unclear
- Identify learning goals for Day 4

LESSON LENGTH: 30 MINUTES

LOCATION

- Classroom

NOTES FOR PARTICIPANTS

This is an opportunity to reflect on your learning, identify remaining questions, and set learning goals for the next training day.

1. What went well today?

2. What aspects or lessons remain unclear or challenging?

3. Identify one or two learning goals for tomorrow:

LESSON 4
MITS Training

LESSON 4.1
MITS Practice

LESSON OBJECTIVES

- Correctly demonstrate implementation of full MITS SOP

LESSON LENGTH: 120 MINUTES

LOCATION

- MITS Room(s)

NOTES FOR PARTICIPANTS

The goal of today's MITS practice is to further reinforce the sequence of MITS sampling and perfect the skills required to obtain samples.

Remember to use Appendix B: MITS Specimen Collection Form as a job aid as you conduct the procedure.

NOTES FROM MITS PRACTICE

LESSON 4.2
Demonstration of Placental Exam and Sampling

LESSON OBJECTIVES

- Observe correct placenta external examination and sampling technique

LESSON LENGTH: 60 MINUTES

LOCATION

- MITS Room(s)

NOTES FOR PARTICIPANTS

Follow along with the demonstration using the MITS Placenta Examination and Sampling Form and MITS Placenta SOP as references, conduct full examination and sampling of placenta.

NOTES FROM MITS PRACTICE

LESSON 4.3
MITS Practice-Placenta Exam and Sampling

LESSON OBJECTIVES

- Demonstrate correct procedure for examining the placenta and obtaining MITS placenta samples.

LESSON LENGTH: 90 MINUTES

LOCATION

- MITS Room(s)

NOTES FOR PARTICIPANTS

The goal of this MITS practice is to practice the sequence and skill required to carry out the Placenta Examination and Sampling SOP.

NOTES FROM MITS PRACTICE

LESSON 4.4
Review of Facility Schematics

LESSON OBJECTIVES

- Incorporate group feedback to troubleshoot and optimize project setup for MITS

LESSON LENGTH: 45 MINUTES

LOCATION

- Classroom

FEEDBACK FROM MITS SKETCH:

LESSON 4.5
Day 4 Debrief

LESSON OBJECTIVES

- Identify and describe the highlights of the day
- Identify the challenges or aspects that remain unclear
- Identify learning goals for Day 5

LESSON LENGTH: 30 MINUTES

LOCATION

- Classroom

NOTES FOR PARTICIPANTS

This is an opportunity to reflect on your learning, identify remaining questions, and set learning goals for the next training day.

1. What went well today?

2. What aspects or lessons remain unclear or challenging?

3. Identify one or two learning goals for tomorrow:

LESSON 5
MITS Training

Lesson 5.1
Practice MITS-Teaching MITS

LESSON OBJECTIVES

- Facilitate dissemination of MITS sample collection knowledge to others

LESSON LENGTH: 180 MINUTES (APPROXIMATELY 90 MINUTES EACH GROUP)

LOCATION

- MITS Room(s)

NOTES FOR PARTICIPANTS

The goal of MITS practice today is to prepare participants to disseminate the skills and knowledge acquired during this training to other members of their project.

NOTES FROM MITS PRACTICE

Lesson 5.2
Training Conclusion, Debrief, and Next Steps

LESSON OBJECTIVES

- Identify and ask remaining questions
- Complete training evaluations
- List next steps and key milestones

LESSON LENGTH: 60 MINUTES

LOCATION

- Classroom

NOTES

SLIDES

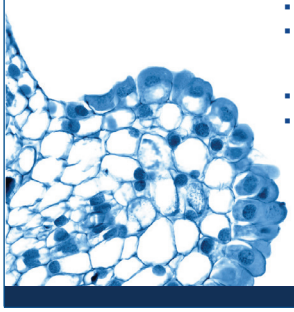
Lesson 5.2 MITS Implementation Moving Forward

Surveillance Site Agreement Milestones and Deliverables

Milestone	Description	Deliverable
1	IRB Approval of the program	IRB Approval Letter
2	Staff trained in MITS	Training certificate
3	25% of MITS utilized	Documentation of MITS used and corresponding QA portal process complete
4	50% of MITS utilized	Documentation of MITS used and corresponding QA process complete
5	75% of MITS utilized	Documentation of MITS used and corresponding QA process complete
6	Program completion report	Program completion report and cost share reporting using the cost share template

2

MITS Kits – Shipment & Customs Reminders



- Secretariat to manage through RTI International logistics, RTI will provide shipping documentation
- Site is responsible for any import permit, as required by country
- Shipment size is generally the number of kits needed for 6 months (in boxes of 10 kits), depending on project storage capacity
- Allow 6 weeks for kit creation and delivery
- Surveillance sites responsible for monitoring inventory, and ordering additional kits using a shipment request form

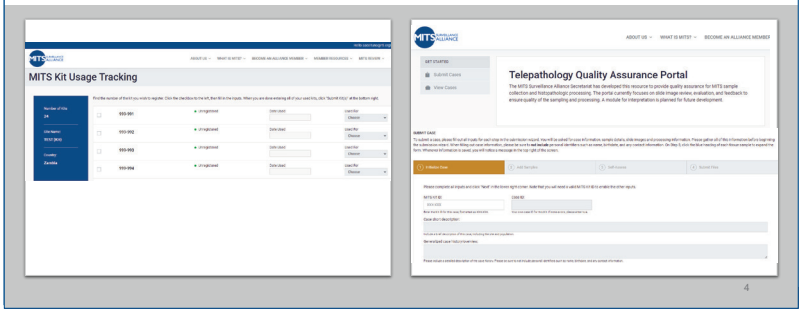
3

NOTES

SLIDES

Documenting kit usage on mitsalliance.org

- Surveillance sites are responsible for documenting kit usage
- Submit 5% cases for quality review via the Telepathology QA Portal
- Milestones 3-5 will be determined using the data entered by the site on mitsalliance.org



Additional Resources

MITSAlliance.org
- Bibliography of MITS references and publications
- Updates on Alliance activities
- MITS in Action features
- Access to member resources

Technical Working Groups (TWGs)
- Monthly meetings
- Receive periodic emails highlighting new MITS resources
- Forum for learning from experienced MITS projects

E-Newsletter
- MITS in Action
- Blog posts
- Recent Alliance member activities

WhatsApp Group
- Forum for posing questions and seeking solutions from entire group
- Opportunity to identify TWG topics

- Thank you for taking part in the MITS training. Please take the time to provide your feedback regarding the training on the course evaluation forms.
- Please note that the MITS Alliance Secretariat will continue to serve as a reference and source of information, and do not hesitate to contact us with questions: MITSAllianceGrants@rti.org.

CONTRIBUTIONS AND ACKNOWLEDGEMENTS

This training represents a collaborative endeavor that includes the University of Nairobi School of Medicine, Kenyatta National Hospital, and RTI International with funding through the Bill & Melinda Gates Foundation.

APPENDICES

Appendix A: MITS Sample Collection SOP

Appendix B: MITS Specimen Collection Form

Appendix C: MITS Placenta SOP

Appendix D: MITS Placenta Collection Form

Appendix E: MITS Histological, Microbiological, and Placenta Sample Transport and Storage Guidance

Appendix F: MITS Histological, Microbiological, (and other) Sample Analysis Guidance

Appendix G: MITS Kit Development Request Form

Appendix H: MITS Kit Shipping Request Form

Appendix A: MITS Sample Collection Standard Operating Procedure (SOP)

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

Version 3.1

January 2023

Version History:

1.0 April 2019

2.0 September 10, 2019

3.0 September 21, 2022

Approvals:

TABLE OF CONTENTS

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
MITS	Minimally Invasive Tissue Sampling
PPE	Personal protective equipment
Filter Paper	*Blood Spot Card*

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 • **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.)

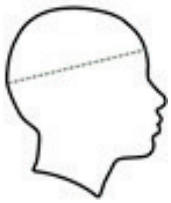
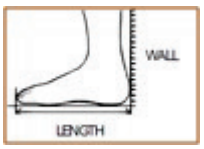
The Specialist places the body on the appropriate type of scale and measures the weight.

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 measureperpendicular 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:

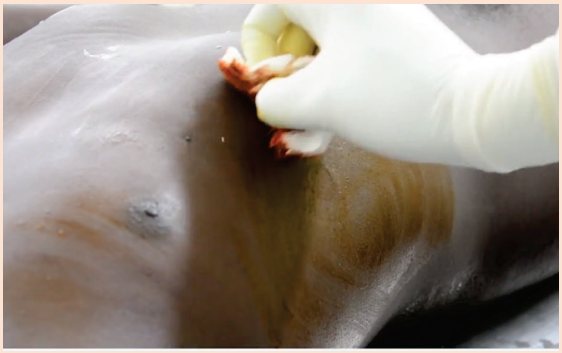
The Specialist cleans with water and then dries with gauze the areas of the body to be punctured. Areas vary by patient age and are indicated for each on the MITS Specimen Collection Form.

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.



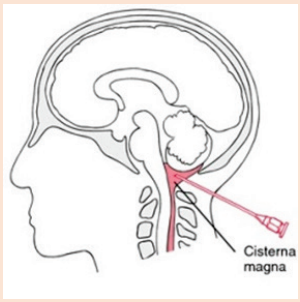
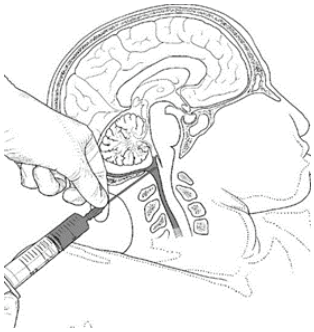
Allow iodine to air dry for at least 5 minutes.

