



# **Saliva Collection and Handling Advice**

3<sup>rd</sup> Edition

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## About Salimetrics

Salimetrics supports thousands of saliva researchers around the world with accurate, high-quality, and relevant salivary assays, collection methods, and saliva testing services. Dedicated to the field of salivary bioscience, Salimetrics assays have been used in more saliva-related published papers than any other assay in the field. Salimetrics is headquartered in the United States with locations in State College, Pennsylvania and Carlsbad, California, as well as offices in Europe and a global distribution network.

Learn more about Salimetrics, their full-service saliva testing service (the SalivaLab), the Centers of Excellence initiative, global partnerships, and what's new in salivary research at [www.salimetrics.com](http://www.salimetrics.com).

## About SalivaBio

At SalivaBio, we recognize the critical role saliva collection plays in providing consistent, accurate results. SalivaBio is dedicated to the creation of a global standard for collection devices and techniques, as well as developing new, cutting edge saliva collection methods to increase ease of use and participant compliance. Exclusively from Salimetrics, all SalivaBio products are extensively tested to minimize or eliminate variability from saliva collection, and have been optimized for collection with specific groups of participants.

Learn more about SalivaBio and the products and techniques at [www.salimetrics.com/collection-supplies](http://www.salimetrics.com/collection-supplies).



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## Graphics Key

Do



Don't Do



Note



Information



## Introduction

Saliva is complex biospecimen. Saliva samples can be collected in a convenient, minimally-invasive, and repeated manner. By following proper saliva collection and handling procedures, researchers can obtain the highest-quality data in their studies. We offer the following advice based on our extensive experience with saliva collection and testing.

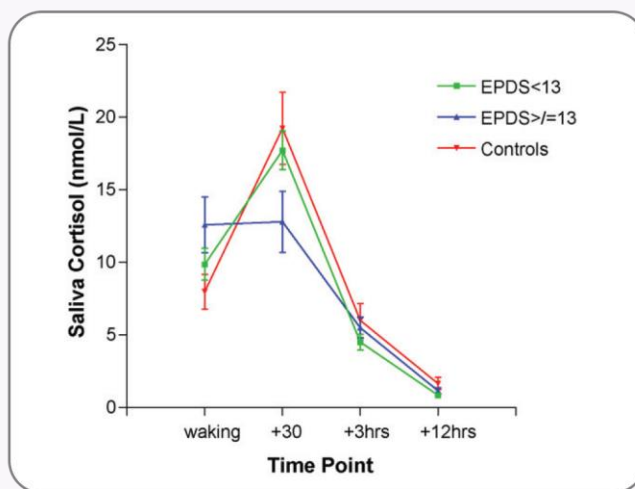
1. Knowledge about saliva testing is rapidly growing and being revised. It is ultimately each researcher's responsibility to make decisions about the best collection methods to use for their specific study. We advise consulting the literature on the analytes to be measured, and when the available literature appears inadequate, we strongly recommend a pilot study.
2. For an additional discussion and current updates on this important topic, we recommend signing up for [The Salivary Bioscience Bulletin](#).

## What to Consider When Designing Your Study

### Variability of Salivary Analytes

Levels of many analytes in saliva do not remain static, and concentrations may change in response to a number of influences (1-5). Several factors may be of importance depending on the analyte of interest and the nature of the study:

- The diurnal cycle of the analyte must be understood. In most cases, sample collection should be made at standardized times.



*Diurnal Rhythm of Salivary Cortisol.* (Taylor, A., et al., [Diurnal pattern of cortisol output in postnatal depression](#). *Psychoneuroendocrinology* (2009), doi:10.1016/j.psyneuen.2009.03.004)

- The response and recovery characteristics of each analyte should be understood so that sample collections are timed to properly capture responses.

- Analytes with pulsatile behavior, such as steroid hormones with episodic secretion patterns, may need to be estimated using with multiple samples over a set period of time.
- In general, the time of day a sample is collected may play a part in the level detected; the time of day for collection should be standardized for all study participants. Consult the literature for guidance on a specific analyte.

