Clinical Performance Evaluation of a Multiplex RT-PCR Assay for Detection of Pathogens Associated with Sexually Transmitted Infections

HEALTHTRACKRx

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INTRODUCTION

Sexually transmitted infections (STIs) are a substantial public healthcare burden not only in the U.S. but also worldwide. Globally, more than 1 million STIs are acquired every day[1]. According to CDC, 1 in 5 individuals suffer from STI, incurring more than 16 billion USD in healthcare costs[2]. In addition, according to recent surveillance data by CDC, more than 2.5 million cases of chlamydia, gonorrhea and syphilis were reported in 2021[3]. While STIs are among the most common infections, their clinical identification is crucial because STIs are often asymptomatic. In addition, in symptomatic patients, due to overlapping clinical presentations, accurate diagnostic testing becomes paramount. Therefore, early detection with a rapid turn-around time is essential to control transmission, improve patient care, and outcomes. Multiplex PCR is an advanced molecular biology technique which allows for simultaneous detection of multiple pathogens in the same sample. In this study, we evaluated the clinical performance of two different multiplex PCR-based platforms: an STI laboratory-developed test (LDT) that is based on a TaqMan® real time OpenArray® PCR platform and the TrueMark™ STI Select Panel Combo Kit for the simultaneous detection of *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG), *Mycoplasma genitalium* (MG), *Trichomonas vaginalis* (TV).

MATERIALS & METHODS

This retrospective study was performed using 100 urine and vaginal swabs (55 urine and 45 vaginal swabs total) submitted for STI testing at HealthTrackRx (n=20, positive for each pathogen). In addition, 20 specimens that were negative for CT, NG, MG, and TV were tested as well.

Testing was performed at HealthTrackRx laboratory located in Denton, TX. Sample extraction was performed using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation kit (ThermoFisher, California, USA). Thereafter, all samples were tested in parallel using the TaqMan® real time OpenArray® PCR platform (Thermofisher Scientific, San Francisco, USA) and the TrueMark™ STI Select Panel Combo Kit (Thermofisher Scientific, San Francisco, USA) for detection of CT, NG, MG, TV as per manufacturer's instructions.

Results were analyzed for concordance between the platforms as well as for the detection of the four pathogens as well the internal control (RNAse P). All statistical analyses were performed using R version 3.6.0.

