Clinical STI testing research

Evaluation and characterization of microorganisms that cause sexually transmitted infections

Introduction

Each year over one billion people are affected worldwide by urogenital infections, according to the Centers for Disease Control and Prevention [1]. Urethritis is a common urogenital infection often caused by sexually transmitted bacterial infections from *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and *Mycoplasma genitalium* [2]. Early detection of the infectious pathogen is important in order to treat these patients. Often, infectious disease researchers opt for a faster and more sensitive molecular method like qPCR over traditional methods like bacteria culturing.

Accessible evaluation and characterization of disease-causing microorganisms through various sample types like swabs and urine are needed to support research in syndromic effects for individuals at high risk of contracting sexually transmitted infections (STIs) and urinary tract infections (UTIs).

Background

Nucleic acid isolation on Thermo Scientific™ KingFisher™ sample extraction and purification instruments using Applied Biosystems™ MagMAX™ bead-based technologies provides semiautomated solutions that increase versatility and flexibility within STI and UTI workflows while reducing time and operator burden. Recovery of DNA and RNA from viral particles and gram-negative bacteria from samples such as biofluids and swabs in transport media can be achieved using MagMAX kit chemistries with KingFisher instruments. Figure 1 depicts a general workflow for molecular biology research applications, from sample collection to analysis with Applied Biosystems™ MagMAX™ Viral/Pathogen kits and Applied Biosystems™ TrueMark™ panels.

Nucleic acid isolation from difficult-to-lyse pathogens such as gram-positive bacteria, yeasts, and fungi can be achieved with flexible and scalable workflows using various MagMAX kits, based on your lab's needs. Table 1 indicates the compatibility of various pathogens and sample types across three MagMAX kits. Here we evaluate the performance of various sample matrices, including urine and viral transport medium (VTM) contrived with various pathogen targets, across three different MagMAX Viral/Pathogen kits to accurately detect a wide variety of sexually transmitted pathogens. To assess the extracted nucleic acid, RT-qPCR was performed using the Applied Biosystems™ TrueMark™ STI Plus Panel, Applied Biosystems™ TrueMark™ STI Select Panel, and Applied Biosystems™ TaqMan™ Assays [3,4].

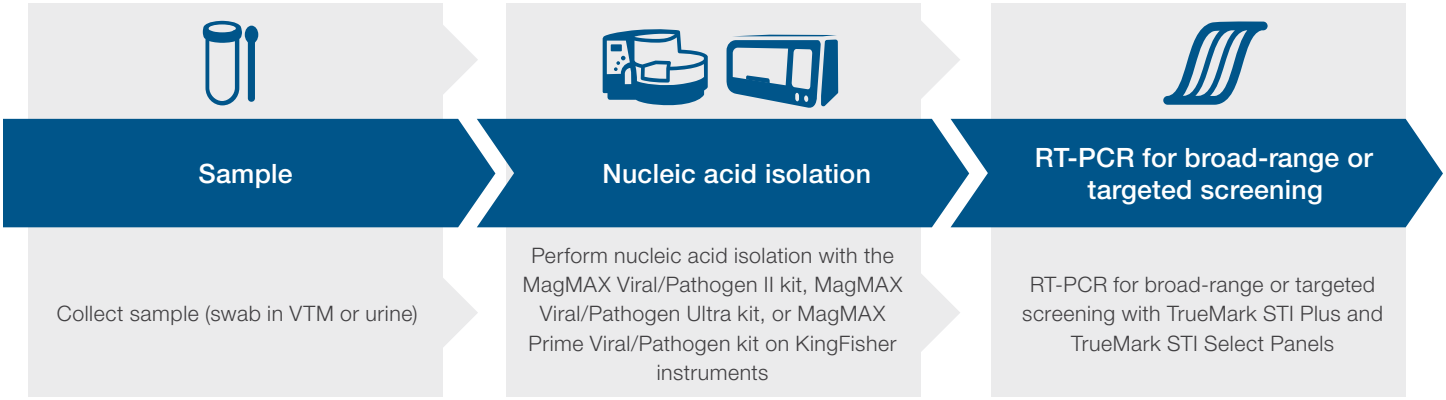

Figure 1. Workflow options for nucleic acid isolation followed by RT-PCR.

Table 1. Pathogens and sample types compatible with MagMAX Viral/Pathogen kits.

