



Collection and preserving of saliva

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Read all instructions prior to collection.

Intended use: For the collection of human DNA from saliva samples.

Contents: Kit contains stabilizing liquid and precautions: Wash with water if stabilizing liquid comes in contact with eyes or skin. Do NOT ingest.

- **Saliva has been used to detect caries risk, periodontitis, oral cancer, breast cancer, salivary gland diseases, and systemic disorders such as hepatitis.**
- **Of course, successful measurement of salivary analytic requires optimal collection, processing, and storage procedures and conditions**
- **Oral samples include saliva, gingival crevicular fluid (GCF), oral swabs and dental plaque. Recent years has seen an increasing interest in the use of oral fluids as diagnostic tools, especially whole or total saliva.**

- **Saliva has many important roles in oral health like lubrication, physical protection, cleansing, buffering, tooth integrity, maintenance of pH and antibacterial action.**
- **When compared to other body fluids, saliva is readily available, can be non-invasively collected, with no risk of cross infection. Moreover, salivary analysis can detect changes that are reflective of both oral and systemic conditions.**
- **Despite its advantages as a diagnostic fluid, lack of enough information regarding the methods of collection and storage of samples.**

- **Saliva is composed of more than 99% water and less than 1% solids, mostly electrolytes and proteins, the latter giving saliva its characteristic viscosity**
- **The term saliva refers to the mixed fluid in the mouth in contact with the teeth and oral mucosa, which is often called 'whole saliva'**
- **Normally the daily production of whole saliva ranges from 0.5 to 1.0 litres**

- **The greatest volume of saliva is produced before, during and after meals, reaching its maximum peak at around 12 am., and falls considerably at night and during sleep.**
- **Some research indicated that saliva secretion was increased with an increase in temperature, hence, the seasons of year is consider as effective factor in saliva collection.**

- **Factors affecting stimulated saliva include gland size, food intake, smoking, gag reflex and type of stimulation given. Stimulated saliva represents the secretion during food intake (physiological stimulation), and is present in the mouth for up to 2 hours. Various stimulants like paraffin wax, unflavoured chewing gum base, cotton puff and rubber bands bring about masticatory stimulation.**

Collection of whole saliva

There are 4 methods for collection of whole saliva:

- 1. Spitting method**
- 2. Draining method**
- 3. Suction method**
- 4. Swabbing method**

Spitting method

- **Ask the patient to spit in the spittoon before starting collection.**
- **Clean the mouth with a sterile piece of gauze**

This considered the zero time.

- **Take a sterile beaker and weight it.**
- **Due to the saliva is allowed to accumulate in the floor of the mouth, ask the patient to spit inside the beaker, e.g.: each 30 sec. interval for about 5 min.**
- **Then the spitting is stopped and the beaker is weighted after that, subtract the weight of beaker when its empty and after completion of spitting , the result is multiplied by the specific gravity of saliva to get the volume of saliva within 5 min.**

Draining method

- **Repeat the first 3 points in the previous method.**
- **Ask the patient to sit with the head bent down and the mouth open to allow the saliva to drip passively from the lower lip into the graduate sterile beaker below his mouth for about 3-5 min.**
- **After that ask the patient to spit what is found in his mouth in the spittoon, and get the volume by the same previous method.**



Suction method

- **Saliva is allowed to accumulate in the floor of mouth and aspirated continuously by using syringe, micropipettes or saliva ejector or an aspirator**
- **Repeat the first 3 steps in the 1 st. method.**
- **Bring the beaker and put in it a rubber stopper which is penetrated from one side by saliva ejector and from the other side by a connector to the vacuum apparatus , when the pressure created inside the beaker by the vacuum lead to more saliva sucked from the oral cavity , e.g. for about 4-5 min, then the volume of saliva collected is getting as previously mentioned.**



Dental suction tubes



Automated micropipette for aspirating saliva

Swabbing Method

- Repeat the first 2 steps in the first method.
- It is performed by introducing a synthetic gauze sponge, pre-weighed swab or cotton pad into the mouth, at the orifices of major salivary glands.
- Asked the patient to chew such that the sponge gets soaked within the saliva. Saliva soaked sponge is removed and placed in a sterile test tubes.
- Though this method is less reliable, it helps in the assessment of the level of oral dryness. It is mainly used in the monitoring of drugs, hormones or steroids.



