

Attachment L – Protocol for Zika virus specimen collection

Specimen Collection

Specimen Collection Sequence Overview

Following completion of informed consent and the questionnaire, the participant is then ready for the specimen collection. As much as possible, all specimens will be collected at the same time and in the same order. Initially visit, all specimens will be collected. In Puerto Rico, participants will be provided with the option to self-collect specimens at home or the study site. Participants will be instructed to bring the specimens to the study state within two hours after specimen collection or to collect the specimens within two hours before the pre-arranged home visit. After 4 weeks (4 collections), if a particular body fluid has two negative RT-PCR results, it will not be collected any more. The participant will continue specimen collection all body fluids have had two consecutive negative RT-PCR results. The blood specimen will be collected at each visit regardless of which body fluid is being collected.

Suggested order of specimens to be collected from women:

1. Blood (4 mL EDTA purple-top tube)/ (5 mL tiger-top tube in Puerto Rico)
2. Urine (also to be used for pregnancy test in Puerto Rico)
3. Saliva/oral swab
4. Vaginal secretion

Suggested order of specimens to be collected from men:

1. Blood (4 mL EDTA purple-top tube)/ (5 mL tiger-top tube in Puerto Rico)
2. Urine
3. Saliva/oral swab
4. Semen

Specimen Labeling and Set-up in Collection Room

The laboratory technician will assemble the necessary materials for specimen collection, depending on which specimens will need to be collected from the participant on that day including:

- A. Blood
 1. (1) 5 mL tiger-top tube
 2. (1) 21 gauge hypodermic needle
 3. (1) 5 mL syringe/vacutainer
 4. (1) Tourniquet
- B. Urine
 1. (1) Specimen cup
 2. Pregnancy dipstick test
- C. Saliva/oral swab
 1. VTM swab
- D. Vaginal swab (vaginal fluid)
 1. VTM swab
- E. Semen specimen
 1. 50 mL conical tube

The specimen tubes will be taken to the research assistant to affix the pre-printed labels to each tube.

For a full visit, this includes:

- 1 Blood tube

- 1 specimen cup
- For women, 2 VTM swabs
- For men, 1 VTM swabs
- For men, 1 50 mL conical tube

Each specimen label has a numbering scheme as follows:

01	01	0001	01	01
(Study	Site	Participant ID	Specimen ID	Visit Serial #)

It is very important to verify that the correct label is placed on the specimen tube, and that the correct specimen is put into the tube that is labeled for that specimen.

Standard Operating Procedure for Blood Collection

Instruction for blood collection by lab technician

- Invite the participant to have a seat in private clinic room or at participants home.
- Check that the participant ID matches the label on the blood tube and that the date is correct.
- Apply tourniquet on upper arm or forearm to identify peripheral vein.
- Clean the skin of participant with alcohol swab.
- Insert needle into the peripheral vein and gently withdraw adequate amount of venous blood into the syringe (or apply tube to vacutainer until filled).
- Release the tourniquet and withdraw the needle gently from the vein.
- Apply dry cotton wool with firm pressure on the injection site.
- Place the syringe through the cap of the blood tube (step not needed if vacutainer is used).
- Dispose of the collection equipment into a sharps container, following biohazard procedure.
- Place the blood tube upright in the designated blood tube rack, within the cooler that is designated for the blood specimens. This cooler does NOT need ice packs.
- Any blood spills are cleaned with absorbent materials and chlorine water or other disinfectant.

Standard Operating Procedure for Urine Collection

- Provide instructions on urine collection.
- Ask female participants if they would like to do a pregnancy test.
- Show the participant where the specimen is to be handed over once they have completed collection.
- Answer any questions.
- Walk the participant to the participant toilet and instruct him/her to wipe the container with disinfectant wipe after collecting the specimen and while still in the restroom, dispose of tissue, and bring the specimen cup with them back into the collection room.
- Stress to the participant the importance of reporting any spillage in the bathroom during collection.
- Assure that the laboratory technician (for males) or nurse (for females) is waiting when the participant returns.