Product	MagMAX Viral/Pathogen II kit	MagMAX Viral/Pathogen Ultra kit	MagMAX Prime Viral/Pathogen kit
Pathogen			
DNA virus	●	●	●
RNA virus	●	●	●
Gram-negative bacteria	●	●	●
Gram-positive bacteria	×	●	●
Yeasts and fungi	×	●	●
Parasites	×	●	●
Sample type			
Biofluid (plasma, serum urine, saliva, BAL)	●	●	●
Swab in transport media	●	●	●
Dried blood spot (DBS)	×	×	●
Stool and fecal swab	×	×	●

● Contains feature for extraction
× Does not contain feature for extraction

Materials and methods

Vaginal swabs from four healthy donors were collected and stored in Aptima™ Multitest Swab transport medium, Amies transport medium, and Stuart transport medium. Collected samples were contrived with eight targets, as listed in Table 2, and processed in triplicate using all three MagMAX Viral/Pathogen kits with their respective workflow instructions for 200 µL VTM (MAN0024756, MAN0018074, and MAN0029683) on the Thermo Scientific™ KingFisher™ Flex Purification System. The eluates from replicates (across donor, media type, and extraction chemistry) were pooled and mixed. The eluate pools were tested for the targets listed in Table 2 using both the TrueMark STI Plus Panel, Combo Kit (MAN0026671) and the TrueMark STI Select Panel, Combo Kit (MAN0028493). Amplification of various targets across the three separate extraction workflows and three different collection media was analyzed using quantification cycle (C_q) values. Both PCR assays utilized RNase P as the internal sample control.

To confirm the compatibility of TrueMark STI kits with urine, a pool of urine from healthy donors was contrived with three STI targets, including HSV, *M. hominis*, and *T. vaginalis*. The same targets were contrived in pooled Amies medium from vaginal swab samples of healthy donors. Using the basic workflow of the MagMAX Prime Viral/Pathogen kit (MAN0029683), one sample plate was prepared and both 200 µL and 400 µL of the same contrived urine and vaginal samples were processed in triplicate with one extraction run. Urine samples were evaluated in duplicate with the TrueMark STI Plus Panel, and both urine and vaginal samples were evaluated using Applied Biosystems™ TaqMan™ Assays to evaluate their concordance.

Table 2. Experimental parameters—media, extraction chemistries, and TrueMark STI panels used in this study—and the quantity of target added into each extraction are listed.

Collection medium	Extraction kit	TrueMark STI panel	Target	Abbreviation	Quantity added to each extraction	Unit of measure	No. of samples
Aptima, Amies, or Stuart transport medium	MagMAX Viral/Pathogen II, MagMAX Viral/Pathogen Ultra, or MagMAX Prime Viral/Pathogen kit	TrueMark STI Plus Panel*	*Chlamydia trachomatis*	CT	2.73×10^7	IFU	48
			Neisseria gonorrhoeae	NG	2.04×10^7	CFU	
			Mycoplasma genitalium	MG	2.50×10^4	Bacterial cells	
			Trichomonas vaginalis	TV	3.40×10^6	Cells	
			Ureaplasma urealyticum	UU	5.00×10^3	CCU	
			Mycoplasma hominis	MH	1.50×10^5	CFU	
			Herpes simplex virus 1	HSV1	1.44×10^7	$TCID_{50}$	
			Herpes simplex virus 2	HSV2	2.00×10^7	$TCID_{50}$	
		TrueMark STI Select Panel	*Chlamydia trachomatis*	CT	2.73×10^7	IFU	24
			Neisseria gonorrhoeae	NG	2.04×10^7	CFU	
			Trichomonas vaginalis	TV	3.40×10^6	Cells	
			Mycoplasma genitalium	MG	2.50×10^4	Bacterial cells	
			RNase P	RNase P	NA	NA	

* Note: *Ureaplasma parvum* (UP) was not spiked with a known concentration into the samples as it was naturally present in the samples of all four donors. Nine targets in total are present in each donor/collection device sample.

Results and discussion

All three MagMAX Viral/Pathogen kits showed correlation in C_q values across the nine targets sampled from four donors and detected by the TrueMark STI Plus Panel, Combo Kit. Figure 2 details a box and whisker plot spanning C_q values, means, ranges, and outliers across four donors and three collection media for all three extraction workflows. C_q values shown for each of the targets are derived from all four donors and three collection media, for a total of 12 individual data points per target extracted by each chemistry. Among the three extraction kits, the trends are similar and the ranges of C_q values overlap. Similarly, Figure 3 details the same trends with the TrueMark STI Select Panel, Combo Kit across the five targets evaluated.

