WOMEN'S INTERAGENCY HIV STUDY
SECTION 13: ORAL PROTOCOL

THE ORAL PROTOCOL WAS DISCONTINUED AT VISIT 21.

I. ORAL PROTOCOL RESEARCH GOALS AND OBJECTIVES

Assess the prevalence, and prognostic significance of oral lesions as outlined by the following questions:

1. What are the most prevalent oral lesions in HIV-1 seropositive subjects?
2. Do prevalence rates differ among women by age, race, and ethnicity?

   Do prevalence rates differ among women by risk factors for HIV transmission, e.g., by drug injection, heterosexual sex, etc.?
3. What are the associations among oral diseases/lesions and:

   - seroconversion
   - immunologic status
   - medications (anti-viral, anti-fungal, antibacterial)
   - pregnancy
   - smoking
   - menstrual cycle/hormonal status
   - dental care utilization
   - CDC classification
   - bio-load of the oral cavity (as characterized by the microflora present in saliva)
   - presence of CDC-defined opportunistic infections
   - age of subject
4. Relationship of oral lesions to HIV-disease progression (to AIDS/DEATH).
5. Relationship between oral lesions and saliva (flow, constituents).
6. Relationship between oral lesions and systemic conditions.

Study fungal and viral lesions as outlined by the following questions:

Fungal lesions:

1. Is there a correlation between the occurrence of oral and vaginal erythematous and pseudomembranous candidiasis in HIV-1+ women?
2. Is there a correlation between the occurrence of oral candidiasis in HIV-1+ women and the menstrual cycle (as determined by menstrual history)?
3. How does oral candidiasis in HIV-1+ women correlate with CD45/CD14, CD3/CD4, CD3/CD19, CD16 or CD56 (hereto referred to as immune status)? with direct HIV quantitation?
4. What is the most common clinical type of oral candidiasis in HIV-1+ women? Does the clinical type of oral candidiasis change with respect to change in HIV disease longevity? with respect to change in immune status? With respect to change in direct HIV quantitation?

5. In HIV-1+ women with repeat episodes of oral candidiasis, are different species of candida emerging over time (population change over time) as determined from serially obtained samples? Are changes in oral candida populations over time related to vaginal population changes?

6. Is clinical evidence of treatment resistant candidiasis correlated with a change in candida population?

Viral lesions:

1. What is the prevalence of oral Herpes Simplex Virus (HSV) infection (ulceration) in HIV-1+ women? How does the prevalence change with duration of HIV infection? What is the correlation with immune status? With direct HIV quantitation? Are the ulcerations more commonly due to HSV 1 or 2?

Aphthous ulcers:

1. What is the prevalence of aphthous ulcers in a population of HIV-1+ women? How does the prevalence correlate with immune status? With direct HIV quantitation?

Neoplasms:

1. How does the presence of oral neoplasms correlate with immune status? With the presence of other soft tissue lesions? With direct HIV quantitation in HIV-1+ women?

Study periodontal diseases as outlined by the following research questions:

1. What is the progression of periodontal disease severity throughout HIV infection as determined by accepted clinical indices and laboratory findings?

2. What are the periodontal disease-associated organisms found in subgingival sites in HIV+ and HIV- women as analyzed via culture and PCR assays?

3. What are the differences in species/colonies of organisms associated with advanced periodontitis and gingivitis/mild periodontitis in HIV+ women using AP-PCR assays?

4. What is the identity of organisms (bacterial, fungal and viral) present in sub-gingival plaque? Over time is there a change in the identity of these organisms as detected by culture and PCR assays?

5. What are the associations among the severity of periodontal disease and:

    - seroconversion
    - immunologic status
    - medications (anti-viral, anti-fungal, antibacterial)
    - pregnancy
    - smoking
    - menstrual cycle/hormonal status
    - oral hygiene measures (self care or professionally provided)
    - CDC classification
    - bio-load of the oral cavity (as characterized by the microflora present in saliva)
    - presence of CDC-defined opportunistic infections
    - age of subject
    - subgingival plaque microbiota

Study saliva as outlined by the following questions:

1. What is the prevalence of subjective xerostomia in HIV+ women?

2. What is the prevalence of salivary gland enlargement in HIV+ women?

3. What is the prevalence of salivary gland hypofunction (objective xerostomia) in HIV+ women?

4. Are there consistent longitudinal changes in the microflora that are consistent with immune suppression, and that are predictive of the development of oral lesions?

Assess decayed, missing and filled coronal and root surfaces using DMFS and Root Caries Index.

Assess functional status as evidenced by tooth count, number of occluding pairs, and prostheses presence/use.

Assess psychosocial and functional morbidity caused by oral lesions using a <u>BRIEF</u> questionnaire.

II. GENERAL ORAL SUBSTUDY OPERATIONS

A. RECRUITMENT STRATEGIES

WIHS participants should be approached uniformly and informed about the opportunity to be part of the Oral Protocol. For example, some sites are incorporating a description of the oral component into the consent form used to participate in the full study.

B. ELIGIBILITY

Four sites are participating in the Oral Protocol: Bronx, LA, San Francisco, and Chicago. Any woman who is enrolled into the WIHS at one of these sites is eligible to participate in the Oral Protocol component, if she signs the oral consent form. In other words, there are no exclusionary criteria from the Oral Protocol for WIHS participants.

Eligibility to participate in the expansion of the oral substudy during visits 15 and 16 will be restricted to HIV positive women only.

C. ENROLLMENT

A woman is considered "enrolled" into the oral component after she signs the consent form. The data management system (WDMS) will track who enrolls into this study via the **Participation Notification Form (OPNOTI)**. Clinic staff should complete this form immediately after the consent form is signed and forward it to the Data Manager.

Starting with visit 15, the oral substudy will expand in association with the WIHS core expansion. Original enrollment in the oral substudy was 504 HIV-positive women and 122 HIV-negative women. During the expansion process, a total of 200 HIV-positive women will be recruited into the oral substudy. The oral substudy questions do not require additional HIV-negative participants and therefore no additional HIV-negative participants will be recruited. The following expansion targets have been determined for each site participating in the oral cohort:

SITE	NUMBER OF HIV POSITIVE PARTICIPANTS ORGINAL RECRUITS	NUMBER OF HIV POSITIVE PARTICIPANTS NEW RECRUITS	TOTAL NUMBER OF WOMEN ENROLLED IN SUBSTUDY
Bronx	60	48	**108**
Chicago	40	41	**81**
Los Angeles	60	68	**128**
San Francisco	40	57	**97**
TOTALS	**200**	**214**	**414**

New HIV positive participants will be recruited sequentially as they enroll in the core study. The target date for full enrollment is at the end of visit 16, September 30, 2002. Initially, HIV-positive women already participating in the WIHS core study will be excluded from the expansion of the oral substudy, however if the target enrollment numbers are not being met, enrollment will be opened to HIV-positive women already participating in the WIHS core study who have not previously been enrolled in the oral substudy.

D. SCHEDULING APPOINTMENTS

Oral visit appointments should be scheduled to occur within two weeks of the core visit. Oral teams should work closely with the core WIHS teams to develop referral/scheduling procedures that enable this to occur. See Section III.B for more information on scheduling windows.

