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Evaluation of a Virus, Chlamydia and Mycoplasma Transport, Storage and Recovery System by Molecular Techniques

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ABSTRACT

Background:
The aim of this study was to evaluate the efficacy of a novel swab transport system, namely the Sigma VCM® Transport System manufactured by Medical Wire & Equipment Co. Ltd., in the transport, preservation and recovery of a comprehensive range of micro-organisms, to include viruses, Chlamydia species, Mycoplasma and Ureaplasma species and *Neisseria gonorrhoeae*.
Traditional swab transport systems tend to be designed to preserve the integrity of either single micro-organisms or groups of similar organisms. This can occasionally lead to an inappropriate swab being placed in inappropriate medium for the specific investigation. The result is deterioration in patient care with delayed or invalid laboratory results, distress to the patient with repeat sampling often necessary and economic wastage.
Molecular techniques have recently become the cornerstone of infectious disease diagnosis in this laboratory, and therefore the suitability and stability of the Sigma VCM® system to enable the recovery and detection of the range organisms above was tested by various molecular techniques employing a range of chemistries, extraction and amplification platforms. The system must allow the detection of both DNA and RNA and be free from inhibitory material.
Methods:
Swabs were rolled in known positive clinical material before being placed in the transport medium. The nucleic acid was then extracted on one of two different automated platforms. In-house Real Time PCR was used for the detection and discrimination of Influenza A (H3N2, H1N1), Influenza B, Respiratory Syncytial Virus A and B, Parainfluenza 1, 2, 3 & 4, Adenovirus, Rhinovirus, human Metapneumovirus and Herpes Simplex Viruses1 and 2. Commercial assays were used for VZV, *C. trachomatis* and *N. gonorrhoeae*.
Results:
All target nucleic acids were successfully detected with no inhibition in any assay.
Conclusions:
In conclusion, the Sigma VCM® transport system is an efficient, stable, robust and versatile system for the transport and preservation and recovery of nucleic acid from a range of micro-organisms.

Introduction

Molecular techniques have become the corner stone of infectious disease diagnosis in the modern clinical microbiology laboratory. It is essential that any clinical material transport system must be robust, sufficiently stable and free of inhibitors to allow for the recovery and detection of microbiological nucleic acids in the clinical setting. The Σ-VCM® swab and preservation system was tested against a range of known positive clinical material containing a range of viruses (both DNA and RNA), *C. trachomatis* and *N. gonorrhoeae*.

References

1. Newcastle HPA Laboratory PCR Standard Operating Procedures 2010.

Disclaimer: Use of trade names is for identification only and does not imply endorsement by Health Protection Agency.

Methods

Method for Multiplex Respiratory PCR:
Under Containment Level 3 conditions the Σ VCM swabs were rolled in 200µl of respiratory positive control and immediately placed into the preservation medium. This positive control contains known tissue culture isolates of RSV A, Parainfluenza types 1-4, Rhinovirus and Adenovirus. The Σ VCM® swabs were also rolled in 200µl of positive clinical material containing HMPV, RSV B, Influenza B, Influenza H1N1v and Influenza H3N2 to complete the respiratory screen. These swabs were inactivated using manufacturer's lysis buffer and spiked with an internal control target prior to nucleic acid extraction. Extraction was performed by either the **Biomerieux easyMAG** or **Qiagen Qiasymphony**. The resulting eluates were tested in the in-house multiplex respiratory PCR assay,in duplicate, using the **ABI Taqman7500 FAST Real-Time PCR platform**. This is based on Taqman hydrolysis chemistry and the positive data is represented as CT values.

Method for HSV 1, HSV 2 and VZV detection:
The Σ VCM® swabs were rolled in 200µl of positive clinical material containing HSV 1, HSV 2 and VZV (as described in the method above). These swabs were inactivated using **Biomerieux easyMAG** lysis buffer and extracted using the **Biomerieux easyMAG** platform. The resulting elute was tested using in-house duplex HSV 1/2 PCR or **Qiagen Artus** VZV assay using the **Roche LightCycler** instrument. This is based on hybridisation chemistry and the positive data is represented as CT values.

Method for *Chlamydia trachomatis (CT) & Neisseria gonorrhoeae (GC)*
The Σ VCM® swabs were rolled in a known CT positive urine, known CT positive endocervical swab, and a known GC positive endocervical swab. These swabs were immediately placed into the preservation medium. 1ml of the medium was transferred into either a **Aptima Urine** or **Aptima Swab** collection tube which are compatible with the **Tigris DTS** system. The **Chlamydia Aptima** tubes were tested using the **Aptima Genprobe 50** assay and the **GC Aptima** tube was tested using the **Aptima Genprobe Combo 2** assay. Both of these assays were performed using the fully automated **Tigris DTS** system, which employs TMA PCR chemistry. Positive results are represented as RLU values.

Results

Table 1: Respiratory Virus PCR Results

Target	Nucleic Acid	CT value 1	CT value 2	Result
RSV A	RNA	27.60	28.28	Detected
RSV B	RNA	27.96	26.89	Detected
Parainfluenza 1	RNA	35.75	26.19	Detected
Parainfluenza 2	RNA	35.50	36.39	Detected
Parainfluenza 3	RNA	32.96	33.93	Detected
Parainfluenza 4	RNA	33.43	34.37	Detected
Rhinovirus	RNA	30.08	30.95	Detected
Adenovirus	DNA	23.37	24.10	Detected
BMV (IC)	RNA	30.34	30.35	Detected
Influenza B	RNA	32.92	35.79	Detected
Influenza A H3N2	RNA	20.90	Not Tested	Detected
Influenza A H1N1v	RNA	H1 29.07 N1 29.46	Not Tested	Detected

Table 2: Herpes Virus PCR Results

Target	Nucleic Acid	Ct Value	Result
HSV 1	DNA	27.56	Detected
HSV 2	DNA	34.84	Detected
VZV	DNA	20.20	Detected

Table 3: *C.trachomatis* and *N.gonorrhoeae* PCR Results

Target	Nucleic Acid	Specimen Type	RLU value	Result
CT	rRNA	Urine	7149	Detected
CT	rRNA	Endocervical swab	6663	Detected
GC	rRNA	Endocervical swab	770	Detected

Discussion

All target nucleic acids were successfully detected with no inhibition in both in-house and commercial PCR assays, irrespective of extraction platform.
In conclusion, the Σ VCM transport system is an efficient, stable, robust and versatile system for the transport and preservation of nucleic acid from a range of micro-organisms.
The flexibility of this swab transport system for a range of micro-organisms rather than a single group will have positive clinical impact. This will be manifested by a reduction of confusion in the clinical setting and hence less taking of inappropriate swabs for specific investigations, leading to a decrease in the need for repeat sampling and thus greater patient and economic benefit..