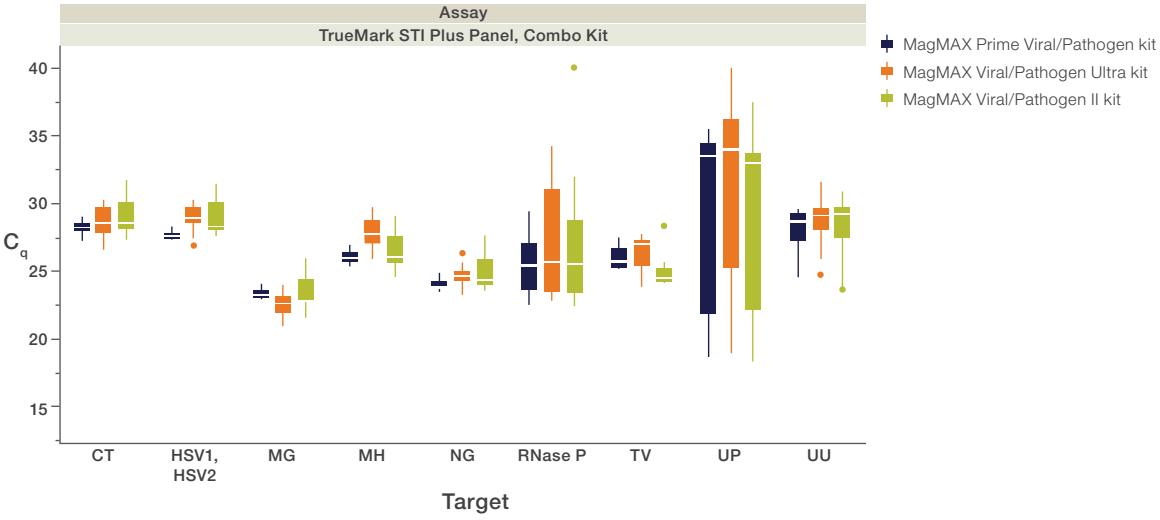

Figure 2. C_q values determined for targets of the TrueMark STI Plus Panel, Combo Kit, from contrived samples processed with the MagMAX Prime Viral/Pathogen, MagMAX Viral/Pathogen Ultra, and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kits.

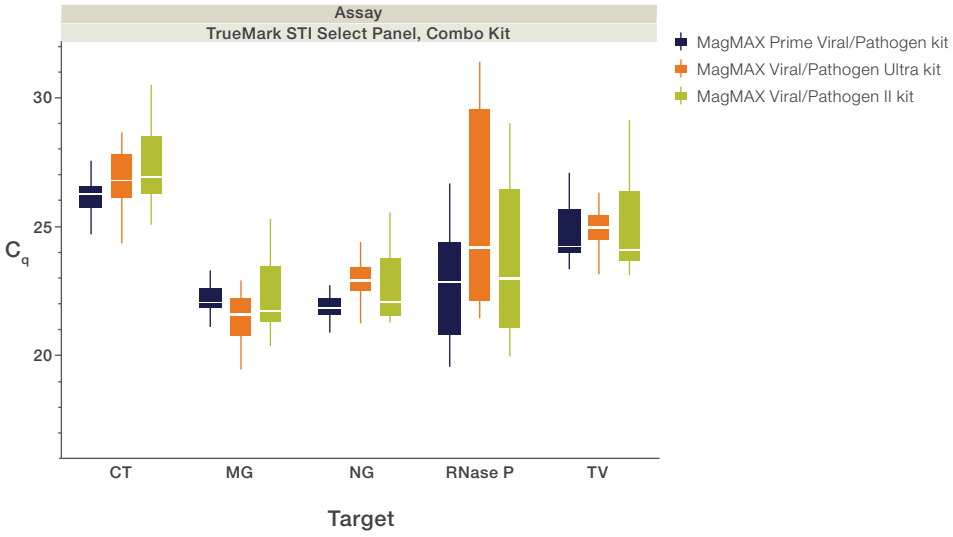

Figure 3. C_q values determined for targets of the TrueMark STI Select Panel, Combo Kit, from contrived samples processed with the MagMAX Prime Viral/Pathogen, MagMAX Viral/Pathogen Ultra, and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kits.