E. FORMS

Each site will be sent updated forms for each visit via CD.

F. STORING DATA

Two photocopies of completed oral forms should be made. One set should be forwarded to the consortium Data Manager and one set should be retained in the dental office. The originals will be forwarded to the consortium Data Manager.

All WIHS core visit forms are kept intact in one file folder, in a locked cabinet, so that there is an official permanent WIHS file, which is easily accessible when the data needs to be reviewed and edited for quality control. The oral forms should also be stored in that master file. Edit reports should be attached to the form to which they refer within the official WIHS file.

Linking the WIHS ID number to the participant's name should not occur except on lists used to contact participants for scheduling and follow-up. Anything that links the WIHS ID number with identifying information should be kept in a separate locked file with limited access. The dental office may want to retain copies of the WIHS oral forms as inserts into the participant's clinical chart or for study purposes. To insure confidentiality, the WIHS ID should be blacked out. If hard copy lab results, which list the participant's name on them, are stored with official WIHS data, the participant's name must be blacked out.

G. INCOMPLETE VISITS

If all of the required procedures are not done for a participant, it is not necessary to complete the forms associated with each exam. For example, suppose the woman is too sick to complete the exam or needs to leave because of time constraints after the papillary assessment (Form OP09) is done. It is not necessary to complete Forms OP10–OP14. Document the situation by writing a memo to the Data Manager listing the forms that were completed as well as the forms that were not completed. Be sure to clearly state why some forms were not completed so that those forms can then be coded as "missing" within the WDMS. This memo should be retained in the participant's file with other study records.

III. FOLLOW-UP VISITS

A. VISIT NUMBERS

Record the core visit number on all forms that request the visit number. The core visit number should also be indicated on specimens collected through the oral protocol. If the visit number is mislabeled, it will be difficult to match up data records for analysis or retrieval of saliva samples from the central repository. Consult the calendar-based visit system schedule in Section 7 of the Manual of Operations if you need to determine the core visit number for the participant.

B. SCHEDULING WINDOWS

Beginning October 1, 1998, WIHS visit windows will be defined by a fixed period of calendar time. For example, all women seen between October 1, 1998, and March 31, 1999, for their CORE visits will be attending visit 9, regardless of how many visits they have had previously.

All oral protocol visits (i.e., baseline and all follow-up visits) are to be scheduled within a two-week window after the participants core WIHS visit(s).

Oral visits should be done within two weeks after the core visit has been initiated. If the core visit is separated over several appointments, the oral visit must happen within two weeks of the first appointment of the core visit. The oral visit may be scheduled on the same day as core study components, but it must be done after the WIHS interview has been completed. Only in the case of extreme scheduling conflict should an oral visit occur before the core visit. If an oral visit is scheduled prior to a core visit, then oral visit should still be within 2 weeks of the core visit.

1. HANDLING ORAL VISITS OUTSIDE OF THE COMPLETION WINDOW

   While it is always preferred that participants' oral visits are conducted within the defined *completion window* (two weeks after the core visit has been initiated), there are times when this is not possible. Rather than miss the opportunity to collect study data, the following provisions should be made:

   - Participants may be scheduled for dental appointments for up to two months after the *scheduling window* closes for the visit. In other words, the participant may be seen anytime between the date of core visit and two months after the date of the core visit.
   - If the woman does not come in for her visit within two months after the scheduling window closes, the visit should be considered missing. Arrange an appointment for the next visit during the appropriate scheduling window and complete a Missed Visit Form.

2. DISENROLLING PARTICIPANTS FROM THE ORAL PROTOCOL

   If a participant states an interest in disenrolling from the Oral Protocol, a Disenrollment Form should be completed. If a Missed Visit Form has already been entered for that visit, it must be deleted before the Disenrollment Form can be entered.

   If an oral participant is disenrolled from the core WIHS protocol, **the completed Disenrollment Form must be photocopied and sent to the oral team/coordinator**. Sites should develop procedures to communicate disenrollment information in a timely manner.

C. LABORATORY ISSUES

- Possible contamination of plaque transport and leakage of saliva blue top tube. Leak-proof blue top tubes are sent by USC to all the sites. If transport media is contaminated, this will be detected and noted when specimens are processed. Sites should try to use both saliva tubes and plaque transports within three months of when they are received. Transport media that are older than six months should be discarded.
- It is imperative that visit information be written on the requisition form for all oral lab specimens as well as participant identification number, specimen collection date, specimen collection type (saliva, papillary or gingival), and tooth number.

IV. SEQUENCE OF PROCEDURES

A. MEDICAL EVALUATION (OP01)

The Medical Evaluation serves as a clinical evaluation (as opposed to a formal "research interview") of the participant's health as it relates to the oral examination. Specifically, it provides a list of conditions and indications which require prophylactic antibiotics to be administered. The information on this form should be obtained by the dental clinician and should serve as a tool for clinical assessment. If in the opinion of the dental clinician, other medical conditions exist which warrant prophylaxis, antibiotics should be administered and recorded.

B. QUESTIONNAIRE (OP02)

The Oral Interview can be administered by either the dental clinician or a research assistant. Unlike the medical evaluation (OP01), this instrument is a formal research interview. Therefore, it is imperative that the questions be read exactly as they are worded on the form to assure standardization of data collection. Read each question and response category exactly as worded and circle the appropriate response code. Be sure you have read **all** the responses to the participant before she gives her answer when indicated. Refer to Section 8 of the Manual of Operations for more detailed instructions on conducting a research interview. Note that prompts appear in bold capital letters on the Oral Interview Form to assist you.

C. SALIVA SAMPLE COLLECTION (OP03)

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 is more comfortable for the participant to chew with her removable dental prosthesis, she may go ahead and replace it. (This should be consistent at every visit; if the participant removes her dental prosthesis at baseline, she should remove it again at for each follow-up visit.)

2. Subjects should rinse thoroughly with deionized water and rest for five minutes (no talking or reading) before saliva collection begins.

3. Subjects are asked to tilt head 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 and discard the sample.

   NOTE: Sample collection must take place for at least two minutes. If sample collection time is under two minutes because of accidental swallowing, forceful sneezing or coughing, start over with a new test tube after a brief (two-minute) rest period. If collection time is between two and five 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 and is approximately 70 chews per minute. The subject is asked to expectorate saliva into the graduated test tube once per minute. (Remind subjects not to spit out the gum base at end of each minute.)

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

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* two minutes (whichever comes first) of stimulated saliva is collected and discarded prior to proceeding to the next step (step #7).

   If the participant ID number indicates that the sample should be submitted for microbiologic evaluation (again, please refer to saliva log), the first 2 ml *or* two 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 eight hours, ship with polar packs to laboratory at USC. Using the preprinted mailing labels provided by the lab, these first 2 mls/two minutes of simulated whole saliva are to be shipped overnight to USC (see address below):

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

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

   Guidelines for shipping to salvia 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. 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 5:00 p.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 ml is collected, stimulated saliva is collected for an additional three minutes in a graduated test tube on ice for flow rate determination. Record the volume to the nearest 1/4 ml.