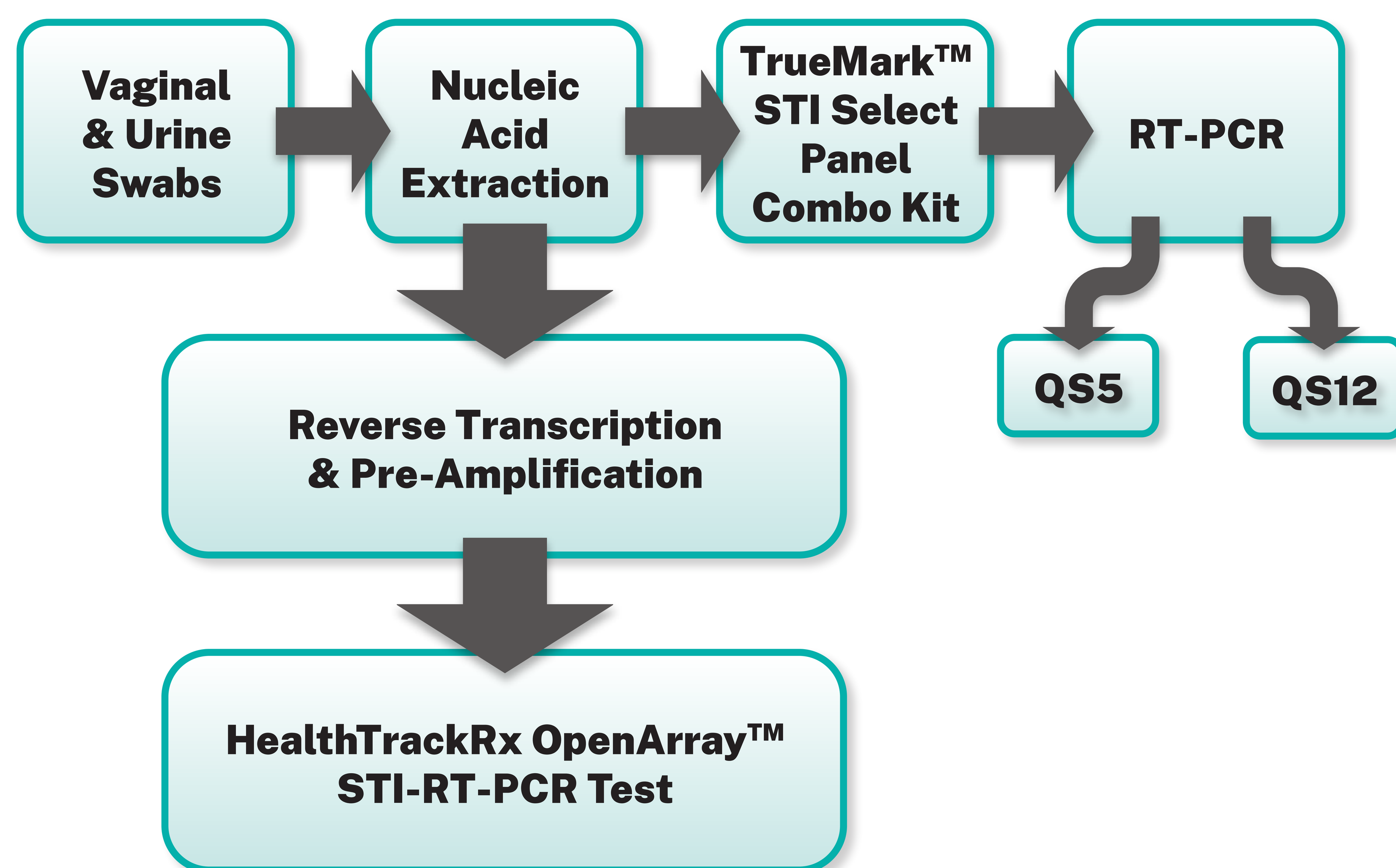

Figure 1: Schematic of the Study design

RESULTS

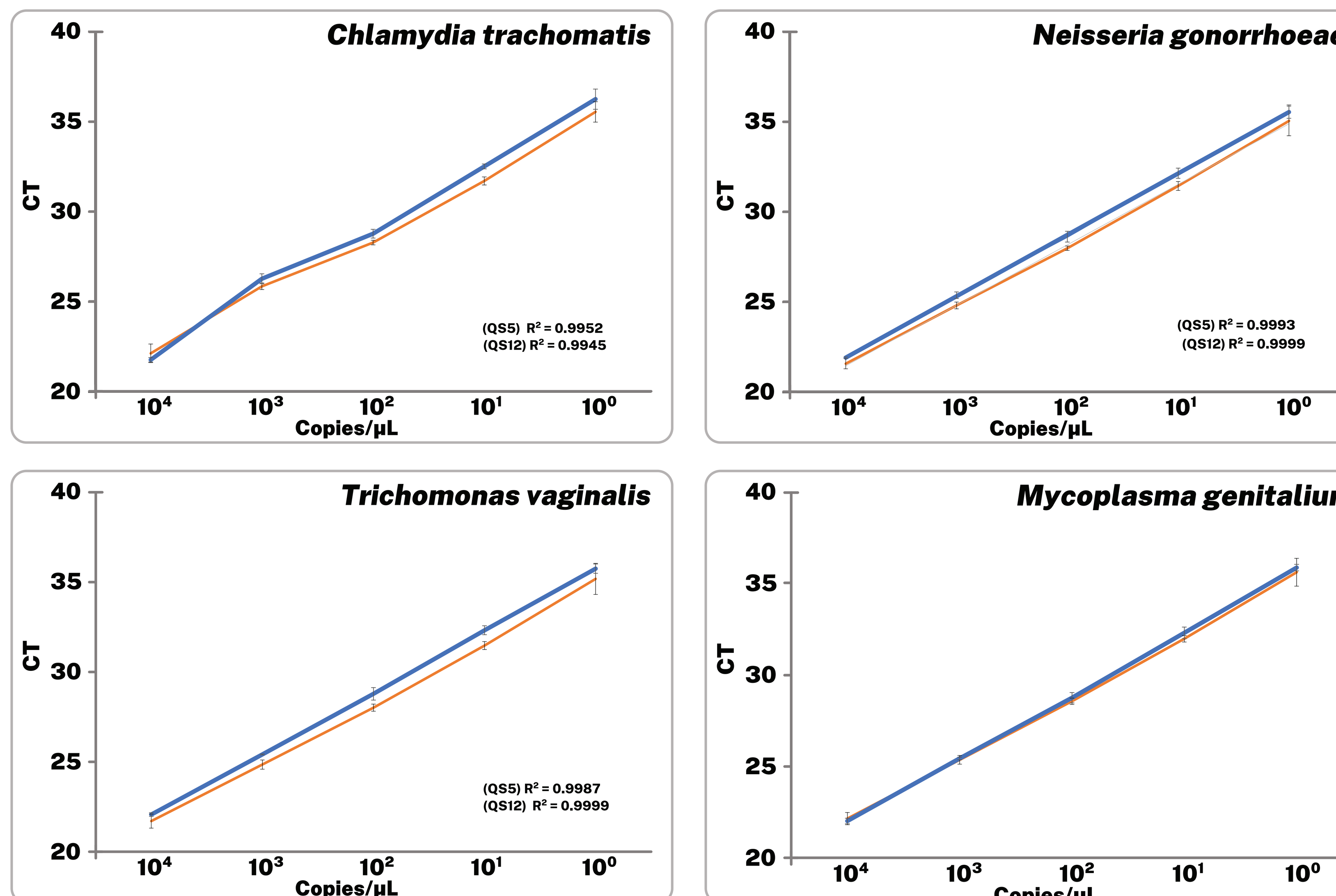

Figure 1: Analytical sensitivity (Limit of detection, LoD) of the multiplex STI assay. RT-PCR was performed using 10-fold serial dilution (10^4-10^0 copies/µl) of positive control nucleic acid provided by the manufacturer. LoD estimation was performed on both QuantStudio™ 5 and QuantStudio™ 12K flex Systems. Cycle Threshold (CT) values presented are averages (n = 20). Error bars represent standard deviation.

