

**STANDARD OPERATING PROCEDURE (SOP):
ORAL - SALIVARY ASSESSMENT**

Unstimulated Whole Saliva Flow Rate and Collection

Materials:

Balance

Pre-weighed and labeled 50 ml tubes and lids

Clock or timer

1. Subjects shall be instructed to discontinue the use of a parasympathomimetic (i.e., Salagen®/pilocarpine, Evoxac®/cevimiline) for 12 hours, and artificial salivas for 3 hours prior to the collection of saliva.
2. Participant should not have eaten or had anything to drink for 90 minutes prior to the collection procedure. Note: to minimize diurnal variation, patients' subsequent flow rates should be measured in the morning hours if their initial measure was made in the morning, and in the afternoon hours if the initial measure was made in the afternoon.
 - a. The unstimulated whole saliva collection procedure should occur **before** the stimulated parotid collection procedure.
 - b. The time of the procedure is recorded in the appropriate box, using a 24 hour clock (00:00-23:59).
 - c. If the participant has eaten or has had something to drink within the 90 minute period, please have the participant wait the requisite amount of time before starting the unstimulated whole saliva collection procedure. Please note that some participants may need to occasionally wet their mouth with water due to severe oral dryness. This wetting is acceptable as long as they are not swallowing the water and using only enough water to coat the oral surfaces.
 - d. During the collection period, the subject shall be seated straight up with eyes open and head tilted slightly forward. See Appendix 1 for sample instructions for the patient regarding collection of unstimulated whole saliva.
3. The subject will be instructed to minimize oro-facial movements to minimize influence on salivary flow (the subject should not swallow and should not speak during the collection process).
4. Immediately before the collection begins, the subject is instructed to swallow.
5. Then the patient allows the saliva to accumulate in the floor of the mouth for 60 seconds without swallowing.
6. The patient empties the entire accumulated saliva into the pre-weighed container.
7. The procedure is then repeated 4 more times for a total collection time of 5 minutes.

8. Subjects are instructed not to swallow during the entire 5-minute collection period. The time of the procedure is recorded in the appropriate box, using a 24 hour clock (00:00-23:59)
9. The collection vial is weighed both before and after collection, and both pre and post-collection weights are recorded in the appropriate boxes on the Oral-Salivary Assessment Form.
 - a. Prior to obtaining the post-collection weight of the tube, the tube is dried or wiped to remove residual water clinging to the outer surface of the tube which can affect the post-collection weight.
10. There are several methods to determine the weight of the pre- and post-collection tube. One method is as follows:
 - a. Determine the weight of a container/cup.
 - b. Add the tube (\pm saliva) to the container/cup (point a, above) and determine the **total** weight of the tube (\pm saliva) and the container/cup.
 - c. Subtracting the weight of the container/cup (point a, above) from the **total** (point b, above) will give the weight of the tube (\pm saliva).
11. To determine the volume of saliva from the weight of saliva, it is assumed that saliva is similar to water where 1 gram of water at 4° = 1 ml (milliliter) of saliva.
12. Record the weight or volume of the saliva collected over the 5 minute period.

Salivary gland palpation

1. The face of the participant will be observed for swelling suggestive of salivary gland enlargement.
2. Each parotid and submandibular gland should be palpated to assess the absence or presence of enlargement and the nature of the enlargement present in any of those four glands should be recorded (see point 3, below).
3. The nature of the observed and/or palpated enlargement, if present, is indicated as:
 - a. “soft”: enlarged normal tissue or the texture of tofu
 - b. “firm”: a texture like that of an orange
 - c. “hard”: a texture like that of an apple
 - d. “nodular”: one or more defined mass(es) within the gland that are firm or hard
 - e. “diffuse”: the presence of enlargement throughout the gland
 - f. “fluctuant”: a fluid-filled space, such as a cyst
4. The presence or absence of tenderness in any of these glands during palpation should also be noted.
5. The above observations will then be recorded in the appropriate boxes on the Oral-Salivary Assessment Form.

Saliva expressed from ducts

1. The right parotid duct will be dried with cotton gauze. The examiner will then observe the duct while pressing on the gland with a sweeping motion (from posterior to anterior) for a few seconds. The character of saliva expressed

through the duct is then recorded by checking the: “clear,” “cloudy,” or “thickened” box on the form.