   If the participant experiences difficulty providing additional saliva for flow rate determination, this collection time can be increased to as long as five minutes. However, if the total volume of stimulated whole saliva collected is less than 2 ml, the entire specimen should be sent to USC and none of it should be aliquoted for BRinc. storage.

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 two-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, 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 baseline 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.

D. ORAL MUCOSAL TISSUE, LYMPH NODE, AND SALIVARY GLAND EXAMINATION (OP04)

1. LYMPH NODE EXAMINATION

   a. Equipment

      - 2 mouth mirrors
      - 2 x 2 gauze squares

   b. Procedures

      1. Stand in front of the patient.
      2. Examine the nodes bilaterally using the flat aspects of finger pads and tips of the first, second and third fingers. Palpate each of the sites in the order outlined below for the superficial lymph nodes.
      3. Touch each area lightly, then increase pressure. Vary the touch pressure among the fingers. This will allow the node to demonstrate movability.
      4. Maintain skin contact while moving in small circles along each lymph node chain.
      5. Look for any tenderness, enlargement > 1cm, and consistency (hard or soft).
      6. Examine the nodes in the following order:

         A. Postauricular nodes over the mastoid process.

         B. Preauricular nodes in front of the ear.

         C. Start at the angle of the mandible and move forward under the jaw until the hands meet, thus palpating the submandibular (posteriorly) and the submental (anterior aspect) nodes.

D. Begin at the base of the skull to palpate the occipital nodes, moving from there into the posterior cervical triangle, palpating the entire contents of the triangle down to the clavicle (use the borders of the sternomastoid and the trapezius muscles as the boundaries). Move into the supraclavicular fossae to palpate those nodes.

E. Return to the angles of the mandible and palpate down the anterior edges of the sternomastoid muscles until the clavicle is reached for the anterior cervical nodes.

Referral reminder: Consider referral for any lymph node that is hard, fixed or > 1cm in diameter. Note this action on Form OP16.

2. ORAL LESIONS

a. Procedures

The examination procedure follows a systematic assessment of the lips; labial mucosa and sulcus; commissures, buccal mucosa and sulcus; gingiva and alveolar ridge, tongue; floor of the mouth; hard and soft palate; salivary glands, and lymph nodes.

1. Begin examination by observing the lips with the mouth both closed and open. Note the color, texture and any surface abnormalities of the upper and lower vermilion borders.

2. With the mouth partially open, visually examine the labial mucosa and sulcus of:

   a. the maxillary vestibule and frenulum, and

   b. the mandibular vestibule.

   Observe the color and any swelling or other abnormalities of the vestibular mucosa and gingiva.

3. Using the two mouth mirrors as retractors and with the mouth open wide, examine first the right, then the left buccal mucosa extending from the labial commissures and back to the anterior tonsillar pillar. Note any change in pigmentation, color, texture, mobility and other abnormalities of the mucosa, make sure that the commissures are examined carefully and are not covered by the mouth mirrors during retraction of the cheek.

4. Next, examine the gingiva and alveolar ridges (processes).

   a. Buccal and Labial Aspects

   Start with the right maxillary posterior gingiva and alveolar ridge and move around the arch to the left posterior gingiva. Continue with the left mandibular and move around the arch to the right posterior gingiva.

   b. Palatal and Lingual Aspects

   Same as above except on the palatal for the maxillary (right to left) examination and on the lingual for the mandibular (left to right) examination.

5. With the tongue at rest, and mouth partially open, inspect the dorsum of the tongue for any swelling, ulceration, coating or variation in size, color or texture. Also note any change in the pattern of the papillae covering the surface of the tongue and examine the top and the tip of the tongue. The subject should then protrude the tongue, and the examiner should note any abnormality of mobility. With the aid of mouth mirrors, inspect the margins of the tongue. Grasping the tip of the tongue with a piece of gauze will assist full protrusion and will aid examination of the margins. Then observe the ventral surface.

6. With the tongue still elevated, inspect the floor of the mouth for swellings or other abnormalities.

7. With the mouth open and the subject's head tilted backward, gently depress the base of the tongue with a mouth mirror. First inspect the hard, and then the soft palate.

b. Oral lesion and destination of samples

(A) Angular Chelitis – samples sent to BRinc.
(B) Pseudomebranous Candidiasis – samples sent to BRinc.
(C) Erythematous Candidiasis – samples sent to BRInc. and the Oral Pathology Laboratory in Flushing, New York (see below)
(G) Herpetic Ulcer Intraoral (HSV culture)
(H) Aphthous Ulcer Major (HSV culture)
(N) Other Ulcer (HSV culture)

c. Procedures for mucosal smear of Erythematous Candidiasis (lesion code C only)

- The smear kit provided contains a wooden spatula, a cotton swab, two glass slides and a packet of fixative. The directions in the kit are for taking vaginal samples and should therefore be ignored.

- Open the packet and remove the packet of fixative, the wooden spatula and cotton swab.

- Tear the covering of the packet along the perforation so the slide part of the packet can be handled separately. Do not remove slides from the packet.

- Label both slides on frosted end with WIHSID in pencil.

- Using the wooden spatula, scrape over the surface of the erythematous area(s) and spread the sample over the two slides.

- Immediately, open the packet of fixative and wet the smeared area of both slides with fixative. Let slides dry.

- Close the packet and place a pre-printed label (with participant's ID) on the outside of the packet. Record the collection date and the visit number on the label.

- Place pre-printed label with WIHSID and date of smear on the laboratory slip. If there is no pre-printed label available, write the WIHISID and date of smear on the laboratory slip.

- Place the packet with the slides enclosed in the special self-addressed mailing envelope provided. The address of the Oral Pathology Laboratory is:

  Oral Pathology Laboratory
  56 – 26 Main St.
  Flushing NY 11355

- When additional smear kits are needed, please contact Dr. Joan Phelan at:

  Phone: (516) 261-4400 ext. 7415
  FAX: (516) 266-6020
  Email: phelan.joan@northport.va.gov

3. SALIVARY GLAND EXAMINATION

Finally, evidence of major gland swelling and/or tenderness upon palpation and failure of saliva to be elicited from either Wharton's or Stensen's ducts are evaluated. Scores are recorded as "0" (absence of sign or symptom) or "1" (presence of sign or symptom).

1. Inspect patient's face and score presence or absence of parotid gland enlargement.

   0 = no enlargement
   1 = enlargement

   Palpate left and right parotid glands. Ask the participant to open her mouth. Retract left cheek with the ball of thumb. Pick up a piece of 2 x 2 gauze with your hand and gently dry the area around the orifice of the parotid duct. Now use the other hand to gently massage the side of the face from the ear lobe forward. Continue to retract the left cheek with your hand and repeat the procedure one more time. *Look for the presence or absence of clear flow of saliva*. If flow is limited to one to two drops or if it is viscous or contaminated with puss or blood, score "absent" (i.e., score = 1). To examine the right parotid, repeat the same procedure, switching hands if necessary.