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 **Note: Projects working with perinatal cases may prefer a smaller 10 mL syringe**	Kit Bag 2
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 75º. 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**

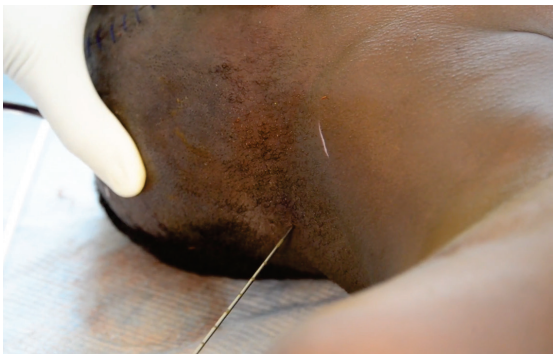
h. The Assistant caps the tube.

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

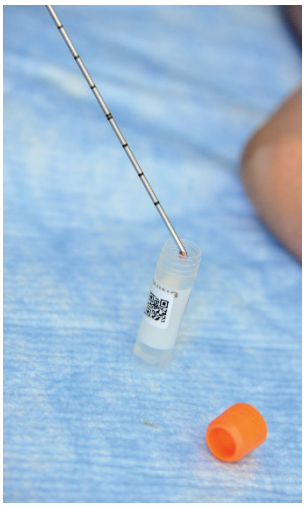
Posterior/Occipital approach-All cases

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	

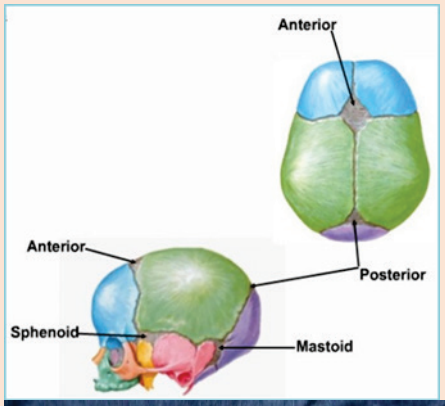
Fontanelle approach – Stillbirth, Neonates, and Infants with OPEN fontanelle Only

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.

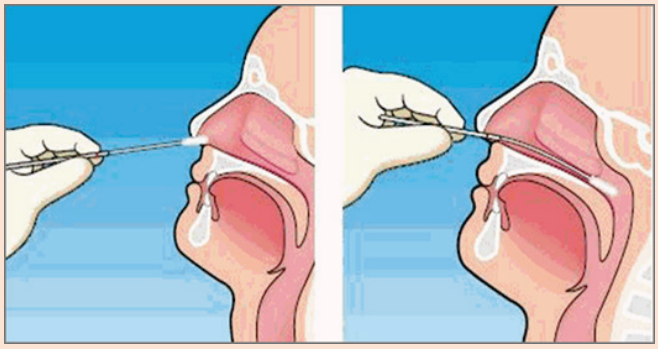
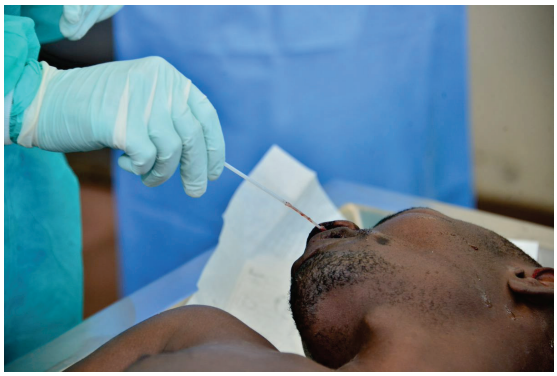


b. Using the same needle that was used for the occipital puncture. The Specialist obtains 6 samples by puncturing the anterior fontanelle and puts them into the same BRAIN/CNS, formalin jar. The Specialist aims the needle in different directions/depths to obtain samples from different regions of the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

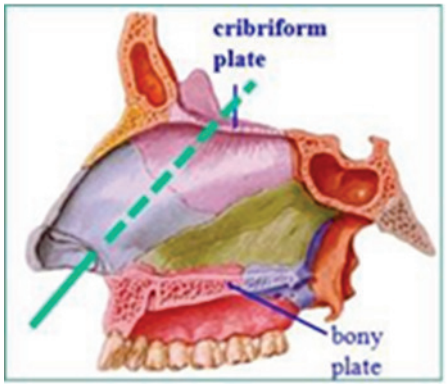
9.5. BRAIN/CNS MITS for Histology Trans-nasal approach – Infant with closed fontanelle, Child, Adult Cases

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.**

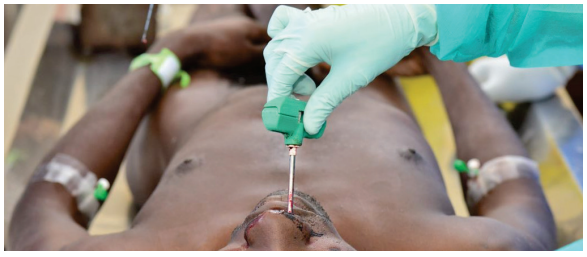
a. The Assistant takes the bone puncture trephine and opens the container of a bone marrow biopsy needle.



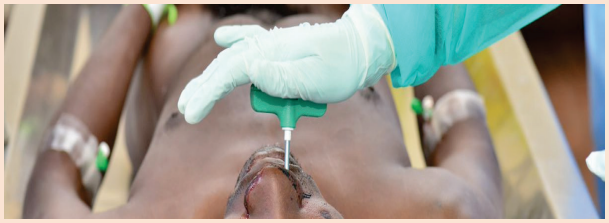
b. The Specialist inserts the stylet into trephine.

c. The Specialist introduces the needle with the internal guide into the nasal cavity with and angle of 45º, 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 45º, 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.

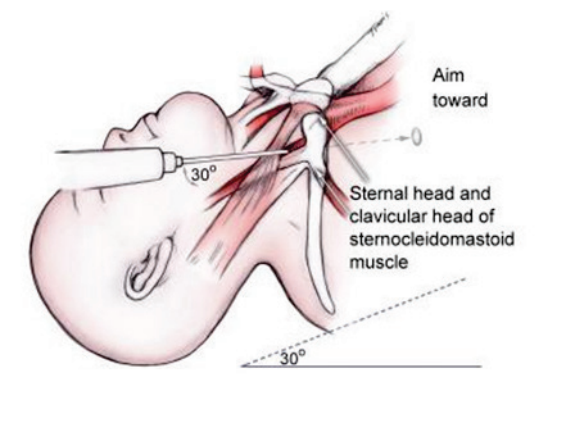
j. Specialist repeats steps f through i, advancing the needle in different directions/depths to obtain specimens from different regions of the brain. At least 6 specimens should be obtained.

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 30º 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 infra-clavicular 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.

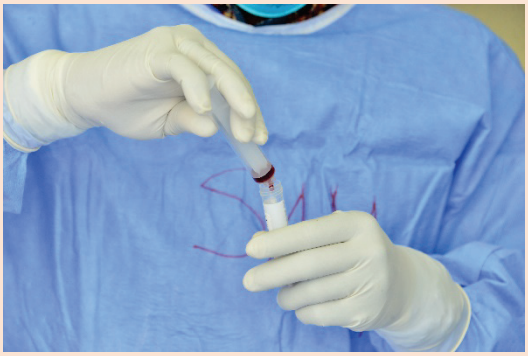
h. Aspirate and redirect the needle as needed until obtaining blood.

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

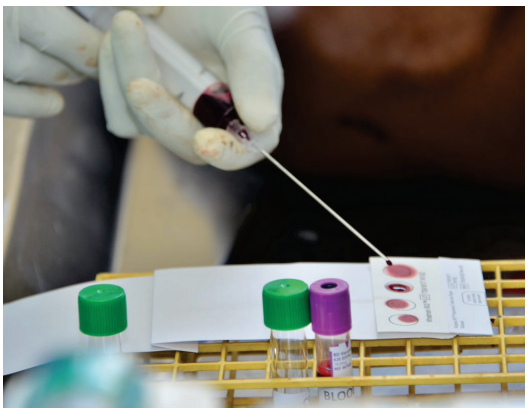
In case serum is obtained instead of blood, try again. In the case that no blood is obtained the second time, put the serum in the EDTA container only.

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.



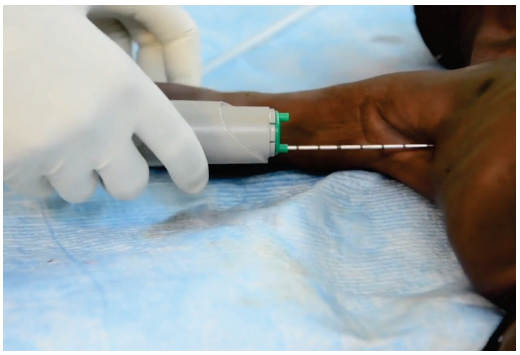
l. The Assistant fills the required information on the MITS Specimen Collection form.

