

**WOMEN'S INTERAGENCY HIV STUDY**  
**ORAL PROTOCOL**  
**FORM OP10: SUBGINGIVAL PLAQUE SAMPLES**

COMLETING THE FORM

GENERAL INFORMATION

Affix the Participants ID label in the space indicated.

Record the visit number, which should be the same as the WIHS core visit.

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

Record your initials.

Record the date.

**SUBGINGIVAL PLAQUE SAMPLES**

If participant is edentulous (OP 6 #1 is coded as 1). Form OP 10 is left blank.

- 1.** Indicate if specimens were collected for positive gingival banding scores on the facial or lingual aspects.
- 1a.** Record the tooth number from which these samples were collected.
- 2.** Indicate if specimens were collected from papilla sites with scores of 3 or 5.
- 2a.** Record the tooth numbers (i.e., the most mesial tooth bounding the interdental space) from which the sample was collected.
- 2b.** Indicate the Papillary Assessment score associated with the sample by circling the appropriate code.
- 3. These samples should be taken at all visits.** Indicate if samples were taken from sites exhibiting a  $\geq 2$ mm change in attachment since the WIHS Oral visit prior to **last**.
- 3a.** Indicate the tooth numbers from which these samples were taken.

Refer to samples in the appendix section of this manual for the complete and proper way to fill out the laboratory request forms that are to accompany each plaque sample collected and shipped to the lab. All three copies of the triplicate should be sent with the specimen.

**EQUIPMENT**

- Fine sterile paper points
- Cotton rolls
- Sterile curette
- Sterile cotton forceps
- 4 vials (2 plastic vials for PCR analysis and 2 glass vials with anaerobic medium) for affected site.

- \*4 vials (2 plastic vials for PCR analysis and 2 glass vials with anaerobic medium) for control site.
  - \*Plastic shipping tube and envelop
  - \*Special waterproof pens for writing on sample vials
- \* **Equipment items marked by asterisk will be provided by Dr. Slot's laboratory at USC.**

## PROCEDURES

1. After assessing the whole mouth using the gingival banding and papillary assessment score, select samples from sites meeting any of the following criteria:
  - a. Positive score for gingival banding (GB) for facial and lingual sites (contralateral samples also taken). All GB samples must have a control sample.
  - b. Papillary assessment (PA) scores of “3” (ulcerated) or “5” (exposed bone).
  - c. Loss of attachment of 2 mm or greater since the previous oral visit.
2. After collecting samples, send overnight or priority mail (must get to lab in two to three days after sampling for viability). As stated above, mailing materials will be provided by Dr. Jorgen Slots at USC. All specimens should be shipped to the following address:

Dr. Jorgen Slots  
 The Oral Microbiology Testing Laboratory  
 USC - School of Dentistry, Room #4111  
 West 34th Street  
 Los Angeles, CA 90089

Telephone: (213) 740-3163  
 Fax: (213) 740-2194

Lab contact personnel:

Pauline Chang– Lab Manager

### Positive score for gingival banding (GB)

- a. Only **one** involved tooth and **one** uninvolved contralateral tooth are sampled regardless of the number of positive scores.
- b. Choose the mid-buccal of the most mesially involved tooth beginning in the **upper left** segment. If there is no involved tooth in this segment, then select the anterior segment. If there is no involved tooth in this segment, sample from the most mesially involved tooth in the **upper right** segment. If there is no involved tooth in this segment, then select the anterior segment. If there is no involved tooth in the upper arch, proceed to sample from the most mesially involved tooth in the **lower left** segment. If there is no involved tooth in this segment then select the anterior segment. If there is no involved tooth in this segment, sample from the most mesially involved tooth in the **lower right** segment

- c. Prepare the chosen tooth by removing supragingival plaque with a periodontal scaler taking care not to push plaque into the subgingival area. Isolate the site with cotton rolls or gauze pads.
- d. With moderate pressure, insert four sterile paper points at once to the depth of the gingival crevice (at the midline) as far as possible. Do not try to do more than one site at a time.
- e. Leave the paper points in place for ten seconds.
- f. Remove all paper points at once and insert tips down into the glass vial with transport medium..
- g. Repeat steps d and e above with a second set of four paperpoints.
- h. Remove the second set of four paper points and place into the plastic vial for PCR analysis.

**NOTE:** When finished there will be four paper points from one tooth in each of the two vials.

- i. When using the glass vial, remove the lid only during actual placement of the paper points into the vial (no longer than 15–20 seconds). A slightly blue coloring of the surface of the anaerobic medium after placement will not compromise the analysis. If the blue color extends beyond the surface layer, that vial should not be used. The plastic vials used for PCR analysis are empty and do not require special precautions.
- j. You will be provided with foil-backed specimen labels preprinted with the participant's ID number to be affixed to the vial. Record the visit number, collection date, tooth number, and a designation of sample site (for example, PA3 = papillary assessment code = 3, GB = gingival banding) as appropriate.
- k. Place both vials (one glass, one plastic) into the mailing container and insert the container into mailing envelope. Send overnight or priority mail (must get to lab in two to three days after sampling for viability) to Dr. Jorgen Slots at USC.

