SDC 1. Standard operating procedure for conducting minimal invasive tissue sampling:

Safety (appendix)

Wear standard PPE (gloves, lab coat, respiratory and eye protection). Dispose of needles and all waste generated during procedure in appropriate container as per laboratory protocols.

**Cleaning and sterilization of the body**

a. Palpate the head to determine whether the fontanels (anterior and posterior) are opened.

b. The Medical Officer/Clinical Associate cleans with water the areas of the body to be punctured (nuchal area, thorax, upper abdomen, iliac crests, anterior and posterior fontanel if opened at palpation and urethral opening in males and females).

c. The Medical Officer/Clinical Associate dries the above-mentioned areas with gauze.

d. The Medical Officer/Clinical Associate cleans with abundant alcohol the areas of the body to be punctured. The cleaning should be made with circular movements from the center to the periphery. The alcohol should act for at least 5 minutes.

e. The Medical Officer/Clinical Associate cleans with abundant iodine solution the areas of the body to be punctured. The cleaning should be made with circular movements from the center to the periphery. The iodine solution should act for at least 5 minutes.

**Blood sample collection**

a. The Nurse/Research Assistant prepares a new and sterile 18 G spinal puncture needle, a 20 mL syringe, the EDTA-containing vacutainer, the pediatric blood culture bottle, and the filter paper from the MITS kit box.

b. The Nurse/Research Assistant opens the container of the 20 mL syringe and the 18 G spinal needle and gives them to the Medical Officer/Clinical Associate.

c. The guide of the needle should be withdrawn before the puncture.

d. The Medical Officer/Clinical Associate locates the midpoint of the clavicle (mid-way between the sternal notch and acromioclavicular joint) and insert needle 1 cm lateral and superior or inferior to the clavicle.

e. For supraclavicular approach use an angle of 30 degrees above the clavicle and aim inferior, attempting to first aim for the clavicle. Once the clavicle is passed, connect the syringe and pull the plunger to make negative pressure.

f. Maintaining the negative pressure with the plunger, continue 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.

g. In case no blood is obtained with this supraclavicular approach, use an infra-clavicular puncture aiming to reach the subclavial vein behind the clavicle.

h. Fill the syringe with as much blood as possible. In stillbirths and neonates, the amount of blood collected may be very small.

i. The Nurse/Research Assistant takes the pediatric blood culture bottle.

j. The Medical Officer/Clinical Associate inoculates 5 mL (or half of the collected blood) in the pediatric blood culture bottle. If less than

k. The Nurse/Research Assistant takes the EDTA-containing vacutainer.

l. The Medical Officer/Clinical Associate inoculates 5 mL (or half of the collected blood) into the EDTA-containing vacutainer.

m. The Nurse/Research Assistant takes the filter paper.

n. The Medical Officer/Clinical Associate deposits four large drops of blood onto the labeled filter paper.

o. In the case that no or very little blood is obtained following this procedure, perform a heart puncture, using the same needle, aiming to obtain an adequate sample.

p. In this case, puncture the thoracic region left central, fifth intercostal space (palpate the ribs to identify the intercostal spaces) in a parasternal location using the same needle and syringe. The needle should penetrate 5-6 cm in a sagittal direction. Aspirate and move the needle until obtaining blood.

q. The Nurse/Research Assistant fills the required information on blood in the body fluid section of the MITS specimen collection form.

r. In some cases (many stillbirths, some neonates, a few children) the amount of blood that can be collected is very small. In these cases, this small amount should be prioritized:

i. Culture (1.5 ml)

ii. EDTA vacutainer (0.5 ml)

iii. Filter paper

**Nasopharyngeal sample collection**

a. The Nurse/Research Assistant takes the nasopharyngeal Swab™ Screw Cap container 12 X 80mm with 1 ml ViCUM from the MITS kit.

b. The Nurse/Research Assistant opens the Nasopharyngeal Swab Screw Cap container.

c. The Medical Officer/Clinical Associate tilts the body head back 70 degrees and inserts swab into the nostril (until resistance is met at turbinates).

d. Insert the swab into nostril. The swab should reach depth equal to distance from nostrils to outer opening of the ear.

e. Leave swab in place for several seconds to absorb secretions.

f. Slowly remove swab while rotating it.

g. Swab both nostrils with same swab.

h. The Medical Officer/Clinical Associate places the swab into the previously identified container deep enough that the medium covers the cotton tips. The Medical Officer/Clinical Associate breaks off the top portion of the stick and discards it.

i. The Nurse/Research Assistant fills the required information on nasopharyngeal sample in the body fluid section of the MITS specimen collection form.

