

**WOMEN'S INTERAGENCY HIV STUDY
ORAL PROTOCOL
FORM OP03: SALIVA SAMPLE**

COMPLETING THE FORM

GENERAL INFORMATION

Affix the Participant ID label in the space indicated.

Record the visit number.

Be sure the form version is the most current version date.

Record your initials.

Record the date of the saliva collection procedure.

SALIVA SAMPLE

1. Determine whether or not the Participant has eaten within the last 90 minutes (1 1/2 hours)
2. Determine whether or not the Participant has brushed, flossed or rinsed with mouthwash in the last 90 minutes (1 1/2 hours).

Unstimulated Whole Saliva Collection

- 3a. Indicate whether unstimulated whole saliva has been collected by circling the yes or no response code. If yes, follow skip pattern to question 3c (time collection started). If no, proceed to question 3b (reason for not collecting sample). If the oral team attempted saliva collection, but the participant was unable to salivate, oral staff should code this question as "yes" and record the amount collected as zero in question 3e.
- 3c. Record the time at which unstimulated whole saliva collection began in HH:MM format. Do not use military time. Indicate AM or PM by circling the appropriate response code. Proceed to question 3d.
- 3d. Record the time at which unstimulated whole saliva collection ended in HH:MM format. Do not use military time. Indicate AM or PM by circling the appropriate response code. Proceed to question 3e.
- 3e. Record the volume of unstimulated whole saliva collected (must be recorded to the nearest 1/4 ml).

Stimulated Whole Saliva Collection

- 4a. Indicate whether the **first 2 mls/2 mins.** of stimulated whole saliva is to be submitted for microbiologic evaluation by circling the yes or no response code. Proceed to question 4b.
- 4b. Indicate whether the first 2ml/2 min. of stimulated whole saliva were collected.
- 5a. Indicate whether stimulated whole saliva for flow rate determination is to be submitted for HIV viral load testing by circling the yes or no response code. Proceed to question 5b.

- 5b.** Indicate whether stimulated saliva for flow rate determination has been collected (3–5 minutes) by circling yes or no. If yes, skip to question 5d. If no, proceed to question 5c and record the reason stimulated saliva for flow rate determination was not collected. If the oral team attempted saliva collection, but the participant was unable to salivate, oral staff should code this question as “yes” and record the amount collected as zero in question 5f.
- 5d.** Record the time at which stimulated whole saliva collection began in HH:MM format for **flow rate determination**. Do not use military time. Indicate AM or PM by circling the appropriate response code.
- 5e.** Record the time at which stimulated saliva collection ended in HH:MM format. Do not use military time. Indicate AM or PM by circling the appropriate response code. (The time should be three minutes later than the time recorded at question 5c.)
- 5f.** Record the volume of stimulated whole saliva collected (must be recorded to the nearest 1/4 ml).

PROCEDURES

1. Subjects are asked to remove lipstick with a 2 x 2 gauze square and any removable dental prosthesis. For both stimulated whole saliva collections: If it would be more comfortable for the participant to chew with her removable dental prosthesis, she may go ahead and replace them (this should be consistent at every visit, if the participant removes her dental prosthesis at baseline, she should remove them again at follow-up visits).
2. Subjects rinse thoroughly with deionized water and rest for five minutes (no talking, or reading) before saliva collection begins.
3. Subjects are asked to tilt forward with eyes open.
4. After a one-minute practice collection, which is discarded, whole, unstimulated saliva is collected over a five-minute interval by the draining method.^{1,2} Saliva is allowed to drip off the lower lip into a graduated test tube which is fitted with a funnel and kept on ice. At the end of the collection period (5 min.), the subject is asked to expectorate into the test tube. Record the volume to the nearest 1/4 ml (at question 3e) and discard sample. [Note: Sample collection must take place for at least 2 minutes. If sample collection time is under 2 minutes because of accidental swallowing, forceful sneezing or coughing, start over with a new test tube after a brief (2 minute) rest period. If collection time is between 2-5 minutes, record the exact time and volume].
5. Whole stimulated saliva is collected by the spitting method using a standard-size gum base as a stimulant. The frequency of stimulation is controlled by a metronome at about 70 chews/minute. The subject is asked to expectorate saliva into the graduated test tube once/minute. [Remind subjects not to spit out gum base at end of each minute].
6. If the participant ID number does not indicate that the sample should be submitted for microbiologic evaluation (please refer to saliva log), the first 2 ml or 2 minutes (whichever comes first) of stimulated saliva is collected and discarded prior to proceeding to the next step (step #7).

