Clinical STI testing research

# Evaluation and characterization of microorganisms that cause sexually transmitted infections

## Introduction

Each year, over one billion women are affected worldwide by urogenital infections according to the Centers for Disease Prevention and Control [1]. Sexually transmitted infections (STIs) like trichomoniasis, chlamydia, and urethritis caused by *Mycoplasma genitalium* can occur in both men and women and can be spread through sexual interaction [2]. Molecular biological techniques for the study of organisms causing STIs and vaginal infection can lead to faster turnaround times for results when compared to culturing techniques.

Accessible evaluation and characterization of microorganisms that cause STIs and vaginal disease are needed to support research in syndromic effects for individuals at high risk of contracting STIs.

## Background

Nucleic acid isolation on Thermo Scientific™ KingFisher™ purification instruments using Applied Biosystems™ MagMAX™ bead–based technologies provides semi-automated solutions to increase versatility in workflow, and reduce time and operator burden. The Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit and Applied Biosystems™ MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit are equally suitable kits to recover both RNA and DNA from viral particles and gram-negative bacteria from samples such as blood, swabs, urine, and transport media, utilized for various research workflow solutions. In addition, the MagMAX Viral/Pathogen Ultra kit includes a unique enzyme mix to enable lysis and nucleic acid recovery from difficult-to-lyse pathogens such as gram-positive bacteria, yeast, and fungi. A flexible, scalable, and reliable solution, from sample preparation to downstream RT-PCR application, allows clinical research labs to further understand microorganism characterization associated with sexually transmitted disease applications in various sample types.

Here, we evaluate the performance of three collection and storage matrices with various pathogen targets across two different MagMAX extraction chemistries and downstream applications to accurately detect a wide variety of sexually transmitted pathogens. To assess the extracted nucleic acid, RT-PCR was performed using the Applied Biosystems™ TrueMark™ STI Plus Panel and the Applied Biosystems™ TrueMark™ STI Select Panel, Combo Kits. Figure 1 illustrates the general workflow evaluated in this study showcasing the versatility associated with common STI sample types, targets, and research assays.