Saliva oral swab

The SOS features a proprietary, synthetic (non-organic), hygienic, individually wrapped oral fluid collection device, specifically designed for both increasing participant compliance and saliva analysis. Medical-grade packaging minimizes the possibility of environmental contaminants, and the swab material provides ready-to-process samples by filtering out unwanted mucins, cells and other aggregates from oral fluid specimens.

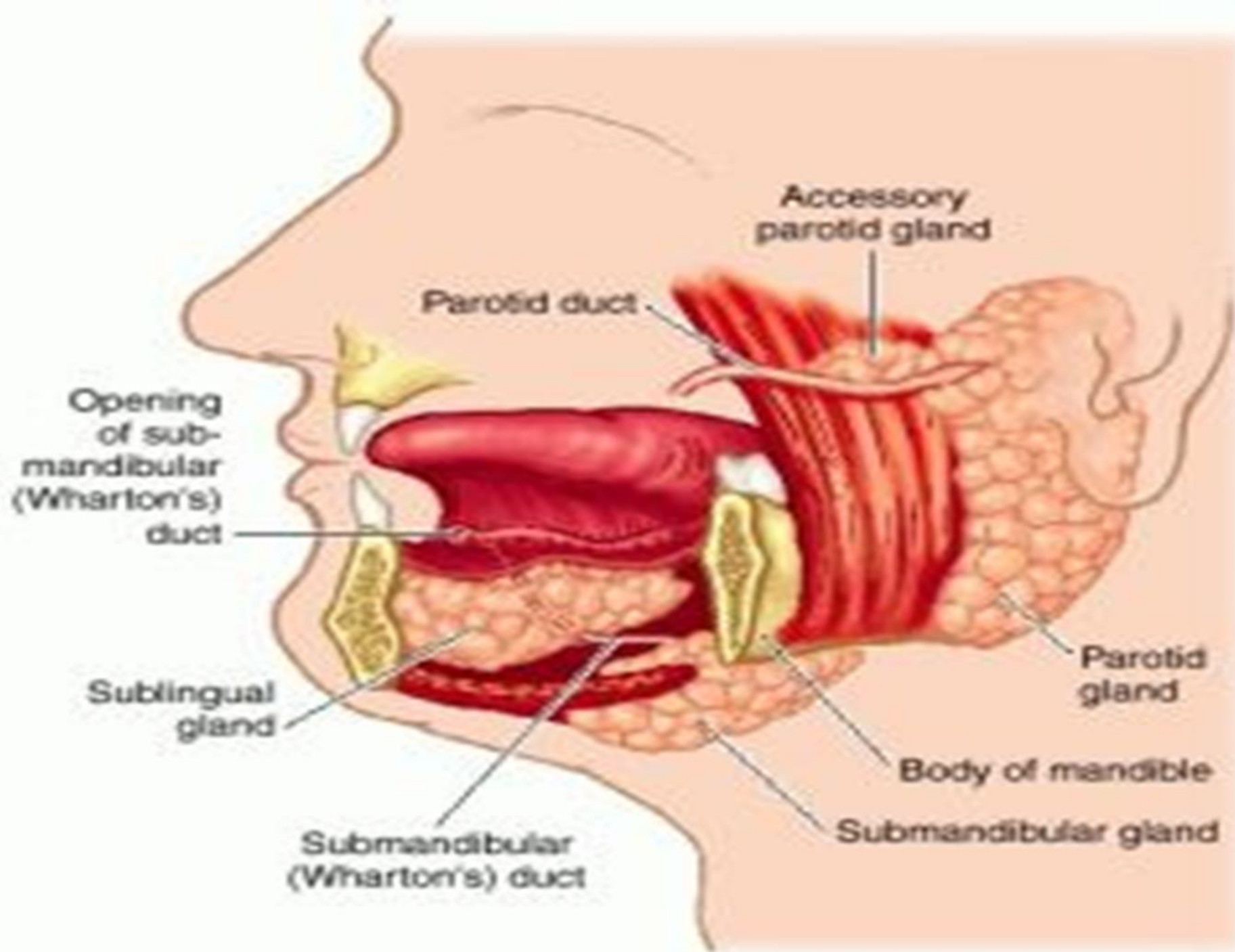


Collection the glandular Saliva

Glandular saliva is collected from a specific site of the oral cavity. It includes parotid saliva, submandibular/ sublingual saliva and secretions from minor salivary glands.

