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Recommended Testing Plan for Initial Evaluation of SalivaGard™ DNA Tubes

With multiple options for commercially available saliva collection tubes, key performance characteristics can now be compared including: DNA yield, purity and integrity, robustness to temperature and shipping stresses, and compatibility with automated liquid handling for increased throughput and decreased contamination risks. The right saliva collection device can ensure sufficient yield of intact and high quality DNA, even from low DNA donors. The wrong choice can dramatically decrease throughput, complicate workflows, and risk sample rejection due to insufficient yield of high quality DNA.

This protocol guide will help you select the right collection device by accounting for real-world environmental stresses and assessing overall robustness of performance. A key error in selecting the correct device is failing to include shipping time and stresses, temperature stresses, and assessment of workflow complexity and throughput.

Collection

Collect saliva from multiple donors into one conical tube each and store on ice for a maximum of 2 hours. Aliquot saliva from each donor[1] into SalivaGard HT DNA devices and any other relevant commercially available devices (e.g., Oragene Dx or other devices with which an assay was originally developed).

Testing

Controls	Environmental Stress
Set baseline Process at least one saliva sample per donor immediately from conical tube. This will serve as the baseline comparator for each donor. Remember that this sample will be straight saliva and thus will have an initial yield higher than the saliva/stabilizer mixture collected from devices. Relevant metrics to assess are described on the next page.	**Subject to stresses** Subject remaining tube(s) for each donor to real-world environmental stresses to mimic the conditions that samples will experience prior to downstream testing. **Recommended stress tests:** - One-way shipping, with temperature monitoring - High temperature stresses Example testing plan see page 3 Relevant metrics to assess are described on the next page.

[11] By aliquoting from donor-specific pools, it is possible to compare DNA yield, purity, and integrity between devices. Because multiple donations from the same donor can have differing DNA yields, and because there is high donor-to-donor variability in saliva DNA content, comparisons between devices are only possible using a shared donor-specific pool.

Relevant metrics to assess include:	
DNA preservation metric	**Indicator of poor DNA preservation**
DNA yield (concentration of extracted DNA by pico green or other fluorescence-based DNA quantitation technique[2])	Loss of yield from the same donor
DNA purity (qualitative: A260/A280)	A260/A280 outside of the acceptable range for downstream test(s) of interest
DNA integrity and degradation by agarose gel electrophoresis	Loss of high molecular weight genomic DNA band and/or significant smearing within a lane
DNA integrity by long-range PCR[3]	Loss of long-range PCR product, as visualized by agarose gel electrophoresis
Target detectability in relevant downstream tests	Poor preservation = false negatives; Target modification/damage = false negatives or false positives
Workflow considerations	
How long does the manufacturer's workflow take, and are there any additional incubation steps[4]?	
Is the saliva collection device compatible with my automated liquid handler, and are there any additional or more complex automation steps (e.g., decapping)[5]?	

[2] DNA yield quantitation by absorbance at 260 nm is not always accurate, as it can be confounded by RNA absorbance.

[3] Minimum 8 kilobase amplicon; Biomatrica can provide primer design and polymerase recommendation upon request

[4] E.g., OrageneDx devices require an additional 1 hour incubation at 50 °C prior to DNA extraction.

[5] See https://youtu.be/-c88xCwSxaU for high-throughput piercing of SalivaGard HT DNA devices with Hamilton Star liquid handler.

Example Testing Plan, Incorporating Shipping & Temperature Stresses (5 donors per environmental stress condition):

Donor	Device	Shipping	Temperature[a]	Time Point[b]
1	Conical tube	No	N/A	Day 0
1	Oragene Dx	Yes	monitor	Day 7
1	SalivaGardHT	Yes	monitor	Day 7
2	Conical tube	No	N/A	Day 0
2	Oragene Dx	Yes	monitor	Day 7
2	SalivaGardHT	Yes	monitor	Day 7
3	Conical tube	No	N/A	Day 0
3	Oragene Dx	Yes	monitor	Day 7
3	SalivaGardHT	Yes	monitor	Day 7
4	Conical tube	No	N/A	Day 0
4	Oragene Dx	Yes	monitor	Day 7
4	SalivaGardHT	Yes	monitor	Day 7
5	Conical tube	No	N/A	Day 0
5	Oragene Dx	Yes	monitor	Day 7
5	SalivaGardHT	Yes	monitor	Day 7

Donor	Device	Shipping	Temperature[a]	Time Point[b]
6	Conical tube	No	N/A	Day 0
6	Oragene Dx	No	37 °C	Day 7
6	SalivaGardHT	No	37 °C	Day 7
7	Conical tube	No	N/A	Day 0
7	Oragene Dx	No	37 °C	Day 7
7	SalivaGardHT	No	37 °C	Day 7
8	Conical tube	No	N/A	Day 0
8	Oragene Dx	No	37 °C	Day 7
8	SalivaGardHT	No	37 °C	Day 7
9	Conical tube	No	N/A	Day 0
9	Oragene Dx	No	37 °C	Day 7
9	SalivaGardHT	No	37 °C	Day 7
10	Conical tube	No	N/A	Day 0
10	Oragene Dx	No	37 °C	Day 7
10	SalivaGardHT	No	37 °C	Day 7

[a]Temperature for shipped samples can be monitored using a temperature logger inserted in the package next to the tubes. Samples can be stored in incubators to mimic temperature stresses.

[b]A baseline comparator sample (Day 0 sample processed immediately from conical tube) should be assessed for every donor. This baseline comparator sample will be straight saliva and thus will have an initial yield higher than the saliva/stabilizer mixture collected from devices. If stabilization is required for more than 7 days post-collection, the time point can be modified to the required specification.