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EVALUATION OF OPTIMAL SWAB TYPES, TRANSPORT MEDIA, AND TRANSPORT CONDITIONS DETECTING CORONA VIRUSES AND OTHERS BY PCR

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Abstract:

Introduction: *Different viral infections such as the Middle East respiratory syndrome (MERS) and influenza A (H1N1) are endemic in Saudi Arabia. It is critical to transport these viral etiologies in appropriate media so that the optimal amount of nucleic acid could be extracted for detection during an outbreak. The present aims to evaluate the optimal types of swab, transport media, and transport condition to detect viruses by PCR in Saudi Arabia.*

Methodology: *This study carried out a comprehensive evaluation of swabs with or without transport media optimal for the detection of five viral etiologies (MERS-CoV, H1N1, adenovirus, herpes virus, and enterovirus) at 4°C, room temperature (RT), and 37°C.*

Results: *The results showed that PCR products were optimal when the samples were transported without any transport medium at 4°C. Linear regression analysis revealed that the Ct values were almost identical for dry swabs maintained at 4°C. Universal Transport Medium (UTM) showed better Ct values at 4°C and RT when the two popular viral transport media (VTM) were compared for H1N1 and MERS-CoV. However, UTM showed better Ct value at 4°C; while, VTM showed better results at RT in the case of the enterovirus. Similarly, UTM was ideal at 4°C and VTM was ideal at RT for the herpes virus.*

Conclusions: *The findings of this study may help the healthcare industry in planning and tackling further outbreaks in an efficient manner.*

Key words: *Optimal swab; transport media; transport condition; polymerase chain reaction (PCR)*

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INTRODUCTION:

The Middle East respiratory syndrome (MERS) is a respiratory disease that was first detected in 2012 in Saudi Arabia. It is caused by a coronavirus called Middle East respiratory syndrome coronavirus (MERS-CoV) that causes disease ranging from common cold to severe acute respiratory syndrome (SARS). MERS has been endemic in Saudi Arabia and the typical symptoms include pyrexia, cough, diarrhea, and/or shortness of breath, and pneumonia in some cases. According to government statistics, approximately 36% of the affected patients have died due to MERS-CoV [1]. MERS is a zoonotic disease transmitted primarily from animals to humans and the camels act as the major reservoir host for the virus [1].

According to WHO, incidences of MERS in the Middle East are likely to increase in the near future and may spread to the member states through people who contract the disease from other infected individuals or animals [2]. In this context, disease monitoring becomes extremely crucial; therefore, molecular biology laboratories need to employ efficient methods to transport the viral samples and use appropriate specimen transport media. This mechanism is likely to ensure optimal conditions for the accurate detection of virus by PCR. The conditions in the swabs and the transport media need not be optimal for the virus to replicate and the viral nucleic acid should be preserved intact [3].

Influenza A (H1N1) is another major viral infection that has been endemic in Saudi Arabia. The first major incident of swine influenza was reported in 1918 when more than 500 million people were infected with the virus and around 50 million people died [4]. By April 2009, WHO declared H1N1 infection as a public health emergency of international concern following the first incidence in the United States [5, 6]. By the middle of 2009, the infection was spread to more than 100 countries and caused more than 260 deaths across the globe and by the end of 2009; more than 200 nations reported swine flu cases [7]. However, Saudi Arabia reported H1N1 as an epidemic with a high mortality in 2009 [8].

There was a substantial increase in the number of samples with influenza during 2009 influenza A H1N1 pandemic [9]. There was a shortage of biological swabs and transport medium due to lack of systematic data about their suitability. It is important to preserve nucleic acid; however, there is no requirement of molecular detection method to replicate competent virus. Type of swab and transport medium to identify viruses has been examined by previous studies [10-12]; however, factors like temperature and transit time between data collection and testing have assessed by Dures et al [3]. The information related to effect of temperature on nucleic acid detection by PCR is lacking.