- **Parotid saliva:** The parotid duct opening is located on the buccal vestibule, opposite to the first and second molars.
- Un-stimulated parotid salivary flow is very low or even absent, hence it is collected under stimulation. Citric acid solution (2-4% weight/ volume) is used for stimulation.
- Parotid saliva is collected using a cannula or Lashley or Carlson Crittenden cups

The device has an outer and inner chamber. The inner chamber is attached to a plastic tubing. The outer chamber is attached to a rubber bulb or a suction device via plastic tubing and the cup is placed over the ductal opening.

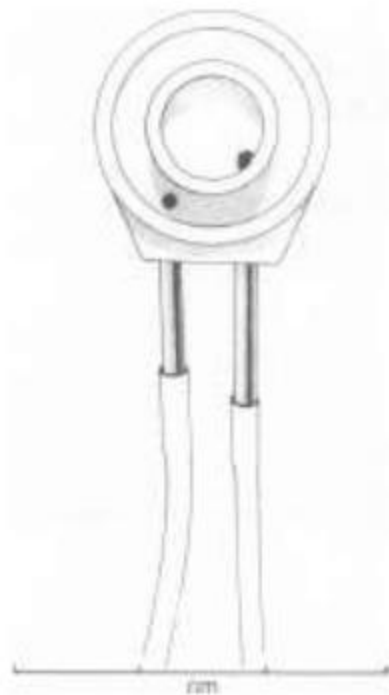




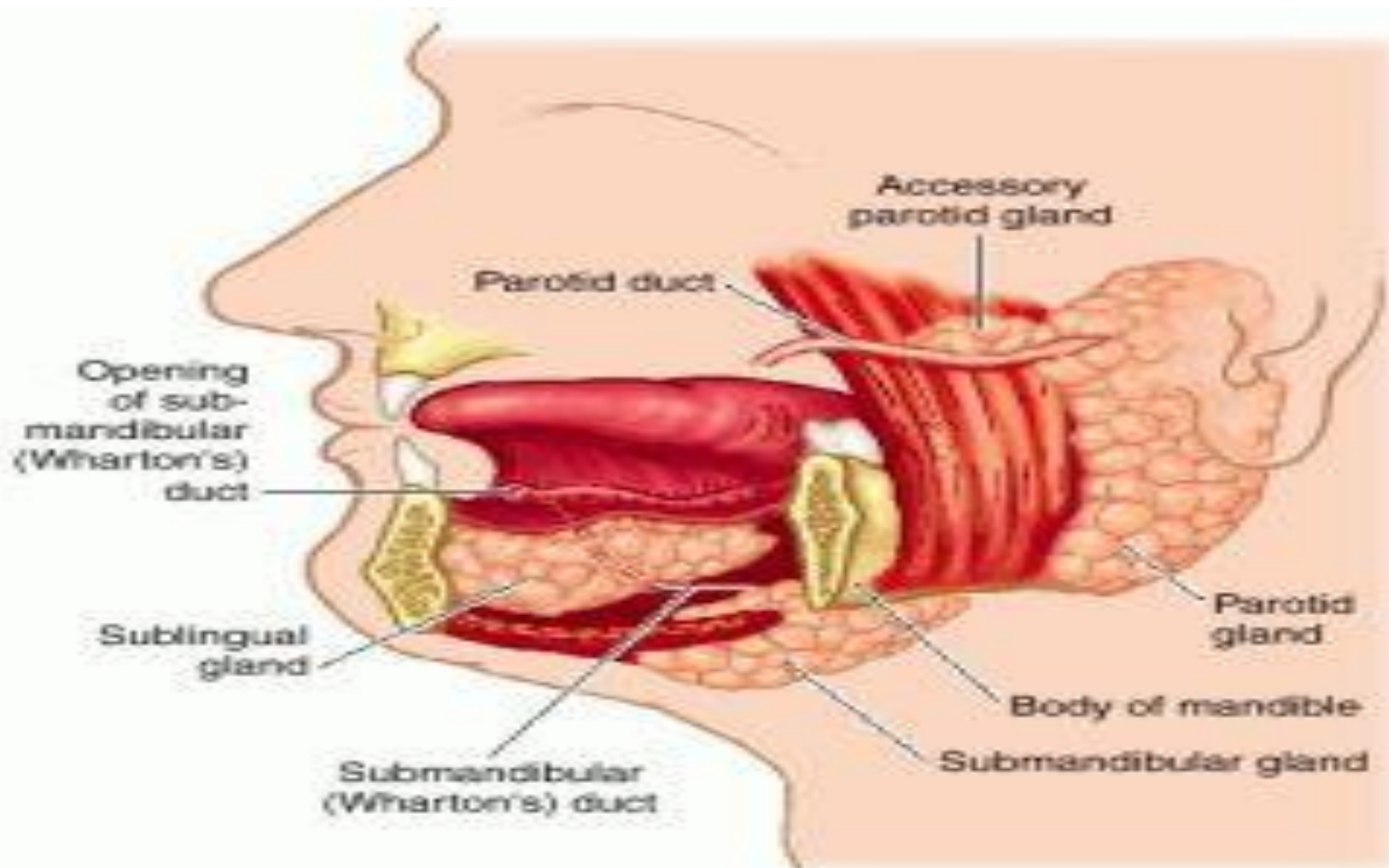
Carlson Crittenden cups

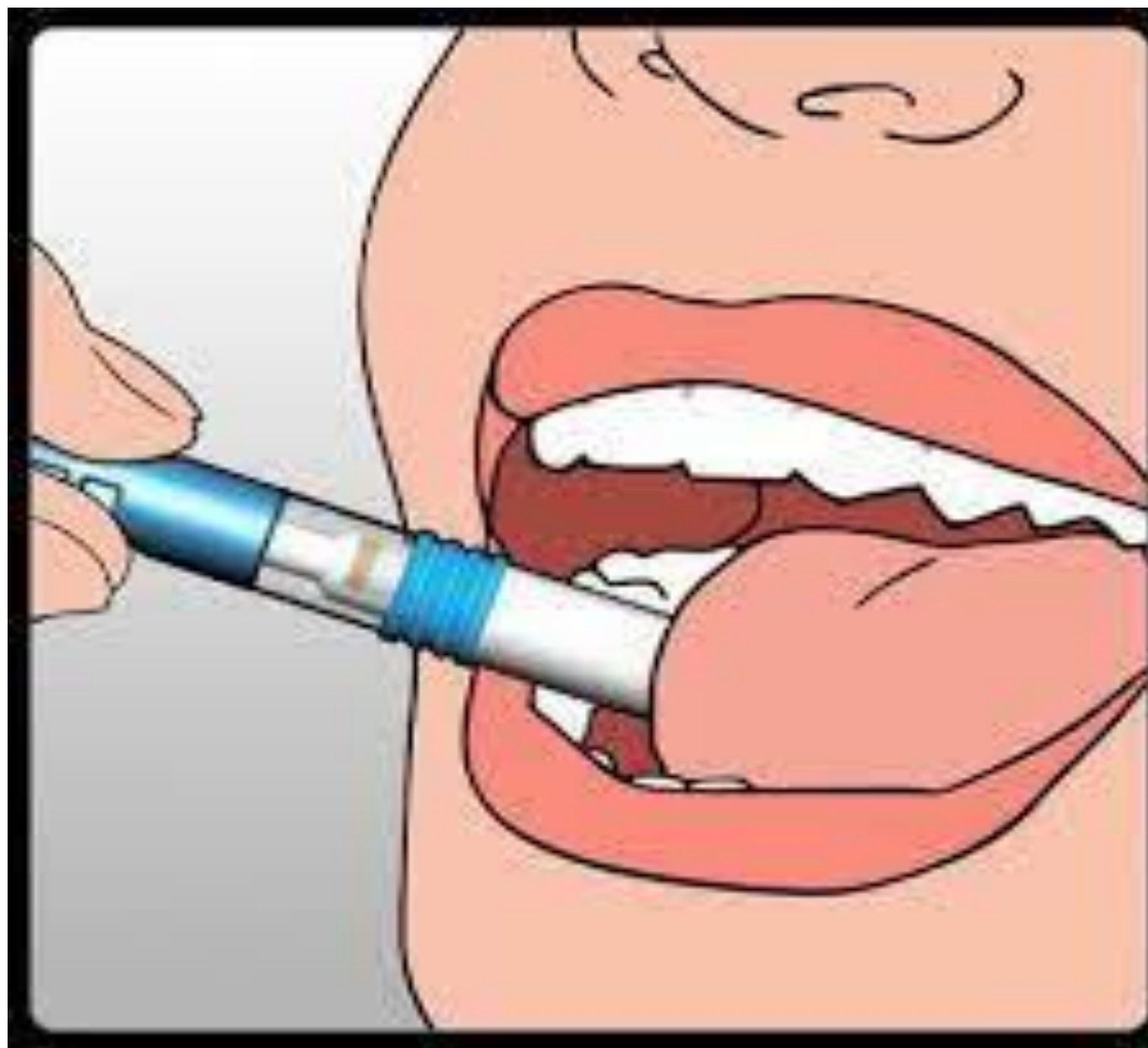
Lashley cup/ Carlson – Crittenden Collector

- Used for parotid
- designed by carlson - crittenden
- 2 metal cups with 2 outlet tubes
- Inner cup collects saliva
- Outer cup connected to vacuum
- Basic design was modified by lashley



The thin duct which is prone to rupture poses to be the biggest disadvantage of this .





