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SHORT COMMUNICATION

Comparison of Four Swab and Transport Media Combinations for the Detection of Respiratory Viruses

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SUMMARY

Background: **We compared four combinations of nasopharyngeal swabs and transport media for their ability to transfer and recover viruses under different storage conditions.**
Methods: **Each swab was immersed in culture supernatants of influenza A virus (IAV), respiratory syncytial virus, and adenovirus, placed in transport medium, and stored at -20℃, +4℃, +20 to 25℃, and +37℃ for 5 days. On each day, virus culture and real-time PCR were performed for each virus.**
Results: **All samples under different storage conditions showed positive results up to 5 days using both virus culture and real-time PCR. Real-time PCR showed that samples stored at -20℃, 4℃, and 20 - 25℃ were within two cycle thresholds (Cts) up to 5 days, but IAV at 37℃ showed that viral titer decreased after 3 days.**
Conclusions: **Our results indicate that these swab and transport media maintained the stability of the above viruses for 5 days at room temperature, refrigerated, and frozen storage conditions.**
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KEYWORDS

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INTRODUCTION

Appropriate collection and transport of viral specimens to the laboratory is important for accurate diagnosis of viral infection. For the diagnosis of respiratory viral infection, nasopharyngeal swabs and transport media are commonly used for virus culture, viral antigen, and viral nucleic acid amplification tests. Nasopharyngeal swabs are composed of a 14 - 16 cm long shaft portion and a 15 - 30 mm x 1 - 4 mm head portion at one end of the shaft [1,2]. The flocked swabs are considered the gold standard for nasopharyngeal sampling because of the design, which has maximized the surface area and increased sample release [1-3]. A breaking point at 6 - 7 cm shaft portion allows the shaft head to be placed in

transport media in the tube after sample collection and to be transported with sealing. Several viral species lose their viability when exposed at room temperature for some time; thus, it is necessary for them to be transported in transport media [4,5].

Owing to the shortage of nasopharyngeal swabs and transport media during the COVID-19 pandemic, several new products were developed and are currently being used; however, the performance of these new products has not been well verified and compared. In this study, we compared the performance of four combinations of nasopharyngeal swabs and transport media, including three new products, for the detection of respiratory viruses for their ability to transfer and maintain the stability of viral particles and recover viruses under different sample storage conditions.

MATERIALS AND METHODS

Comparative evaluation of swabs and transport media

Four swab and transport medium combinations were used for evaluation, as follow: 1) Universal Transport Medium (CTM-RT) and FLOQSwabs (Copan Diagnostics Inc., Brescia, Italy); 2) Clinical Virus Transport Medium (CTM) and NFS-SWAB applicator (Noble Bio, Hwaseong, Korea); 3) Transport Medium (IS2) and I-Swab2 (Asan Pharm, Korea); and 4) ALLTM set including SG Brush (SG medical, Seoul, Korea). All nasopharyngeal swabs have a flocked nylon fiber tip with a breaking point. The I-Swab2 from Asan Pharm had the longest swab head of 21.4 ± 0.4 mm (average length of 10 measurements), and the remaining swabs had a similar length of 15 - 16 mm. The composition of transport media in transport tubes was similar, including Hanks Balanced Salt Solution (HBSS) with bovine serum albumin, an antimicrobial agent (antifungal and antibacterial), protein, and sugars with a pH indicator [6, 7].

Swab elution test

Three virus culture supernatants (influenza A virus (H1N1) (KBPV-VR-76 from Korea Bank for Pathogenic Viruses) in MDCK cells (KCLB10034 from Korean Cell Line Bank), respiratory syncytial virus A (KBPV-VR-73) in Hep-2 cells (KCLB10023), and adenovirus type 3 (KBPV-VR-62) in Hep-2 cells (KCLB10023)) were used for the swab elution test. Each swab was immersed in the diluted virus culture supernatants of influenza A virus (IAV), respiratory syncytial virus (RSV), and adenovirus (ADV) for 5 seconds, placed in 1 mL of saline, and vortexed for 5 seconds. The adsorption amount of each swab was calculated as the difference of the weight before and after adsorption. Real-time PCR was performed to amplify IAV, RSV, and AdV using the Seegene Allplex Respiratory Panel 1/2 (Seegene, Seoul, Korea), and results of the cycle thresholds (Ct) values were compared. All procedures were performed in duplicate.