The detection rates for respiratory viruses are similar in both anterior nasal swab specimens and samples, when using highly sensitive PCR assays. These samples are collected via traditional method of nasopharyngeal aspiration [13]. Similar results have been presented for parent-collected anterior nasal swab specimens, along with nasopharyngeal aspirates [14, 15]. A study conducted by O'Grady et al. [16] highlighted the impact of transporting samples for extended periods at higher temperatures; however, sample transport showing mailing swabs at ambient temperature was exhibited by few of the previous studies [17, 18].

The significance of the viral transport medium for optimal nucleic acid detection becomes very critical. The present study endeavors to determine the optimal temperature and medium for the transport of viral swabs and samples. Viruses including herpes virus, enterovirus, and adenovirus were also evaluated along with MERS and H1N1. The main objective of the study is to evaluate swab types, transport media, and transport conditions for optimal detection of viruses such as MERS, H1N1, herpes virus, enterovirus, and adenovirus by PCR. Universal Transport Medium (UTM) is a highly stable transport medium that is routinely used for maintaining viral samples such as H1N1 under frozen conditions for a long time. UTM preserves the properties of the virus so that they remain ideal for future cell culture and further molecular assays. Similarly, commercially available viral transport medium Virocult is routinely used to transport viral samples such as herpes and H1N1. This medium is considered ideal for long-term survival of viruses to ensure optimum Ct values.

MATERIAL AND METHODOLOGY:

This study has explored the stability of four viruses in several transport and swab medium combinations with different physiochemical properties. The viruses include A/New Caledonia/20/99 (H1N1), herpes simplex virus type 2 (HSV-2), adenovirus type 7, enveloped and non-enveloped RNA viruses, and an enterovirus (echovirus type 30). The influence of viral transport medium, the duration and temperature of transport, and swab type was examined in this study.

Swabs were bought from Copan (Brescia, Italy). Virus transport medium swabs in a medium-soaked sponge (VTM), flocked swabs with universal transport medium (UTM), flocked nylon fiber swabs with liquid Amies medium (E swabs), plain swabs without medium, and swabs with Amies gel comprising charcoal. A 2% fetal bovine serum was comprised in virus stocks to be prepared in

minimum essential medium for giving cycle threshold values of between 10,000 to 1,000 nucleic acid per ml. The immersion process of swabs last for 2 seconds and swabs related with transport media were quickly placed in that medium. The gel was used to resheath Amies gel swabs. A tube comprising 2ml sterile saline was used for plain swabs, which were either resheathed in their original housings regardless of medium.

Replacements of each swab type exposed to the original viruses were quickly placed at -70°C as time zero controls. Similarly, the remaining swabs were distributed into ambient temperatures (20°C to 22°C), 3 groups and held at 4°C or 37°C. For each treatment condition, duplicate swab/medium combinations were then gathered and stored at -70°C for 7 days every 24h in the laboratory. In addition, the incubation process for all swabs was based on 7 days at -70°C and afterward thawed at 4°C overnight. The medium for nucleic acid extraction was directly used on UTM, VTM, saline swabs, and E where the volume in VTM swabs was increased by 2 ml with MEM2%. Regardless of transport medium and the Amies gel swabs, plain swabs were positioned in 2 ml MEM2% quickly before the extraction.

Qiagen DX extraction kits (Qiagen Sciences, Germantown, MD) and a QIAxtractor robot (Qiagen, Hilden, Germany) were used to extract total nucleic acid in 200µl. cDNA was obtained by linearizing 10µl extract at 65ºC for 10 minutes. It was then quenched on ice and added to 12µl reverse transcription (RT) master mix that contained random hexamers, deoxynucleoside triphosphates, enzymes, and buffers. This solution was later incubated at 42ºC for 30 minutes and at 100ºC for 10 minutes. 4µl DNA and 16µl ABI Fast master mix was used during PCR that contained probes at 0.2µM and primers at 0.9µM. 7500 fast real-time system (Applied Bio-systems, CA) was used to set the cycling conditions at 95ºC for 2 minutes, then 45 cycles for 2 seconds, and then 45 cycles for 39 seconds at 60ºC. Real-time PCR analysis was conducted to detect influenza virus RNA from the swabs that were kept at 4ºC, room temperature, and 37ºC for 7 days. There was no replication as CT of 45 indicated the results.