- **About 1-1.5 ml of parotid saliva can be collected in 10-15 minutes. It must be noted that the protein and organic material concentration of parotid saliva is twice as high as submandibular and sublingual saliva.**
- **The disadvantages of this procedure are that it is complex, slow and invasive. It requires skilled personnel and expertise.**
- **Parotid salivary flow rate has been shown to reach its peak value, seasonally during the winter and shows circadian in the afternoons.**

2. Submandibular/ sublingual saliva: This glandular saliva can be collected by cannulation, segregator methods and suction methods. The simplest method for collection of submandibular saliva is the suction method.

Suction method: It is collected by blocking the Stensen's duct (parotid duct) using cotton roll or Lashley cup. Then, the saliva which gets accumulated in the floor of the mouth is aspirated using a syringe or micropipette or with gentle suction.

Cannulation: Tapered polyethylene tubing can be used for cannulation of the Wharton's duct (submandibular duct or submaxillary duct).

Segregator method:

An apparatus capable of collecting submandibular and sublingual saliva with masticatory as well as gustatory stimuli has been reported. The collector is placed on the lower jaw and the polyethylene tube connects the chamber to the collecting tube.

The central chamber collects the sub- mandibular saliva, while the two lateral chambers collect sub-lingual saliva, this is prevent further eliminate the contamination by parotid saliva and minimize the intermixing of submandibular and sublingual saliva.

The procedure is time consuming because the device has to be fabricated and adjusted on an individual basis.

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Collection of saliva from minor glands:

- **Saliva from minor salivary glands includes palatine saliva, buccal and labial saliva.**
- **Palatine saliva is collected using filter paper (periopaper) or pipette method or by a collecting prosthesis.**
- **Labial and buccal saliva can be collected using the periopaper or sialopaper absorbent method.**



Sialopaper, used for saliva collection from minor salivary gland



Graduated sterile tubes with conical end



Plastic conical tubes used for collecting saliva

Factors affecting salivary flow

- Patient position
- Hydration
- Diurnal
- Seasonal variation

Guidelines for collection of saliva:

- The subject should be made to sit comfortably in a calm and isolated room.**
- He / she should rinse the mouth thoroughly using distilled water or deionized water to remove any food debris.**
- The patients are then asked to spit out the saliva that has been collected in the initial 30 seconds.**
- They are also trained to collect the saliva in the floor of the mouth for whole saliva collection.**
- High quality tubes or vials should be used for collection.**

- **Collection should be made at a standard time, preferably between 8 to 11 am. The subject should preferably be in the fasting state or two hrs after breakfast.**
- **The participants should not brush their teeth for a duration of 45 min prior to the sample collection.**
- **The participants should not drink alcohol or drink anything on the day of saliva collection.**
- **The participants should not consume dairy products, acidic or sugary food within 30 min before saliva collection.**
- **Denture wearers should remove their dentures prior to saliva collection.**

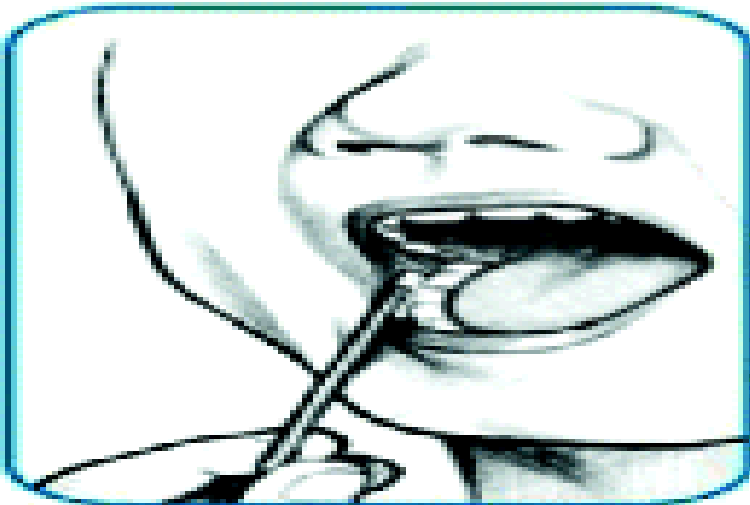
- **Dental work or oral examination should not be performed within 24 hrs prior to the sample collection.**
- **Participants should be screened for any oral health problems or injuries. Visibly contaminated samples with blood should be discarded.**
- **The subjects should avoid smoking for at least two hours prior to saliva collection.**