2. Palpate left and right submandibular/sublingual glands. Ask the participant to open her mouth. Dry the floor of the mouth with 2 x 2 gauze. Place your index finger under the left side of the jaw and press up against skin covering the inner side of the mandible. Place your index finger between the lower left molar teeth and bring your finger forward, massaging the duct of submandibular gland. Repeat one more time. *Look for clear flow of saliva and score its presence or absence.*

   To examine the right submandibular gland, while the participant's mouth remains open, dry the floor of the mouth again and repeat the examination on the other side.

E. HSV SWABS OF ORAL ULCERS OR FISSURES (OP05)

- Using a sterile, dacron-tipped swab moistened with viral transport medium, obtain the material from the base of any HSV ulcers and/or fissures (lesion codes **G**, **H**, and **N** on Form OP04).
- Record the following information on the preprinted (with the participant's ID) foil-backed label: the collection date, the visit number, the code "O/D" and the location number (from the diagram on Form OP04). For example, if an ulcer was found at location #13, record "O/D13" on the label. Be sure the lab reports this number on result form.
- Check with your local lab regarding the need for placement in transport medium or refrigeration. Send to local lab for immediate culture. HSV typing is not required on positive cultures (although those codes appear on the result form). Isolates will not be stored.

F. TOOTH COUNT & NUMBER OF OCCLUDING PAIRS (OP06)

1. TOOTH COUNT

Beginning in the **upper left** quadrant, count the number of teeth in the Maxillary arch and record in the space provided. Repeat for the Mandibular arch. Record leading zeros for single digit numbers. **NOTE**: third molars, deciduous teeth, pontics and retained roots should not be included in the tooth count.

2. NUMBER OF OCCLUDING PAIRS

Next, ask the patient to bite down and, starting on the left side of the patient's mouth and moving to the right, count the number of occluding pairs of teeth and record the total in the space

provided. **NOTE**: third molars, deciduous teeth, pontics and retained roots should not be included in the calculation of the number of occluding pairs.

3. SELECTION OF RANDOM QUADRANTS FOR PERIODONTAL ASSESSMENT FOR PATIENTS WITH ≥10 NATURAL TEETH

   The sixth and seventh digits of the participant's ID number (that is, the last two digits of the four-digit participant number [refer to Section 11 of the Manual of Operations for an explanation of the ID structure]) will be used to select random quadrants as follows:

   The sixth digit of the participant's ID number will be used to select the upper quadrant. If this number is even, the right side will be used. If this number is odd, the left side will be used.

   Similarly, the seventh digit of the participant's ID number will be used to select the lower quadrant. If this number is even, the right side will be used. If this number is odd, the left side will be used.

   The quadrants selected will then be indicated on the Tooth Count Form (OP06), as well as on the Plaque Index (OP07), Gingival Bleeding Score (OP13) and Loss of Attachment (OP14).

   For example, if the participant's ID number is 1-23-4567-8, this would represent a right upper and left lower designation for the participant.

1	2	3	4	5	6	7	8
					upper right	lower left	

G. PLAQUE INDEX (OP07)

1. EQUIPMENT

   - mirror
   - NIDCR probe

2. SITES TO BE EXAMINED

   a. Participant presents with **ten or more** natural teeth: examine each of the teeth from the random half-mouth selected for periodontal assessments.

   b. Participant presents with **fewer than ten** natural teeth: examine each tooth in the participant's mouth.

   In all cases, assess four sites per tooth: i.e., three on the buccal (distal [D], midbuccal [MB], and mesial [M]) and one on the lingual (midlingual [L]).

3. PROCEDURE

   The randomly selected **upper** arch is examined first, beginning with the buccal sites. The examination begins at the **DB** of the **most distal tooth** and proceeds to the midline. **Lingual** sites (i.e., the midlingual [L] of each tooth) of the upper arch are examined next using the same sequence. The procedure is then repeated for the **lower** arch.

   If the participant presents with fewer than ten natural teeth, call codes for each tooth in her mouth beginning with the upper arch. Indicate missing teeth by coding Y.

   The quadrant is dried with air and examined using a surface reflecting mirror and an NIDCR probe. The examiner first observes the site to determine whether or not plaque is visible. If plaque is not visible, the examiner runs the NIDCR probe across the surface to determine if the surface has plaque that could be detected only by an instrument. The probe should be kept

parallel to the contour of the tooth in the area adjacent to the gingiva. When plaque adheres to the probe, it must be wiped from the probe prior to assessing the next site. A single score is recorded for each tooth site.

H. GINGIVAL BANDING SCORE (OP08)

1. EQUIPMENT

   - Dental mirror

2. PROCEDURE

   Facial and lingual aspects of each arch are divided into three segments: one anterior segment and a left and right posterior segment. The anterior segment extends from canine to canine and the posterior segments comprise all teeth distal to the canines, up to and including the second molar. A single score is assigned for each of the twelve segments of the mouth.

   The gingival margin is assessed in each segment beginning with the facial aspect of the upper left posterior segment, followed by the anterior segment and the right posterior segment. Next, the lingual segments of the upper arch are examined in the same order. The sequence is repeated in the lower arch.

   The gingiva is examined visually for the presence of a continuous band of erythema at the gingival margin, at least one millimeter in width, which extends from the mesial to the distal line angle of the tooth surface.

I. PAPILLARY ASSESSMENT SCORES (OP09)

1. EQUIPMENT

   - Dental mirror

2. PROCEDURE

   An interdental papilla is that part of the free gingiva occupying the gingival embrasure between adjacent teeth. All interdental papillae anterior to the second molars are examined from the buccal and lingual aspects. One or more score(s) is/are assigned to each papilla, as appropriate. Scores are to be recorded under the column labeled as the tooth that is mesial to the papilla. The papilla between the two centrals is scored under the column labeled midline.

   A maximum of 13 papillary areas are available for observation in each dental arch. Each papilla is examined visually from both the buccal and lingual aspect for signs of redness, swelling, necrosis, cratering and exposed bone.

   The examination begins in the **upper left** quadrant and proceeds around the arch to the **upper right**. The procedures are then repeated for the **lower** arch.

   Each papillary site is considered as a single unit, to be examined from both buccal and lingual aspects. If one or more of the conditions below is/are present on either aspect, the site is scored accordingly. If no clinical changes are observed on either aspect, the site is scored 0. Scores are not recorded separately for buccal and lingual views. Multiple conditions **should** be recorded when they occur, i.e., the presence of <u>each</u> condition in the scoring system should be recorded as present or absent on each site. Any combination of scores is permitted, except that sites scored 0 (normal) may not have any additional scores. Papillary sites are scored as "Y" if either of the adjacent teeth is missing unless another tooth has moved forward to form a papilla.

J. SUBGINGIVAL PLAQUES SAMPLES (OP10)

1. EQUIPMENT

- *****4 packets of fine sterile paper points (approximately 20 paperpoints in all)
- 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.**

2. 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. For each category of positive score (GB,PA,LOA), take a sample from ONE, most severely, involved tooth and one uninvolved contralateral tooth (control) regardless of the number of teeth with positive scores:

   a. Positive score for gingival banding (GB) for facial and lingual sites (contralateral samples also taken).

   b. Papillary assessment (PA) scores of "3" (ulcerated) or "5" (exposed bone).

   c. Loss of attachment (LOA) of 2 mm or greater change 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

3. 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. All gingival banding plaque samples should have a control sample.