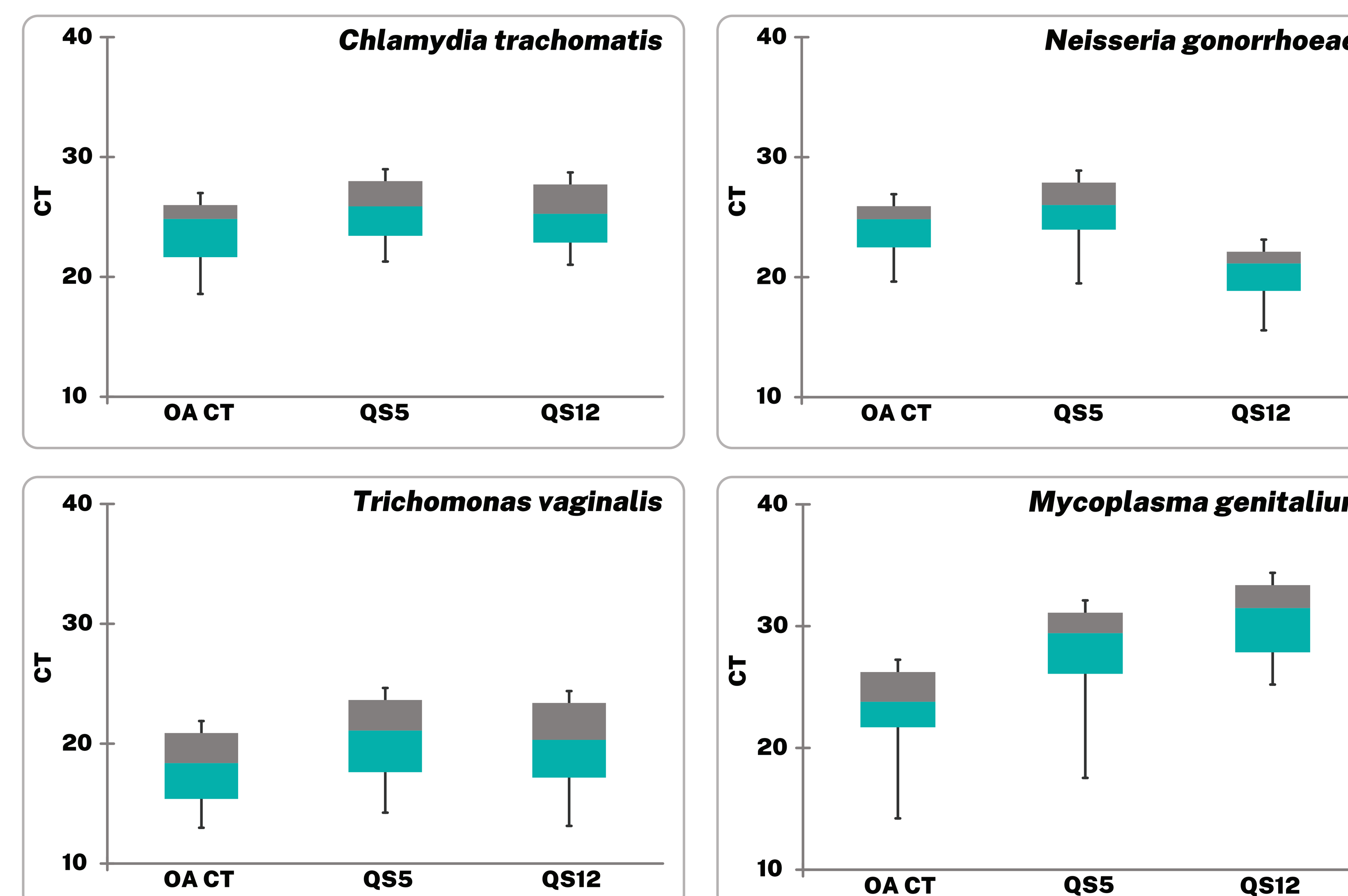

Figure 2: CT value distribution obtained from positive patient samples (n=20 for each pathogen). No significant difference in CT values was observed across the three real time-PCR systems tested (OA = OpenArray™, QS = QuantStudio™) standard deviation.

Organisms z	Samples	Result for CT, NG, TV & MG
Peptostreptococcus anaerobius	18	Negative
Eschericia coli	14	Negative
Bacterial vaginosis associated bacteria (BVAB)	7	Negative
Ureaplasma urealyticum, parvum	5	Negative
Streptococcus agalactiae (Group B Strep.)	4	Negative
Gardnerella vaginalis	2	Negative
Atopobium vaginae	2	Negative
Bacteroides fragilis	2	Negative
Enterococcus faecalis, faecium	2	Negative
Human papillomavirus (HPV)	1	Negative
Psuedomonas aeruginosa	1	Negative
Staphylococcus aureus	1	Negative
Treponema pallidum	1	Negative
Klebsiella pneumoniae, oxytoca	1	Negative
Enterobacter aerogenes, cloacae	1	Negative
Candida sp.	1	Negative
Candida glabrata	1	Negative

Table 1: Cross reactivity. The new STI assay demonstrated no cross-reactivity in patient samples positive for the viral and bacterial pathogen commonly detected in vaginal and urine swab samples.

CONCLUSIONS

The new STI multiplex assay developed at HealthTrackRx is compatible to be used on both systems (QS5 and QS12, 96 well format) which is demonstrated by the analytical sensitivity test and cross-reactive test results.

Good linear correlation and high sensitivity (100.1 copies/µl) was observed for all targets on both PCR platforms (Fig. 2)

Overall, the positive cohort for all the four tested pathogens spanned a wide CT range (14.2 to 33.3) with an average CT value of 25.1. Results obtained from QuantStudio™ 5 System and QuantStudio™ 12 Flex System showed 100% agreement for positive and negative samples.

For each test, no cross-reactivity with 17 other common micro-organisms was observed *(Table 1)*.

While expanded panels are recommended for in-patient settings for STI detection and associated coinfections. The TrueMark™ STI Select Panel Combo Kit was used to create LDT for the detection of the common STI pathogens in both urine and vaginal swab samples. Using targeted multiplex PCR panels for the detection of common STI pathogens can help expedite routine screening and contribute to better patient outcomes and overall public health.

REFERENCES

1. https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis) (last accessed 8-22-2023)
2. https://www.cdc.gov/std/statistics/prevalence-incidence-cost-2020.htm (last accessed 8-22-2023)
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