Viral recovery test

The viability of the virus at different incubation periods and temperatures was evaluated. Stains of IAV, RSV, and AdV were used for swab and media validation. Each swab was immersed in the diluted virus culture supernatants for 5 seconds, and then placed in 2 mL of each transport media. The media containing the swabs were stored at 20 - 25℃, 4℃, -20℃, or 37℃ for 5 days. On each day, real-time PCR and virus culture using stored media were performed for IAV, RSV, and AdV. Nucleic acid extraction was conducted on Qiagen QIAcube using the QIAamp Viral RNA Mini kit (Qiagen, Germany). Real-time PCR was also performed for IAV, RSV, and AdV using the Seegene Allplex Respiratory Panel 1/2 reagents (Seegene, Seoul, Korea) and Bio-Rad CFX96 real-time PCR Detection System (Bio-Rad, USA). All procedure was performed in duplicate. Virus culture was performed using the conventional culture technique. On each day, 100 μL of transport media containing each virus were inoculated into monolayer cultured cells and incubated for 5 days. The viability of each virus after culture was determined using fluorescent antibody staining for each virus with the D^3 Ultra 8 DFA Respiratory Virus Screening and Identification Kit (Quidel Company, San Diego, CA, USA) and a fluorescence microscope. Virus culture was interpreted as positive, weakly positive, or negative. Weakly positive results indicated that > 90% reduction in fluorescing infected cells.

RESULTS

Swab elution test

The absorption amount of each swab is shown in Table 1. The largest absorption amount was observed in I-Swab2 from Asan Pharm, showing 104.5 mg for IAV, 80.5 mg for RSV, and 114 mg for AdV, among all swabs tested. The relative amount of virus after elution from I-Swab2 from Asan Pharm showed the lowest Ct values, of 24.1 mg for IAV, 20.4 mg for RSV, and 16.0 mg for AdV, among all swabs tested (Table 1).

Viral recovery test

All samples from different swabs and transport media showed positive results up to 5 days in real-time PCR and virus culture techniques under different storage conditions (-20℃, 4℃, 20 - 25℃, and 37℃). Real-time PCR results showed that all samples stored at 4℃, 20 - 25℃, and -20℃ were within two Ct values when comparing the average Ct values at day 0 until day 5. However, after 3 days of storage at 37℃, Ct values for the influenza virus tended to increase, and the viral titer observed using virus culture and fluorescence staining decreased (Supplemental Table S1 and Figure 1). The Ct

Table 1. Swab elution test with four swabs and transport media combinations.

Virus culture supernatant	Swab and transport media	Swab length (mm, mean)	Amount of adsorption (mg)	Real-time PCR result after adsorption and elution (Ct)	ΔCt
Influenza A virus (Ct 25.4)	Copan	16.0	59.95	27.7	2.3
	Noble Bio	15.9	56.35	27.2	1.8
	Asan Pharm	21.4	95.45	26.7	1.5
	SG medical	15.1	65.30	27.4	2.0
Respiratory syncytial virus (Ct 19.3)	Copan	16.0	59.65	21.2	1.9
	Noble Bio	15.9	59.70	21.6	2.3
	Asan Pharm	21.4	79.70	20.4	1.1
	SG medical	15.1	54.05	21.6	2.3
Adenovirus (Ct 11.8)	Copan	16.0	64.10	16.2	4.4
	Noble Bio	15.9	59.25	16.6	4.8
	Asan Pharm	21.4	96.15	16.0	4.3
	SG medical	15.1	56.90	17.2	5.4

Ct - threshold cycle of real-time PCR for each virus.