**Liver and abdominal MITS**

a. The Nurse/Research Assistant takes the biopsy needle Bard Monopty 16G, 100 mm from the MITS specimen collection kit.

b. The Nurse/Research Assistant takes the cryovials #1 and #2 and the formalin jars #1 and #2 from the MITS specimen collection kit.

c. The Medical Officer/Clinical Associate rotates the handle of the needle until it is ready for the biopsy (arrow in the top slot).

d. The Medical Officer/Clinical Associate 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 2-5 cm depending on the age of the patient.

e. The Medical Officer/Clinical Associate engages the biopsy needle by pressing with the thumb the button at the back end of the handle.

f. The Medical Officer/Clinical Associate removes the needle.

g. The Medical Officer/Clinical Associate opens the needle to obtain the sample by rotating the handle in a clockwise direction.

h. The Medical Officer/Clinical Associate checks that a correct tissue cylinder has been obtained (the cylinder should be about 20 x 1 mm and show a brownish color).

i. Put the sample in the cryogenic tissue storage vial #1.

j. At least 2 samples from different areas should be put in the cryovial.

k. Repeat the same procedure (steps c to g) for the cryogenic tissue storage vial #2. Use 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.

l. At least 2 samples from different areas should be put in the cryovial.

m. Repeat the puncture, using the same entry point but changing the direction of the needle, to obtain 6 biopsies of the liver. Put them in the formalin jar #1.

n. Repeat the procedure (steps c, e f, g) using two different approaches in the abdomen, one postero-lateral right and the other postero-lateral left, aiming to obtain kidney, spleen and other abdominal organs. Put them in the formalin jar #1. At least 2 samples should be obtained.

o. Repeat the procedure (steps c, e f, g, k, l) and put the samples in the formalin jar #2. Use the same entry points used in the previous punctures. At least 6 samples from the liver and 2 form other abdominal organs should be obtained.

p. The Nurse/Research Assistant fills the required information on liver in the liver tissue section of the MITS specimen collection form.

q. In the case liquid leakage is observed though the puncture points, indicating that ascites fluid may be present, the Nurse/Research Assistant will take a 20 mL syringe, a 18 G spinal needle and a 10 mL sterile vial form the MITS backup box

r. The Nurse/Research Assistant identifies the 10 mL sterile vial with one of the extra labels

s. The Medical Officer/Clinical Associate will withdraw the guide of the needle and connect the syringe

t. A new puncture will be performed using the same entry point that shows ascites fluid leakage.

u. Fill the syringe with 10-20 mL of ascites fluid.

v. The Nurse/Research Assistant opens the 10 mL sterile vial, and the Medical Officer/Clinical Associate transfers the ascites fluid from the syringe to the vial.

w. The Nurse/Research Assistant fills the required information in the MITS specimen collection form.

**Right thorax MITS for microbiology**

a. The Nurse/Research Assistant takes and prepares the second, new, automatic Monopty 16 G biopsy needle from the MITS specimen collection kit.

b. The Nurse/Research Assistant takes the cryovials #3 and #4, from the MITS specimen collection kit.

c. The Medical Officer/Clinical Associate rotates the handle of the needle until it is ready for the biopsy (arrow in the top slot).

d. The Medical Officer/Clinical Associate performs the puncture with the automatic needle in the mid-axillary line, thoracic upper region, 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, and the movement of penetration should be quick. After reaching the limit, the needle should be retracted 2-3 cm.

e. The Medical Officer/Clinical Associate engages the biopsy needle by pressing with the thumb the button at the back end of the handle.

f. The Medical Officer/Clinical Associate removes the needle after removing the needle from the chest.

g. The Medical Officer/Clinical Associate opens the needle to obtain the sample by rotating the handle in a clockwise direction.

h. The Medical Officer/Clinical Associate 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). 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.

i. Put the first tissue sample in the cryovial #3.

j. Repeat steps c-h for middle and lower right lung lobes. Use the same entry point used for the first puncture, modifying the direction of the needle (no angle for the middle lobe, 15º caudal for the lower lobe). Put the sample in the cryovial #3. Make sure that a sample from each lobe is included in the container.

k. Repeat the punctures in the right thoracic region (upper, middle and lower lobes) using the same needle and following the same procedure.

l. Put the samples in cryovial #4 (duplicate for CPL).

m. The Nurse/Research Assistant fills the required information on the cryovial sample from the right thorax puncture in the lung section of the MITS specimen collection form.

n. The punctures of the lung can also be obtained using a posterior approach (back of the body, below the inferior angle of the scapula). For this approach, the Nurse/Research Assistant should help the technician 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 lobes.