Data Analysis

The total nucleic acids from the samples (200µL) was extracted from the samples using QIAxtractor robot and Qiagen DX extraction kits). The elution volume was 60µL. The procedure followed for obtaining cDNA is as follows;

Linearization of 10µL extract at 65°C for 10 min
Quenching on ice

Addition of to 12µL reverse transcription master mix containing random hexamers, deoxynucleoside triphosphates (dNTPs), and avian myeloblastosis virus (AMV)-RT enzyme and buffers

Incubation at 42°C for 30 min and at 100°C for 10 min.

PCR was performed using 4µL DNA and 16µL ABI Fast master mix containing 0.µM primers and 0.2µM probes in an ABI 7500 fast real-time system. PCR cycling conditions included an initial denaturation at 95°C for 2 min followed by 45 cycles of 95°C for 2 seconds and 60°C for 30 seconds.

RESULTS:

Ct values obtained for H1N1, MERS-CoV, Enterovirus, Herpes virus, and Adenovirus under the conditions (4°C, room temperature, and 37°C) have been shown in figure 1, 2, 3, 4, and 5 respectively. For all the five viral samples, Ct values in PCR detection were higher for swabs without transport media. The results of present study have shown statistically significant difference in Ct values over the time between the saline swabs held at either Amies gels that were consistently higher for these three viruses (Enterovirus, Herpes virus, and Adenovirus). Use of UTM and VTM was consistently associated with an amplifiable product.

The results have shown that Ct values became identical by day 6 at 37°C. This indicated that PCR products were optimal when the samples were transported without any transport medium at 4°C and RT compared to that with a transport medium at the same temperature. Linear regression analysis revealed that the Ct values were almost identical for dry swabs maintained at 4°C. UTM showed better Ct values at 4°C and RT, when the two popular viral transport media (UTM and VTM) were compared for H1N1 (Figure 1) and MERS-CoV (Figure 2). However, UTM showed better Ct value at 4°C in the case of the enterovirus (Figure 3); while, VTM showed better results at RT. Similarly, UTM was ideal at 4°C and VTM was ideal at RT for the herpes virus (Figure 4). Similar result was observed for adenovirus (Figure 5) leading to the assumption that this difference may be influenced by the nucleic acid type.