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

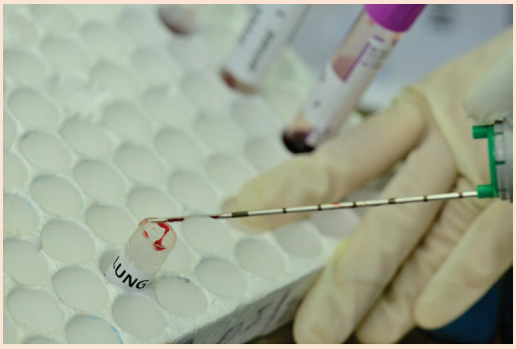
m. The Assistant stores the EDTA tube in the MITS test tube rack and the blood spot card on the MITS tray.

9.7. Lung/Thorax for MITS for Microbiology

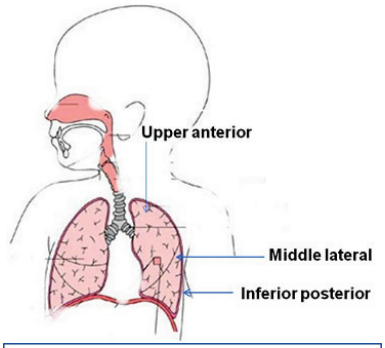
Materials Needed	MITS KIT Location
Bard Monopty 16G 100mm (unlabeled)	Kit Bag 1
1 LUNG 2mL cryogenic vial (pre-labeled)	Kit Bag 2

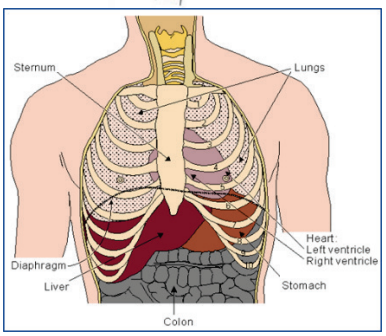
MITS Specimen Collection
Note: That if pleural effusion is suspected the fluid should be collected as extra sample.
a. The Assistant prepares the second, new, disposable Bard Monopty 16 G biopsy needle, and the LUNG cryogenic vials from the MITS kit.
b. The Specialist performs the puncture with the automatic 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.

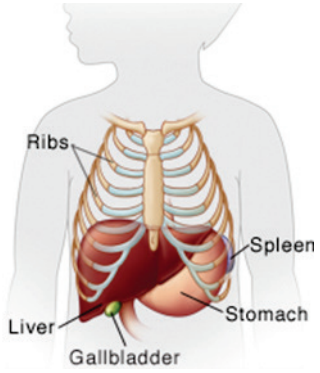
9.9. Liver MITS for Microbiology

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



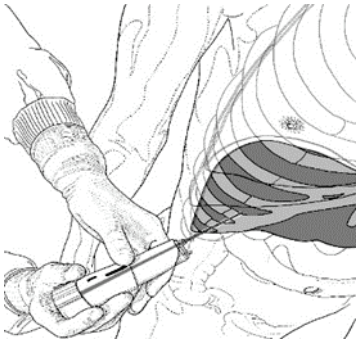
b. The Specialist performs a puncture with the needle in the mid-axillary line, in one of the three last intercostal spaces.

- The needle should be oriented 30º in a cranial and 15º 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

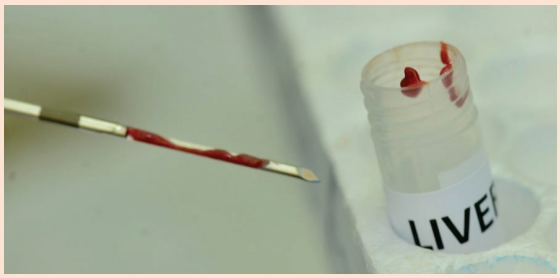
d. The Specialist removes the needle

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

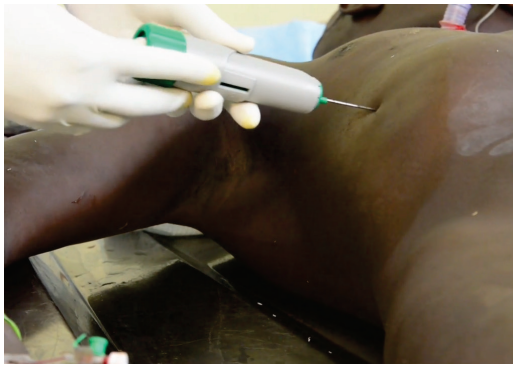
9.10. Liver MITS for Histology

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.

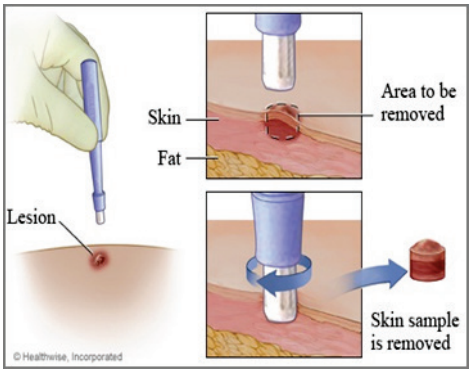
9.11. Rectal Stool Sample

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
a. The Assistant takes the rectal brush from the MITS kit and a 10mL sterile tube from the MITS box.
b. The Assistant opens the protective wrap of the rectal brush and gives it to the Specialist.
c. The Assistant positions the head and leg as needed.
d. The Specialist inserts the rectal brush 3 to 4 cm into the rectum and gently rotates. 
e. The Specialist removes the brush. 
f. If sample is collected, the Assistant opens the protective wrap of the 10mL sterile tube, labels the tube and gives it to the Specialist.
g. The Specialist places the brush into the vial and breaks off the top portion of the stick and discards it.
h. 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

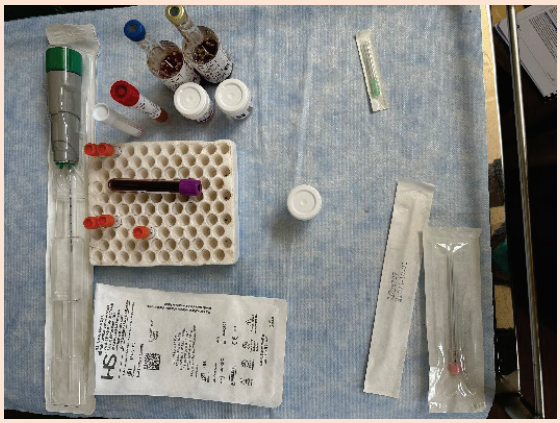
10.1. MITS Specimen Collection Form

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

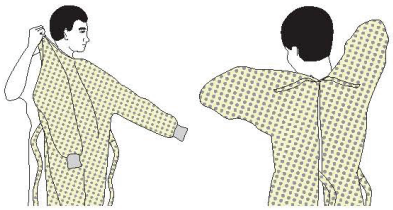
10.5. Donning and Removing Personal Protective Equipment

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

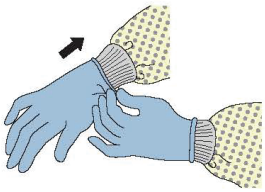
- Secure ties or elastic bands at middle of head and neck
- Fit flexible band to nose bridge
- Fit snug to face and below chin
- Fit-check respirator

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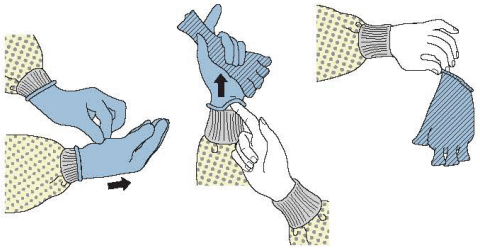
HOW TO SAFELY REMOVE PERSONAL PROTECTIVE EQUIPMENT (PPE) EXAMPLE 1