- If participant requests a pregnancy test, place a labeled pregnancy test in the specimen collection area

Instruction on urine collection: Study participant

- Do not clean the penis (for men) or labia (for women) prior to collecting the urine specimens.
- Urinate into the cup provided.
- Do not put anything else into the collection cup.
- Screw the cap on properly and tightly.
- Wipe the outside of the collection cup with paper towel, moistened with chlorine solution or other disinfectant.
- After use, throw the paper towel away in the bin provided.
- Wash your hands with soap and water or alcohol gel before you touch anything else.
- The participant will carry the urine cup to the back specimen collection room.
- The nurse/technician will collect the container and place it in the designated area on the counter.
- For women who have agreed to the procedure, the nurse will then conduct a pregnancy test after all samples have been collected.

Standard Operating Procedure for Oral Fluid Collection

- Laboratory technician will ensure that the collection materials are properly labelled with the participant's ID and date, and place the collection materials – swab and VTM tube – in the collection room.
- The nurse/technician will provide clear spoken instructions on the procedure on how to collect the oral fluid (see below).
- Answer any questions.

Collection of Oral Fluid

- The nurse/technician will explain the procedure below.
- Remove the swab from the wrapper.
- Gently place the collection swab between the lower cheek and gum and gently rub back and forth, repeat between the upper cheek and gum, for a total of about 1 minute.
- Carefully remove swab.
- Insert swab into the correct tube.
- Carefully snap the swab stick at indentation and cap the tube.
- Tighten cap to ensure a tight fit.
- Place in designated rack on table.

Standard Operating Procedure for Semen Collection

- Laboratory technician gives instructions.
- Laboratory technician will ensure that the semen collection container is properly labeled with the participant's ID and date, and place the tube in the collection room in the rack with the cap removed and placed on the table.
- A small amount of lubricant will be placed on a piece of paper towel and placed in the counter inside the room.
- Paper towels that are moistened with 0.05 % chlorine water or other disinfectant will be placed

in a wash basin on the table for the participant to wipe the specimen tube with.

- Laboratory technician will provide spoken instruction on the procedure on how to collect the semen specimen (see below). This will include how to screw the plastic cap onto the tube properly, and will ask the participant to practice doing this with a spare tube.
- Answer any questions.

Leave the patient in the room and instruct him to open the door and notify you when he finishes. Turn the radio to full volume to ensure audio privacy.

Instruction on semen collection: Study participant (posted in back exam room).

- If you need to use a lubricant, apply the lubricant in the body (shaft) of penis. Avoid it from touching the head of the penis.
- Masturbate.
- Once you ejaculate, collect the entire specimen in the semen container. Do not put anything else into the tube. Screw the cap on properly.
- Wipe the outside of the container with chlorine solution or other disinfectant on a paper towel. Throw the paper towel away in the bin provided.
- Leave the tube in the rack provided in the room.
- Wash your hands with soap and water or alcohol gel before you touch anything else.
- Clean up any spills. Spray bottle of disinfectant in the area with spills, clean with paper towels and put paper towels in the bin. Make sure to not step on any spills.
- If you spilled any semen on yourself, you can use the alcohol gel and paper towel. After use, dispose of used towels in the biohazard waste.
- Clean hands with alcohol gel which is located behind the screen. Wait for hands to dry.
- Open the door and tell the technician that you are finished.

Laboratory Technician (collection room)

- Check that ALL specimen tube caps are properly threaded onto the tube (or correct it if not properly threaded).
- Place the VTM into one zip lock biohazard bag with absorbent paper.
- Place semen and urine into a separate zip lock biohazard bag with absorbent paper.
- Remove gloves
- Each bagged specimen will be placed in the cooler with ice packs.
- The specimens are transferred to the study's refrigerator located in the hospital lab at least every 2 hours.

Standard Operating Procedure for Collection of Vaginal Secretions

Self-collection

- Undress from the waist down.
- Get in a position where you can easily reach your vagina (squat, put one leg up, or lie on your back)
- Remove the swab from the wrapper, but be careful to NOT lay it down on any surface.
- While separating the labia with one hand, use the other hand to hold the plastic swab shaft between thumb and forefinger and insert the soft tip of the swab into the vagina approximately 4-5 cm (about the length of one's little finger). Move the swab for 10 seconds, attempting to touch all walls of the vagina.
- Carefully withdraw the swab and place it in the paper wrapper

- Hold out swab and wait for nurse to take swab from hand.
- Ask the nurse if you are done.
- If you are done, wash hands with alcohol gel which is located behind the screen. Wait for hands to dry.

Put clothes back on and come from behind the screen.