Collection media were evaluated for performance across the MagMAX and TrueMark STI Plus Panel, Combo Kit chemistries. Figure 4 details trends in C_q values that indicated the best performance with Aptima and Stuart media samples from all four healthy donors. Although the Amies medium performed similarly across the MagMAX kits, delayed amplification was observed with the Amies medium when compared to the Aptima and Stuart media across four donors. The variation between the collection media devices is most notable with RNase P targets. Donor-to-donor variation is expected to occur across all samples and can be seen with variation in C_q values per target.

A similar trend is observed with the TrueMark STI Select Panel, Combo Kit. Figure 5 details the same observed variation in RNase P target between Amies, Aptima, and Stuart media, with Amies medium indicating a potential lag in amplification.

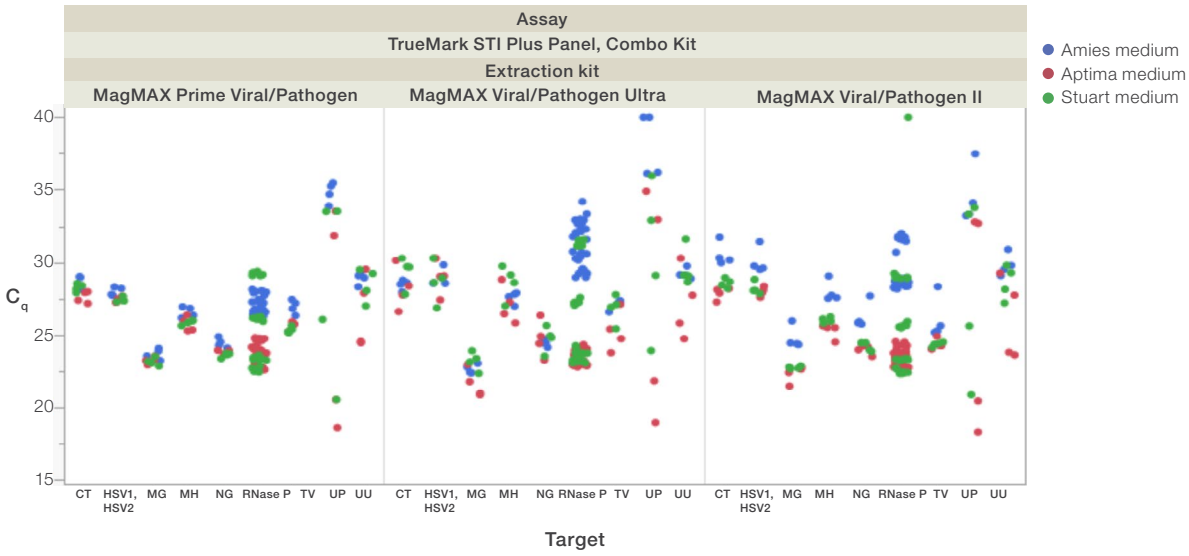

Figure 4. C_q values determined with the TrueMark STI Plus Panel, Combo Kit, from contrived samples stored in Amies, Aptima, and Stuart devices and processed with the MagMAX Prime Viral/Pathogen, MagMAX Viral/Pathogen Ultra, and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kits.