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

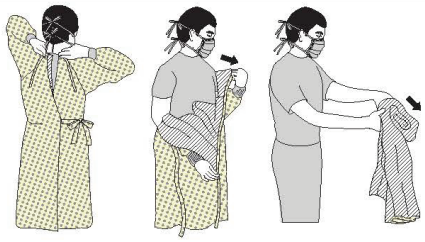


2. GOGGLES OR FACE SHIELD

- 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

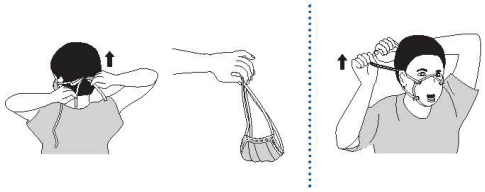
3. GOWN

- 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

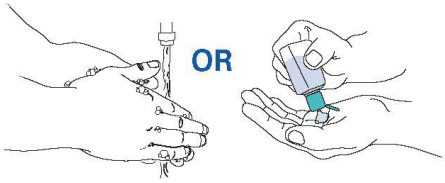


4. MASK OR RESPIRATOR

- 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
0.1	Tape Measure	1
0.2	Disposable placemat	2
	Bag 1	**Units**
1.1	Bard Monopty needle 16G, 100mm *Lung/thorax, liver*	2
1.2	Bard Monopty needle 16G, 160mm *Brain*	1
1.3	Bone Marrow Trephine *Brain transnasal approach if closed fontanelle*	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) *Perinatal/infant CSF collection*	1
2.2	21G 1.5" intramuscular needle (green) *Perinatal/infant blood collection*	1
2.3	18G 3.5" spinal puncture needle (pink) *Child/adult CSF and blood collection*	2
2.4	20mL syringes *CSF, blood collection*	2
2.5	10mL EDTA vacutainer (purple top, pre-labeled) *Blood*	1
2.6	10mL sterile tube (prelabeled) *CSF, rectal swab*	2
2.7	2mL cryogenic storage vials (pre-labeled) *Brain, lung/thorax, liver*	3
	BAG 3 – Histology	**UNITS**
3.1	Large (120 ml) screw cap jar (pre-labeled) *Storage of fixed tissue cassettes in ethanol*	1
3.2	Tissue cassettes (unlabeled) *Brain, right lung/thorax, left lung/thorax, liver*	4
3.3	20 mL jars (prefilled 10% formalin) (pre-labeled) *Brain, right lung/thorax, left lung/thorax, liver*	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 *2 placenta sampling, 1 back-up*	3
E.5	Millimeter ruler (5 cm) *2 placenta sampling, 1 back-up*	3
E.6	Scalpel blade #24 *2 placenta sampling, 1 back-up*	3
0.1	Tape Measure *2 back-up*	2
0.2	Disposable placemat *2 placenta sampling, 2 back-up*	4
	Bag 1	**Units**
1.1	Bard Monopty needle 16G, 100mm *Lung/thorax, liver*	2
1.2	Bard Monopty needle 16G, 160mm *Brain*	1
1.3	Biopsy punch needle 5mm *Skin*	2
1.4	Bone Marrow Trephine *Transnasal approach if closed fontanelle*	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) *Perinatal/infant CSF collection*	1
2.2	21G 1.5" intramuscular needle (green) *Perinatal/infant blood collection*	1
2.3	18G 3.5" spinal puncture needle (pink) *Child/adult CSF and blood collection*	2
2.4	20mL syringes *CSF, blood collection*	2
2.5	10mL EDTA vacutainer (purple top, pre-labeled) *Blood*	1
2.6	10mL sterile tube (prelabeled) *CSF, rectal swab*	2
2.7	2mL cryogenic storage vials (pre-labeled) *3 back-up, 4 extra samples (e.g. skin), 2 placentas* 3 samples*	13
3.1	Forceps *2 placenta sampling*	2
3.2	Scalpel handle *Placenta sampling (reusable)*	1
3.3	Large (120 ml) screw cap jar *2 placenta sampling, 1 back-up*	3
3.4	Tissue cassettes *4 back-up, 2 extra samples (e.g. skin), 2 placentas* 4 cassettes*	14
3.5	20 mL jars (prefilled 10% formalin) *same as cassettes*	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	

Appendix B: MITS Specimen Collection Form

MITS Specimen Collection (01) (2023/03/21 v2.0)

Study-Specific/Autopsy ID: _____

PLACE MITS KIT ID HERE

Form Instructions: Complete one form per MITS case, marking positive findings.

1. Preparation

Ensure appropriate personal protective equipment (PPE) is worn by all MITS personnel prior to beginning the MITS procedure: ☐ gown ☐ cap ☐ mask ☐ goggles ☐ shoe covers/boots ☐ gloves (x2) ☐ None

☐ Confirm MITS kit contains all items in the list of contents, arrange items on disinfected surface, and apply labels to tubes as needed. Gather all materials and reagents not included in the MITS kit prior to starting the MITS specimen collection. Check supplies available in backup box.

☐ **Check name and informed consent.**

2. Overview

Site/Location _____ MITS Specialist _____ MITS Assistant _____

Time and date of birth _____ (24 hour) ☐ Unknown _____ (DD/MM/YYYY) ☐ Unknown

Time and date of death _____ (24 hour) ☐ Unknown _____ (DD/MM/YYYY) ☐ Unknown

Confirm MITS Case Type ☐ Stillbirth ☐ Neonate ☐ Infant (1-11 months) ☐ Child/Pediatric (1-16 years) ☐ Adult (> 16 years)

If **Stillbirth** or **Neonate**, provide Gestational Age (GA) in weeks _____ ☐ Unknown

Method of GA Determination? ☐ Sure LMP ☐ Early ultrasound ☐ Uncertain LMP ☐Late ultrasound ☐ Other, specify _____

Circumstances preventing MITS? ☐ Y ☐ N If yes, describe: _____

Time and date MITS initiated _____ (24 hour) _____ (DD/MM/YYYY)

Placenta submitted? ☐ Y ☐ N ☐ N/A *If yes, complete Placenta Collection and Examination Form (08)*

Photographs taken (***check all that apply***): ☐ Front ☐ Back ☐ Side ☐ Nails/Hand ☐ Other gross lesions ☐ No photos

If No photos taken, explain why: _____

If other photos, describe: _____

Is face in full view in the frontal picture? ☐ Y ☐ N

**If additional space is required for description use the "Other notes" in Section 6.*

3. Anthropometric Measurements

Date of measurements _____ (DD/MM/YYYY)

Weight _____ ☐ g ☐ kg ☐ NA (only > than 16 years)	Height/length _____ (cm)
MUAC (6month- 5years) _____ (cm)	Head circumference (up to 12 months)_____ (cm)
For Stillbirth ONLY: Right lower leg length _____(cm)	Right foot length _____(cm)

4. Body Inspection and Palpation

Date of gross findings _____ (DD/MM/YYYY)

Sex ☐ Male ☐ Female ☐ Indeterminate

Evidence of trauma(*check all that apply*): ☐ No ☐ Bruises ☐ Lacerations ☐ Abrasions ☐ Burns ☐ Fractures ☐ Bleeding ☐ Fragmentation (stillbirths) ☐ Other

If present, describe: _____

Appearance of bodyweight ☐ Normal ☐ Obese ☐ Cachectic ☐ Thin

Petechiae ☐ Y ☐ N ☐ No exam If yes, location: _____

Rash ☐ Y ☐ N ☐ No exam If yes, location: _____

Other skin/mucosal lesions ☐ Y ☐ N If yes, describe: _____

Bleeding from(*check all that apply*): ☐ No bleeding ☐ Mouth ☐ Ears ☐ Nose ☐ Rectum ☐ Other

Gross facial abnormalities ☐ Y ☐ N ☐ No exam If yes, describe: _____

Jaundice/Icterus ☐ Y ☐ N ☐ No exam

Abdominal distention ☐ Y ☐ N ☐ No exam

Lymphadenomegaly ☐ Y ☐ N ☐ No exam If yes, location of enlarged lymph nodes: _____

Hepatomegaly ☐ Y ☐ N ☐ No exam

Splenomegaly ☐ Y ☐ N ☐ No exam

Umbilicus abnormal ☐ Y ☐ N ☐ No exam If yes, describe: _____

Other significant gross findings ☐ Y ☐ N If yes, describe: _____

**If additional space is required for description use the "Other notes" in Section 6.*

Study-Specific/Autopsy ID: _____

5. Body Cleaning and Sterilization

Clean the areas of the body to be punctured before beginning the MITS procedure.

Specific areas to be cleaned are indicated in Section 6 for each category of MITS case.

☐ 1. Clean with water. Dry with gauze.
☐ 2. Clean with abundant alcohol, using circular movements from the center to the periphery. Wait 5 minutes.
☐ 3. Clean with abundant iodine, using circular movements from the center to the periphery. Wait 5 minutes.

6. Specimen Collection

Note/Reminder: Numbers in parentheses indicate the number of cores to be taken

☐ **Stillbirth** Maceration level: ☐ None, fresh ☐ Grade 1 ☐ Grade 2 ☐ Grade 3	☐ **Neonate** ☐ **Infant, open fontanelle**	☐ **Infant, closed fontanelle** ☐ **Child/Pediatric** ☐ **Adult**
Cleaning completed: ☐ nuchal area ☐ anterior fontanelle ☐ thorax ☐ axillae ☐ abdomen	**Cleaning completed:** ☐ nuchal area ☐ anterior fontanelle ☐ nares ☐ thorax ☐ axillae ☐ abdomen	**Cleaning completed:** ☐ nuchal area ☐ nares ☐ thorax ☐ axillae ☐ abdomen
CSF ☐ Y ☐ N Total volume collected: ____ (ml) ☐ Clear ☐ Turbid ☐ Purulent ☐ Hematic	**CSF** ☐ Y ☐ N Total volume collected: ____ (ml) ☐ Clear ☐ Turbid ☐ Purulent ☐ Hematic	**CSF** ☐ Y ☐ N Total volume collected: ____ (ml) ☐Clear ☐ Turbid ☐ Purulent ☐ Hematic
CNS, posterior - cryovial (3) ☐ Y ☐ N	**CNS, posterior - cryovial** (3) ☐ Y ☐ N	**CNS, posterior - cryovial** (3) ☐ Y ☐ N
CNS, posterior - formalin jar (6) ☐ Y ☐ N	**CNS, posterior - formalin jar** (6) ☐ Y ☐ N	**CNS, posterior - formalin jar** (6) ☐ Y ☐ N
CNS, fontanelle - formalin jar (6) ☐ Y ☐ N	**CNS, fontanelle - formalin** jar (6) ☐ Y ☐ N	CNS, fontanelle: DO NOT COLLECT
If any of the brain, posterior and fontanelle, specimens were not collected, specify which and why they were not collected: _____	If any of the brain, posterior and fontanelle, specimens were not collected, specify which and why they were not collected: _____	If any of the brain, posterior specimens were not collected, specify which and why they were not collected: _____
NP swab: DO NOT COLLECT	**NP swab** ☐ Y **label tube* ☐ N	**NP swab** ☐ Y **label tube* ☐ N
CNS transnasal: DO NOT COLLECT	CNS transnasal: DO NOT COLLECT	**CNS, transnasal - formalin jar** (6) ☐ Y ☐ N If any of the brain, trans-nasal, specimens were not collected, specify which and why they were not collected: _____
Blood ☐ Y ☐ N Total volume collected: _____ (ml) Location collected from: ☐ Supraclavicular ☐ Heart ☐ Other: _____ 1. Culture tube - 0.5-5 ml ☐ Y ☐ N 2. EDTA tube - 1.5-9 ml ☐ Y ☐ N **invert EDTA tube after filling* 3. Blood spot card circles ☐ Y ☐ N **completely fill circles*	**Blood** ☐ Y ☐ N Total volume collected: _____ (ml) Location collected from: ☐ Supraclavicular ☐ Heart ☐ Other: _____ 1. Culture tube - 0.5-5 ml ☐ Y ☐ N 2. EDTA tube - 1.5-9 ml ☐ Y ☐ N **invert EDTA tube after filling* 3. Blood spot card circles ☐ Y ☐ N **completely fill circles*	**Blood** ☐ Y ☐ N Total volume collected: _____ (ml) Location collected from: ☐ Supraclavicular ☐ Heart ☐ Other: _____ 1. Culture tube - 0.5-5ml ☐ Y ☐ N 2. EDTA tube - 1.5-9 ml ☐ Y ☐ N **invert EDTA tube after filling* 3. Blood spot card circles ☐ Y ☐ N **completely fill circles*