Saliva storage:

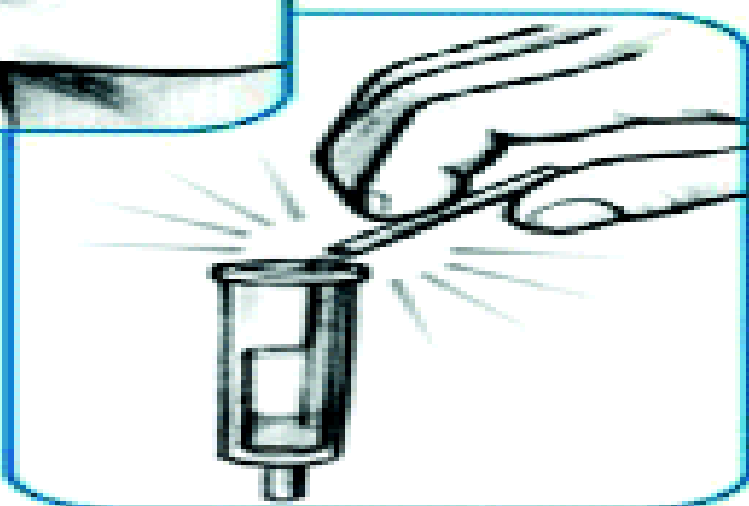
- Freeze samples at or below -20°C (temperature of a household freezer) immediately after collection.
- If freezing is not possible, refrigerate immediately at 4°C and maintain at this temperature for no longer than necessary (ideally less than 2 hours) before freezing at or below -20°C to minimize degradation and to prevent bacterial growth.
- Samples can be frozen in the swab for up to 6 months with no decline in levels.

- If it is known ahead of time that samples may need to be stored for longer than 6 months, we recommend expressing the saliva out of the swab either by centrifugation or squeezing through a syringe immediately after collection, and storing the expressed saliva in cryovials, ideally at -80 C.
- Samples can be stored at -80°C for several years, many samples that have been stored properly for over four years have shown little or no degradation.

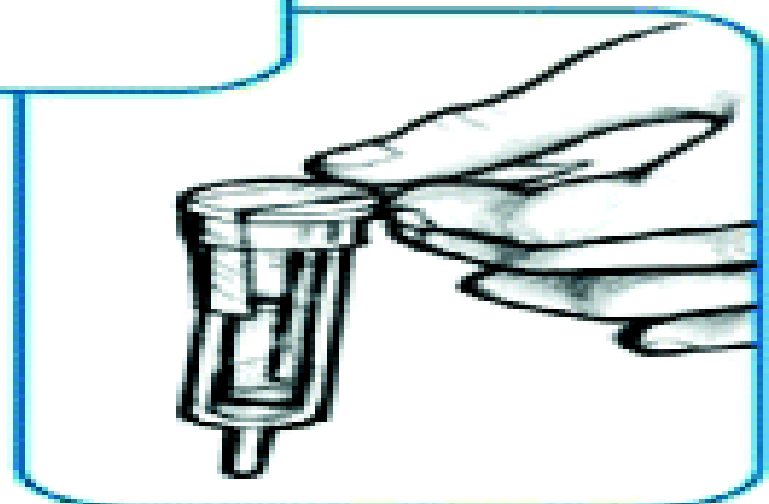




Swap



Snap



Seal

PRIOR TO SAMPLE TESTING

- On the day samples are to be assayed, bring samples to room temperature, vortex, and then centrifuge at 1500 x g for 15 minutes.
- If the samples appear viscous, centrifuge at a higher speed such as 2500 x g or break up the clot with a pipette tip and re-centrifuge.
- Assays should be performed using only clear saliva, avoiding the pellet formed at the bottom of the tube.
- When pipetting viscous solutions such as saliva, greater accuracy is obtained by aspirating slowly to avoid the formation of bubbles.

- Vortex and re-centrifuge tubes following each freeze-thaw cycle since additional precipitates. If samples will be used for genetic analysis, it is important to keep the cell sediment at the bottom of your whole saliva sample or contained in the swab device, may develop upon refreezing.