### Effects of Mouth Location and Flow Rate on Salivary Analytes

Two common, well-documented methods of saliva collection are: (1) the passive drool technique, and (2) the absorbent device technique. In order to maintain consistency in the type of sample collected, some researchers prefer to use the unstimulated, whole saliva that pools on the floor of the mouth, collected by the passive drool technique. On the other hand, use of an absorbent device that can be placed in the mouth often allows for studies with small children or other individuals that have difficulty with the passive drool technique. Researchers should understand, however, that mouth location placement of the absorbent devices may collect localized saliva rather than whole saliva, which may affect results for many analytes.

Mouth location and flow rate are not of concern for most steroid hormones. However, salivary levels of proteins such as  $\alpha$ -Amylase (sAA) and Secretory IgA (SIgA) do vary according to mouth location (6-8). In addition, levels of a few analytes such as Dehydroepiandrosterone Sulfate (DHEA-S) and SIgA decrease as saliva flow rates increase, and sAA may also be similarly affected (see below) (9, 10).



*An examination of the effects of flow rate and mouth location on sAA measurement found that the type of swab used, location of the swab in the mouth, and duration of the collection all interacted to affect estimates of Alpha-Amylase activity (6). It is important that these factors are carefully considered and standardized, in order to maintain a consistent basis within and between subjects.*

The study on flow rate and mouth location also noted that sAA activity in samples of passive drool generally decreased as flow rates increased, suggesting that this marker may also be affected by flow in a manner similar to SIgA and DHEA-S (6). Additional research is needed on this question; we currently advise that researchers should covary the secretion rate (mL/min) for analytes such as sAA, SIgA, and DHEA-S.

We also advise recording the total time necessary to collect the desired volume of saliva. The assay results can then be multiplied by the flow rate (mL/min) in order to express the results as a secretion rate (output per unit of time).

**Example (SIgA):  $205.60 \text{ ug/mL} \times 1.33 \text{ mL/min} = 273.45 \text{ ug/min}$**

If an absorbent device is used to collect the saliva for determination of an analyte that is flow rate sensitive, the volume of saliva collected by the swab can be determined by weighing the device and storage tube together before and after collection. (An approximate value of 1.0 g/mL may be assumed for the density of the saliva.) If the length of time the swab is in the mouth is also recorded, the flow rate can then be estimated.



*The device must be removed from the mouth before it reaches its absorbance capacity; after that point, the estimate of flow rate will not be accurate—the ceiling effect (6).*

Saturation is especially a concern for smaller devices, which can reach their limit fairly quickly. A preliminary study may be necessary to determine the optimum collection period that is most likely to work for all participants.

## Sample Volume and Salivary Stimulants

Modern immunoassays are designed to use small sample volumes (less than 100 µL), and in most cases, stimulants are not required to collect adequate sample volume. We recommend against the use of oral stimulants when collecting saliva samples due to the possibility of causing assay interference or alteration of levels of some analytes (11); the goal should be to minimize unnecessary sources of variation in saliva test results. For example, even chewing on unflavored paraffin/wax could affect flow-dependent analytes.

If stimulants are absolutely necessary (no saliva is able to be collected without their use) they must be used sparingly and in a consistent manner throughout the entire study (12). We also recommend a pilot study be conducted to ensure that the use of the material does not cause interference in the assays to be used.

Prior to sample collection, researchers are encouraged to contact Salimetrics concerning the necessary sample volume based on the number and type of assays to be performed. Generally, Salimetrics recommends a maximum of three tests per sample vial. For multi-analyte testing where freeze thaw is a concern, an alternative is to collect a large sample in to one vial and then aliquot the saliva in to smaller vials.

## Combining Salivary Analyte and DNA Analysis

If you are planning to include DNA analysis in your study, or if you think that you may want to analyze the samples for DNA at some future date, please see the advice in the **Collection for DNA Analysis** section of this handbook. In many cases, it may also be possible to perform DNA analysis on existing samples that have been archived from previous studies. Please contact Salimetrics for details.

## Blood Contamination in Saliva

Contamination of saliva samples with blood can also be a concern because the levels of many analytes are higher in the general circulation than in saliva. Blood can leak into saliva under certain conditions (21-24).

We recommend the following:

- Participants should not brush their teeth within 45 minutes prior to sample collection (23).
- Dental work should not be performed within 24 hours prior to sample collection.
- Research participants should be screened for oral health problems or injuries.
- Saliva samples visibly contaminated with blood should be discarded and recollected (22).
- Samples collected from populations at high risk for oral health problems, may be screened with the Salimetrics Blood Contamination Assay Kit (Salimetrics Item No. 1-1302; 1-1302-5).