Study-Specific/Autopsy ID: _____

R & L lung - cryovial (6) ☐ Y ☐ N If the right and left lung specimen was not collected, specify why it was not collected: _____	**R & L lung - cryovial** (6) ☐ Y ☐ N If the right and left lung specimen was not collected, specify why it was not collected: _____	**R & L lung - cryovial** (6) ☐ Y ☐ N If the right and left lung specimen was not collected, specify why it was not collected: _____
Left lung - formalin jar (6) ☐ Y ☐ N If any of the left lung specimens were not collected, specify which and why they were not collected: _____	**Left lung - formalin jar** (6) ☐ Y ☐ N If any of the left lung specimens were not collected, specify which and why they were not collected: _____	**Left lung - formalin jar** (6) ☐ Y ☐ N If any of the left lung specimens were not collected, specify which and why they were not collected: _____
Right lung - formalin jar (6) ☐ Y ☐ N If any of the right lung specimens were not collected, specify which and why they were not collected: _____	**Right lung - formalin jar** (6)☐ Y ☐ N If any of the right lung specimens were not collected, specify which and why they were not collected: _____	**Right lung - formalin jar** (6)☐ Y ☐ N If any of the right lung specimens were not collected, specify which and why they were not collected: _____
Liver - cryovial (3) ☐ Y ☐ N	**Liver - cryovial** (3) ☐ Y ☐ N	**Liver - cryovial** (3) ☐ Y ☐ N
Liver - formalin jar (6) ☐ Y ☐ N	**Liver - formalin jar** (6) ☐ Y ☐ N	**Liver - formalin jar** (6) ☐ Y ☐ N
If any of the liver specimens were not collected, specify which and why they were not collected: _____	If any of the liver specimens were not collected, specify which and why they were not collected: _____	If any of the liver specimens were not collected, specify which and why they were not collected: _____
DO NOT COLLECT	**Rectal swab** ☐ Y ☐ N	**Rectal swab** ☐ Y ☐ N

Additional samples collected (*e.g., skin, effusions, etc.)*

☐ Bone Marrow ☐ Hair ☐ Skin ☐ Urine

Describe any additional samples collected: _____

Other notes: _____

_____

7. Sample Collection Summary

Check all samples taken below.

	Histology	Microbiology
CSF	-	☐
Blood	-	☐
Brain/CNS	☐	☐
Left Lung/Thorax	☐	☐
Right Lung/Thorax	☐	☐
Liver	☐	☐
Extra 1: _____	☐	☐
Extra 2: _____	☐	☐
Other: _____	☐	☐
Other: _____	☐	☐

8. End of Procedure

MITS procedure end time: _____ (24 hour)

☐ Check for excessive seepage or bleeding. Clean body with water as needed.

☐ Confirm all the containers and jars are properly labeled and closed.

☐ Confirm any unused, labeled materials either have their labels removed or are disposed of in a biowaste container.

☐ Confirm any unused, unlabeled materials are added to a backup box.

☐ Confirm all sharps are disposed in sharps container.

☐ Confirm MITS rack with all the used cryovials in the MITS cool box.

☐ Confirm MITS tray with the used formalin jars, the unused large screw-cap jar, any tissue cassettes in the MITS kit box.

☐ Confirm all used surfaces are washed and sterilized, digital camera is stored/charging.

Appendix C: MITS Placenta SOP

Placenta
Gross Examination and Sampling Standard Operating Procedure (SOP)

Incentive Grant Awardees

Version 2.0
October 28, 2019

Version History:
1.0 – September 10, 2019

Table of Contents

1. Purpose

The purpose of this Standard Operating Procedure (SOP) is to describe the procedures for placental gross examination and sampling.

2. Scope

This document is relevant to all placentas from stillbirths and preterm neonates.

This document also includes instructions for sampling for Placenta Microbiology and Placenta Histology (Section 4 and Section 7). Please note this information is not represented in the Placenta Collection Form.

NOTE: Placenta gross examination and sample collection can occur even if MITS is not done, as long as the mother provides consent.

3. Safety

- Don personal protective equipment (PPE).
- Dispose of needles and all waste generated during procedure in appropriate container as per laboratory protocols.

4. Sampling for Microbiology

Note: All sampling for microbiology should occur as soon as possible to avoid cross-contamination and must occur before any contact with formalin (fixation).

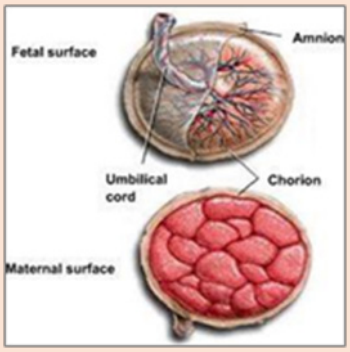
Materials Needed	MITS Kit Location
Placenta cryogenic vial	Backup Box Bag 1
Membrane cryogenic vial	Backup Box Bag 1
5 cm measuring ruler	Backup Box Envelope
Scalpel blade #24 (3)	Backup Box Envelope
Scalpel Handle *provided in box BU001 only, reusable	Backup Box Bag 3
Forceps (3)	Backup Box Bag 1
MITS Placental Collection Form	Backup Box Envelope

Microbiology Specimen Collection
After the gross examination, the placenta should be sampled.
a. The Assistant takes placenta and MEMBRANE cryogenic vials from the MITS kit.
b. The Specialist cuts with the first scalpel blade two pieces of tissue 2mm3 from the: • Placenta parenchyma maternal side. • Placenta parenchyma maternal side.
c. Using the forceps, the Specialist places two pieces in the placenta cryogenic vial.
d. Using a new set of forceps, the specialist places two pieces in the membrane cryogenic vial.
e. Using a new scalpel blade, the specialist cuts two thin transverse sections of the cord (one from the fetal end and one from the maternal end).
f. Using new forceps, the specialist places the two pieces of cord in the cord cryogenic vial.

5. Gross Examination

Materials Needed	Location
Scale	Project- specific
Large receptacle to transport placenta	Project- specific
Label for receptacle	Project- specific
Photo ID Card	MITS Backup Box
Knife	Project- specific
5 cm measuring ruler	MITS Backup Box Envelope
MITS Placental Collection Form	MITS Backup Box

5.1. Placenta Disc and Description

Placenta Disc
a. Following delivery, the cord and placenta should be collected.
NOTE: Samples for PCR/Microbiology are collected prior to placement in formalin.
b. The Assistant takes photos of the maternal and fetal sides, cord and membranes, and other gross lesions including the photo card in the setting. 
c. The Specialist performs a thorough visual inspection and determines if the fetal and maternal sides are normal. If abnormal, the Assistant describes abnormalities on the Placental Collection Form.
d. The Specialist measures the maximal linear length and width (Greatest dimension of the axis perpendicular to this linear measurement) of the placenta disc. 

e. The specialist measures the cord from the insertion site to the nearest placental margin.

f. The Specialist removes the cord by using the scalpel to trim near the insertion site. Using the scissors the specialist then removes the membranes and sets both the membranes and cord aside.

g. The Specialist places the whole placenta (cord and membranes removed) on the scale.

h. The Assistant records if the pregnancy was a singleton, twin, triplet, or other on the Placenta Collection Form.

i. The Specialist determines if there is subchorionic fibrin, masses, the presence of hematoma, or any other gross findings.

j. The Assistant records the information on the Placenta Collection Form.

5.2. Umbilical Cord

Umbilical Cord
a. The Specialist measures the diameter, length, and distance between the insertion site and the nearest placental margin.
b. The Specialist describes the appearance of the umbilical cord. The Specialist examines and notes the presence of thrombosis, strictures, discoloration, and the number of vessels present on the umbilical cord.
c. The Assistant records the information on the Placenta Collection Form.

5.3. Membranes

Membranes
a. The Specialist describes the color and opacity of the membranes
b. The Specialist determines if the membranes are complete
c. The Specialist determines if the membranes are Circumvallate (%), Circummarginate (%), Neither, and/or other.
d. The Assistant records the information on the Placenta Collection Form Section 4.