   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.

4. Papillary assessment (PA) scores of "1,",2","3" or "5"

   a. A maximum of six papilla with scores "1,"2,","3" or "5" may be sampled. Insert four sterilepaper 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.

K. CORONAL CARIES ASSESSMENT (OP11)

1. EQUIPMENT

   - Dental Mirror
   - Number 23 Explorer

2. PROCEDURE

The examiner starts with the **Maxillary Left** quadrant beginning with the central incisor through the left third molar, followed by the **Maxillary Right** quadrant, **Mandibular Left** quadrant, and the **Mandibular Right** quadrant in the same sequence.

The anterior tooth sites are examined in the following order: lingual, facial, mesial, distal. The posterior tooth sites are examined in the following order: occlusal, lingual, buccal, mesial, distal. The third molar is scored as present or absent.

It is not advisable to call out individual surface diagnostic codes as each tooth surface is examined, as this can be confusing to the recorder. It is better for the examiner to mentally accumulate surface diagnoses for a given tooth until all surfaces have been examined before dictating the diagnostic codes to the recorder. **For any given tooth surface, caries takes precedence over restorations.**

3. GUIDELINES FOR CORONAL CARIES ASSESSMENT

a. Decayed Tooth Surfaces (The **"D"** component of the index)

Advanced lesions are detected as gross cavitation and thus present few problems in diagnosis. Early lesions, on the other hand, are more difficult to diagnose consistently. Early lesions may be subdivided into three categories according to location, each with the following special diagnostic consideration:

i. Pits and fissures on occlusal, buccal and lingual surfaces

These areas are diagnosed as carious when the explorer catches after insertion with moderate, firm pressure and when the catch is accompanied by one or both of the following signs of caries:

(1) Softness at the base of the area, and/or

(2) Opacity adjacent to the area providing evidence of undermining or demineralization.

In other words, a deep pit or fissure in which the explorer catches is not in itself sufficient evidence of decay; it must be accompanied by at least one of the above signs.

ii. Smooth areas on buccal (labial) or lingual surfaces

These areas are carious if they are decalcified or if there is a white spot as evidence of subsurface demineralization and if the area is found to be soft by:

(1) Penetration with the explorer, or

(2) Scraping away the enamel with the explorer.

These areas should be diagnosed as sound when there is only visual evidence of demineralization.

iii. Proximal surfaces

For areas accessible to direct visual and tactile examination, as when there is no adjacent tooth, the criteria are the same as those for smooth areas on buccal or lingual surfaces. For areas not available to direct examination, other criteria must be applied. In anterior teeth, transillumination can serve as a useful aid in discovering proximal lesions. Transillumination is achieved by placing a mirror lingually and positioning the examining light so that it passes through the teeth and reflects into the mirror. If a

characteristic shadow or loss of translucency is seen on the proximal surface, then this is indicative of caries on the surface. Ideally, the actual diagnosis should be confirmed by detecting a break in the enamel surface with the explorer; however, clear visualization of a lesion by transillumination can justify a positive diagnosis. In posterior teeth, however, visual evidence alone, such as undermining under a marginal ridge, is not sufficient proof for diagnosing a proximal lesion. A positive diagnosis is made only if a break in the enamel surface can be detected with the explorer.

b. Missing Tooth Surfaces (the **"M"** component of the index)

This component usually includes only those permanent teeth which have been extracted as a result of caries. It is essential to distinguish between teeth extracted because of caries and those extracted or missing for other reasons. The code "E" is used to indicate teeth extracted because of caries or perio, and "ER" is used for teeth extracted because of caries or perio and subsequently replaced with a fixed or removable appliance. A different code, "M", is used for teeth missing due to trauma, orthodontic treatment, or other non-disease related causes. "MR" is used for teeth missing due to trauma, orthodontic treatment, or other non-disease related causes and subsequently replaced with a fixed or removable appliance. Unerupted or congenitally missing teeth (code "U") must also be correctly identified.

c. Filled Tooth Surfaces (the **"F"** component of the index)

The "F" component represents a tooth surface that has been filled with either a permanent or a temporary restoration as a result of caries involvement. Here also it is necessary to distinguish between surfaces restored for caries and those restored for other reasons, such as trauma, hypoplasia or malformation.

d. Guidelines for Diagnosing Coronal Caries

The following conventions have been adopted in the interest of achieving diagnostic consistency:

Third molars are not scored. When examining second molars it is important to be aware that a drifted third molar may occupy the space of a missing second molar. In such cases, the diagnosis and score must relate to the status of the missing second molar, not the third molar. If the second molar, for example, was extracted due to caries and the space is now occupied by a sound third molar, the second molar is scored as extracted and not replaced (E), and the third molar is scored as present or absent.

If both a deciduous and a permanent tooth occupy the same tooth space, only the permanent tooth is scored.

A tooth is considered to be in eruption when any part of its crown projects through the gum. This criterion is easier to standardize than one based on a more advanced stage of eruption.

In the case of supernumerary teeth, only one tooth is scored for the tooth space. The examiner must decide which tooth is the "legitimate" occupant of the space.

Incisal edges of anterior teeth are not considered to be separate surfaces. If a lesion or restoration is confined solely to the incisal edge, its score should be assigned to the nearest adjacent surface. Thus, anterior teeth have only four scorable surfaces (mesial, distal, labial, and lingual). The inclusion of the occlusal surface for posterior teeth gives those teeth five surfaces. Therefore, a total of 128 surfaces are examined and diagnosed for each subject.

When a caries lesion extends beyond the line angle onto another surface, the other surface is also scored as affected. However, a proximal filling on an anterior tooth is not considered to involve the adjacent labial or lingual surface unless it extends at least one-third of the

distance across the labial or lingual surface. The reason for this criterion is that tooth structure on adjacent surfaces must often be removed to provide access for the restoration of a proximal lesion on anterior teeth. Also, to guard against a similar possibility for overestimating the amount of disease in posterior teeth, a proximal restoration should extend at least a millimeter past the line angle before it is considered to involve the adjacent buccal or lingual surface.

If a permanent tooth has a full crown restoration placed because of caries, the tooth will be coded as "C," which represents the maximum number of surfaces for the tooth type, i.e., four surfaces on anterior teeth and five surfaces on posterior teeth. By convention, all crowns on posterior teeth, including abutment teeth for fixed or removable prostheses, are considered to have been placed as a result of caries. If a tooth has been restored with less than full coverage, all surfaces not involved should be scored in the usual manner. On anterior teeth, however, the examiner should make a determination of the reason for crown placement. If the crown was placed for any reason other than caries, such as fracture, malformation or esthetics, the tooth is coded "Y." This rule applies only to those anterior teeth with full crowns or jackets. If a tooth has been restored with less than full coverage, all surfaces not involved should be scored in the usual manner.

Retained carious roots of posterior teeth should be called X, 0, 1, 2, 3. In the coronal caries assessment, carious roots of anterior teeth should be called 0, 1, 2, 3.