## Sample Collection Materials



Use only high quality polypropylene collection tubes and vials. Polystyrene tubes may adversely affect the measured values of analytes. All SalivaBio collection tubes and vials are made from high quality polypropylene.

## Sample Identification and Labeling

Clear and legible labels are necessary for proper sample identification, organization and handling.

1. **Assembling packets of supplies for in-home collection:** place label on tubes PRIOR to sample collection. Study participant will have all supplies ready-to-use.
2. **Sample collection in lab:** Have necessary supplies at hand (tubes, labels, collection aids, etc.) & collect sample. Place appropriate label on tube immediately prior to or immediately post collection.

Labeling Tips:


- **Under no circumstances should a collected sample be left UNIDENTIFIED!**
- If a label is not readily available, use a permanent marker to write the information on the tube.
- Place pre-printed label on tube PRIOR to freezing.
- Use only labels recommended for freezing (cryolabels); do not use ordinary paper labels as they will likely fall off when the samples are thawed.
- **Apply bar-coded labels vials HORIZONTALLY, so that they can easily be read with bar-code readers.**



### Label formatting: Keep numbering simple!

- If collecting multiple time points per subject, consider subject ID followed by time point shown in the following example:  
001-1  
001-2  
002-1  
002-2
- If collecting multiple waves, assign subject numbers, followed by wave number. For example:  
001W1  
001W2

## Sample Storage and Handling

 It is critical that sample storage conditions are researched prior to initiation of sample collection. Some analytes are unstable and can change at room temperature; you should consult the literature for information on your specific analyte of interest. In addition, samples can vary substantially between research participants.

The effects of freeze thaw on most biological measures, regardless of biospecimen type, can be dramatic. Analytes in oral fluid are not distinct or different in this way. As a general rule, multiple freeze-thaws should be avoided. The most practical way to address this concern is by aliquoting samples after collection. We recommend that investigators consult the literature for the analytes of interest or contact Salimetrics.





## Before Saliva Collection

Good saliva collection requires documenting items which may affect results, as well as following procedures which avoid the possibility of contaminating saliva with substances that could interfere with the immunoassay. Before starting saliva collection from a research participant, we recommend the following precautions:

1. Avoid foods with high sugar or acidity, or high caffeine content, immediately before sample collection, since they may compromise the assay by lowering saliva pH and increasing bacterial growth (13, 14).
2. Document consumption of alcohol, caffeine, nicotine, and prescription/over-the-counter medications within the prior 12 hours (15-18).
3. Document vigorous physical activity and the presence of oral diseases or injury (19, 20).
4. Do not eat a major meal within 60 minutes of sample collection.
5. Rinse mouth with water to remove food residue and **wait at least 10 minutes** after rinsing to avoid sample dilution before collecting saliva.

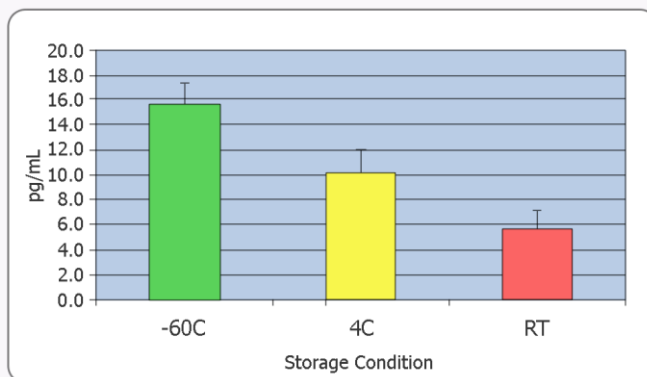


## After Saliva Collection

It is always best to freeze samples at or below  $-20^{\circ}\text{C}$  immediately after collection to preserve the sample for possible use in future studies. If freezing is not possible, to minimize degradation of unstable analytes and to prevent bacterial growth (25), refrigerate immediately at  $4^{\circ}\text{C}$  and maintain at this temperature for no longer than necessary (ideally less than 2 hours) before freezing at or below  $-20^{\circ}\text{C}$  (temperature of a regular household freezer).