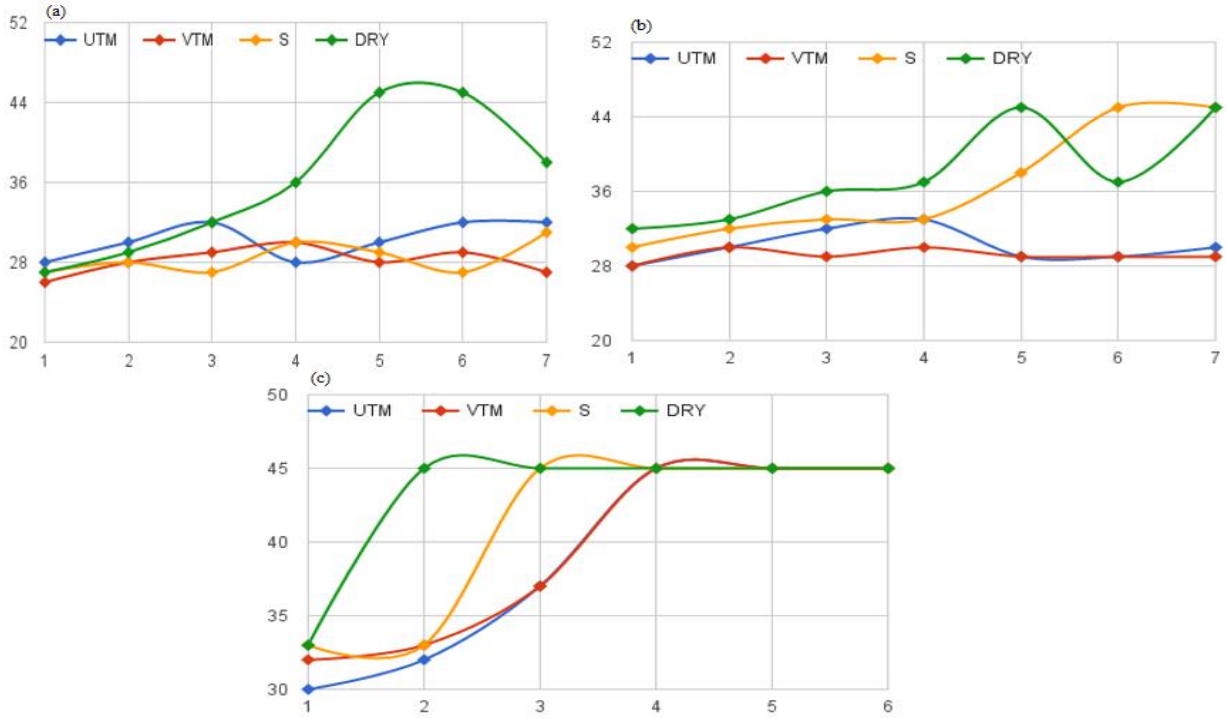

Figure (1) Cyclic threshold values obtained from uniformly inoculated swabs of H1N1 (a) at 4°C; (b) room temperature; (c) 37°C for 7 days (Note: UTM = Unified Transaction Model; VTA = Ventral Tegmental Area)