Teeth that are banded or bracketed for orthodontic treatment are examined in the usual manner and all visible surfaces are scored.

Certain teeth, notably first bicuspids, may have been extracted as part of orthodontic treatment. These teeth are coded "M" and will be excluded from the DMFS analysis. The examiner must make the determination that the teeth were in fact extracted for orthodontic reasons, although this is not usually difficult because of the typically symmetric pattern of these extractions. For the sake of uniformity, all orthodontically extracted bicuspids are scored as first bicuspids. Teeth other than bicuspids may also be extracted for orthodontic reasons. In many cases, the subject will have good recall of the reason for the extractions, and can help in making the correct determination.

Non-vital teeth are scored in the same manner as vital teeth. If, however, a restoration on a non-vital tooth was placed solely to seal a root canal and not for caries, that restoration is not scored. If no other lesions or restorations are present, the tooth will be called sound (code "S").

Hypoplastic teeth are scored in the usual manner. However, for anterior teeth, if it can be determined that a restoration on such a tooth was placed solely for esthetic reasons and not for caries, that restoration is not scored. If a hypoplastic tooth is restored with a full crown, it is coded "Y."

Malformed teeth are scored in the usual manner except when they have been restored with a full crown for esthetic reasons, in which case they are coded as "Y."

When the tooth surface is both carious and filled, only the caries is scored.

Fractured or missing restorations are scored as if the restoration were intact unless caries is found to be present. In that case, the involved surface is scored as carious rather than restored.

Stain and pigmentation alone should not be regarded as evidence of caries as either can occur on sound teeth.

L. ROOT CARRIES ASSESSMENT (OP12)

1. EQUIPMENT

   - Dental Mirror
   - Number 23 Explorer

2. PROCEDURE

   Root caries is summarized by the DFS Index (Decayed and Filled Permanent Root Surfaces), missing teeth being ignored. By convention, each tooth is considered to have four root surfaces: mesial, buccal (labial), distal and lingual.

   The sequence of examination is exactly the same as for coronal caries. All exposed portions of a tooth's root surface should be carefully examined. The most difficult areas to examine are interproximal surfaces in posterior teeth, particularly those that contain restorations. Subgingival inspection is not appropriate because few lesions are confined subgingivally and it may produce bleeding.

   The tooth and surface codes for root caries are identical to those for coronal caries with the exception of the "R" score, which is equivalent to the "S" score for coronal caries, indicating a tooth for which all root surfaces are sound.

3. GUIDELINES FOR ROOT CARIES ASSESSMENT

   The following conventions and notes have been adopted to promote consistency of diagnoses.

   In some incipient lesions the carious area of the root surface may merely be discolored without cavitation, but the area will be soft to exploration. Cavitation with jagged margins and a roughened, but soft floor or base usually occurs in advanced lesions. Normal cementum is softer than enamel, and frequently will yield to pressure from the tip of an explorer. Areas of root caries, however, are softer than surrounding cementum; therefore, it is possible to differentiate sound cementum from carious cementum based on tactile sense. In the presence of root caries, an explorer penetrates the tissue but usually can be removed easily. However, if the explorer penetrates but resists withdrawal or "sticks," the surface is usually sound cementum.

   Areas of abrasion or erosion in root surfaces rarely become carious because they are generally kept clean and are free of plaque. Root caries frequently occurs beneath plaque, but rarely beneath calculus. Accumulations of plaque which obstruct the examination procedure should be removed. Surfaces covered entirely by calculus are considered sound.

   Whenever both a coronal and root surface are affected by a single caries lesion that extends at least 1 mm past the CEJ in both cervical-incisal and cervical-apical directions, both surfaces should be scored as decayed. However, for a lesion affecting both crown and root surfaces that does not meet respective 1 mm extent of involvement, the surface on the side of CEJ that involves more than 50 percent of the area of the lesion should be scored. When it is impossible to apply the ">50% rule," i.e., when both coronal and root surfaces appear equally affected, both surfaces should be scored "decayed." For restorations, the same rules apply. **NOTE**: Retained roots of posterior teeth should be called 0, 1, 2, 3. Retained carious roots of anterior teeth should be called 0, 1, 2, 3.

   Because of the constricted anatomy of the root surfaces of lower incisors, few lesions will be confined solely to the lingual surface – only small lesions at the midpoint. Most lingual lesions will also affect the adjacent mesial and/or distal root surfaces. However, lesions of the mesial and distal surfaces which extend lingually but do not reach the midline are only scored as interproximal lesions.

On all other teeth, when root caries appears to wrap around the line angle of the root, the more involved surface is considered the primary site of the lesion and is scored carious, whereas the adjoining surface is only scored as carious when the lesion clearly extends at least 1 mm past the line angle.

Defective margins of fillings should be checked with an explorer for recurrent decay. The criterion for scoring "decayed and filled" root surfaces is the same as for coronal surfaces, that is, decay takes precedence over a filling. Full crown coverage is considered to have been placed for coronal caries even if the margin of the crown extends onto the root surface. Thus, root surface with a crown margin free of recurrent decay should be scored sound or "R" (no caries or restorations).

M. GINGIVAL BLEEDING SCORE (OP13)

1. EQUIPMENT

   - mirror
   - NIDCR probe

2. SITES TO BE EXAMINED

   a. Patient presents with ten or more natural teeth: examine each of the teeth from the random half-mouth selected for periodontal assessments.

   b. Patient presents with fewer than ten natural teeth: examine each tooth in patient's mouth.

   In all cases, assess four sites per tooth: i.e., three on the buccal (distal [D], midbuccal [MB] and mesial [M]) and one on the lingual (midlingual [ML]).

3. PROCEDURE

   The randomly selected upper arch is examined first, beginning with the buccal sites. The examination begins at the D of the most distal tooth and proceeds to the midline. Lingual sites of the upper arch are examined next using the same sequence. The procedure is then repeated for the lower arch.

   The gingival assessment is made using a modification of the Gingival Index proposed by Löe and Silness. The teeth should be dried with air (or gently with gauze) before beginning the examination of each quadrant.

   The periodontal probe is inserted no more than 2mm into gingival sulcus, at the distal of the most posterior tooth and than moved gently into the mesial interproximal area. Care must be taken to minimize pressure on the gingival tissue. This "sweeping" motion of the probe is continued in the same manner for each fully erupted permanent tooth in the quadrant until the central incisor is reached. The bleeding points in that quadrant are then scored. A score of 0 or 1 (or Y) is made for each of the three buccal sites per tooth (i.e., D, MB, M) beginning with the second molar and continuing to the central incisor. Lingual sites (i.e., the midlingual [L] of each tooth) of the upper arch are examined next using the same sequence. The procedure is then repeated for the lower arch.

4. GUIDELINES

   Guidelines for scoring bleeding points on the buccal are as follows:

   a. Bleeding points at or distal to the distal line angle of the tooth are scored under the column labeled D.

b. Bleeding points at or mesial to the mesial line angle of the tooth are scored under the column labeled M.

c. All other bleeding points on the buccal are scored under column labeled MB.