Samples stored for more than 4 months should be frozen at  $-80^{\circ}\text{C}$ . Freezing and centrifugation also helps precipitate mucins in the samples, which makes pipetting easier. We recommend samples are expressed or centrifuged to remove saliva from swab collections as soon as possible and prior to freezing at temperatures of  $-20^{\circ}\text{C}$  or below in order to minimize freeze-thaw cycles. However, samples can be frozen in the swab for up to 6 months.

**Note that freeze-thaw cycles should be minimized for some analytes (e.g. DHEA, Progesterone, Estradiol).** It is critical that storage conditions are researched prior to initiation of sample collection. Contact Salimetrics for details.



Effect of storage for 96 hours at three different temperatures on Salivary Estradiol levels (n=10).



# How to Collect Saliva: Collection Methods and Devices

## Collection Methods and Devices: Adults & Children 6 + Years of Age

### Passive Drool

*Passive drool is highly recommended because it is both cost effective and approved for use with almost all analytes.* If research participants are not willing or able to drool saliva into a vial, the SalivaBio Oral Swab may be used as an alternative collection method, but only for certain analytes ([see Passive Drool instructions](#)).



To avoid problems with analyte retention or the introduction of contaminants, use only high quality polypropylene vials for collection, such as our 2 ml cryovials (Salimetrics Item No. 5002.01). The vials used must seal tightly, must be able to withstand temperatures down to -80°C, and must be externally threaded to allow for use of the Saliva Collection Aid (SCA – Salimetrics Item No. 5016.02) to effectively guide drool directly into the cryovial.



*If you are collecting saliva for biomarker analysis and think that the sample may be used at some point for genetic analysis, please see **Collection for DNA Analysis** before proceeding.*

### Materials List:

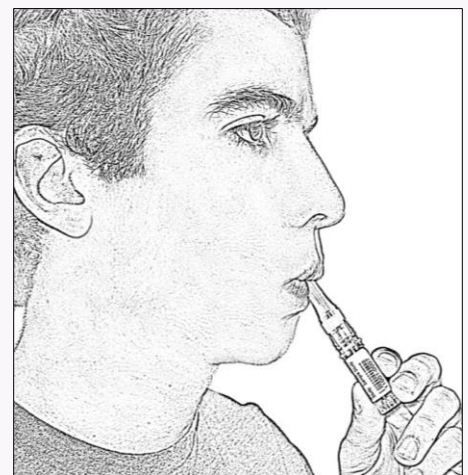
1. Cryovials
2. Saliva Collection Aid
3. Bar-Coded Labels
4. Cryostorage Box (2")



*Secretory IgA & DHEA-S concentrations in saliva are affected by saliva flow rate, and  $\alpha$ -Amylase may also be affected. (6,9,10) We recommend recording mL/min during collection. See **Effects of Flow Rate and Mouth Location**, or contact us for details.*

### Instructions for Collecting Saliva:

1. Remove cap from cryovial
2. Remove SCA from packaging and place securely into cryovial.
3. Instruct participants to allow saliva to pool in the mouth. Some find it helpful to imagine eating their favorite food.
4. With head tilted forward, participants should drool through the SCA to collect saliva in the cryovial. (We advise using a vial with twice the capacity of the desired sample volume.)
5. Repeat until sufficient sample is collected. **Reserve air space in the vial to accommodate the expansion of saliva during freezing.** Collection of samples to be analyzed for multiple analytes may require additional cryovials.
6. Replace cap onto cryovial.



## The SalivaBio Oral Swab (SOS)

For certain analytes ([see SOS instructions](#)), the SalivaBio Oral Swab (SOS) (Item No. 5001.02) can be an excellent alternative to passive drool because of its ease of use. The SOS also helps filter large macro molecules and other particulate matter from the sample, which may help improve assay results. All SalivaBio swabs are made from the same non-toxic, inert polymer which is guaranteed for consistency across all lots, making it ideal for longitudinal and multi-participant group studies.

Due to its small size, the SOS is considered a possible choking hazard and is not recommended for small children. However, SalivaBio also offers infant and child-appropriate collection devices (See **Collection Methods and Devices: Infants and Small Children**).



