A New Oral Sampling Device for the Collection of Human DNA: Collection Time, Long Term Storage Studies, and Sample Collection in Children

Moses S. Schanfield, Ph.D.[1], Nancy B. Schanfield, B.S.[1], Shirley A. Miller[1], Stephen Zelik, B.S.[1] and J. Richard George, Ph.D.[2]
[1] Analytical Genetic Testing Center, Inc., 7808 Cherry Creek South Drive, Suite 201, Denver, Colorado 80231
[2] Epitope, Inc., 8505 Southwest Creekside Place, Beaverton, Oregon 97008

The OraSure™ oral sampling device was originally developed for HIV screening and is being evaluated for its utility as an oral method of collection for drug testing. Since this system is simple and easy to use in a non-laboratory collection environment, the OraSure™ device was evaluated as a means of collecting DNA for clinical, forensic identification, and paternity testing.

In initial studies (presented at the American Academy of Forensic Sciences Meeting, February, 1998, San Francisco, CA), comparison of the OraSure™ oral sampling device, the OmniSwab™ (FITZCO, Maple Plain, MN) and regular Dacron swabs (FITZCO) indicated that the OraSure™ oral sampling device had significantly less degradation than either Dacron swab or OmniSwab™. Sufficient DNA was obtained from the OraSure™ swab for detection of restriction fragment length polymorphism loci using chemiluminescent detection (D2S44) as well as, PCR based STR Multiplex™ (CTTv, Promega Corp., Madison, WI) and VNTR loci (D1S80, Perkin Elmer Corporation, Foster City, CA). The question arose as to the optimal length of time for sample collection, storage conditions, and the suitability of the device in sample collection from children for paternity cases.

Per the package insert, the OraSure™ device should be kept in the mouth for two minutes, with a maximum collection time of five minutes. These parameters were established based on testing for the HIV-1 antibodies. To evaluate whether this amount of time was necessary for DNA sample collection two males and two females from the original sample group were asked to collect samples for 15, 30, 60, and 120 seconds. To simplify processing one half of the swab was extracted. To allow the results to be comparable to previous results, the results per microliter was adjusted to one quarter swab. The results of testing are presented in Table 1.

Table 1. DNA yield per microliter by collection time and donor

Time	JVB	MSS	SAM	NBS	Mean
15 sec.	31.3	6.4	2.0	6.5	11.5
30 sec.	14.4	39.8	1.9	4.4	15.1
60 sec.	37.5	28.0	3.1	12.5	20.3
120 sec.	32.2	41.5	1.5	2.3	19.4
Mean	28.9	28.9	2.1	6.4	16.6

Non-parametric analysis of variance indicated that there was significant variance among donors, regardless of length of time of collection ($H=11.338$, $p=0.01$), but not among time of collection among donors ($H=0.463$, $p=0.93$). There were no

To further evaluate the usefulness of OraSure™oral sampling device for genetic testing, forensic identification and paternity testing, storage studies were performed. OraSure™ samples were collected from five individuals and stored for 1, 7, and 28 days frozen, room temperature, and 37°C with (wet) and without (dry) the sample preservation liquid, prior to testing. In addition, a series of samples collected for FDA validation purposes and stored at room temperature, dry, without preservation liquid, and samples collected and stored frozen for one year (part of the original study samples), without preservation liquid were analyzed. Five µl of extracted sample were amplified for STR GammaSTR™ (Quadriplex, Promega Corp., Madison, WI) and LTR loci (D1S80, Perkin Elmer Corporation, Foster City, CA).

The summary results are presented in Table 2., as the fraction of loci amplified over the samples tested. Non-parametric analysis of variance was performed as above. The p value of the H tests is presented.

Table 2. Distribution of test results by duration and storage condition.

Duration	Storage Condition				p among conditions
	N	-20°	RT	37°	
1 day	10	0.880	0.580	0.800	0.247
7 days	10	1.000	0.680*	0.900	0.284
28 days	10	1.000	0.840	0.500**	0.051
1 year	10	1.000	0.860	n.d.	0.450
Total		0.970	0.740	0.733	0.004***
p Over time		0.577	0.252	0.119	

* Indicates marginally significant differences between wet and dry storage.
** Indicates significant heterogeneity within the wet and dry groups.
*** Marginally significant after correction for the number of tests performed.

Though there was variation among samples within groups, at each storage time (1, 7 and 28 days and 1 year) there was no significant difference between storage conditions. However, when the results were pooled there was a significantly higher success rate for samples stored at –20°C (significant before correction for tests, marginally afterwards). The 37° storage was the only storage condition that seemed to provide a negative correlation with time, however it was not significant. However, previous studies indicate that increasing the amount of DNA amplified to 15 µl would probably produce a result.

To study the application of the OraSure™ oral sampling device in children, children between one week and fifteen years were collected (N=18). DNA was extracted and amplified for GammaSTR™ quadriplex (Promega) and D1S80 LTR (PE). All samples have amplified via the PCR process.

In conclusion, the OraSure™oral sampling device provides a method of collecting sufficient DNA for paternity or forensic testing by both PCR and RFLP in adults and children. Prolonged storage of dry pads at RT yielded degraded DNA only testable by PCR. This system appears to be a good sample collection system for the collection of samples in remote locations for testing elsewhere with little concern for sample collection parameters or special shipping or handling precautions.