- a. “Clear” refers to a non-viscous, clear secretion that rapidly spreads on the buccal mucosa
 - b. “cloudy” refers to saliva that does not spread across the mucosa, hangs at the duct opening and contains white material;
 - c. “thickened” refers to a viscous, inspissated mucus secretion;
 - d. “none” refers to the absence of saliva at the duct opening during this procedure.
2. The same procedure will then be repeated for the left parotid gland and both submandibular glands (SMG).
 3. The SMG are palpated by gently moving the index finger along the floor of the mouth, next to the mandible, to the distal border of the mylohyoid muscle and pressing downward into the SMG while pressing upward on the skin below the SMG with the other hand.
 4. This procedure should be done before the collection of stimulated parotid saliva so as to not change the quality of the initial saliva expressed from the salivary ducts.

Oral mucosa examination

1. The standardized oral mucosal tissue examination will be conducted using a mouth mirror and cotton gauze.
2. The oral examination protocol is abstracted from the standardized oral examination method recommended by the World Health Organization, and should take no longer than 5 minutes [1]. The procedure is as follows:
 - a. Examine lips, particularly looking for cracks, fissures or erythema at the commissures
 - b. Pull lower lip to examine lower labial mucosa
 - c. Pull upper lip to examine upper labial mucosa
 - d. Examine buccal mucosa, hard and soft palate, gingiva
 - e. Examine dorsum and ventral tongue. Use gauze to pull the tongue and examine its lateral borders and foliate papillae area
 - f. Examine the floor of the mouth by having the patient place the tip of their tongue to the posterior roof of their mouth.
 - g. To determine whether salivary pooling occurs in the floor of the mouth, use a piece of gauze to dry the floor of the mouth and gently push the tongue aside to observe whether saliva exits from the submandibular/sublingual glands and pools in the floor of the mouth.
3. On the Oral-Salivary Assessment Form, record the presence or absence (yes/no) of papillary atrophy, erythema, and/or fissures seen on the dorsal tongue.
4. Oral mucosal erythema is defined as:
 - a. “localized” if patchy erythema is observed at one anatomical site (e.g., palate; buccal mucosa; labial mucosa; tongue; floor of mouth).
 - b. “generalized” if patchy erythema is observed at more than one anatomical site.

5. For the clinical diagnosis of oral candidiasis, we adapted the definition and diagnostic criteria developed for the USA Oral AIDS Collaborative Group and the EC-Clearinghouse and WHO Collaborative Center [2,3] for the presumptive diagnosis of oral candidiasis. The definitions are as follows:
 - a. **Pseudomembranous candidiasis:** white maculopapular plaques that may be located in any part of the oral cavity, and can be rubbed off revealing an erythematous surface
 - b. **Erythematous candidiasis:** red areas (patchy erythema) usually located on the palate or dorsum of tongue, but occasionally on the buccal and labial mucosa.
 - c. **Angular cheilitis:** red fissures or cracks located at the commissure of the lips.
6. Mucosal telangiectasias appear as well-defined, flat to slightly raised, usually multiple, polygonal red lesions on the labial vermilion or mucosa of the lips, cheeks, tongue or palate. They represent clusters of dilated superficial blood vessels with active blood circulation. When telangiectasias are covered and pressed on by a transparent object, such as a microscopic slide (called diascopy), they blanch, in contrast to purpura, which do not blanch under pressure.

Dental assessment

1. The total number of teeth present will be recorded in the corresponding box under the “Caries Assessment” section of the Oral-Salivary examination form.
2. The detection of incisal, cervical, and root caries will utilize a dental explorer (curved or straight), but not require radiographs.
3. The presence of caries will be recorded when an area of the tooth exhibits a brown-colored spot or area that feels relatively “soft” to the explorer.
4. Several types of caries are defined as follows:
 - a. **Incisal caries** refers to caries affecting the incisal edge of anterior teeth or the cusp tips of posterior teeth (premolars and molars). Such caries may extend to the horizontal midline of the coronal surface on either the lip or tongue side of the teeth.
 - b. **Cervical and root caries** refers to caries affecting any tooth at the exposed root or gingival margin. Such caries may extend to the horizontal midline of the coronal surface on either the lip or tongue side of the teeth.
 - c. **Recurrent caries** refers to caries at the margin(s) of preexisting restorations. On the Oral-Salivary Examination Form, only recurrent caries occurring around a pre-existing cervical or incisal restoration will be charted.
 - d. **Advanced caries** are detected as gross cavitation and thus present few problems in diagnosis, but will not be noted on the Oral-Salivary Examination Form unless they occur in cervical or incisal locations.
 - e. **Early caries** are more difficult to diagnose consistently. Therefore, the following guideline is provided:
 - i. Incisal, cervical, and root surfaces are carious if they are decalcified with a white, chalky, or brown spot as evidence of