6. Fixation and Sectioning

Materials Needed	MITS Kit Location
Knife (sterilized)	Site -specific
Cutting board	Site -specific
Formalin	Site -specific
Large container	Site -specific
Tray (for photo)	Site -specific
5 cm measuring ruler	Backup Box Envelope
MITS Placental Collection Form	Backup Box Envelope

Placenta Fixation and Sectioning
a. The Specialist uses the scalpel or a knife to 1-2cm "breadloaf" cuts, without cutting all the way through on the maternal surface of the placenta to enhance its preservation in formalin solution. 
b. The placenta, membranes and cord are placed in a large container with 10% formalin solution. **NOTE: Ideal volume of formalin should be 10x volume of tissue**.
c. Fix placenta for 24-48 hours
d. Following fixation. Rinse the placenta with water to remove formalin.
e. Using a knife, the specialist serially sections the disc from the maternal to the fetal surface at 2 cm interval and examines each slice for parenchymal lesions (e.g. infarcts)

f. The Assistant photographs all the sections together. All the placental sections are put on a clean surface and a photo is taken. If placental lesions are identified be sure to document with additional photos.

g. The Specialist re-evaluates the fixed specimen for lesions (infarcts may be more apparent after fixation).

h. The Specialist measures the mural minimal and maximal thickness.

i. The Assistant records the measurements in centimeters on the Placenta Collection form.

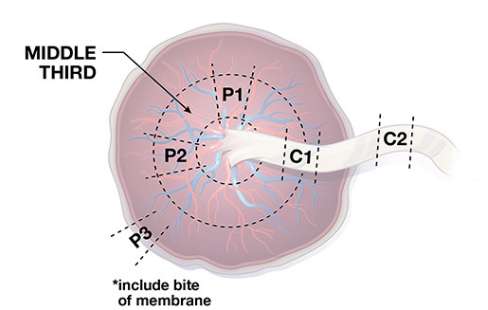
j. The Specialist describes and photographs any lesions.

k. The Specialist re-determines if there is increased parenchymal fibrin, subchorionic fibrin, masses, classification, presence of infarcts, hematoma, or any other gross findings.

l. The Assistant records the information on the Placenta Collection Form Section 5.

7. Sampling for Histology

Materials Needed	MITS Kit Location
Cutting board	Site-specific
5 cm measuring ruler	Backup Box Envelope
Scalpel blade #24 (2 blades)	Backup Box Envelope
Forceps	Backup Box Bag 3
Cassettes	Backup Box Bag 3
MITS Placental Collection Form	Backup Box Envelope

Specimen Collection	
a. After the gross examination. The Specialist should sample the following from the placenta.	
b. Extra placental membrane roll	
c. Umbilical cord – 5mm fetal end	
d. Membrane roll-1-2mm sections	
e. Full thickness normal appearing placenta 1-2mm section Ideally these are from middle third of the placenta....thirds being concentric rings. If the transmural thickness is greater than the length of the cassette, divide the gross slice and submit it in two cassettes: The upper third (chorionic plate and subjacent tissue) and lower third (basal aspect) of the parenchyma.	 *https://www.cdc.gov/zika/hc-providers/test-specimens-at-time-of-birth.html
f. Placental lesion 1 (Describe)	
g. Placental lesion 2 (Describe)	
h. Placental lesion 3 (Describe)	

8. End of the Procedure and Collection Form

8.1. Placenta Collection Form

After the Placenta Collection
a. The Assistant reviews the form and stores it in the project specific location.

8.2. Containers and Tools

After the Placental 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 are stored in a backup box and any unused materials labeled with an ID are disposed of in the biowaste container.
c. The Assistant washes the permanent marker with 70% ethanol and the surfaces used during the process with the standard 10% bleach followed by 70% ethanol to dry.

9. Appendices

9.1. Appendix A: Back-up box Components needed for Placental Sampling

9.2. Appendix B: Labeling systems formalin jars, cryogenic vials, slides

9.1. Appendix A: Backup Box Components Needed for Placental Sampling

Note: Backup 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.4	Photo card *2 placenta sampling, 1 backup*	3
E.5	Millimeter ruler (5 cm) *2 placenta sampling, 1 backup*	3
E.6	Scalpel blade #24 *2 placenta sampling, 1 backup*	3
	NO BAG	**UNITS**
0.1	Tape measure *2 backup*	2
0.2	Disposable placemat *2 placenta sampling, 2 backup*	4
2.7	2mL cryogenic storage vials (pre-labeled) *3 backup, 4 extra samples (e.g., skin), 2 placentas*, 3 samples*	13
	Bag 3 – Histology	**UNITS**
3.1	Forceps *2 placenta sampling*	2
3.2	Scalpel handle *Placenta sampling (reusable)*	1
3.3	Large (120 ml) screw cap jar *2 placenta sampling, 1 backup*	3
3.4	Tissue cassettes *4 backup, 2 extra samples (e.g. skin), 2 placentas*, 4 cassettes*	14
3.5	20 mL jars (prefilled 10% formalin) *Same as cassettes*	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).

9.2. Appendix B: Labeling Systems Formalin Jars, Cryogenic Vials, Slides

I. Formalin Jar Labels (Histology)

Tissue Specimen Description	Label Sample ID	Label Text	Label location
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
Placental disc membrane junction	1##-###-08-V	Placenta	In Kit envelope
Umbilical cord	1##-###-09-V	Cord	
Placenta	1##-###-10-V	Placental Memb.	
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
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	

10. Reference

Khong TY, Mooney EE, Ariel I, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. Arch Pathol Lab Med. 2016; 140(7):698-713.

Appendix D: MITS Placenta Collection Form

Placenta Collection and Evaluation (08) (2023/01/10) (v1.0)

Place MITS KIT ID and Placental ID HERE

Study-Specific/Autopsy ID: _____

Name of person who completed the form: _____

Date of completion: __ __ / __ __ / _ _ _ _ (DD/MM/YYYY)

Form Instructions: Mark only what is present

☐ Following delivery, the cord and placenta should be collected.

☐ Samples for PCR/Microbiology are collected prior to placement in formalin and as soon as possible.

☐ Weight and photographs should be taken PRIOR to fixation of the placenta in formalin for > 24 hours.

1. Overview

Site ID _____ MITS specialist _____ MITS assistant _____

Date of exam _ _ /_ _ /_ _ _ _ (DD/MM/YYYY) Time of exam __ __ : __ __ (24 hour)

2. Placental Disc and Description

Note: Measurements should be taken prior to fixation

Photographs taken (check all that apply): ☐ Maternal side ☐ Fetal side ☐ Cord and membranes ☐ Other gross lesions

Is the fetal side normal: ☐ Yes ☐ No - If No, Describe:_____

Is the maternal side normal: ☐ Yes ☐ No - If No, Describe:_____

Weight (*cord and membranes removed*) _____ (g)

Maximal linear length _____ (cm)

Maximal linear width _____ (cm)

Pregnancy was: ☐ Singleton ☐ Twin ☐ Triplet ☐ Other, specify:_____

Presence of hematoma: ☐ Yes ☐ No If yes, Specify:_____

If hematoma:

% of maternal surface involved _____ (%)

Subchorionic fibrin: ☐ Yes ☐ No If yes, Specify:_____

Masses: ☐ Yes ☐ No If yes, Specify:_____

Other gross findings: ☐ Yes ☐ No

If yes, Describe:_____

3. Umbilical Cord

Diameter of umbilical cord _____ (cm)

Length of umbilical cord _____ (cm)

Distance between insertion and nearest placental margin _____ (cm)

Velamentous insertion of umbilical cord: ☐ Yes ☐ No If yes, Specify:_____

Presence of knots: ☐ Yes ☐ No If yes, Specify:_____

Appearance of umbilical cord: ☐ Hypercoiled ☐ Hypocoiled ☐ Other, Specify:_____

Presence of thrombosis: ☐ Yes ☐ No If yes, Specify:_____

Presence of strictures: ☐ Yes ☐ No If yes, Specify:_____

Presence of discoloration: ☐ Yes ☐ No If yes, Specify:_____

Number of vessels: ☐ 2 ☐ 3 ☐ Other specify:_____

4. Membranes

Describe color: ☐ White ☐ Brown/Green ☐ Red/purple ☐ Other, Specify:_____

Describe opacity: ☐ Clear ☐ Slightly opaque ☐ Opaque ☐ Other, Specify:_____

Are membranes complete: ☐ Yes ☐ No ☐ Other (specify): _____

Are the membranes:

☐ Circumvallate: _____% ☐ Circummarginate_____%

☐ Neither ☐ Other, Specify:_____

☐ Make membrane roll

Placenta Collection and Evaluation (08) (2023/01/10) (v1.0)

5. Placenta Fixation and Sectioning

☐ Fix placenta for 24 hours. *There should be a 10:1 ratio formalin to placenta*

☐ Following fixation rinse placenta with water to remove formalin

☐ Section

1. "Breadloaf" entire placenta at 1-2 cm intervals
2. Photograph the sections together (*all on the same tray)*
3. Re-evaluate fixed specimen for lesions (*infarcts may be more apparent after fixation*)

Mural minimal thickness _____ (cm)

Mural maximal thickness _____ (cm)

Any additional lesions: ☐ Yes (If yes, photograph) ☐ No ☐ Other, Specify:_____

If lesions: Total number_____

Location ☐ Central/paracentral ☐ Peripheral ☐ Diffuse

☐ Other, Specify:_____

Increased parenchymal fibrin: ☐ Yes ☐ No If Yes, Specify:_____

Subchorionic fibrin: ☐ Yes ☐ No If Yes, Specify:_____

Masses: ☐ Yes ☐ No If Yes, Specify:_____

Calcification: ☐ Yes ☐ No If Yes, Specify:_____

Presence of infarcts: ☐ Yes ☐ No If Yes, Specify:_____

If infarcts: Total number_____

% of total parenchymal volume_____ (%)

Presence of hematoma: ☐ Yes ☐ No ☐ Other, Specify:_____

If hematoma: ☐ Marginal ☐ Retroplacental ☐ Subchorionic

% of maternal surface involved _____ (%) ☐ N/A

Other gross findings: ☐ Yes ☐ No

If yes, Describe:_____

6. Placental Sample Collection

Check all samples taken below.