N. LOSS OF ATTACHMENT ASSESSMENT (OP14)

1. EQUIPMENT

- mirror
- NIDCR probe

2. SITES TO BE EXAMINED

a. Patient presents with ten or more natural teeth: examine each of the teeth in the random half-mouth selected for periodontal assessments

b. Patient presents fewer than ten natural teeth: examine each tooth in patient's mouth.

In all cases, assess four sites per tooth: i.e., three on the buccal (distal [D], midbuccal [MB], and mesial [M]) and one on the lingual (midlingual [L]).

3. PROCEDURE

The randomly selected upper arch is examined first, beginning with the buccal sites. The examination begins at the DB of the most distal tooth and proceeds to the midline. Lingual sites (i.e., the midlingual (L) of each tooth) of the upper arch are examined next using the same sequence. The procedure is then repeated for the lower arch.

Attachment Level Assessment

The periodontal attachment level assessment is made for the same maxillary and mandibular quadrants, and at the same four sites (i.e., D, MB, M, and L) as the gingival bleeding and plaque assessments. Attachment Levels are measured using the method described by Ramfjord. Only teeth in full eruption (excluding third molars) are measured. The distance from the free gingival margin (FGM) to the CEJ and the distance from the FGM to the bottom of the sulcus ("pocket depth") are measured using the periodontal probe. **Measurements are rounded downward to the nearest whole millimeter before they are recorded.**

The probe should be held with a light grasp and pointed toward the apex of the tooth or the central axis of multirooted teeth. At the interproximal sites, the probe should be kept parallel to the long axis of the tooth and as close to the contact point as possible, even if the adjacent tooth is missing. Generally, there are four situations encountered in the measurement of attachment level. Where the gingival margin has receded and the CEJ is exposed, the distance from the CEJ to the gingival margin is scored as a negative value, and pocket depth is scored as usual (non-negative). When the epithelial attachment is located at the cemento-enamel junction, the first and second measurements are identical and non-negative. When the free gingival margin is at the CEJ, the first measurement is zero. Pocket depth may also be zero or positive. Finally, where there is pocketing without evidence of gingival recession (i.e., the epithelial attachment is below the CEJ, and the free gingival margin is above), both measurements will be non-negative. The level of attachment is later calculated by subtracting the recorded distance from the FGM to CEJ from the distance FGM to base of sulcus.

Special Considerations:

- Calculus at the mesial or buccal sites which obscures the CEJ or interferes with the correct placement of the probe should be removed using the curette.

- Subginvival plaque samples are to be taken from sites exhibiting 4 mm or greater change in attachment level since previous WIHS visit.

O. DENTAL PROSTHESES ASSESSMENT (OP15)

The examiner should assess whether the participant has any prostheses (i.e., upper full denture, upper partial denture, lower full denture, and/or lower partial denture). In addition, the examiner will determine whether or not any of the participant's prostheses are an apparent source of trauma, irritation or infection.

P. TREATMENT NEEDS / REFERRAL ASSESSMENT (OP16)

If the participant has any treatment needs, Form OP16 should be completed to indicate whether or not the participant requires a referral for any of the following reasons: preventive dentistry, restorations, crowns or fixed bridges, endodontics, periodontics, surgery, removable partial, complete denture, oral lesion or lymph node, or any other reason.

V. SALIVA HIV VIRAL LOAD PROTOCOL

A. LOCAL LABORATORY PROCEDURES FOR UNSPUN SALIVA AND TO SEPARATE CELLULAR AND SUPERNATANT COMPONENTS PROCESSING

Listed below are the necessary laboratory supplies required to complete the stimulated saliva processing:

- 50 ml conical specimen tubes containing collected stimulated saliva specimen
- 1.5 ml cryovials
- 5.0 ml sterile pipette
- Chemstrip-OB (Boehringer Mannheim Diagnostics, Cat # 417144)
- RPMI 1640/IL2
- Round Cell Stain Kit (Humagen, Charlottesville, VA, Cat. # 112-RCS) and hemacytometer

B. PREPARATION OF SALIVA SPECIMEN BY THE LOCAL LABORATORY

Immediate sample processing (within six hours) by the local laboratory is required for Oral/WIHS II saliva study specimens to insure the stability of the RNA.

1. Note the volume (a minimum of 3.0 ml sample of stimulated saliva is expected from each woman), color, and presence of gross blood.

2. Gently invert the specimen to thoroughly mix the cellular and fluid components.

3. Using a sterile pipette tip and pipettor, vortex 50 µl unspun saliva directly onto the Chemstrip-OB reagent pad to check for presence of blood and leukocytes.

4. To perform the Round Cell Stain assay use a vial of 0.01 M benzidine and a vial of 0.038% peroxide. In a separate clean vial, mix 20 µl benzidine and 20 µl peroxide. Add the 20 µl unspun saliva. Mix gently. Incubate at room temperature (20–25°C) for five to ten minutes. Transfer 3 µl of mixture to a hemacytometer and count the number of stained vs. unstained round cells (see package insert). This kit stains the granules of phagocytic cells (e.g., monocytes, eosinophils, etc.) a dark brown, but will not stain lymphocytes or basophils. A light microscope may also be used to view the stained cells; a phase microscope is not recommended as it may provide too much depth to adequately view the granules.

5. If a woman produces $\leq$ 3.0 ml, aliquot at least one 1.0 ml aliquot of unspun saliva for HIV-RNA quantitation/proviral DNA detection. Proceed to Section D to process remaining saliva specimen.

Remove two 0.5 ml aliquots of unspun saliva before apportioning the remaining 2.0 ml volume for the HIV-RNA quantitation/proviral DNA detection (two 1.0 mL aliquots; see step 5a below). Proceed to Section D to process remaining saliva specimen.

a. **Reserve aliquots**

For saliva specimens which are > 3.0 ml, stimulated saliva will be aliquoted into two 0.5 ml volumes in 1.5 ml cryovials. Label each 0.5 ml aliquot with the appropriate reserve label, freeze at -70º C and batch ship to the BRinc. specimen repository on dry ice. This specimen will remain in the repository as a reserve specimen until needed for an authorized use.

b. **HIV RNA quantitation and proviral DNA detection**

Prepare two aliquots (1.0 ml in 1.5 ml cryovials) with the appropriate HIV-RNA/proviral DNA label, freeze at -70º C and batch ship on dry ice to address below to perform HIV RNA assay:

Marek Nowicki, Ph.D.
University of Southern California
Maternal-Child Virology Research Laboratory
1801 E. Marengo Street – GLB 1G8
Los Angeles, CA 90033
Telephone (323) 226-4161
Fax (323) 226-4168

On the day specimens to be tested for HIV-RNA are shipped, Patty Yepez at Maternal-Child Virology Research Laboratory at LAC/USC, 1801 East Marengo Street, GLB 1G8, Los Angeles, CA 90033, must be notified by FAX that a shipment is en route (323-226-4161, FAX: 323-226-4168). The FAX should include the Federal Express Airbill number and the number of specimens to be shipped. The ambient temperature specimen shipment FAX also serves as a packing slip. At the bottom of the FAX is an area to which you are to apply the pink packing slip labels. Attach to this area the PINK label(s) with BLACK lettering that match the barcode number on the labels attached to the specimen tube(s). The laboratory will, upon receipt of the specimens, check the sample(s) against the packing slip label(s) to identify missing or damaged specimens, and will notify the WDMAC study manager of any problems.

