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APPLICATION NOTE

Utilization of a mechanical homogenization-based direct-to-PCR method for influenza A virus detection.

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Summary

Respiratory viruses, both novel and known, are a significant focus of public health efforts across the globe. With the mainstay of many public health efforts to combat these pathogens relying on accurate detection of the virus within human or animal populations, the need for clinical research to improve testing has driven innovation in the field of nucleic acid detection of these viruses.

The current gold-standard method for detection of respiratory viruses, such as influenza, is polymerase chain reaction (PCR) amplification of a specific gene product. This method has two fundamental parts, the sample preparation step involving nucleic acid extraction and purification, and the PCR amplification of the targeted viral gene. While this method has been shown to have high specificity and reproducibility, the workflow is time intensive and costly. Hence, research is being done on improving diagnostic testing for respiratory viruses like Influenza A (IAV) and SARS-CoV-2 through a direct-to-PCR (dPCR) method to lower the time and resources needed for viral detection over traditional extraction-based PCR.

Herein, we demonstrate the utility of a mechanical homogenization-based dPCR method for IAV detection utilizing a set of performance-verified universal IAV probes targeting the matrix protein (MP) gene to confirm presence of IAV on spiked swabs simulating nasopharyngeal swabs.

For research use only. Not for use in diagnostic procedures.

Omni Bead Ruptor Elite™ bead mill homogenizer

Materials and methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite 2 mL Tube Carriage (Cat # 19-373)
- 2 mL low binding tubes with screw caps and silicone O-rings (Cat # 19-660-1000)

Procedure

Swab viral spike

Sterile cotton swabs (Fisher Scientific, Cat # 22-029-488) were submerged for 5 seconds in IAV viral solutions ranging from 10^9 to 10^5 PFU/mL. The swabs were exposed in a serial dilution pattern, with three to five swabs being exposed at each concentration log to evaluate the detection capabilities of this method. The saturated swabs were then placed in a 2 mL screw capped tube (Cat # 19-660-1000) prefilled with 1 mL of viral transfer buffer. The stem of the swab was then broken off at a level even with the top of the tube to allow for the cap to be screwed on for transporting and processing. The samples were prepared at 23 °C and then incubated for 1 hr at 23 °C prior to processing.

Shaker-Mill Swab Processing for Viral Lysis

Twenty-four 2 mL screw cap tubes containing the virally spiked swabs were processed on the Omni Bead Ruptor Elite (Cat # 19-042E) for 30 s at 4.2 m/s. This processing generated froth within the tube which was allowed to settle prior to removal of 1 µL of lysate for RT-qPCR.

IAV nucleic acid extraction

All samples processed in the standard nucleic acid extraction workflow underwent nucleic acid extraction utilizing a commercially available universal RNA extraction kit.

IAV RT-qPCR

IAV matrix gene (M gene) was selected as a proposed universal influenza A target for RT-qPCR from Nagy et al. The M gene was targeted with forward primer 5′-GGCCCCCTCAAAGCCGA-3′, reverse primer 5′-CGTCTACGYTGCAGTCC-3′, and probe 5′FAM-3′BHQ-1 with sequence 5′-TCACTKGGCACGGTGAGCGT-3′. 3 µL of sample lysate was added to create a final reaction volume of 20 µL using the proportions of primers, probe, sample, reaction mix, RT, and DEPC-treated H_2O as laid out in the New England Biolabs Luna Universal Probe One-Step RT-qPCR Kit (NEB, Cat # E3006S). Amplification of sample was performed for 50 cycles. Out of abundance of caution, the loading of the PCR plate with viral lysate should be completed in a biosafety cabinet to protect the user from any potentially viable virus particles remaining following homogenization.

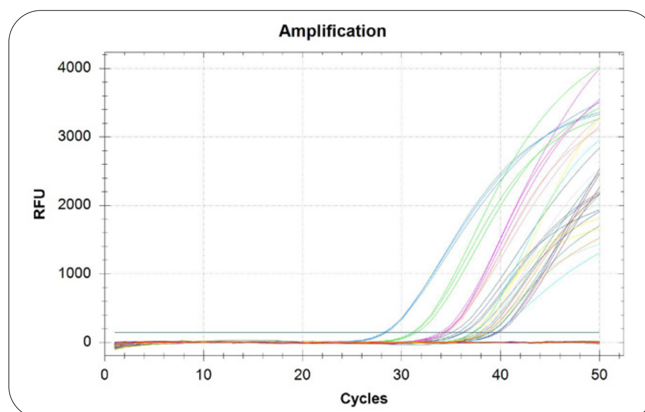

Figure 1: Amplification of all tested samples as denoted by RFU per PCR cycle. Light blue, lime, pink, and yellow lines represent the extracted samples, while the navy, green, gold, and orange lines represent the dPCR samples.

Table 1: Average Ct values following RT-PCR for M gene amplification at each given concentration.

	10^9	10^8	10^7	10^6
dPCR Workflow	36.05	38.52	38.88	39.76
Extraction Workflow	28.28	31.23	34.33	34.18

Conclusions

Herein, we have shown a useful workflow for sample lysis that is sufficient in releasing intracellular viral RNA for RT-qPCR amplification of M gene. This finding allows for decreased run time in traditional PCR-based protocols and reduces the reagents and plastics required for each sample, ultimately reducing the cost and sample turn around time when compared to traditional extraction-based PCR-based methods.

References

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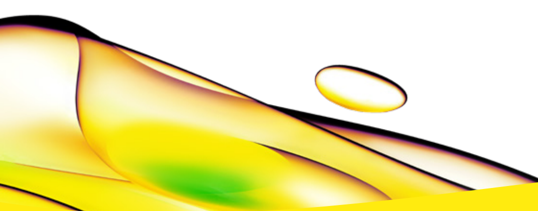

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