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Detection of Influenza A (Pandemic H1N1v), RSV, Rhinovirus and other respiratory viruses in different populations using Sigma-Virocult®

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Abstract

During the height of the Influenza A H1N1v pandemic in 2009 the Σ-Virocult® swab system proved useful as a robust, stable sampling and transport system that allowed the recovery and detection of viral nucleic acids, leading to percentage positivity rates of 6.9% and 37.5% in two diverse populations investigated. The same sample and transport system performed with equal merit when used in the detection of a range of viral targets including Influenza viruses, Respiratory Syncytial Virus, Rhinovirus and Adenovirus in a local trust setting.

Introduction

The use of less invasive methods for producing clinical samples has progressed rapidly in recent years, with the production of more specialised sampling techniques that cause little or no distress to the patient but yield high quality clinical material that may be examined for the presence of pathogens. These samples must lend themselves to the ever more novel methodologies that are increasingly becoming routine in the diagnostic laboratory.

The detection of viral pathogens relies heavily on molecular technologies requiring extraction of target nucleic acid that must be of a high quality, purity and free of inhibitors. Therefore, the generation of the primary sample is very important. Σ-Virocult® swab system from MWE (Medical Wire and Equipment Co Ltd) provides a combined viral specimen collection and transport system that achieves these goals providing high quality, stable samples.[1,2,3].

The Σ-Virocult® swab system is routinely used in many laboratories for the detection of a range of viral agents, both DNA and RNA, that typically cause infection of the respiratory and genital tract. A “look-back” exercise of data generated from 2009 Influenza A H1N1v pandemic and respiratory virus detection is presented here.

Methods

Swabs taken for respiratory sites were sent to the Newcastle Health Protection Agency Laboratory from three main sources. During summer 2009, 1056 respiratory swabs were submitted from two geographically distinct sites, (site A, n=453 and site B, n=603). These were only screened for the presence of Influenza A, Influenza A H1v (and N1 if results were discordant) and Influenza B, in an attempt to detect the circulating Influenza A H1N1v pandemic virus. From 02/01/10 – 30/04/10, 294 respiratory swabs from symptomatic patients aged 6 days to 85 years were submitted from the local trust (189 nose/throat, 92 throat, 11 nasal and 2 nasopharyngeal). These were screened by a number of real-time PCR assays designed to detect a range of viral pathogens including Influenza A and B, RSV A and B, Parainfluenza 1,2,3 and 4, Rhinovirus (hRV), human Metapneumovirus (hMPV) and Adenovirus.

These swab samples were first inactivated at Containment Level III and “spiked” with an extraction control target prior to undergoing an automated total nucleic extraction method on either the Biomerieaux easyMAG or Qiagen QIAsymphony instrument as per the manufacturers’ protocol. The resultant eluate was then tested in a number of PCR assays in an attempt to detect one or more of the respiratory targets.

All respiratory PCR assays were performed using the ABI 7500 FAST Real-Time PCR Platform, employing “TaqMan” hydrolysis chemistry with fluorescently labelled probes that allow detection of the viral targets. Data analyses were performed post-PCR using the instrument software and results collated.

Positive control material was derived from wilt type target virus grown in tissue culture prior to extraction. Σ-Virocult® swabs were also challenged with the same positive control material and examined as with clinical samples.

Results

Positive results were obtained for all targets when swabs were challenged with known control material, the results were comparable with the positive control results.

Swabs submitted for Influenza A, H1N1v and Influenza B screening from site A and site B yielded 170 (37.5%) and 42 (6.9%) confirmed Influenza A H1N1v positive results respectively as shown in table 1. No positive results were obtained for other Influenza A strains or for Influenza B. Table 1. shows the results based on target combination to provide a confirmed result.

		Number of samples positive per location	
		Site A	Site B
Amplification Target	A + H1	163	40
	A + N1	4	2
	H1 + N1	3	0
	Influenza B	0	0

Table 1. distribution of results when screening respiratory swabs for Influenza A,H1N1v and Influenza B

Of the 294 samples submitted from the local trust, eight different viral pathogens were detected in 42 samples (14.3%). Table 2. shows the distribution of viral targets detected in the cohort of 294 respiratory samples submitted from the local trust for complete viral screening.

		Sampling site			
		Nose and Throat (n=189)	Throat (n=92)	Nose (n=11)	Nasopharynx (n=2)
Viral Target	Influenza A	5	0	0	0
	Influenza B	0	0	0	0
	Parainfluenza 1	0	0	0	0
	Parainfluenza 2	0	0	0	0
	Parainfluenza 3	0	0	1	0
	Parainfluenza 4	0	1	0	0
	RSV A	0	2	0	1
	RSV B	5	9	0	0
	Adenovirus	5	1	0	0
	hMPV	3	0	0	0
	hRV	8	1	0	0

Table 2. distribution of viral targets when screening respiratory swabs from different respiratory sites for common respiratory viruses.

Discussion

On examination of those results generated from the Σ-Virocult® swabs taken from respiratory sites and submitted for Influenza A H1N1v screening, it can clearly be seen that a stable, high quality sample has been presented for analysis. The difference in percentage positivity rate does not represent any inadequacies of the sample or transport system but merely reflects epidemiological distribution of the virus. It was well evidenced that certain areas in the UK showed high levels of virus activity compared to others. This was probably down to local factors such as population density and local sampling policy.

The data obtained from samples taken in the local trust catchment area compare well with that of previous years, and when testing different sample types e.g. nasopharyngeal secretions or washings especially for those infants where a nasopharyngeal aspirate is considered the sample of choice for the detection of RSV. A wide variety of viral targets were detected, with little difference in percentage positivity rate based on site.

The detection of the extraction control in all eluates provides evidence that the constituents of the swab and transport media did not inhibit the PCR reaction.

In conclusion, the Σ-Virocult® swab system, when used as directed, is able to provide high quality clinical material that may be analysed with confidence in downstream molecular assays following nucleic acid extraction. The purified nucleic acid derived from the swabs may be examined for both RNA and DNA targets.

References

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