### Materials List:

1. SalivaBio Oral Swab (SOS)
2. Swab Storage Tube\*
3. Bar-Coded Labels
4. Cryostorage Box (4")

*\* If centrifugation is not available, saliva from the swab may be expressed into a cryovial (Item No. 5002.01) using a needle-less 5 cc plastic syringe. However, expressing samples will result in smaller sample volumes.*

### Instructions for Use

1. Remove SOS from outer packaging and place into proper mouth location based on research question. Keep SOS in place for 1-2 minutes to ensure that it is saturated. (If collecting from the parotid glands in the cheek, saliva flow will be low, and collection time should be extended for up to 5 minutes to ensure adequate volume.) Due to location effects for certain analytes, we recommend that the SOS should not be moved around in the mouth.
2. Place SOS into the swab storage basket insert (upper portion of the tube).
3. Replace cap and snap securely onto tube.



*Before storage for periods longer than 6-12 months, we recommend that the specimen be removed from the SOS by centrifugation or compression.*

### SOS Cautions:

- **Use only as directed.**
- These devices are packaged clean, not sterile.
- Store out of reach of children.
- Do not use this device for children under the age of 6.
- These devices are not toys and are intended for collection of saliva.
- Investigators who use the SOS for biomarkers not approved by Salimetrics do so at their own risk.
- The SOS may cause temporary dryness of mucosal membranes or oral cavity.
- Instructions for use must be distributed to each device user.



## Collection Methods and Devices: Infants and Small Children

Some older preschoolers are able to provide saliva samples by the passive drool method, but the use of absorbent devices is more customary when collecting saliva from small children. Due to the potential for choking when collection devices are placed in the mouth, collecting saliva from infants and children under the age of six requires special consideration.

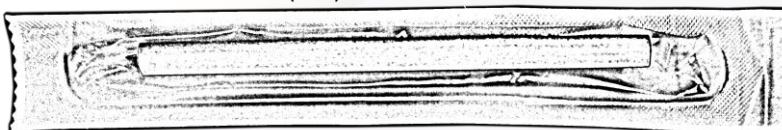
### The Children's Swab and Infant's Swab

We recommend the SalivaBio Children's Swab (SCS) (Item No. 5001.06), for children under the age of 6, and the SalivaBio Infant's Swab (SIS) (Item No. 5001.08), for infants under 6 months of age.

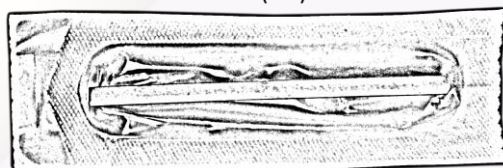


*The extra-length SalivaBio Children's Swab (SCS) (Item No. 5001.06), may also be used for saliva collection from infirmed adult patients to avoid any danger of choking. Follow the instructions provided in the section **Collection Methods and Devices: Infants and Small Children**.*

SalivaBio Children's Swab (SCS)



SalivaBio Infant's Swab (SIS)



All SalivaBio swabs are made from the same non-toxic, inert polymer which is guaranteed for consistency across all lots, making it ideal for longitudinal and multi-participant group studies. These devices are manufactured in longer lengths and narrower widths to allow one end of the swab to be held by a parent or technician while the other end is placed in the child's mouth. The diameters are appropriate for the size of children's mouths. The polymer material is durable and can withstand chewing, and its taste and texture are also acceptable to children. The volume of sample recovered from the SCS and SIS is typically in the range of 200-1000  $\mu$ L. Like the adult SOS, samples collected with either the SCS or the SIS may be tested for various analytes ([see SCS instructions](#), [see SIS instructions](#)).



*Be sure you've collected enough volume. Too little volume may make it impossible to perform the test.*

### Materials List:

1. SalivaBio Children's Swab (SCS) **OR** SalivaBio Infant's Swab (SIS)
2. Swab Storage Tube
3. Bar-Coded Labels
4. Cryostorage Box (4")