subsurface demineralization and if the area is found to be soft by penetration with the explorer or by scraping away the enamel with the explorer. ***For purposes of SICCA, only a brown-colored spot (not chalky or white colored lesions) or spot/area that feels relatively “soft” to the explorer will be recorded as caries.***

5. The presence or absence of a removable maxillary or mandibular partial or full denture will be recorded in the relevant box.

Stimulated Parotid Flow Rate and Collection

2% Citric acid solution (2 grams in 100 ml sterile water, kept refrigerated)

15 ml screw-top tubes (pre-weighed; tubes and tops labeled for collection from the right and left parotids); use smaller 1.8 ml cryovial if only a small volume of saliva is expected.

Parotid saliva collector (right and left)

Tygon tubing (10.2 cm for the collecting tube from the inner circle of the parotid saliva collector, and 30.5-40.6 cm to connect the parotid collector to the suction bulb)

Suction bulb (a luer-lock syringe attached to a blunt cannula may be substituted for the suction bulb).

Medium Binder Clip (for use with the syringe method to create suction)

Ice and container

Timer or clock

Balance

1. Subjects shall be instructed to discontinue the use of a parasympathomimetic (i.e., Salagen®/pilocarpine, Evoxac®/cevimiline) for 12 hours, and artificial salivas for 3 hours prior to the collection of saliva.
2. If the subject has completed the unstimulated whole saliva collection procedure, then he/she may be offered a single oral rinse of water.
3. The orifice of the parotid duct is located bilaterally on the buccal mucosa opposite the upper second molar tooth. If you have difficulty visualizing the orifice, dry the area with gauze.
4. The parotid collector is placed on the mucosa so that the inner ring surrounds the duct orifice.
5. The collector is held on the mucosa by suction from the outer ring, created by squeezing and holding the deflated bulb during placement over the duct orifice, then releasing the bulb when the cup is in place. As an alternative, a 3ml luer-lock syringe attached to a blunt cannula may be used in place of the red bulb. Suction is obtained by pulling back on the syringe and allowing the pressure to come to equilibrium.
 - a. The bulb/syringe can then be rested on the patient's shoulder.
 - b. A medium binder clip is attached to the tygon tubing going from the collector to the syringe to “lock-in” the air in the tubing. The suction created should be sufficient to hold the cup in place without occluding the inner chamber of the parotid collector with tissue (i.e., not too much suction).

6. Saliva from the parotid gland then flows passively into the inner ring and through the attached tubing.
7. The subject should avoid unnecessary movement of their head or jaw to prevent dislodging this cup.
8. The flowing saliva will be collected into an ice-cooled pre-weighed and pre-labeled container.
9. 2% citric acid solution is applied to the posterior lateral surfaces of the tongue, bilaterally, with a cotton swab for 5 seconds every 30 seconds to stimulate secretion. See Appendix 3 for flow chart on the collection of stimulated parotid saliva.
10. Flow may not begin for a minute or two after stimulation has been applied.
11. A maximum of 5 minutes is allowed for saliva to appear in the clear portion of the tubing.
12. If saliva flow is observed during this 5-minute period, an additional 5 minutes is allowed for the saliva to reach the end of the tubing and *the 5-minute collection period begins when saliva begins to exit the Tygon tube.*
13. If no saliva is seen after the 5-minute waiting period, the salivary collection apparatus is removed to assure correct placement over the duct orifice (an impression of two concentric circles on the buccal mucosa with the parotid orifice observed in the middle of the inner circle verifies correct placement).
14. If the positioning was incorrect, the saliva collector is replaced and a second 5 minute collection period is initiated. If no saliva flows during that period, the post-collection flow rate will be the same as the pre-collection weight.
15. The citric acid stimulation should continue for 30-second intervals during the entire collection procedure: a) only the first 5 minutes of saliva collection is weighed, b) any excess saliva does not need to be weighed, c) if the total value in the tube is less than 0.5 ml, empty the residual saliva in the right or left collecting tube into a separate tube.
16. The saliva is collected into chilled pre-weighed tubes and kept on ice until frozen.
17. The collection vials are weighed both before and after collection, and the right and left pre- and post-collection weights are recorded in the appropriate boxes on the Oral-Salivary Assessment Form. In addition, the total volume of saliva collected is determined by subtracting the post collection weight from the pre-collection weight. See points 11 and 12 of Unstimulated Whole Saliva for directions on determining the pre- and post-collection weight of the tube and volume of saliva.
 - a. It is recommended that the screw-top **and** tube be labeled.
 - b. If multiple tubes are used during the saliva collection process, the paired tube and screw-top need to be simultaneously tracked to ensure that the same tube and screw-top are measured before and after collection.
18. Trouble shooting:

If one of the parotid collectors falls off during the collection process and the other collection cup remains intact:

 - a. Complete the 5 minute collection period (recall that the 5 minute collection period starts when the saliva exits the tubing) for the parotid collector that

remains intact. Re-apply the collector to the opposite side and restart the procedure.

19. The actual time of saliva collection should be recorded for the right and left parotid glands so a salivary flow rate may be calculated. If technical problems were encountered and it is not possible to calculate a salivary flow rate, enter 00 minutes and 00 seconds for actual collection time.
 - a. For example, if saliva was collected from the right parotid for 5 minutes, then the post-collection weight of the vial should be entered along with a collection time of 5 minutes. If saliva was collected simultaneously from the left parotid gland, but there were technical problems and it is not possible to determine an accurate salivary flow rate from the left parotid gland, enter the post-collection weight and an actual collection time of 0.000 minutes as well as indicating the pertinent technical problem (i.e., cup placement, cup retention, patient cooperation).
 - b. Even if technical problems are encountered and it is not possible to calculate a salivary flow rate, please enter the post collection weight of the tube so the net volume of saliva may be determined *and send any collected saliva to the bank for freezing*, no matter how small. The 00 minutes 00 second collection time will indicate that a salivary flow rate should not be calculated.
 - c. Please check “yes” for “technical problems” only if a salivary flow rate cannot be determined (Question #33). If technical problems are overcome during the salivary collection process, then it is not necessary to indicate that you had technical problems.

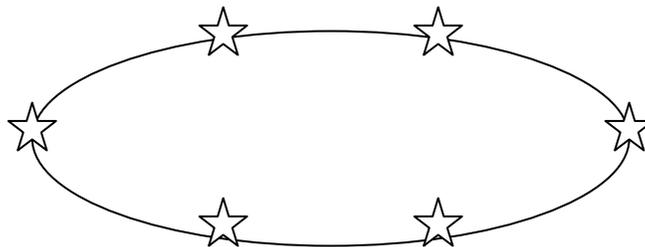
Labial Salivary Gland (LSG) Biopsy

Materials:

Topical anesthetic
Local anesthetic with vasoconstrictor
Syringe and disposable needle
Scalpel, #15 blade
Adson tissue forceps (1 x 2 teeth)
Iris scissors
Needle holder
Suture scissors
Suture material, 0000 plain gut
Cotton gauze

1. The biopsy is done through mucosa of the lower lip that appears normal clinically. The normal appearance is important because mucosal inflammation, of various causes, could result in inflammatory cell infiltration of submucosal salivary glands.
2. Topical anesthetic is applied at the site of local anesthetic injection, usually at the site of the medial extent of the proposed incision (see #5 below).