If participant ID number indicates that the sample should be submitted for microbiologic evaluation (again, please refer to saliva log), the first 2 ml or 2 minutes (whichever comes first) of stimulated saliva is collected in a special container and submitted for microbiologic evaluation. Record the collection date and the visit number on the foil-backed specimen label, preprinted with the Participant's ID number. Immediately store in the refrigerator and within 8 hours, ship with polar packs to laboratory at USC. Using the preprinted mailing labels provided by the lab, these first 2 mls/2 minutes of simulated whole saliva are to be shipped overnight, according to the following guidelines to USC (see address below):

¹. NAVAZESH, M. & C.M. CHRISTENSEN. 1982. A comparison of whole mouth testing and stimulated salivary measurement procedures. J Dent Res. 61:1158-1162.

²NAVAZESH, M. Methods for collecting saliva. Reprinted from Saliva as a Diagnostic Fluid. Vol. 694 of the Annals of the New York Academy of Sciences. September 20, 1993.

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Guidelines for shipping saliva specimen to the USC Oral Microbiology Testing Laboratory

- Place test tubes into the compartmentalized plastic canister provided by USC Laboratory. Make sure that all containers are tightly capped and secure to avoid spillage. It is important that a lab request form (in triplicate) provided by the USC laboratory be filled out correctly and accompany each saliva sample that is shipped. Refer to the appendix section of this protocol manual for the proper way of filling out these forms. All three pages of each form must be sent with the saliva specimen to the laboratory (if a site needs a copy for their files, it must be photocopied).
- Place canister in styrofoam fiberboard box (provided by USC laboratory) and secure with shipping tape.
- Place appropriate airbill and biohazard sticker as indicated in the appendix section of the protocol.

Please note: The laboratory is closed on weekends and major holidays, and service is not available after 5:00p.m. on Fridays. The laboratory at USC will provide several polar-tech packs. Additional packs can be ordered by calling Polar Tech at (312) 697-1400 or 1-800- ICE-BRIX.

7. After the first 2 milliliters is collected, stimulated saliva is collected for an additional 3 minutes in a graduated test tube on ice for flow rate determination. Record the volume to the nearest 1/4 ml (at question 5f).

If the participant experiences difficulty providing additional saliva for flow rate determination, this collection time can be increased to as long as 5 minutes.

If the participant ID number does not indicate that the sample should be submitted for HIV viral load evaluation (please refer to saliva log), proceed to the next step (#8).

If participant ID number indicates that the sample should be submitted for HIV viral load evaluation, send saliva specimen to local lab for processing. Saliva specimen must be processed and frozen within 6 hours after collection. To assure this, the person collecting the saliva specimen must make arrangements to deliver saliva from the collection site (clinic) to the local lab within approximately 2 hours. The transport vial with saliva must be wrapped with 2-3 layers of paper towel and placed with an ice pack in the cooler or other insulated container as soon as possible after collecting. Please refer to saliva HIV viral load protocol for local lab saliva preparation.

8. Vortex the specimen and aliquot into cryotubes appropriate for shipment to **BRinc.**, the central repository for long term storage. The cryotubes for **BRinc** should be flat-bottom screw cap 2 ml tubes. The tube height cannot exceed that which will fit into 2 inch high boxes. Record on the foil-backed specimen label (preprinted with the Participant's ID), the specimen collection date, the volume to the nearest 1/4 ml., and the code "SS" to identify the specimen as stimulated saliva from the oral visit. Affix the label to the cryotube. Immediately store in the refrigerator until either it can be shipped on dry ice to **BRinc.** or stored frozen at -70° C until shipment.

General Notes on Saliva Collection:

- Whenever possible, saliva collection should take place between 8:30 and 11:30 a.m. and subjects should be instructed to fast (**i.e., no eating, no smoking, flossing, toothbrushing, or drinking anything besides water**) for 90 minutes prior to the collection visit.
- Whenever possible subjects should be appointed at the same time of day (i.e., a.m. or p.m.) for follow-up visits as they were for the base-line visit. For example, if subject was seen for the first collection visit in the morning, efforts should be made to schedule the participant in a.m. hours for all subsequent visits.
- All salivary samples should be collected on ice and stored in the refrigerator until shipped or frozen as indicated.
- Record all volumes to the nearest 1/4 ml.
- Immediately after finishing saliva collection, label the tubes with the preprinted labels provided. Record on each label the volume, specimen date and the study visit number.