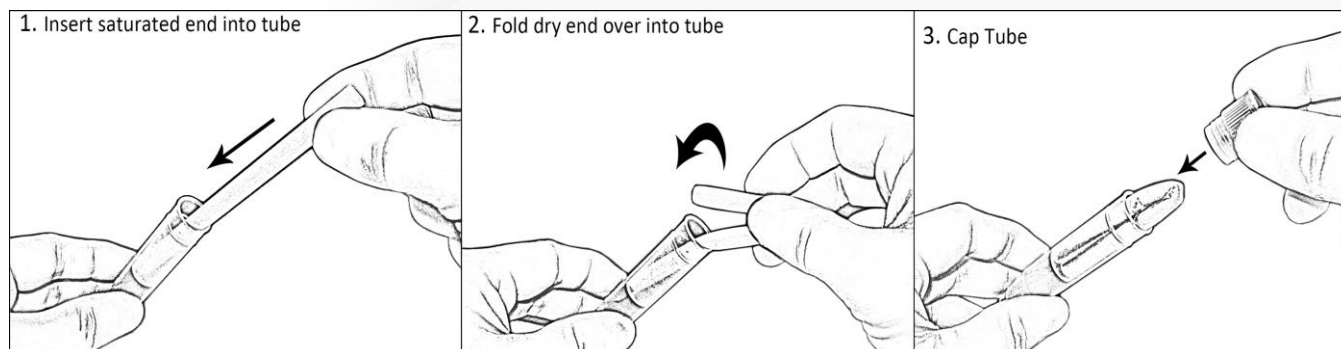


## Instructions for Use

1. For the SCS or SIS, peel open the outer package and remove the device.
2. Securely hold one end of the device and try to place the other end under the child's tongue. With infants it may only be possible to collect pooling saliva (often at the corners of the mouth or under the tongue). You can try to collect for the full 60-90 seconds at once by resting the swab inside the mouth, or collect in intervals by re-introducing the swab into the mouth as needed until the lower third of the swab is saturated (60-90 seconds total).
3. Place the saturated SIS or SCS into the Swab Storage Tube for recovery by centrifugation, or use a 3-5 mL syringe for immediate compression.

The compression method allows the researcher to determine if sufficient saliva has been collected on the first attempt, and the procedure can be repeated if necessary. Some researchers prefer to cut free the saturated portion of the swab before placing it in the centrifuge tube or syringe.

If the swab is used to collect samples for analytes that are affected by saliva flow, however, we advise placing the entire swab into the tube or syringe, in order to estimate saliva flow rates, as described above under ***Effects of Mouth Location and Flow Rate on Salivary Analytes***. The entire swab may be placed in the Swab Storage Tube by inserting the saturated end first, followed by doubling over the dry end into the opening, and finally using the cap or plunger to push the entire swab into the interior space.



### SCS/SIS Cautions:

#### Use only as directed.

- These devices are packaged clean, not sterile.
- Adult assistance and supervision is **required** during use.
- Inspect device for tears or imperfections. **DO NOT USE** if cuts or tears are present.
- When not used as directed these devices may represent a choking hazard for children.
- Store out of reach of children. These devices are not toys and are intended for collection of saliva.
- Use instructions must be distributed to each device user.

## Collection Methods and Devices: Non-Human Species

Hormones and other biomarkers in saliva are increasingly being used to monitor the health and well-being of animals. Cotton ropes, swabs, or pads, either plain or flavored, have been used to collect saliva from deer, Guinea pigs, dogs, and primates (29-36). Hydrocellulose eye spears have also been used to collect saliva from dogs (34). Saliva has been collected from pigs and primates by allowing them to chew on larger sponges attached to poles (36). Saliva has even been collected from large and dangerous animals such as the rhinoceros by using a plastic spoon to scoop up several milliliters at a time from the lower lip (37).

Salimetrics' tests on the durability of the SCS suggest that it may be appropriate for use as a collection device for small, domestic animals, as long as the device is used in a supervised manner. The polymer used in the swab is very resistant to chewing, and even when the device was partially cut during testing, (similar to what might occur during vigorous chewing by a dog) tearing off a portion was difficult. To date, the SCS has been used successfully in collecting from many different animals. We recommend consulting the literature or contacting Salimetrics for more information.

The literature also contains numerous descriptions of techniques for saliva collection from mice and rats. These include capillary tubes, filter paper strips, plastic pipettes, and more sophisticated suction devices. Salimetrics does not have direct experience with such methods and cannot advise on their use.

### SCS/SIS Cautions:

#### Use only as directed.

- These devices are packaged clean, not sterile.
- Adult assistance and supervision is **required** during use.
- Inspect device for tears or imperfections. **DO NOT USE** if cuts or tears are present.
- When not used as directed these devices may represent a choking hazard.
- Store out of reach of children. These devices are not toys and are intended for collection of saliva.
- Use instructions must be distributed to each device user.