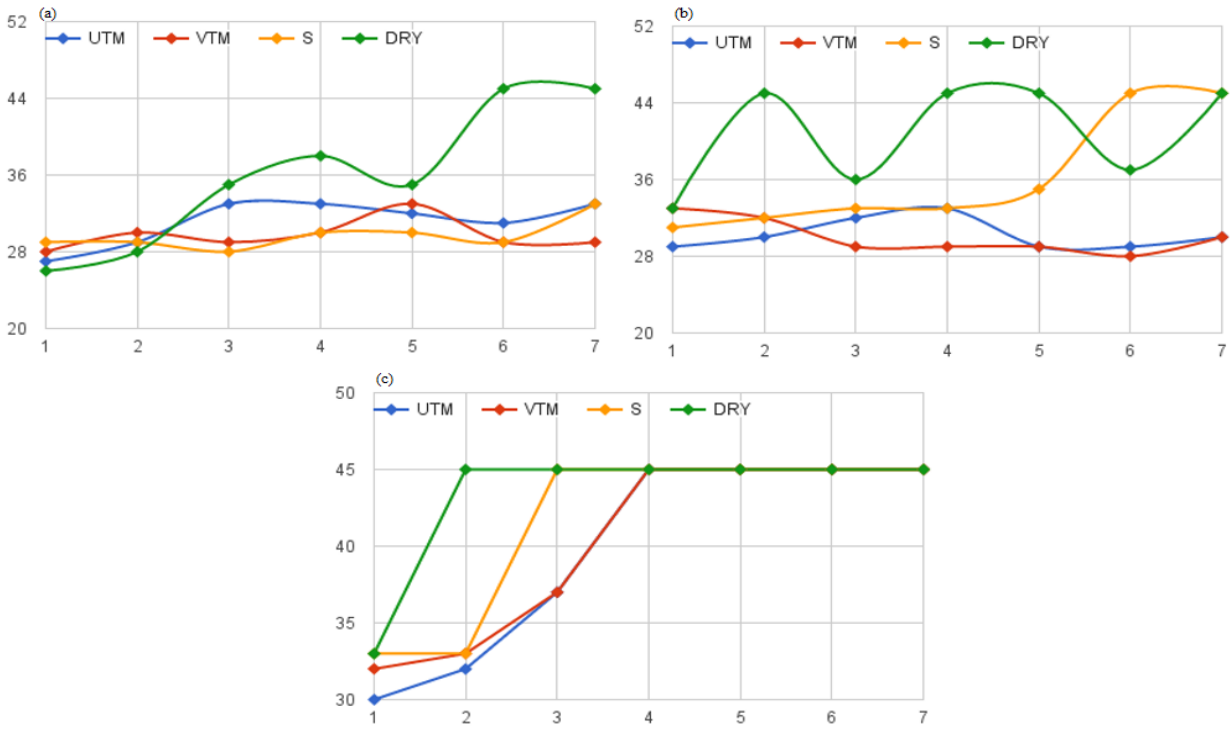

Figure (2) Cyclic threshold values obtained from uniformly inoculated swabs of MERS-COV (a) at 4°C; (b) room temperature; (c) 37°C for 7 days (Note: UTM = Unified Transaction Model; VTA = Ventral Tegmental Area)

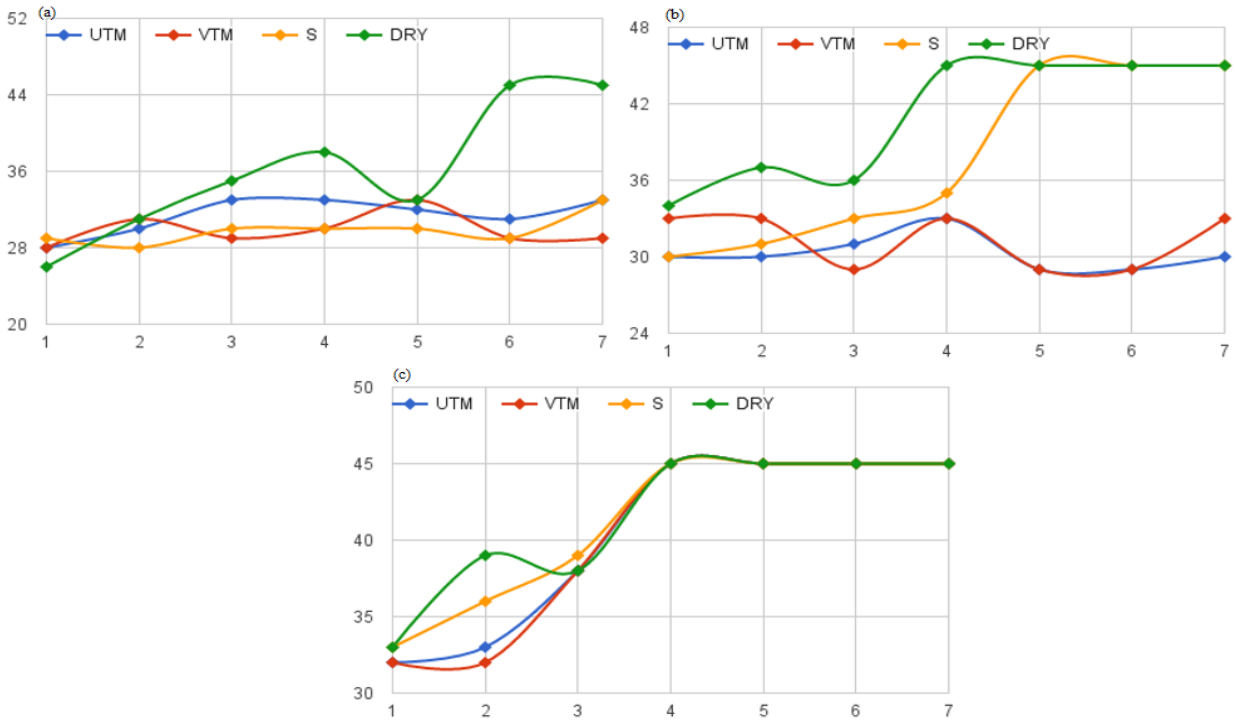

Figure (3) Cyclic threshold values obtained from uniformly inoculated swabs of enterovirus (a) at 4°C; (b) room temperature; (c) 37°C for 7 days (Note: UTM = Unified Transaction Model; VTA = Ventral Tegmental Area)

