

## **iSWAB Microbiome Extraction-less Sample Stability at Ambient Temperature**

### **SCOPE AND OBJECTIVE**

The scope of this experiment is to test the extent of how long iSWAB-Microbiome-EL can stabilize the viral RNA at ambient and 37°C temperature stability of viral RNA before it gets degraded in Azureseq kit, direct one step RT-PCR assay for COVID-19 testing, (SeqOnce). And to test equivalency of collection efficiency of nasal samples between the well-established Copan oral flocked swab and Mawi’s molded swab, NextSwab measured by quantification of RNA in the sample.

### **MATERIALS and METHOD**

Samples were collected using iSWAB Microbiome Extraction-less collection devices (ISM-T-NEL), samples were collected with **NASAL MID-TURBINATE** area, half the samples were collected with oral flocked swabs (Copan, Italy) and the other half with Mawi’s NextSwab. The AzureSeq One-Step Universal RT-qPCR Kit for SARS-CoV-2 supplied by SeqOnce Biosciences was used for all qPCRs. Ct values for the RNA Stability test were determined using a MyGo Mini real-time PCR instrument running MyGo Mini PCR Software V3.5.

### **CONTROLS**

Each qPCR run included a no template Negative Control as well as a Positive Control containing SARS-CoV-2 RNA (conc. 40 c/μl) supplied by SeqOnce.

### **SAMPLE PREPARATION**

Nasal samples were collected using ISM-T-NEL. One swab was inserted into each nostril and rotated 10 times before being removed. The swab was then inserted into the iSWAB collection device and mixed with the Mawi stabilizing buffer following the protocol provided by Mawi DNA Technologies.

<b>Sample</b>	<b>Description</b>
<b>Flock swab Incubated @ 37° C</b>	Sample collected 10-7-2020 using ISM-T-ELv2 with one flock swab, incubated @ 37°C
<b>Flock swab kept @ RT</b>	Sample collected 10-7-2020 using ISM-T-ELv2 with one flock swab, kept @ room temperature
<b>NextSwab Incubated @ 37° C</b>	Sample collected 10-7-2020 using ISM-T-ELv2 with one NextSwab, incubated @ 37°C
<b>NextSwab kept @ RT</b>	Sample collected 10-7-2020 using ISM-T-ELv2 with one NextSwab, kept at room temperature
<b>C+ Stock (40 c/μl) Frozen</b>	SARS-CoV-2 RNA stored at -20°C (Positive control)

**REACTION SETUP**

The Direct no extraction protocol for the AzureSeq Kit was used following the reaction set-up for 20 µl reactions.

**THERMAL CYCLING CONDITIONS**

Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
RT Incubation	1	1	50°C	15 minutes
Enzyme Activation	2	1	95°C	2 minutes
Amplification**	3	45	95°C	3 seconds
			60°C**	30 seconds

\*\*Collect fluorescence during annealing/extension phase (55°C) step on FAM, HEX, and ROX channels (or equivalent channels).

**RESULTS**

**Table 1: Copan oral flocked swabs collected from nasal mid-turbinate area (Average CT of two duplicates)**

Day	Flock swab kept @ RT			Flock swab Incubated @ 37° C			C+ Stock (40 c/µl) Frozen		
	N1	N2	Rnase P	N1	N2	Rnase P	N1	N2	Rnase P
<b>0</b>	35.1	35.2	31.8	35.6	35.7	30.3	37.3	37.7	34.4
<b>1</b>	35.8	36	29.8	35.6	35.9	29.1	37.5	38.1	34.4
<b>3</b>	35.4	35.6	30.3	36.3	36.3	30.1	36	36.2	31.9
<b>7</b>	34.8	34.8	29.3	36	36.3	29.8	35.9	36.4	31.7
<b>10</b>	35.6	35.7	28	35	35.2	27.9	36.4	36.2	32.2
<b>14</b>	36.3	36.8	29.2	36.7	36.5	28.7	35.7	36.1	32.5
<b>21</b>	35.5	35.5	28.9	35.2	35.6	28.2	37.2	38.1	33.8

**Table 2: Mawi’s NextSwab swabs collected from nasal mid-turbinate area (Average CT of two duplicates)**

Day	NextSwab kept @ RT			NextSwab Incubated @ 37° C			C+ Stock (40 c/μl) Frozen		
	N1	N2	Rnase P	N1	N2	Rnase P	N1	N2	Rnase P
<b>0</b>	36.7	37.2	30.8	36.5	36.5	30.6	37.3	37.7	34.4
<b>1</b>	36.3	36.7	31.8	35.8	36.1	28.8	37.5	38.1	34.4
<b>3</b>	35.3	35.5	29.7	35.1	35.3	29.4	36	36.2	31.9
<b>7</b>	34.7	34.9	28.9	33.8	34.1	27.7	35.9	36.4	31.7
<b>10</b>	36.1	36.4	26.9	34.9	35.2	28.1	36.4	36.2	32.2
<b>14</b>	36.6	36.9	31.6	35.5	35.6	29.4	35.7	36.1	32.5
<b>21</b>	36.1	36.2	31.8	36.2	36.3	29.5	37.2	38.1	33.8

**Summary and Conclusion: After 21 days of incubation at 37° C in the iSWAB-Microbiome-EL stabilization buffer, samples did not show any significant changes in CT value.**