3. Local anesthetic is infiltrated into the subepithelial area that will be incised. Using local anesthetic with vasoconstrictor (at 1:100,000 dilution) is beneficial because it reduces bleeding in the surgical field and significantly improves visibility for the surgeon.
4. The assistant everts the lip so the mucosa is exposed. Note: tension on the lip is minimized if the patient has their teeth together, or nearly so.
5. A horizontal incision will be made to the right or left of the midline, approximately halfway between the vestibule and the vermilion border and halfway between the midline and the labial commissure.
6. A 1.5 to 2.0 cm horizontal linear incision is made through the epithelium only, not into the underlying connective tissue. Incision through the epithelium is confirmed when the incision margins separate, creating an elliptical shape. The depth of incision is important because the minor salivary glands are located within the lamina propria. If the incision goes beyond the epithelium and through the lamina propria, the minor salivary glands will retract with the epithelial margins and dissecting the glands becomes very difficult.
7. Blunt dissecting the lamina propria adjacent to the epithelial margins is the next step. This releases minor salivary glands from lamina propria beyond the incision and brings them into the operating field. Blunt dissection should not be done more than necessary to avoid damage to the glands and surrounding tissue. Excessive blunt dissection will increase post-operative pain, swelling and purpura. Blunt dissection should be done at six sites: at the two corners of the ellipse, and at points one third of the way along each side of the epithelial incision (as noted by stars in the diagram below).



8. Blunt dissection is done by grasping the epithelium with the Adson forceps at the area of the incision indicated by a star on the diagram above and inserting the iris scissors horizontally into the lamina propria below the Adson forceps. The curve of the scissor blades must be up and the points closed. With the scissors penetrating horizontally 0.5 to 0.7 cm into the lamina propria, open the scissors and withdraw them. Repeat this process at the remaining 5 sites.
9. Blotting the field with gauze should now clearly reveal minor salivary glands and any nerve fibers passing through the field.
10. Approximately 10 (see #11 and #12 below) minor salivary glands should be removed, one at a time. This is done by grasping and lifting them slightly with Adson forceps, gently dissecting with the iris scissors, and then cutting the remaining attachment with the scissors. This should be done with care to avoid excessive damage to the glands and protect any sensory nerve fibers in the field.

11. A number of LSGs should be placed in neutral buffered formalin that will provide a minimum gland section area 12-15 mm² for microscopic focus scoring and studies using paraffin-embedded sections. The number of glands needed to provide that area can be estimated from the diameter of each gland:

<u>LSG diameter</u>	<u>LSG section area (in middle 1/2 of gland)</u>
1	~1 mm ²
1.5	~2 mm ²
2.0	~3 mm ²
2.5	~5 mm ²
3.0	~7 mm ²