C. ALIQUOTING THE STIMULATED SALIVA SUPERNATANT

1. Centrifuge the remaining unspun saliva at 1,500 x g for 20 minutes at 4° C.
2. Carefully aspirate supernatant off the saliva cell pellet (about 0.2 ml saliva cell pellet volume is expected) with 5 ml pipette. Reserve cell pellet for further processing (see Section E).
3. Note total volume of supernatant and prepare the corresponding number of vials.
4. Remove 0.5 ml supernatant into a 1.5 ml cryovial. Label with the appropriate label with participant's PID/SID and set aside.

D. ALIQUOTING THE STIMULATED SALIVA VIABLE CELLS

1. RESERVE SALIVA CELLS – INSTRUCTIONS FOR CRYOPRESERVATION OF VIABLE SALIVA CELLS

   a. For the saliva cell pellet to be cryopreserved as viable cells, resuspend the saliva cells to a concentration of 1 x 10^6 /ml with cold Cryoprotective Medium (after counting them using Round Cell Stain kit and hemacytometer). The cryoprotective medium is added drop-wise, with constant mixing, over one to two minutes. Make sure cells are totally resuspended.

b. Dispense 0.5 mL aliquots of the cell suspension into separate 1.5 mL cryovials. Each cryovial will contain approximately 0.5 x 10^6 viable cells. Label all cryovials with appropriate label and store at -70° C until batch shipment on dry ice to the BRinc. specimen repository.

2. FREEZING INSTRUCTIONS FOR CRYOPRESERVED VIABLE CELLS

Place the cryovials in a styrofoam container in the bottom of a -70° C freezer. **NOTE**: Store at -70° C for no more than two to three months (optimal is to ship monthly to the BRinc. specimen repository).

E. CRYOPRESERVATION MATERIALS AND METHODS

1. MATERIALS

- Round Cell Stain Kit and hemacytometer
- RPMI 1640
- 200 mM L-glutamine
- GIBCO PBS pH 7.4 (Cat # 10010 015) (Ca^{++}, Mg^{++} free)
- DMSO
- FBS (fetal bovine serum)
- 50 ml sterile polypropylene conical plug-seal centrifuge tubes
- (Fisher Cat # 05-539-6)
- 1.8 ml cryovial
- Styrofoam containers for 1.8 ml aliquots
- 56° C water bath

2. CRYOPRESERVATION REAGENT PREPARATION (FETAL BOVINE SERUM (FBS) FROM FLOW, GIBCO, BIOLOGOS, OR HAZELTON MAY BE USED)

a. Thaw the 500 ml bottle of FBS completely.

b. Heat inactivate the FBS by immersing the entire bottle into a 56° C water bath for 30 minutes.

c. Aliquot in sterile 20 ml volumes (optional). Each aliquot should be thawed once then used.

d. Label the bottle with the day of inactivation and store at -20° C for 18 months from the date of receipt.

3. CRYOPRESERVATIVE MEDIUM – PREPARE AS NEEDED (REAGENT GOOD FOR 24 HOURS ONLY)

a. Add 20 ml of heat-inactivated FBS to 25 ml of RPMI 1640.

b. Mix well by inversion.

c. Add 5 ml of DMSO to the mixture.

d. Mix well by inversion.

e. Add 1.4 ml of 200 mM L-glutamine to the mixture.

f. Cool the media to 2–8° C and mix well prior to use.

F. ORDERING SHIPPING AND SPECIMEN SUPPLIES

- Chemstrip OB kit
- Round Cell Stain Kit
- 1-ml plastic cryovials
- **ISS-2 SAF-T-PAK**
- 81-cell boxes
- Federal Express airbills
- Labels for outside of shipping box

G. SCHEDULING SHIPMENTS OF FROZEN SPECIMENS TO THE BBI SPECIMEN REPOSITORY

International Air Transport Association Dangerous Goods Regulation, section 1.3.3.1, requires that the consignee be notified of all shipping details and advance arrangements made to accept any infectious substance for carriage. When specimens are to be shipped, the repository must be notified and be prepared to accept any shipments to ensure compliance with this regulation.

Shipment of frozen specimens to the BBI repository should occur ONLY on Mondays, Tuesdays, Wednesdays, or Thursdays. Specimens should NEVER be shipped on a holiday or the day before a holiday. Because these specimens are perishable, it is critical that the above schedule be followed.

H. PACKAGING THE SPECIMEN SHIPMENTS

The purpose of this section is to describe procedures used to package and ship infectious substances. This manual follows the procedures mandated by the International Air Transport Association Dangerous Goods Regulations – Packaging Instruction 602.

All frozen specimens sent via Federal Express must use the **ISS - 2 SAF-T-PAK Container** . This container can be packed with a maximum of 162 vials of infectious substance since it can hold two 81-cell boxes. However, most of your shipments will contain fewer than 162 vials.

A minimum of one container per month should be sent to the repository. When the repository receives your shipment, they will immediately send you another Bio-Transporter Cargo container for your next shipment. If necessary, you may send more than one container per month in separate shipments.

1. ISS - 2 SAF-T-PAK CONTAINER CONTENTS

   - Two absorbent strips
   - One foam insert
   - Two 81-cell cryovial storage boxes
   - Two cardboard inserts
   - Two Zip-lock bags

2. PACKING THE ISS - 2 SAF-T-PAK CONTAINER

   Adherence to the following procedures will assure a safe package for shipment.

   - After aliquoting the specimens, place the cryovials in an appropriate cryovial box for storage in the freezer. The 1- ml plastic cryovials are stored in an 81-cell box.
   - When you have filled (or nearly filled) with 81-cell cryovial storage boxes, secure the lids of the cryovial storage boxes with tape or a rubber band.
   - Place the boxes, along with two absorbent strips, into the Zip-lock bag, remove air and seal.

- Place the bagged box(s) into the ISS-2 shipping container.
- Fill the ISS-2 approximately one-quarter full with five to ten pounds of dry ice.
- Place the foam insert into the ISS-2 box. Fold flaps 1, 2 and 3 down.
- Enclose a copy of the Specimen Shipment FAX and the Packing Slip in an envelope and place on top of flap
- Close flap 4 and seal on the top and corners with three-inch reinforced tape.

APPENDIX A: LIST OF SALIVA HIV VIRAL LOAD PROTOCOL PARTICIPANTS

40100135
40100147
40100185
40100236
40100313
40100351
40100452
40100527
40100565
40100591
40100604
40203119
40203121
40203222
40203323
40203373
40203385
40203400
40203448
40203599
40203614
40203777
40203804
40203905
40409088
40512075
40924016
40924129
40924131
40924179
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40924460
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40924612
41027053
41027192
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41027609
41027623
41027661
41027673
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41027863
41027902
41027926
41027938
41028005
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60100545
60100608
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60100711
60101004
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60406860
60101016
60101117
60101143
60101321
60101333
60101383
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60406517
60406531
60406721
60100672
60100886
60304193
60406062
60406644