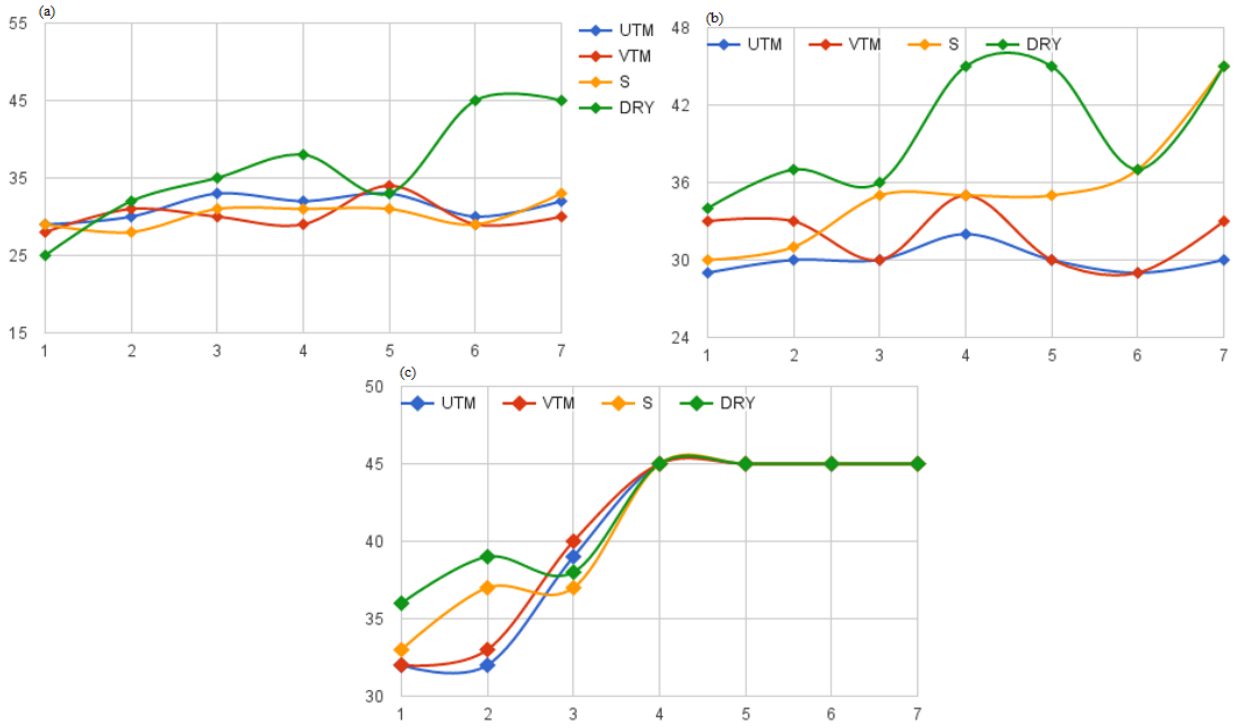

Figure (4) Cyclic threshold values obtained from uniformly inoculated swabs of Herpes virus (a) at 4°C; (b) room temperature; (c) 37°C for 7 days (Note: UTM = Unified Transaction Model; VTA = Ventral Tegmental Area)