**Left thorax MITS for microbiology**

a. The Nurse/Research Assistant takes the cryovials #5 and #6 from the MITS specimen collection kit.

b. The Medical Officer/Clinical Associate repeats the sequence of punctures of the right thoracic region in the left thoracic region using the same needle and following the same procedure.

c. Put these samples in cryovials #5 and #6. 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 heart tissue), the puncture should be repeated using the same needle until an adequate sample is obtained.

d. Make sure that a sample from each lung lobe is included in each container.

e. The Nurse/Research Assistant fills the required information on the cryovial sample from the left thorax puncture in the lung section of the MITS specimen collection form.

f. The punctures of the lung can also be obtained using a posterior approach (back of the body, below the inferior angle of the scapula). For this approach, the Nurse/Research Assistant should help the technician 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 lobes.

**Right and left thorax MITS for DNA extraction and PCR**

a. The Nurse/Research Assistant takes the cryovial #14 containing lysis buffer from the MITS specimen collection kit.

b. Using the same needle used for the previous microbiology punctures, the same entry points and following the same procedure, the Medical Officer/Clinical Associate repeats the sequence of punctures in the right and left thoracic region.

c. Put all the samples obtained (from the right and left lung) in the cryovial #14.

d. The Nurse/Research Assistant fills the required information on the cryovial sample from the left and right thorax punctures in the lung section of the MITS specimen collection form.

**Right thorax MITS for histology**

a. The Nurse/Research Assistant takes the formalin jars, #3 and #4, from the MITS specimen collection kit.

b. Using the same needle used for microbiology punctures, the Medical Officer/Clinical Associate repeats the sequence of puncture approaches of the right thoracic region using the same entry points and following the same procedure and puts two samples of each area in the formalin jar #3 (a total of 6 samples from the lung).

c. The Medical Officer/Clinical Associate repeats the sequence of puncture approaches of the right thoracic region using the same needle and following the same procedure and puts two samples of each area in formalin jar #4.

d. The Nurse/Research Assistant fills the required information on the formalin jar sample in the lung section of the MITS specimen collection form.

e. The punctures of the lung can also be obtained using a posterior approach (back of the body, below the inferior angle of the scapula). For this approach, the Nurse/Research Assistant should help the technician 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 lobes.

**Left thorax MITS for histology**

a. The Nurse/Research Assistant takes the formalin jars, #5 and #6, from the MITS specimen collection kit.

b. Using the same needle used for microbiology punctures, the Medical Officer/Clinical Associate repeats the sequence of puncture approaches of the left thoracic region using the same entry points and following the same procedure and puts two samples of each area in formalin jars #5 and #6. At least 6 samples from the lung/heart should be obtained).

c. The Nurse/Research Assistant fills the required information on the formalin jar sample in the lung section of the MITS specimen collection form.

d. In the case liquid leakage is observed though the puncture points, indicating that pleural effusion may be present, the Nurse/Research Assistant will take a 20 mL syringe, a 18 G spinal needle and a 10 mL sterile vial form the MITS backup box

e. The Nurse/Research Assistant identifies the 10 mL sterile vial with one of the extra labels.

f. The Medical Officer/Clinical Associate withdraws the guide of the needle and connect the syringe.

g. A new puncture is performed using the same entry point that shows pleural effusion leakage.

h. Fill the syringe with 10-20 mL of pleural effusion fluid.

i. The Nurse/Research Assistant opens the 10mL sterile vial, and the Medical Officer/Clinical Associate transfers the pleural effusion from the syringe to the vial.

j. The Nurse/Research Assistant fills the required information in the MITS specimen collection form.

k. The punctures of the lung can also be obtained using a posterior approach (back of the body, below the inferior angle of the scapula). For this approach, the Nurse/Research Assistant should help the technician 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 lobes.

**MITS of the brain parenchyma, occipital and fontanelle approach for histology**

a. The Nurse/Research Assistant selects formalin jars#7 and #8 from the MITS specimen collection kit.

b. The Medical Officer/Clinical Associate repeats steps c to l from the previous section using the same needle and the same entry points.

c. At least 6 samples should be obtained for each jar. Take care to use different penetrations in order to get parenchyma from different areas of the brain.

d. The samples are put in formalin jars #7 and #8.

e. The Nurse/Research Assistant fills the required information in the CNS, occipital and fontanelle section of the MITS specimen collection form.

**MITS of the brain parenchyma, transnasal approach for histology**

a. The Nurse/Research Assistant selects formalin jars#9 and #10 from the MITS specimen collection kit.

b. The Medical Officer/Clinical Associate repeats steps h to l from the previous section using the same needle.

c. The Medical Officer/Clinical Associate puts the samples in formalin jars #9 and #10. At least six samples should be obtained for each formalin jar.

d. The Nurse/Research Assistant fills the required information in the CNS transnasal section of the MITS specimen collection form.