SAMPLE	SAMPLE ID
Extra placental membrane roll	☐
Umbilical cord - 5cm fetal end	☐
Umbilical cord - 5cm from placental insertion	☐
Full thickness normal appearing placenta - 1	☐
Full thickness normal appearing placenta - 2	☐
Disc membrane junction	☐
Placental lesion 1 (Describe)	☐
Placental lesion 2 (Describe)	☐
Placental lesion 3 (Describe)	☐

7. End of Procedure

☐ Confirm all the containers and jars are properly labeled and closed.

☐ Confirm any unused cryovials and jars are disposed of in a biowaste container.

☐ Confirm all used surfaces are washed, and the digital camera is stored and charging.

Appendix E: MITS Histological, Microbiological, and Placenta Sample Transport and Storage Guidance

MITS HISTOLOGICAL, MICROBIOLOGICAL, AND PLACENTA SAMPLE TRANSPORT AND STORAGE GUIDANCE

Histological Sample Storage

1. Put histology samples (except placenta) in buffered formalin for 4-6 hours.
2. After 4-6 hours transfer tissues from formalin jars to tissue cassettes.
3. Place cassettes in a large container filled with distilled water or 70% ethanol/alcohol. Tissues can remain here for a maximum of 5 days.
4. Put cassettes in tissue processor.
5. Prepare the paraffin blocks.
6. Prepare the histological sections.
7. Stain the sections with hematoxylin and eosin.
8. Mount the sections.
9. Allow slides to dry for 1 day.

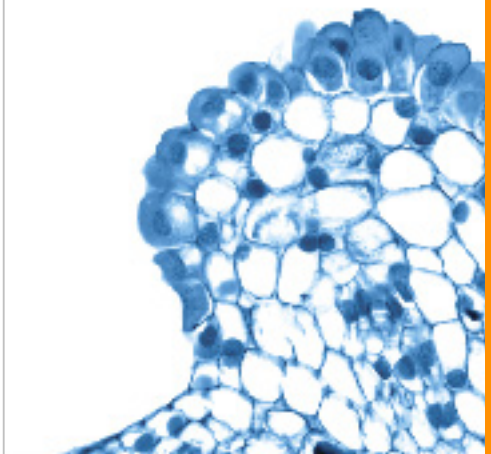
Microbiological Sample Storage

1. Samples in cryovials or tubes should be transported dry or preferably in solution.
   - Culture analysis—transport medium or culture medium
   - Molecular analysis—DNA/RNA stabilization buffer
2. Place all cryovials/tubes in cool box on ice packs immediately following conclusion of MITS procedure.
3. **NOTE**: blood cultures should be stored at room temperature.
4. Please adhere to the cool storage guidelines to maintain optimal sample quality.

CONDITION	TEMP (C°)	MAXIMUM STORAGE TIME (APPROX.)
Cool box with ice packs	2-8°	8 hours
Dry ice	< -80°	Duration of international shipment
Standard refrigerator	2-8°	24 hours
Standard freezer	-20°	1-3 years
Super-cooled freezer	-80°	1-10 years

Placenta Storage

1. Fixation of the placenta.
   - Place placenta in container filled with 10% buffered formalin (ideally 10X the volume of the placenta) for at least 24 hours.
2. Following fixation, rinse with water and proceed with sample collection.
3. Place samples in cassettes.
4. Place cassettes in a large container filled with distilled water or 70% ethanol/alcohol. Tissues can remain here for a maximum of 5 days.
5. Put cassettes in tissue processor.
6. Prepare the paraffin blocks.
7. Prepare the histological sections.
8. Stain the sections with hematoxylin and eosin.
9. Mount the sections.
10. Allow slides to dry for 1 day.



Appendix F: MITS Histological, Microbiological, (and other) Sample Analysis Guidance

1. OVERVIEW

This guidance is intended to support cause of death determination by providing a framework for testing of MITS samples (brain, lung, liver, blood, and cerebrospinal fluid [CSF]) based on histological evaluation. This guidance is not prescriptive and should be adapted to reflect the objectives and resources of the MITS team. This guidance has been organized first by age group and then by type of MITS sample.

Acronyms used in this summary:

- CMV: cytomegalovirus
- CNS: central nervous system
- CSF: cerebrospinal fluid
- GMS: Grocott methenamine silver stain
- H&E: hematoxylin and eosin
- HIV: human immunodeficiency virus
- IHC: immunohistochemistry
- ISH: *in situ* hybridization
- PCR: polymerase chain reaction
- RSV: respiratory syncytial virus
- SARS-Cov-2: severe acute respiratory syndrome—coronavirus
- TORCH group: *Toxoplasma gondii, Treponema pallidum*, parvovirus B19, lymphocytic choriomeningitis virus, rubella virus, cytomegalovirus, and herpes virus

2. MITS IN ADULTS, CHILDREN, AND NEONATES (>24 HOURS OF LIFE)

General considerations to be considered in these age groups:

- Routine hematoxylin and eosin (H&E) stain should always be performed in all the samples collected for histology.
- Microbiological testing should be directed by histological abnormalities identified in each of the main organs. However, several tests can be applied to all cases, depending on epidemiological specificities of the area.
- Several tests are specific to age groups.

A. Blood

Samples suitable for microbiological testing:
Plasma obtained after centrifugation of the whole blood

Microbiological testing (all samples):
Serology for HIV-1/2 antibodies (plasma)

Microbiological testing (depending on epidemiological features of the area):

- Antibodies against Hepatitis C and B virus
- PCR for *Plasmodium falciparum* (in malaria-endemic areas)

Microbiological testing (only in stillbirths and neonates):
PCR for pathogens included in the TORCH group

Other microbiological testing (depending on clinical and pathological findings):

- 16S PCR (if bacterial infection suspected)
- 18S PCR (if fungal infection suspected)
- PCR for *Mycobacterium tuberculosis* (if mycobacterial infection suspected)

B. Liver

Samples suitable for microbiological testing: Liver MITS sample collected for microbiology (frozen fresh tissue)

HISTOLOGICAL FEATURE	POSSIBLE INFECTIOUS AGENTS	MICROBIOLOGICAL TESTS	HISTOLOGICAL STAINS
Neonatal giant cell hepatitis (neonates)	Epstein-Barr virus, CMV, bacteria (*Brucella, Treponema pallidum*)	Specific PCR for each of the viruses, viral serology	IHC (CMV), Epstein-Barr ISH
Hepatitis/cirrhosis	Hepatotropic viruses (A, B, C, D, E)		
Granulomas (caseating or non-caseating)	*Mycobacterium*, fungi (*Histoplasma, Cryptococcus, Schistosoma*, other)	PCR mycobacteria 16S PCR	Ziehl-Neelsen stain, GMS stain, Warthin–Starry stain
Parenchymal abscess/es	Gastrointestinal bacteria (*Clostridium spp, S. aureus*, gram-negative bacteria), parasites (*Entamoeba histolytica*)	16S PCR	Gram stain
Pigment in Kupffer cells	Parasite (*Plasmodium spp*)	PCR for Plasmodium	Polarized light, IHC for malaria, iron stain to differentiate hemosiderin

C. Central Nervous System

Samples suitable for microbiological testing: CSF and CNS samples obtained by occipital approach (frozen fresh tissue)

HISTOLOGICAL FEATURE	POSSIBLE INFECTIOUS AGENTS	MICROBIOLOGICAL TESTS	HISTOLOGICAL STAINS
Meningeal inflammation (leukocytes)	Bacteria (*N. meningitidis, S. Pneumoniae. Haemophilus spp, Listeria, Mycobacteria*), fungi (*Candida, Mucorales, Aspergillus, Cryptococcus*)	16S PCR, 18S PCR	Gram stain, GMS stain
Lymphocytic inflammatory infiltrate (meninges and brain)	Viruses (Herpes viruses, SARS-CoV-2, among other)	Specific PCR, viral serology (plasma)	IHC for Herpes virus 1 and 2, IHC for SARS-CoV-2
Parenchymal abscess/es	Fungi (*H. capsulatum, C. immitis, B. dermatitidis, C. bantiana*), parasites (e.g., Toxoplasmosis), bacteria	18S PCR, serology for Toxoplasma, 16S PCR	GMS stain, IHC for Toxoplasma, Gram stain
Granulomas (caseating or non-caseating)	*Mycobacterium*, fungi (*Aspergillus, Cryptococcus*)	PCR mycobacterium, 18S PCR	Ziehl-Neelsen stain, GMS stain
Pigment in brain capillaries	Parasite (*Plasmodium* spp)	PCR for Plasmodium	Polarized light, IHC for malaria

D. Lungs

Samples suitable for microbiological testing: Lung samples (frozen fresh tissue)

HISTOLOGICAL FEATURE	POSSIBLE INFECTIOUS AGENTS	MICROBIOLOGICAL TESTS	HISTOLOGICAL STAINS
Bronchopneumonia/ Parenchymal abscess/es (neutrophilic inflammation)	Bacteria (*Streptococcus pneumoniae, Haemophilus influenzae*, etc.), fungi (*Pneumocystis, Cryptococcus*, etc.)	16S PCR, 18S PCR	Gram stain, GMS stain
Viral pneumonia (interstitial lymphocytic inflammation)	Virus (Respiratory syncytial virus (RSV), SARS-CoV-2, cytomegalovirus, parainfluenza, etc.)	Specific PCR for each of the viruses, Viral serology	IHC for Herpes virus 1 and 2, IHC for SARS-CoV-2
Granulomas (caseating and non-caseating)	*Mycobacteria*, fungi (*Aspergillus, Cryptococcus*, etc.)	PCR mycobacteria, 18S PCR	Ziehl-Neelsen stain, GMS stain
Pigment in lung capillaries	Parasite (*Plasmodium*)	PCR for Plasmodium	Polarized light, IHC for malaria

3. MITS IN STILLBIRTHS AND NEONATES (<24 HOURS OF LIFE)

General guidance to be considered in these populations:

- Routine H&E stain should always be performed in all the samples collected for histology.
- Microbiological testing should be directed by histological abnormalities identified in each of the main organs. However, several tests can be applied to all cases, depending on epidemiological specificities of the area.