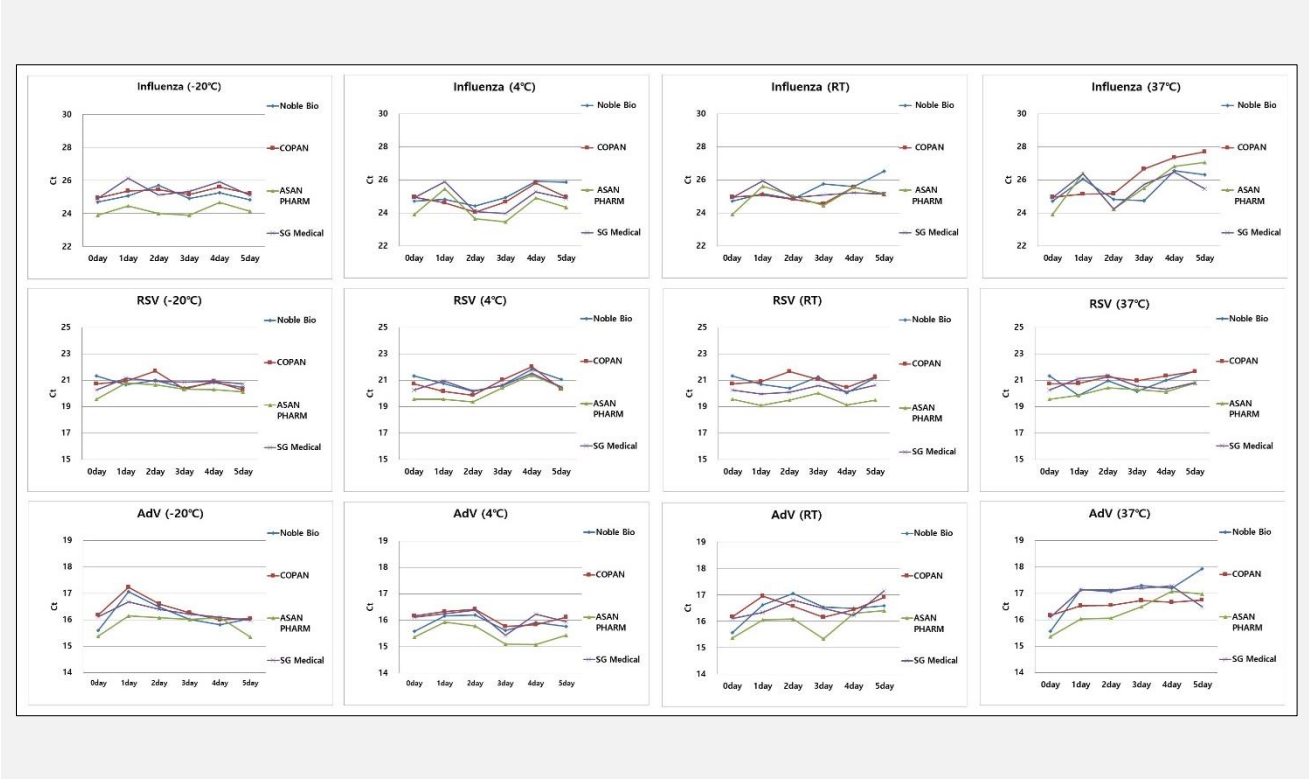

Figure 1. Stability test for 5 days using the different swabs and transport media combinations under different storage conditions.

Real-time PCR was performed to detect influenza A virus, respiratory syncytial virus, and adenovirus. RSV - respiratory syncytial virus, AdV - adenovirus, RT - room temperature.

value of I-Swab2 from Asan Pharm was also the lowest under the same storage conditions, except at 37℃.

DISCUSSION

Three viruses were used to compare and evaluate the performance of four nasopharyngeal swab and transport medium combinations. For the swab absorption and elusion tests, the I-Swab2 swabs from Asan Pharm were able to collect the largest amount of virus solution, and this was thought to be due to the long length of the swab head. The longer or larger the head, the more sample can be collected; however, as this may cause discomfort to the patient, an appropriate length of swab head is favorable.

All samples from different swabs and transport media showed positive results for the three viruses, IAV, RSV, and AdV, up to 5 days using virus culture and real-time PCR techniques under different storage conditions (-20℃, 4℃, 20 - 25℃, and 37℃). Only a few studies have evaluated the viral stability of swabs and transport media using the virus culture technique. For IAV, viral stability was noted at 4℃ or -20℃ for 21 days; however, the viability of the virus gradually decreased after 7 days when stored at 20 - 25℃ and 37℃, especially without the use of transport medium [5,8]. Furthermore, previous studies have shown that the stability of each virus was different depending on the temperature and the type of transport medium used [4,6,9]. Several studies on the stability of transport medium using PCR technique have been reported after the recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak [10-14]. SARS-CoV-2 is stable for up to 7 days when stored in a refrigerator, at room temperature, and -20℃ even without using a transport medium [10]. IAV is stable using UTM and saline, but there is a large difference in Ct values a day after collection with Amies gel [15]. The results of the virus stability test may vary depending on the virus type, test method, transport medium, and storage conditions.

In this study, we only selected RSV, AdV, and IAV that are relatively well cultured. If this study had been performed using viruses with difficult culture conditions, the viability of the virus culture may not have been maintained for 5 days, and recovery may have been greatly reduced for that virus. Nevertheless, our results indicate that these four swab and transport medium combinations, including three new kits, maintained the stability of the three viruses for 5 days at room temperature, refrigerated, and frozen storage conditions, and the amount of the samples collected was related to the size of the swab head. As the stability of a virus may vary depending on the viral species, further tests using other viruses are needed. Our results may provide helpful information in the development and improvement of swabs and transport media for the detection of viral infections.

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Declaration of Interest:
All authors declare no conflict of interest.

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