#### **Guidelines for shipping plaque specimens to the USC Oral Microbiology Testing Laboratory**

- Place the plastic container containing both glass and plastic plaque transport media into the plastic container provided by USC laboratory. Make certain that all containers are tightly capped and secure to avoid spillage.
- A lab request form (in triplicate) provided by the USC laboratory must accompany each plaque sample that is shipped. These must be filled out correctly. All three pages of each form must be sent to the USC laboratory with the plaque specimen. If the site needs a copy of these forms, they must be photocopied.
- Place the canister in the styrofoam fiberboard box (provided by USC laboratory) and secure with shipping tape.
- Place appropriate airbill and biohazard sticker as indicated in the appendix of this protocol.

### **Papillary assessment (PA) scores of “3” or “5”**

- a. A maximum of six papilla with scores “3” or “5” may be sampled. Insert four sterile paper points at once (see 3d for method) into the depth of the distal sulcus of the tooth that is mesial to the involved papilla. Prepare the chosen tooth by removing supragingival plaque with a periodontal scaler or cotton tip applicator taking care not to push plaque into the subgingival area. Isolate the site with cotton rolls or gauze pads.
- b. Leave the paper points in place for ten seconds.
- c. Remove all paper points at once and insert tips down into the glass vial with transport medium..
- d. Repeat step “a” above with a second set of four paperpoints. Do not try to do more than one site at a time.
- e. Remove the second set of four paper points and place into the plastic vial for PCR analysis.

**NOTE:** When finished there will be four paper points from one tooth in each of the two vials.

- f. When using the glass vial, remove the lid only during actual placement of the paper points into the vial (no longer than 15–20 seconds). A slightly blue coloring of the surface of the anaerobic medium after placement will not compromise the analysis. If the blue color extends beyond the surface layer, that vial should not be used. The plastic vials used for PCR analysis are empty and do not require special precautions.
- g. You will be provided with foil-backed specimen labels preprinted with the participant's ID number to be affixed to the vial. Record the visit number, collection date, tooth number, and a designation of sample site (for example, PA3 = papillary assessment code = 3, GB = gingival banding) as appropriate.
- h. Send overnight or priority mail (must get to lab in two to three days after sampling for viability) to Dr. Jorgen Slots at USC.

### **Guidelines for shipping plaque specimens to the USC Oral Microbiology Testing Laboratory**

- Place plastic container containing both glass and plastic plaque transport media into the plastic container provided by USC laboratory. Make certain that all containers are tightly capped and secure to avoid spillage.
- A lab request form (in triplicate) provided by the USC laboratory must accompany each plaque sample that is shipped. These must be filled out correctly. All three pages of each form must be sent to the USC laboratory with the plaque specimen. If the site needs a copy of these forms, they must be photocopied.
- Place the canister in the styrofoam fiberboard box (provided by USC laboratory) and secure with shipping tape.

- Place appropriate airbill and biohazard sticker.

### **5. Severe periodontal breakdown**

- a. For a maximum of six sites (most involved sites) exhibiting attachment loss of 2 millimeters or greater, it is necessary to determine if 2 millimeters or more have been lost since the visit prior to last visit (this should give us a reading of the exam results of approximate one year ago).
- b. Calculate the loss of attachment from Form OP14 by subtracting column “a” from column “b.” Calculate the new loss of attachment by performing the same calculation after measuring the distances. Subtract the two calculations from each other. Select sites exhibiting a 2mm or greater change since the last visit which are present in the random half-mouth examined.
- c. Prepare the chosen tooth by removing supragingival plaque with a periodontal scaler taking care not to push plaque into the subgingival area. Isolate the site with cotton rolls or gauze pads.
- d. Label the specimen type with the code “LA” to indicate “loss of attachment.”
- e. With moderate pressure, insert four sterile paper points at once to the depth of the gingival crevice for each criterion tooth. Do not try to do more than one site at a time.
- f. Leave the paper points in place for ten seconds
- g. Remove all **paper** points from each criterion tooth at once and insert tips down into the glass vial with transport medium.
- h. Repeat step “e” above with a second set of four paper points. Do not try to do more than one site at a time.
- i. Remove the second set of four paper points and place into the plastic vial for PCR analysis.  
**NOTE:** When finished there will be four paper points from one tooth in each of the two vials.
- j. When using the glass vial, remove the lid only during actual placement of the paper points into the vial (no longer than 15–20 seconds). A slightly blue coloring of the surface of the anaerobic medium after placement will not compromise the analysis. If the blue color extends beyond the surface layer, that vial should not be used. The plastic vials used for PCR analysis are empty and do not require special precautions.
- K. Label each vial with the subject’s identifying number sample site and date of sampling.
- L. Place both vials (one glass, one plastic) into the mailing container and insert container into mailing envelope. Send overnight or priority mail (must get to lab in two to three days after sampling for viability) to Dr. Jorgen Slots at USC.

**See guidelines in item 4 above for proper shipping to USC laboratory.**