A. Blood, liver, central nervous system

Microbiological testing can be performed but probably will not contribute to the cause of death attribution considering that the majority of causes of death in this group will be related to maternal conditions and placental abnormalities.

B. Lungs

Samples suitable for microbiological testing: Lung MITS sample collected for Microbiology

HISTOLOGICAL FEATURE	POSSIBLE INFECTIOUS AGENTS	MICROBIOLOGICAL TESTS	HISTOLOGICAL STAINS
Bronchopneumonia	Bacteria (*Streptococcus agalactiae, Salmonella*, etc)	16S PCR	Gram stain
Nuclear inclusions	Virus (CMV, other)	TORCH panel real-time PCR*	IHC for CMV

*If CMV infection is detected by PCR, testing placenta, blood, and other tissues and using IHC to confirm disseminated CMV disease is recommended.

C. Placenta

HISTOLOGICAL FEATURE	POSSIBLE INFECTIOUS AGENTS	MICROBIOLOGICAL TESTS	HISTOLOGICAL STAINS
Chorioamnionitis/ funisitis	Bacteria (*S. agalactiae, E. coli* other), fungi (*Candida*)	16S PCR, 18S PCR	Gram stain, GMS stain
Acute villitis or intervillositis	Bacteria (*S. agalactiae, T. pallidum, Listeria monocytogenes, E. coli*, other), fungi (*Candida*)	16S PCR, 18S PCR	Gram, Warthin–Starry, GMS stains
Massive fibrinous deposition, necrosis of the trophoblast	Virus (SARS-CoV-2)	PCR for SARS-CoV-2	IHC for SARS-CoV-2
Nuclear inclusions	Virus (CMV, other)	TORCH panel real-time PCR	IHC for CMV

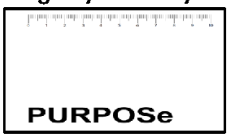
Appendix G: MITS Kit Development Request Form

MITS Kit Development Request Form

We will use the below information to create the following four documents for your approval:

1. MITS SOP
2. Placenta Gross Examination SOP *(if applicable to your study population)*
3. MITS Kit contents list
4. MITS backup box contents list

Once the above documents are finalized, IRB approval is complete, and your team has completed MITS training, you can submit a MITS kit shipping request form to order your first shipment of MITS Kits and backup boxes.

MITS Kit Development Request Form	
Awardee First and Last Name	
Date of MITS Kit development request (dd/mm/yy)	
Shipping Contact/Recipient - Full Name	
Address 1	
Address 2	
Address 3	
City	
State/Region	
Postal Code	
Country	
Shipping Contact/Recipient Phone Number	
Shipping Contact/Recipient Email	
Email addresses for other points of contact: (person other than the primary awardee who should be including on emails related to the shipment of MITS Kits)	
Additional comments: (special instructions for RTI related to shipping or customs documentation)	
Total Number of Kits Awarded	☐ 30 ☐ 50 ☐ 100 ☐ 150
Study Population(s)	☐ Perinatal (stillbirth and neonates < 28 days of age) ☐ Infant (29 days - < 1 year of age) ☐ Child (1-19 years of age) ☐ Adult (> 19 years of age)
Number of kits, including backup boxes, requested per shipment (based on local storage capacity)	☐ 20 ☐ 30 ☐ 40 ☐ 50 ☐ 60 ☐ 70 ☐ 80 ☐ 100
Please chose the short study name (< 20 characters) corresponding to your study for MITS photo cards. **Example:** 	☐ KazCoDe Study ☐ DECODE MAUN ☐ MITZ Study ☐ MITS at CHUK ☐ MITS in Neonates, Ethiopia ☐ EMECaD ☐ MITS-BiNS ☐ MITS at MAHAN ☐ MITS in Belgaum ☐ MITS at CHUB ☐ MITS in Argentina ☐ ZPRIME- MITS ☐ If incorrect/prefer other, specify:_____

MITS Kit Development Request Form

Do you require backup boxes for this shipment? (~ 1 backup box per 10 kits)	☐ Yes ☐ No
Do you want BARD biopsy needles in your backup boxes?	☐ Yes ☐ No -- *Check no if you have sufficient supply remaining from backup boxes from previous shipments.*
Pathology supplies needed?	☐ Yes *If yes, specify* ☐ Unlabeled histology cassettes ☐ Jar to store cassettes in ethanol ☐ No
Do you approve the MITS Surveillance Alliance sample identification code (see page 3)	☐ Yes ☐ No, *specify:* _____ _____ _____ _____
QR codes required on labels? (see photos of labels on page 4 for example)	☐ Yes ☐ No
Location to write study ID or case ID required on labels? (see photos of labels on page 4 for example)	☐ Yes ☐ No
Labels for histology slides needed?	☐ Yes ☐ No

Please check the boxes next to the samples the study will collect for each population, to inform the MITS kit contents:

Sample Type	*Perinatal Microbiology*	*Perinatal Histology*	*Infant Microbiology*	*Infant Histology*	*Child/Adult Microbiology*	*Child/Adult Histology*
Cerebral spinal fluid	☐	*n/a*	☐	*n/a*	☐	*n/a*
Brain	☐	☐	☐	☐	☐	☐
Nasopharyngeal swab	☐	*n/a*	☐	*n/a*	☐	*n/a*
Blood	☐	*n/a*	☐	*n/a*	☐	*n/a*
Lungs	☐	☐	☐	☐	☐	☐
Liver	☐	☐	☐	☐	☐	☐
Rectal swab	☐	*n/a*	☐	*n/a*	☐	*n/a*
Skin biopsy (*of lesions*)	*n/a*	☐	*n/a*	☐	*n/a*	☐
Placenta	☐	☐	*n/a*	*n/a*	*n/a*	*n/a*

If sampling placenta from stillbirths or neonates: Proportion of MITS estimated to have placentas	☐ 10%	☐ 20%	☐ 30%	☐ 40%
	☐ 50%	☐ 70%	☐ 80%	☐ 100%

MITS Kit Development Request Form

MITS Surveillance Alliance Sample Identification Code for MITS samples:

| 1 | 00 | - | 000 | - | 00 | - | Z | - | 0 |

| MITS S.A. | Site ID | - | MITS Kit ID 001+ | - | Sample Code | - | Container Code | – | Duplicate # (optional) |

Sample Code		Container Code		Duplicate Number
01	CSF	P	Blood Spot Paper	1, 2, 3
02	NP Swab	E	EDTA	(if multiple samples are collected for one sample type)
03	Brain/CNS	H	Blood Culture	
04	Blood	T	NP Swab Tube	
05	Lung/Thorax	J	Formalin Jar	1= Left Lung, 2 = Right Lung
06	Liver	V	Cryogenic Vial	
07	Rectal Swab	C	Cassette	
08	Placental Membrane	B	Block	
09	Umbilical Cord	S	Slide	
10	Placenta	X	Extra	
11	Extra 1			
12	Extra 2			

MITS Kit Development Request Form

Example labels with MITS ID, QR Code, and space to write in Study ID:

Appendix H: MITS Kit Shipping Request Form

MITS Kit Shipping Request Form

We will use the below information to create the shipping orders for the MITS Kits.

MITS Kit Shipping Information	
Awardee First and Last Name	
Date of request	
Shipping Contact/Recipient - Full Name	
Address 1	
Address 2	
Address 3	
City	
State/Region	
Postal Code	
Country	
Shipping Contact/Recipient Phone Number	
Shipping Contact/Recipient Email	
Email addresses for other points of contact: (person other than the primary awardee who should be including on emails related to the shipment of MITS Kits)	
Additional comments: (special instructions related to shipping or customs documentation. RTI will cover the cost and logistics of kit shipment and customs clearance)	
Have you received IRB approval? *	☐ Yes ☐ No
Have you completed MITS training? *	☐ Yes ☐ No
Have you approved a final MITS SOP for your study? *	☐ Yes ☐ No
Have you approved final MITS Kit and MITS backup box contents lists? *	☐ Yes ☐ No
Please indicate the number of kits and backup boxes would you like shipped in this shipment. If this is the first shipment, we recommend 18 kits and 2 backup boxes. If any changes need to be made to kit contents or labeling, they can be made for the second shipment.	_____ # MITS kits _____ # MITS backup boxes Ideally the total boxes (kits plus backup boxes) should be a multiple of 5 (e.g., 18 kits + 2 backup boxes = 20 total boxes).

**If any of the above are "NO," please contact the MITS Alliance Secretariat to finalize before submitting a MITS Kit request.*