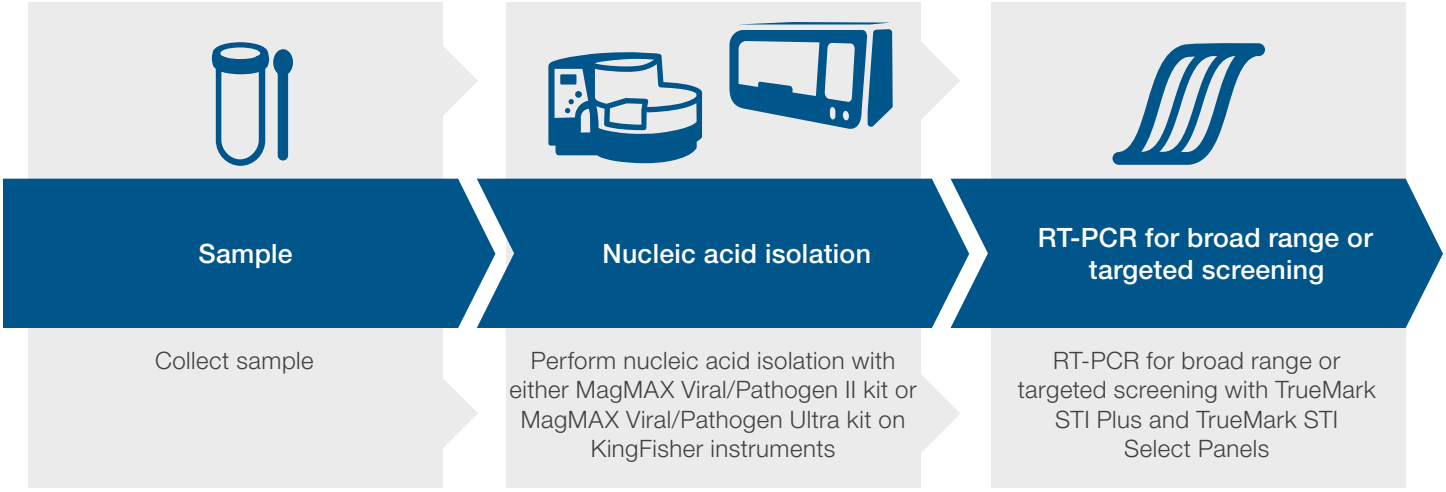


**Figure 1. Workflow options for nucleic acid isolation followed by RT-PCR.**

## Materials and methods

Vaginal swabs from four healthy donors were collected and stored in Aptima™ Multitest Swab transport media, Amies transport media, and Stuart transport media. Collected samples were contrived with eight targets, as listed in Table 1, and extracted in triplicate using both MagMAX Viral/Pathogen II kit 200 µL viral transport media (VTM) (MAN0024756) and MagMAX Viral/Pathogen Ultra kit 200 µL VTM (MAN0018074) workflows on Thermo Scientific™ KingFisher™ Flex instruments following the scripts recommended in their respective workflow. The eluates from each triplicate (across donor, media type, and extraction chemistry) were pooled and mixed. The eluate pools were tested for the targets listed within Table 1 using both the TrueMark STI Plus Panel, Combo Kit (MAN0026671) and the TrueMark STI Select Panel, Combo Kit (MAN0028493). Amplification of various targets across two separate extraction workflows and three different collection devices, or media, were analyzed by cycle threshold values ($C_q$). Both PCR assays utilized RNase P as the internal sample control.

**Table 1. Experimental parameters—media, extraction chemistries, and TrueMark STI panels used within this study—and the quantity of target added into each extraction are listed.**

| Collection device | Extraction kit | TrueMark STI panel | Target | Abbreviation | Quantity added into each extraction | Unit of measure | No. of samples |
|---|---|---|---|---|---|---|---|
| Aptima media | MagMAX Viral/ Pathogen Ultra | TrueMark STI Plus Panel* | *Chlamydia trachmatis* | CT | $2.73 \times 10^7$ | IFU | 48 |
| Amies media | MagMAX Viral/ Pathogen II | | *Neisseria gonorrhoeae* | NG | $2.04 \times 10^7$ | CFU | |
| Stuart media | | | *Mycoplasma genitalium* | MG | $2.50 \times 10^4$ | Bacterial cell | |
| | | | *Trichomonas vaginalis* | TV | $3.40 \times 10^6$ | Cell | |
| | | | *Ureaplasma urealyticum* | UU | $5.00 \times 10^3$ | CCU | |
| | | | *Mycoplasma hominis* | MH | $1.50 \times 10^5$ | CFU | |
| | | | Herpes simplex virus 1 | HSV1 | $1.44 \times 10^7$ | $TCID_{50}$ | |
| | | | Herpes simplex virus 2 | HSV2 | $2.00 \times 10^7$ | $TCID_{50}$ | |
| | | TrueMark STI Select Panel | *Chlamydia trachmatis* | CT | $2.73 \times 10^7$ | IFU | 24 |
| | | | *Neisseria gonorrhoeae* | NG | $2.04 \times 10^7$ | CFU | |
| | | | *Trichomonas vaginalis* | TV | $3.40 \times 10^6$ | Cell | |
| | | | *Mycoplasma genitalium* | MG | $2.50 \times 10^4$ | Bacterial cell | |

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

## Results and discussion

MagMAX Viral/Pathogen Ultra and MagMAX Viral/Pathogen II extraction 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 devices for both MagMAX Viral/Pathogen Ultra and MagMAX Viral/Pathogen II extraction workflows. $C_q$ values shown for each of the targets are derived from all four donors and three collection devices for a total of 12 individual data points per target extracted by each chemistry. The trends between the two extraction kits are similar and overlap in range of $C_q$ values. Similarly, Figure 3 details the same trends with the TrueMark STI Select Panel, Combo Kit across the five targets evaluated.

