

STANDARD OPERATING PROCEDURE (SOP) FOR SALIVA COLLECTION

Equipment and supplies:

- (1) Laboratory vortex mixer
- (2) Refrigerated centrifuge (at least 2600 xg) able to accommodate 15 mL sterile collection tubes
- (3) -80°C freezer

Materials:

- (1) Water to rinse mouth, 10-15 mL (*optional*)
- (2) 15 mL sterile collection tubes
- (3) Cryotubes able to accommodate -80°C temperatures
- (4) Pipettes (2, 20, 1000 μ L) and tips
- (5) Superase-In (Ambion, Cat #AM2696, 10,000 U, 20 U/ μ L) stored at -20°C

Operating room or clinics:

- (1) Preferably aim for 8–11 a.m. and ask the subject to refrain from eating and drinking anything except water for at least 30 minutes prior to saliva collection. Saliva donors should not have brushed their teeth within 1h of collection and should not have undergone dental procedures in the previous days.
- (2) Oral rinse (*optional*-if dried mouth): give the subject the water and ask that they rinse their mouth out well. The subject need then to expectorate or swallow the water.
- (3) **Five minutes after this oral rinse**, ask the subject to spit into a 15 mL sterile collection tube. Remind subject to refrain from coughing up mucus and no nasal mucus, only saliva from mouth.
- (4) Collect approximately 2-5 mL of saliva. Do not take more than 30 minutes to collect. Keep collected saliva **on ice**.
- (5) Processing should occur within a 1-2 h window of collection time.

(NOTE: It is imperative that all saliva sample processing occurs as soon as possible after collection and that all samples remain on ice at all times. Prior to actually collecting the saliva sample from the patient, set the centrifuge to 4°C to make sure that it has reached the proper temperature for processing).

Laboratory:

1. Vortex the whole saliva for 10-15 seconds (to reduce the inherent viscosity-mucous).
2. Centrifuge the tube at 2600 xg for 15 min at 4°C.
 - (a) During centrifugation, label cryotubes (see “Labels” section below).
 - (b) Incomplete separation may occur, if so, centrifuge for an additional 15 minutes.
3. Pipette the supernatant (SN).
 - (a) Pipette 1 mL of saliva SN into a cryotube.
 - (b) Then add 1 μ L Superase-In (final 20U/ml) into 1 mL of saliva SN, cap & invert to mix. (**NOTE: Keep Superase-In reagent on ice during use**)
-- Repeat steps 3.a – b for each extra 1 mL of SN --
4. Discard the pellet remaining in the 15 mL tube.
5. Store the samples in the -80°C freezer in a labeled storage box.

Labels:

Sample/patient ID code + vial # (i.e.: OC1 #1)

FRACTION: SN

DATE: mm/dd/yyyy