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iSWAB™-MB-EL: Enables extraction-less rapid pathogen detection from fecal samples

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Introduction:

In clinical settings, fecal samples are often used to diagnose a patient's condition, whether it involves infectious diseases or similar ailments. However, researchers and technicians commonly encounter a challenge when analyzing fecal samples due to their complex nature, which often contains many inhibitory factors that can complicate an accurate diagnosis especially with rapid tests such as qPCR. Mawi DNA Technologies has developed iSWAB™-Microbiome-Extraction-Less (iSWAB™-MB-EL), a non-alcohol, non-toxic, extraction-less stabilization device where collected sample can be tested using rapid tests such as qPCR without the need for the extraction of nucleic acids. This innovative device not only allows for direct placement of the sample onto a qPCR plate but also effectively overcomes the presence of these inhibitory factors. After successfully validating iSWAB™-MB-EL's performance on saliva and nasal samples[1], we proceeded to test fecal samples to explore its broader applications.

Table 1: Pretreatments tested for SARS-CoV-2 detection.

Dilution Factor	Temperature	Duration (mins)
Undiluted	RT	NA
	95° C	5
1:3	RT	NA
	65° C	2
		5
		10
	95° C	5
1:6	RT	NA
	65° C	2
		5
		10
	95° C	5
1:11	RT	NA
	95° C	5

Method:

One fecal sample was collected and aliquoted (~50 mg each) into iSWAB™-MB-EL devices (**Figure 1a**). The devices were vortexed and then centrifuged at 100 x g for 5 minutes. The supernatant was pooled as a master sample which was then aliquoted and spiked with Heat-inactivated SARS-CoV-2 at concentrations of 0 and 300 cp/µL with duplicates. The samples were rocked on a mixer overnight to simulate transport. The following day, the samples were pretreated with multiple conditions (**Table 1**). 10µL of each pretreated sample was run directly on a BGI RT-qPCR SARS-CoV-2 assay with duplicates at Days 1, 3 and 7.

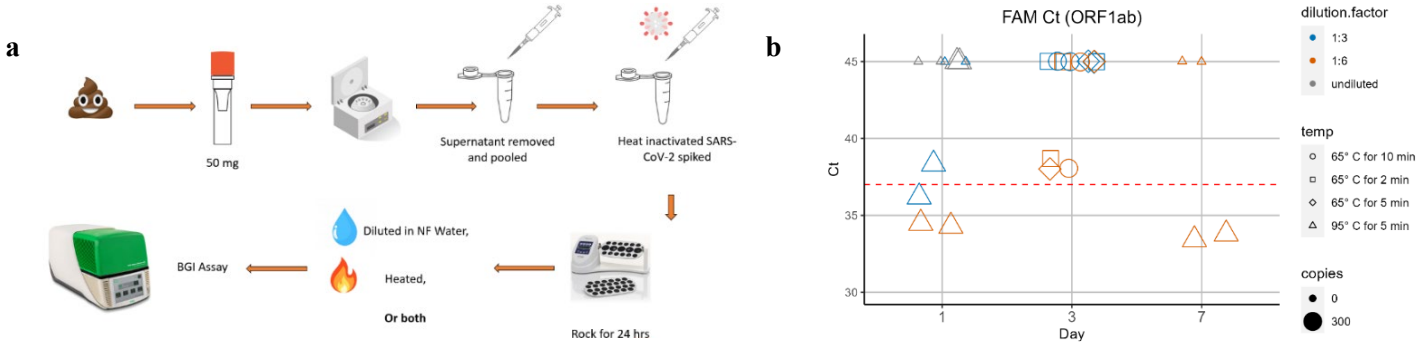

Figure 1a: *Workflow for the detection of SARS-CoV-2 from fecal samples using iSWAB-MB-EL.* ***b****: BGI Assay results on Days 1 and 7. Samples where both replicates did not fall below the threshold were not tested at the Day 7 time point. Samples that failed to amplify were set at 45 cycles. Colors represent different dilution factors. Shapes represent different pretreatment conditions. Sizes represent the concentration of the spiked in SARS-CoV-2 virus. Red dotted line represents the positive detection cut-off for SARS-CoV-2 virus.*

Results and Discussion:

Out of all the pretreatments, samples diluted at 1:6 while heated at 95°C for 5 minutes were consistently detected positive for SARS-CoV-2 (Ct value of FAM (ORF1ab) < 37). The successful detection was reproduced on Days 1 and 7 with averaged Ct values of 34.39 and 33.60 Ct, showing sample stability over the one-week period **(Figure 1b**). However, it is worth noting that the detection of the internal control via HEX (β-actin) exceeded the acceptable threshold (35 Ct) for a positive SARS-CoV-2 sample, with an average of 37.59 on Day 1 and 37.78 on Day 7. This suggests a low HEX gene in fecal samples. A more suitable internal control gene for complicated stool samples may be needed.

Conclusion:

The iSWAB™-MB-EL device simplifies the process of viral detection within stool, given that fecal samples are inherently complex, by eliminating the extraction step altogether. The usage of this device has the potential to expand to streamline other infectious diagnostic tests, as well as the detection of bacteria and parasites in fecal matter.

[1] https://www.mawidna.com/mawi-product/ism-t-el/