Validation of Saliva Samples with the *EasyScreen*™ 3base™ SARS-CoV-2 Detection Kit (RP012)

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Introduction/Background:

The need for accurate SARS-CoV-2 testing remains a priority. While nasopharyngeal (NP) swabs and PCR tests are considered the most sensitive and accurate, there is a need for simpler sample collection. Saliva has been investigated as an alternate sample, but the literature shows great variability in performance of saliva samples.

Objectives:

Validate a saliva sampling method on matched NP and saliva samples using the Genetic Signatures *EasyScreen*™ methodology.

Methods:

Matched NP and saliva samples (N=104 pairs) were collected from known or suspected COVID-19 patients in St. Vincent's Hospital, Sydney. NP samples were collected by standard swab sampling into VTM transport media. Saliva samples were collected using the 3D printed swab from 3DMEDiTech (Victoria, Australia) into 1mL liquid Amies transport media (Figure 1). The saliva was collected in the morning before brushing teeth, after a deep cough with the mouth closed, with the swab placed on the tongue for 30 sec, rotated on the tongue 30 sec, then agitated into the provided liquid Amies media. Nucleic acid was extracted from NP and saliva using the *EasyScreen*™ SP012 Sample Processing Kit on the Genetic Signatures GS1-HT platform. PCR detection was with the *EasyScreen*™ SARS-CoV-2 Detection Kit RP012 (targets both N-gene and M-gene) on a Bio-Rad CFX-384 real-time thermal cycler.

Results:

Of the 104 matched pairs, seven were negative in both NP and Saliva testing. The remaining 97 pairs were positive by at least one test. Of these, 89 were positive by both tests; 92 were positive by the NP test, and 94 were positive by the saliva test. Using the validated NP swab test as a reference, the positive percent agreement (PPA) of the saliva method was 98% (Table 1). Assuming all positives are true positives, the NP and saliva tests had sensitivities of 95% and 97%, respectively (Table 2). These positivity differences are not significantly different (P=0.47, Z-score test). Any discrepant positives had very late Ct values in the PCR tests and presumably represent samples at or below the LoD of the RP012 test.

The Ct values obtained from either the N-gene or M-gene were not significantly different for the saliva or NP test (Figure 2).

The saliva test tended to give earlier Cts than the NP test for samples with NP Ct later than ~31 (Figure 3), suggesting it may be more sensitive for low viral load samples.

Conclusions:

With the saliva sampling method employed here, the *EasyScreen*™ SARS-CoV-2 Detection Kit RP012 is at least as sensitive as the NP swab test on this dataset. Given its less invasive sampling method, saliva sampling is suitable for use with the *EasyScreen*™ methodology.

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Table 1: Positive percent agreement (PPA)

	NP positive	NP negative	Total
Saliva positive	92	2	94
Saliva negative	0	10	10
Total	92	12	104

PPA = 97.9% (95% CI 93-99.4%)

Table 2: Sensitivity

	NP positive	NP negative	Total
Saliva positive	89	5	94
Saliva negative	3	7	10
Total	92	12	104

Sensitivity saliva vs NP = 96.7% (95% CI 90.8-99.3%)

Figure 1: 3D printed swab from 3DMEDiTech

Figure 2: Ct values are not significantly different

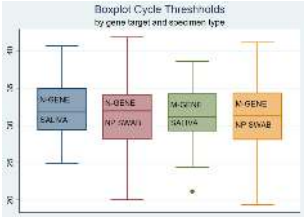
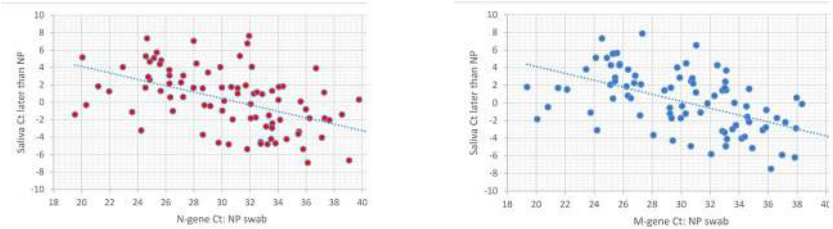


Figure 3: Saliva Ct values trend earlier as viral load decreases



X-axis: Ct value from NP swab. Y-axis: Ct delay for saliva vs NP swab (negative means earlier Ct). Dotted line = best fit.

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