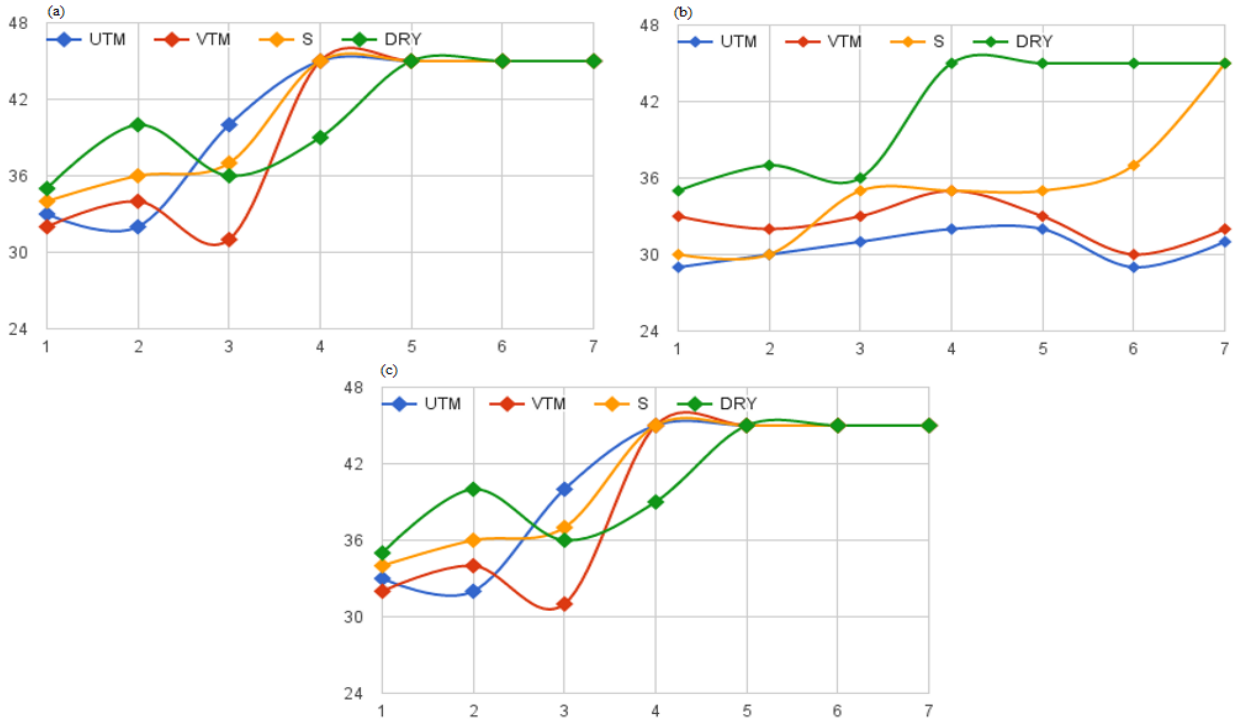

Figure (5) Cyclic threshold values obtained from uniformly inoculated swabs of Adenovirus (a) at 4°C; (b) room temperature; (c) 37°C for 7 days (Note: UTM = Unified Transaction Model; VTA = Ventral Tegmental Area)

Each of the swab/transport medium combinations produced an amplifiable enterovirus, adenovirus product, or HSV-2. On the contrary, Ct values increased after day 3 when the holding temperature was 37°C for each of these viruses on dry swabs, which indicate a rapid loss of nucleic acid integrity. Ct values were consistently higher for these 3 viruses from Amies gels when compared to the result achieved for influenza virus.

DISCUSSION:

The present study has evaluated different transport media that can be used for viral samples under different temperatures. The results have shown that difference in transport medium affect the Ct values, leading to the assumption that the nucleic acid type may influence this difference. This is because H1N1, enterovirus, and MERS-CoV are RNA viruses and adenovirus, and herpes virus are DNA viruses. These results are warranted by Druce [3] as the results showed that Ct values of viral swabs without any transport medium held at 4°C and RT showed a statistical difference at 7th day. The same was observed when compared at a holding temperature of 37°C. Druce [3] also observed that Ct values of all the viruses (with or without any transport medium) increased with each passing day and reached identical levels by day 6. This study indicated that the dry swabs are the most ideal for transportation of viral stocks ensuring optimal PCR products at 4 °C and RT.

Plain swabs in saline also yielded an amplifiable product at each time point and temperature, with Ct values similar to those of the commercial products. This was expected, since this product has been developed for the preservation of anaerobic bacteria, but sometimes used to collect and transport specimens for viral studies. Another study conducted by Moore et al. [19] observed that dry viral swabs without any transport medium are ideal for the transportation of viral samples such as influenza A and influenza B. It can also be a viable alternative to traditional methods involving different transport media without any compromise on the recovery of nucleic acids. Respiratory syncytial virus (RSV) was detected on dry cotton swabs using PCR after 15 days at room temperature by Moore et al. [19]. However, the present study differs from several previous investigations that compared swab types and specimen collection methods. The experiments were conducted in a laboratory setting and did not analyze clinical samples. Therefore, the results of present study may not be entirely accurate from a clinical perspective but do give an indication of the ideal transport conditions.