## Collection for DNA Analysis

### Collection Methods and Sample Volume

Whole saliva samples are preferred for DNA analysis. A volume of 500 µL whole saliva obtained through the passive drool technique is sufficient to gather DNA for multiple polymorphism assays. If collecting samples for genotyping only, we recommend collecting a second saliva sample.

DNA can be obtained from samples that have been collected for another type of analysis, and from all areas of the mouth (38). If your samples have been collected by the passive drool technique or with SalivaBio collection devices, collection of new samples specifically for DNA testing is often unnecessary.

DNA is found in the nuclei of cells. For DNA testing, the pellet formed by centrifugation of your saliva samples contains the cells that provide the DNA. **Do not discard the pellet if genetic testing is desired.** Additional collection devices such as buccal swabs may be acceptable; contact us for the latest advice. When collecting buccal cells, be sure to rub the inside of cheeks for 30-60 seconds with firm pressure.

Take care not to touch the swab or brushes with your fingers.

### Stability of DNA over Time

The DNA sequence of every individual is constant throughout life. Samples can be pulled from different “waves” of your project to submit for DNA testing.

### Storing Saliva for DNA Analysis

DNA can tolerate storage at room temperature for up to 5 days without compromising the quality of the DNA for genetic testing (38). DNA also withstands multiple freeze-thaw cycles without a significant effect on the DNA quality.

If samples are to be tested for analytes in addition to DNA analysis, do not use a device which dilutes the saliva by an unknown amount or has the potential to interfere with the assays. In addition, follow storage instructions for the more sensitive analyte (i.e., the hormone or biomarker) of interest. **For genetic analysis alone, the Oragene•DNA® self-collection kit (OG-500) may be used when long-term room-temperature storage is necessary.** Detailed collection and mailing instructions can be found in the Oragene•DNA kit package or at [dnagenotek.com](http://dnagenotek.com). For young children or people who are unable to drool freely, use the Oragene•DNA assisted collection format (OG-575) to collect whole saliva.

Please visit [www.dnagenotek.com](http://www.dnagenotek.com) for more product and protocol information. Oragene products are also available for DNA collection from animals.



## Avoiding Contamination of Samples

To prevent contamination of saliva samples for DNA analysis, we recommend the following precautions in addition to those recommended for immunoassay testing collection:

1. Employ only single-use materials (disposable forceps, etc.) for sample collection/transfer to prevent possible contamination between research subjects.
2. Those researchers assisting with collections should wear gloves and avoid touching collection device materials and samples.

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## Long Term Sample Storage

Samples can be stored at -80°C for several years; the exact time has not been determined and may vary by analyte. However, many samples that have been stored properly for over four years have shown little or no degradation. We recommend you consult the literature or contact Salimetrics for details.

## 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 for a greater amount of time, or break up the clot with a pipette tip and re-centrifuge. Assays should be performed using only clear saliva, avoiding any sediment present in the bottom of the tube. ***When pipetting viscous solutions such as saliva, greater accuracy in sample volume is obtained by aspirating slowly, in order to avoid the formation of bubbles.*** Re-centrifuge tubes following each freeze-thaw cycle since additional precipitates may develop upon refreezing.



*If samples will be used for genetic analysis, it is important to keep the cell pellet at the bottom of your whole saliva sample. Please see **Collection for DNA Analysis**.*



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## Collection Device Comparison

### Cryovial (Passive Drool)



Size:  
10x46 mm

Used With:  
Saliva Collection Aid  
Expressed Samples



Intended Use:  
Children 6+  
Adults

Average Collection Time:  
Variable

### Oral Swab (SOS)



Size:  
10x30 mm

Used With:  
SST, Syringe

Intended Use:  
Children 6+  
Adults

Average Collection Time:  
60 seconds

### Children's Swab (SCS)



Size:  
8x125 mm

Used With:  
SST, Syringe

Intended Use:  
Children <6,  
animals,  
elderly,  
impaired adults

Average Collection Time:  
60 seconds

### Infant's Swab (SIS)



Size:  
6.5x90 mm

Used With:  
SST, Syringe

Intended Use:  
Infants <6 months,  
animals

Average Collection Time:  
60 seconds