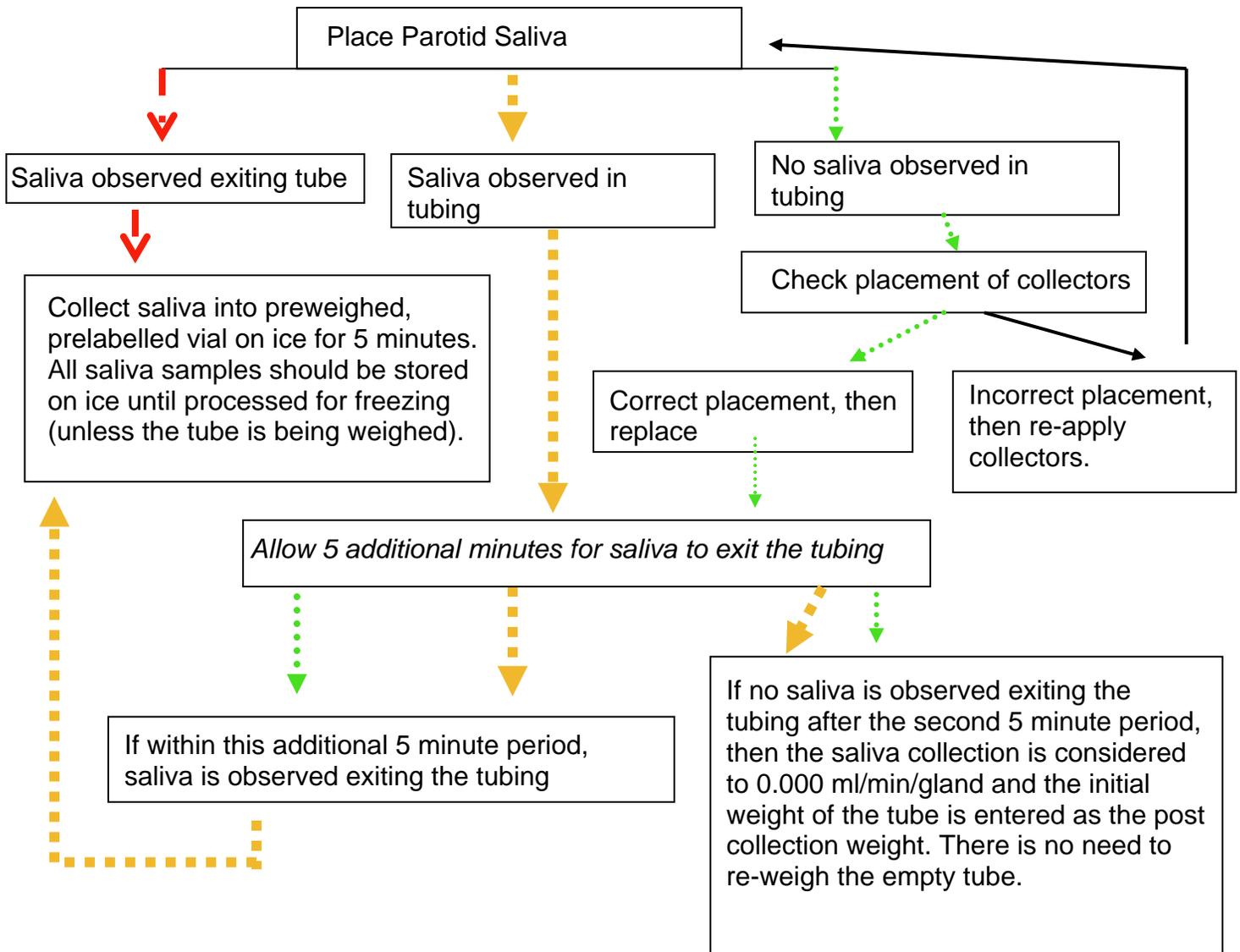
12. Remaining LSGs are prepared for freezing as follows:
- Place them on an aluminum foil boat.
 - The foil and glands are placed into a Nunc vial, capped, and immersed into liquid nitrogen (snap-freezing). The label on the Nunc vial should indicate the number of glands that are in the vial.
 - Depending on the number of glands available for freezing, you may repeat steps a and b as necessary to freeze all of the glands collected.
13. If additional minor salivary glands are present in the field, they should be added to the specimens to be frozen. They are removed to reduce the possibility of the patient developing a mucus extravasation lesion during the healing process.
14. The mucosal incision margins are then repositioned by two or three interrupted sutures using 0000 plain gut or other suture material that will dissolve in 5 days or less. The sutures should be placed fairly close (approximately 2mm) to the wound margins to avoid penetrating salivary glands remaining in the adjacent lamina propria. Each suture should be tensioned to just bring the wound margins into contact, without overlap or rolling.
15. A moistened, folded 2 x 2 cotton gauze pack is placed over the biopsy site between the lip and teeth.
16. An ice pack placed on the skin in the area of the biopsy soon after the procedure will reduce swelling and the possibility of purpura forming on the skin.

References cited

- 1) World Health Organization on the Epidemiology of Oral Mucosal Diseases and Conditions. Reprinted in *Comm Dent Oral Epidemiol*, 1980; 8:1-26
- 2) Greenspan JS, Barr CE, Sciubba JJ, Winkler JR, U.S.A Oral AIDS Collaborative Group. Oral manifestations of HIV infection. Definitions, diagnostic criteria, and principles of therapy. *Oral Surg Oral Med Oral Pathol* 1992; 73:142-144.
- 3) EC-Clearinghouse on Oral Problems Related to HIV infection and WHO Collaborative Centre on Oral Manifestations of the Immunodeficiency Virus. Classification and diagnostic criteria for oral lesions in HIV infection. *J Oral Pathol Med* 1993; 22:289-91.

Appendix 1. Sample Patient Instruction for the Collection of Unstimulated Whole Saliva.

We would like to collect all the saliva that you can empty into a tube for a period of 5 minutes. Immediately before collecting the saliva, you will be asked to clear the saliva in your mouth by swallowing it. For the next 5 minutes, you will be asked to not swallow and to not speak. Every 60 seconds, you will be directed to gently spit any of the accumulated saliva in your mouth into the provided tube. During this time, you should be sitting upright with your head tilted slightly forward. Thank you.



Appendix 2: Collection of Stimulated Parotid Saliva