The study results differ from previous investigations comparing the types of swabs and methods of specimen collection in a laboratory setting [20, 21]. However, no patient or clinical based method was involved in the present study. Moreover, issues

related to association between disease severity and viral load could not be assessed; although, quantitative information was provided based on the CT values. A similar study conducted by Moore et al. [19] showed that PCR could be used to detect respiratory syncytial virus (RSV) on dry cotton swabs after 15 days at room temperature. These results also depicted the use of dry swabs for clinical sampling of different respiratory viruses. However, this approach did not affect the direct comparison to other transport media. The rRT-PCR positive results can be easily confirmed by sequencing as MERS-CoV surveillance partner with the CDC or another qualified reference laboratory was conducted. Genomic sequencing provide confidence to public health authorities for response planning, data essential for monitoring both virus evolution and rRT-PCR assay signature integrity, and avoiding false alarms. MERS-CoV is likely to cause depletion of commercially available stocks of swabs and other consumables, which shows its association with large epidemics. The present study results showing effectiveness of collecting and transporting influenza virus on dry swabs for future pandemic planning.

CONCLUSION:

The present study has evaluated the optimal types of swab, transport media, and transport condition to detect viruses by PCR in Saudi Arabia. The results indicated that dry swabs without any transport medium are optimal for the transportation of viral samples at 4°C and RT. There was a significant difference between the Ct values of swabs with UTM and VTM and swabs without a transport medium, when samples were maintained at 37°C for approximately seven days. The utility of swab/medium combinations for the molecular detection of influenza virus is of considerable importance. This virus is historically associated with large epidemics and is more likely to cause depletion of commercially available stocks of swabs and other consumables during times of high demand. Moreover, the collection and transport of influenza virus on dry swabs in saline is considered appropriate for PCR detection is important for pandemic planning. The isolation would match the sensitivity of PCR analysis under the specific condition selected. It has been suggested that the laboratories need to be mindful for strain characterization, drug resistance phenotyping, and supply of candidate vaccine strains during outbreaks representative virus isolates. It is also important to consider utility of swab/medium combinations for the molecular detection of influenza virus. The utility of swab or medium combinations is of significant importance for the molecular detection of influenza virus. This virus is traditionally related with large epidemics and is more likely as compared to other viruses for causing depletion of commercially available swabs stocks and other consumables throughout high testing demand times. It is observed that transport and collection of influenza virus on dry swabs is adequate in saline for PCR detection for future pandemic planning.

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Figures:

Figure 1. Cyclic threshold values obtained from uniformly inoculated swabs of H1N1 (a) at 4°C; (b) room temperature; (c) 37°C for 7 days (Note: UTM = Unified Transaction Model; VTA = Ventral Tegmental Area)

Figure 2. Cyclic threshold values obtained from uniformly inoculated swabs of MERS-COV (a) at 4°C; (b) room temperature; (c) 37°C for 7 days (Note: UTM = Unified Transaction Model; VTA = Ventral Tegmental Area)

Figure 3. Cyclic threshold values obtained from uniformly inoculated swabs of enterovirus (a) at 4°C; (b) room temperature; (c) 37°C for 7 days (Note: UTM = Unified Transaction Model; VTA = Ventral Tegmental Area)

Figure 4. Cyclic threshold values obtained from uniformly inoculated swabs of Herpes virus (a) at 4°C; (b) room temperature; (c) 37°C for 7 days (Note: UTM = Unified Transaction Model; VTA = Ventral Tegmental Area)

Figure 5. Cyclic threshold values obtained from uniformly inoculated swabs of Adenovirus (a) at 4°C; (b) room temperature; (c) 37°C for 7 days (Note: UTM = Unified Transaction Model; VTA = Ventral Tegmental Area)