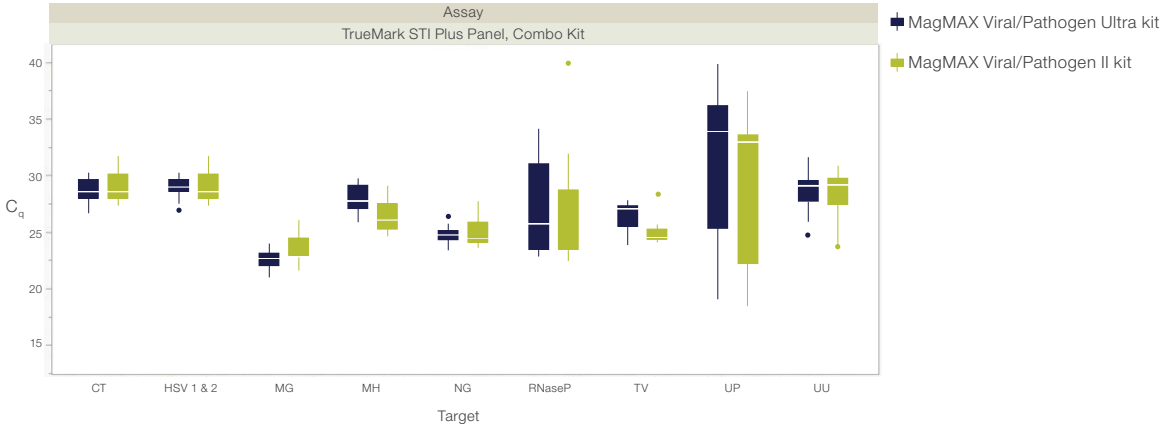


**Figure 2. $C_q$ values determined with TrueMark STI Plus Panel, Combo Kit from contrived samples extracted with MagMAX Viral/Pathogen Ultra and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kits.**

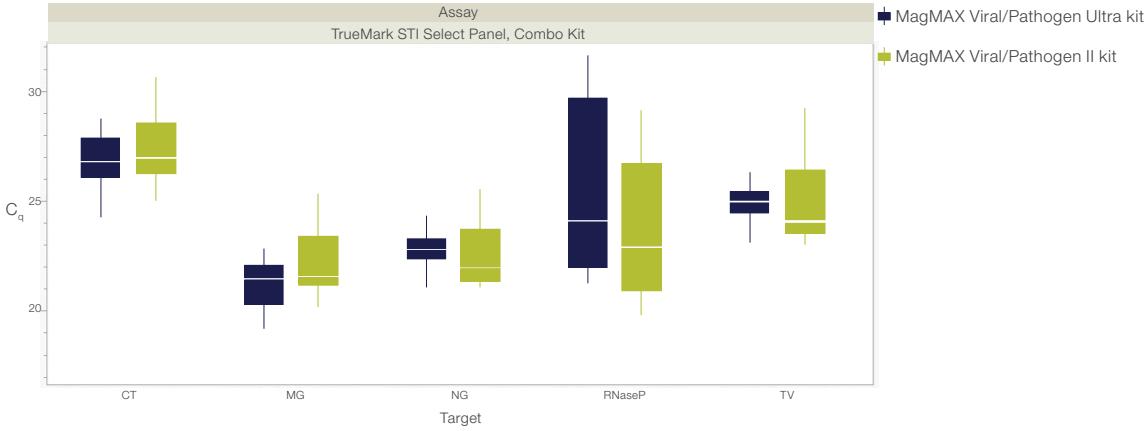


**Figure 3. $C_q$ values determined with TrueMark STI Select Panel, Combo Kit from contrived samples extracted with MagMAX Viral/Pathogen Ultra and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kits.**

Performance of collection devices were evaluated across the MagMAX and TrueMark STI Plus Panel, Combo Kit chemistries. Figure 4 details trends in $C_q$ values that indicated best performance with Aptima and Stuart devices across backgrounds from all four healthy donors. Although Amies device performed equivalently across both MagMAX Viral/Pathogen Ultra and MagMAX Viral/Pathogen II kits, delayed amplification was observed with the Amies device when compared to the Aptima and Stuart devices across four donors. The variation between devices is most notable with RNaseP 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 RNaseP target between Amies, Aptima, and Stuart collection devices, with Amies device indicating a potential lag in amplification.

