Public Health England

An Investigation into the Suitability of a Viral Transport Medium and Swab System for the Diagnosis of Urethritis

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mwe medical wire

INTRODUCTION

Historically, a number of microbiological agents, due to their fastidious nature and the associated difficulties in preparing routine culture systems, have been passed on to the virology laboratory for detection and identification. For example, the diagnosis of Chlamydia species and Mycoplasma species, has often been part of the Virologist's remit. In an attempt to investigate the suitability of swabs stored in viral transport media and submitted for the detection of causes of urethritis, we evaluated the use of the MWE VCM®/PurFlock® Swab system (Medical Wire & Equipment Co.) for the sampling, storage and detection of *Chlamydia trachomatis*, *Neisseria gonorrhoea*, and Mycoplasma genitalium DNA using the Fast-Track FTD Urethritis Basic Kit. A particular focus was placed on specimen storage.

MATERIALS & METHODS

Using commercially sourced DNA (Vircell) for all three targets, a serial dilution was prepared in Nuclisens lysis buffer. DNA was then extracted from 200µl of sample using the bioMérieux EasyMAG instrument. The resulting DNA extracts were then examined for the target DNA using the fast-track FTD Urethritis Basic PCR assay on the ABI 7500 Real-time PCR platform. The data generated in this experiment allowed for the identification of one dilution of each target that gave a relatively low Ct (approx. 22-25) and one that gives a relatively high Ct (approx. 30-33).

From this, the selected target dilutions were used to spike VCM® product by placing PurFlock Swab® into sample then transferring to VCM® tube. Samples were then taken at 0 hours, DNA extracted (from 200µl sample and elute DNA into 55µl elution buffer), and PCR performed in triplicate for individual and pooled targets (pooled targets duplicate testing only). This procedure was repeated following storage of spiked VCM product at room temperature at 24 hours, 48 hours and 72 hours.

The assay includes the use of an internal process control which is co-amplified during the PCR in an attempt to identify any possible inhibition or interference during the assay.

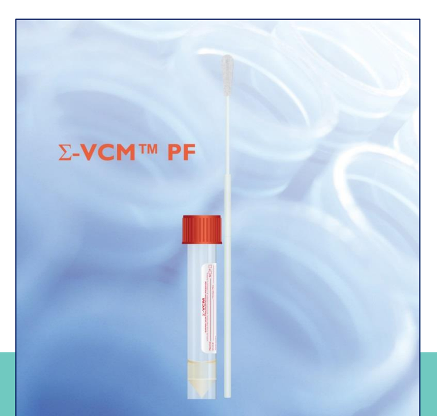
RESULTS

On examination of the resultant data shown in Table 1., it can be seen that very little degradation of sample occurs with storage of up to 72 hours at room temperature, based on the Ct values obtained following PCR for all three targets. The Internal Process Control gave results that correlated well with each sample tested.

Table 1. Ct values for all individual (in triplicate) and pooled (in duplicate) targets at sampling points 0, 24, 48 and 72 hours.

0 Hrs	CT	NG	MG	IPC	24 Hrs	CT	NG	MG	IPC	48 Hrs	CT	NG	MG	IPC	72 Hrs	CT	NG	MG	IPC
Pos C	28.27	29.14	28.03	Neg	Pos C	30.1	29.65	28.68	Neg	Pos C	30.07	29.75	28.48	Neg	Pos C	33.39	31.78	28.56	Neg
Neg C	Neg	Neg	Neg	28.61	Neg C	Neg	Neg	Neg	24.74	Neg C	Neg	Neg	Neg	23.95	Neg C	Neg	Neg	Neg	24.20
CTA	25.18			28.32	CTA	26.53			24.67	CTA	26.54			24.72	CTA	26.34			24.32
CTA	24.91			28.15	CTA	26.33			24.59	CTA	26.38			24.38	CTA	26.40			24.48
CTA	25.10			28.39	CTA	26.53			24.86	CTA	26.48			24.65	CTA	26.38			24.53
CTB	29.99			22.49	CTB	29.61			22.77	CTB	29.42			23.21	CTB	30.20			24.45
CTB	30.14			23.70	CTB	29.98			24.31	CTB	29.87			24.33	CTB	30.24			24.71
CTB	29.73			23.65	CTB	29.66			24.19	CTB	29.95			21.37	CTB	30.07			24.52
NGA		27.12		28.43	NGA		27.05		24.92	NGA		26.96		24.95	NGA		27.15		24.74
NGA		27.01		28.27	NGA		26.98		24.80	NGA		27.03		24.81	NGA		26.93		24.85
NGA		29.91		28.29	NGA		27.00		24.47	NGA		27.21		25.01	NGA		27.04		24.59
NGB		30.28		24.57	NGB		30.58		24.37	NGB		30.52		25.11	NGB		30.40		24.74
NGB		31.23		24.94	NGB		30.45		24.55	NGB		30.40		25.10	NGB		30.36		25.14
NGB		30.43		25.05	NGB		30.24		24.86	NGB		30.55		25.03	NGB		30.50		25.08
MGA			28.23	28.07	MGA			28.29	24.41	MGA			28.27	24.64	MGA			28.25	24.39
MGA			28.14	27.69	MGA			28.29	24.08	MGA			28.25	24.28	MGA			28.29	24.11
MGA			28.08	27.28	MGA			28.32	24.00	MGA			28.23	24.04	MGA			28.21	24.06
MGB			31.24	23.75	MGB			31.34	25.06	MGB			31.76	24.66	MGB			32.05	25.29
MGB			31.38	23.86	MGB			31.43	24.88	MGB			32.18	24.63	MGB			31.48	25.03
MGB			31.34	23.97	MGB			31.39	24.79	MGB			31.71	24.63	MGB			31.92	25.04
Pool A	26.49	27.57	28.20	28.12	Pool A	28.06	28.27	29.53	24.42	Pool A	28.06	28.37	29.52	24.52	Pool A	28.05	28.88	29.67	24.30
Pool A	26.50	28.01	28.13	27.68	Pool A	27.92	28.34	29.34	24.16	Pool A	28.02	28.19	29.44	24.10	Pool A	28.02	28.37	29.58	24.09
Pool B	31.32	32.62	32.73	23.30	Pool B	31.28	32.46	33.37	23.48	Pool B	31.08	31.95	33.14	24.04	Pool B	31.78	32.09	33.97	24.30
Pool B	31.58	32.08	33.41	22.92	Pool B	31.57	33.19	32.80	23.17	Pool B	31.13	32.17	32.19	23.78	Pool B	31.35	32.03	33.19	24.07

Key:
- CTA: *C. trachomatis* low dilution
- CTB: *C. trachomatis* high dilution
- NGA: *N. gonorrhoea* low dilution
- NGB: *N. gonorrhoea* high dilution
- MGA: *M. genitalium* low dilution
- MGB: *M. genitalium* high dilution
- Pool A: pooled low dilution targets
- Pool B: pooled high dilution targets
- IPC: internal process control



CONCLUSIONS

These results indicate that the use of MWE PurFlock® swabs and VCM® liquid medium allows for the detection of target DNA following prolonged storage at room temperature without sample degradation. On examination of the Internal Process Control results, an amplification curve, with acceptable threshold crossing point was observed with every sample. This indicated that the PCR process was not affected by the use of the sample, or the transport and storage system.

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Caption: Angel of the North
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