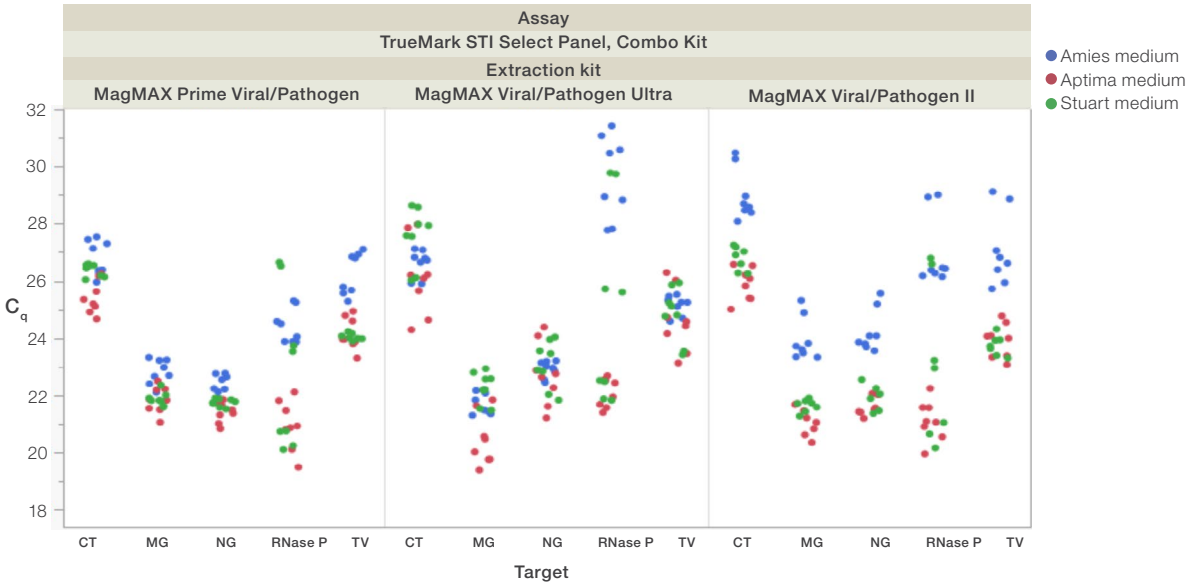

Figure 5. C_q values determined with the TrueMark STI Select Panel, Combo Kit, from contrived samples stored in Amies, Aptima, and Stuart devices and processed with the MagMAX Prime Viral/Pathogen, MagMAX Viral/Pathogen Ultra, and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kits. Duplicate PCR reactions were performed from sample eluates.

C_q values for TaqMan Assays indicated <0.5 cycle variation between contrived vaginal swabs and urine samples across HSV1, *M. hominis*, and *T. vaginalis* (Table 3), which suggests that the MagMAX Prime Viral/Pathogen kit is successfully able to process both sample types from the contrived pools analyzed in this study. Furthermore, the TrueMark STI Plus Panel showed amplification similar to or better than that of the TaqMan Assays for urine, indicating the feasibility of using the MagMAX Prime Viral/Pathogen kit with various downstream assays. The ability of the MagMAX Prime Viral/Pathogen kit to extract nucleic acid from both 200 µL and 400 µL sample inputs on one plate is demonstrated by the similar C_q values across both TaqMan and TrueMark assay chemistries for HSV1, *M. hominis*, and *T. vaginalis*.

Table 3. C_q values from TaqMan Assays and the TrueMark STI Plus Panel using the basic workflow of the MagMAX Prime Viral/Pathogen kit on 200 µL and 400 µL sample inputs with urine and vaginal swabs.

Target		HSV1		*M. hominis*		*T. vaginalis*	
Extraction input volume with the MagMAX Prime Viral/Pathogen kit		200 µL	400 µL	200 µL	400 µL	200 µL	400 µL
TaqMan Assays	Urine	19.9	19.8	21.4	21.8	29.4	29.2
	Vaginal swabs	19.8	19.6	21.5	21.5	28.6	28.9
TrueMark STI Plus Panel	Urine	18.0	18.1	21.9	21.9	28.1	28.1

Conclusions

Thermo Fisher Scientific offers convenient and flexible solutions for broad profiling or targeted research testing of STIs with a choice of MagMAX kits on KingFisher instruments. We demonstrated that the MagMAX Prime Viral/Pathogen, MagMAX Viral/Pathogen Ultra, and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kits are suitable for research on STIs using the TrueMark STI Plus Panel and TrueMark STI Select Panel Combo Kits across different sample types and collection media, with a variety of workflow options available.

Ordering information

Description	Cat. No.
MagMAX Prime Viral/Pathogen Nucleic Acid Isolation Kit	A58145
MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit	A42356
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	A48383R
KingFisher Flex Purification System, KingFisher with 96 Deep-Well Head	5400640
KingFisher Apex Purification System with 96 Deep-Well head	5400930
TrueMark STI Plus Panel, Combo Kit	A56291C
TrueMark STI Select Panel, Combo Kit	A57083

Authors

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References

1. Centers for Disease Control and Prevention. Bacterial vaginosis (BV) statistics. **cdc.gov/std/bv/stats.htm**
2. Centers for Disease Control and Prevention. Diseases characterized by urethritis and cervicitis. **cdc.gov/std/treatment-guidelines/urethritis-and-cervicitis.htm**
3. Thermo Fisher Scientific. TrueMark STI Select Panel: Real-time PCR for research in the detection of sexually transmitted infections. **assets.thermofisher.com/TFS-Assets/GSD/Flyers/global-truemark-sti-select-panel-flyer.pdf**
4. Thermo Fisher Scientific. A complete workflow solution for detecting sexually transmitted infections and vaginal microbiota using TrueMark STI and Vaginal Plus Panels. **assets.thermofisher.com/TFS-Assets/GSD/Technical-Notes/gts-sti-vaginal-plus-tech-note.pdf**

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