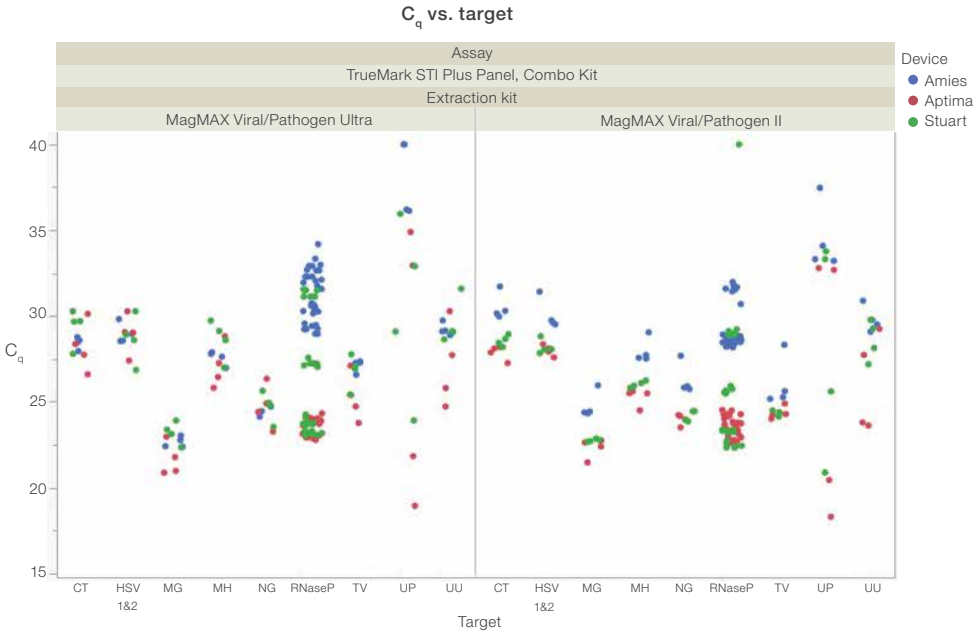


**Figure 4. $C_q$ values determined with TrueMark STI Plus Panel, Combo Kit from contrived samples stored in Amies, Aptima, and Stuart devices and extracted with MagMAX Viral/Pathogen Ultra and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kits.**

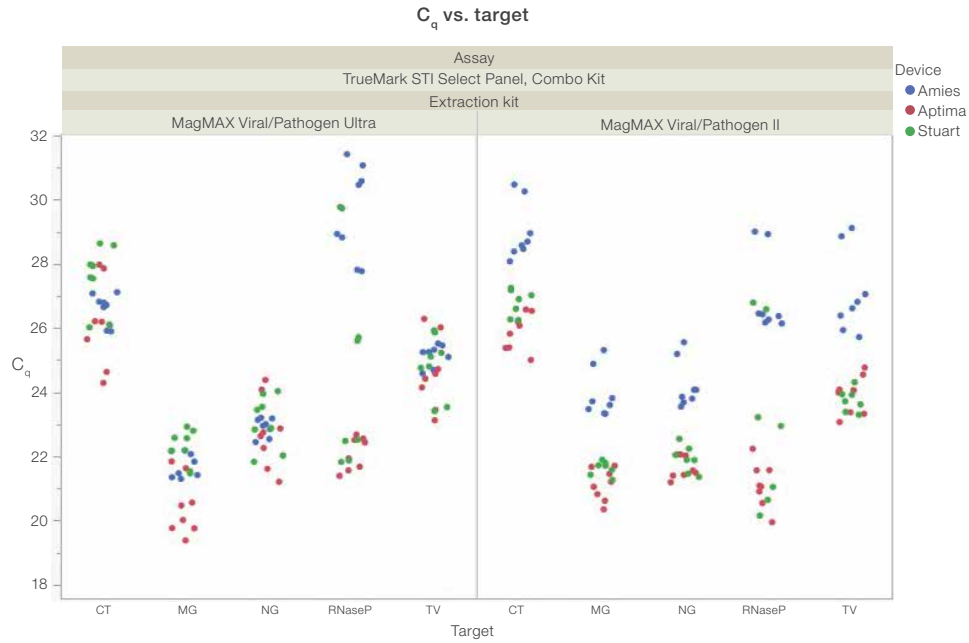


**Figure 5. $C_q$ values determined with TrueMark STI Select Panel, Combo Kit from contrived samples stored in Amies, Aptima, and Stuart devices and extracted with MagMAX Viral/Pathogen Ultra and MagMAX Viral/Pathogen II Nucleic Acid Isolation Kits.** Duplicate PCR reactions were performed from sample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

## Conclusions

Thermo Fisher Scientific offers convenient and flexible solutions for broad profiling or targeted research testing of STIs with Thermo Scientific options of MagMAX chemistry solutions on KingFisher instruments. We demonstrated that both MagMAX Viral/Pathogen II and MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kits are suitable for research of STIs using TrueMark STI Plus Panel and TrueMark STI Select Panel, Combo Kits across multiple sample collection devices.

## Ordering information

| Description | Cat. No. |
|---|---|
| MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit | A48383R |
| MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit | A42356 |
| TrueMark STI Plus Panel, Combo Kit | A56291C |
| TrueMark STI Select Panel, Combo Kit | A57083 |


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[1]Thermo Fisher Scientific, Austin, TX; [2]Thermo Fisher Scientific, San Francisco, CA


### References


1. CDC: Bacterial Vaginosis Statistics
2. CDC: Sexually Transmitted Diseases (STDs)


Learn more at **thermofisher.com/magmaxviralpathogen**