BRIEF REPORT

Comparison between DiaPlexQ™ STI6 and GeneFinder™ STD I/STD II multiplex Real-time PCR Kits in the detection of six sexually transmitted disease pathogens

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Background: The DiaPlexQ™ STI6 Detection Kit (DiaPlexQ; Solgent Co., Ltd., Daejeon, South Korea) is a multiplex real-time PCR assay for the detection of the following sexually transmitted disease (STD) pathogens: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma hominis*, *Trichomonas vaginalis*, *Ureaplasma urealyticum*, and *Mycoplasma genitalium*. We compared the performance of the DiaPlexQ assay with the GeneFinder™ STD I (CT/NG/UU) and STD II (MG/MH/TV) Multiplex Real-time PCR Kits (GeneFinder; Infopia Co., Ltd., Anyang, South Korea).

Methods: We evaluated the performance of the DiaPlexQ assay in comparison to that of GeneFinder using 1106 clinical specimens (542 genital swabs and 564 urine samples). The analytical performance of the DiaPlexQ assay, including the limit of detection (LOD) and analytical specificity, was evaluated using reference strains.

Results: The positive percent agreement, negative percent agreement, and kappa value between the two assays were 96.6%-99.4%, 98.2%-99.8%, and 0.93%-0.99%, respectively. No cross-reactivity was observed in a collection of 41 different microorganisms and the LOD of the DiaPlexQ assay ranged from 1 to 10 copies/reaction for each microorganism.

Conclusion: The DiaPlexQ assay showed comparable performance to that of the GeneFinder assay so that it can be used for the screening and diagnosis of non-viral curable STD pathogens.

KEYWORDS

multiplex real-time PCR, sexually transmitted disease, validation

1 | INTRODUCTION

Sexually transmitted diseases (STDs) impose substantial strain on the budgets of both households and national health systems, and have an adverse effect on the overall well-being of individuals. The World Health Organization (WHO) estimated that there are 357 million new cases of gonorrhea, chlamydia, syphilis, and trichomoniasis annually among people aged 15-49 years.¹ Several methods are

available for detecting non-viral curable STD pathogens, including culture, microscopic identification, antigen detection testing, and nucleic acid amplification tests (NAATs).²⁻⁴ NAAT is a highly sensitive method with excellent specificity, and therefore it is the preferred method for detecting STD pathogens. Moreover, multiplex molecular assays have an additional advantage in screening and diagnosis since they enable simultaneous diagnosis of several sexually transmitted infections (STIs). Several commercial multiplex PCR kits have been developed, and a few studies have evaluated their performance.⁵⁻¹³

Huh and Ki contributed equally to this article.

Recently, the DiaPlexQ™ STI6 Detection Kit (DiaPlexQ; Solgent Co., Ltd., Daejeon, South Korea), a European Conformity-In Vitro Diagnostic marked multiplex real-time PCR assay, was developed and received Korean Ministry of Food and Drug Safety (MFDS) approval. The assay comprises two reaction tubes and targets six STD pathogens: set 1 includes *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Mycoplasma hominis* (MH); set 2 includes *Trichomonas vaginalis* (TV), *Ureaplasma urealyticum* (UU), and *Mycoplasma genitalium* (MG).

The purpose of this study was to evaluate the analytical performance of the DiaPlexQ assay and compare the results with those of a comparative method, the GeneFinder™ STD I (CT/NG/UU) and STD II (MG/MH/TV) Multiplex Real-time PCR Kit (GeneFinder; Infopia Co., Ltd., Anyang, South Korea).

2 | MATERIALS AND METHODS

This retrospective study was approved by the Institutional Review Board of Samsung Medical Center. A total of 1100 patients who were suspected of having an STI and who visited gynecology or urology hospitals were enrolled. A total of 1106 clinical specimens were obtained from a total of 542 genital swabs (531 vaginal swabs and 11 urethral swabs) and 564 urine samples collected from Feb. to Nov. 2016. Patient ages ranged from 13 to 90 years old. DNA was extracted using a QIAamp DNA Mini Kit (Qiagen cat#51304; Qiagen, Hilden, Germany) according to the manufacturer's instructions.

For the DiaPlexQ assay, extracted DNA (5 µL) was added to a tube containing 15 µL of PCR premix (10 µL of 2X Multiplex Real-Time PCR Smart mix and 5 µL of Primer & Probe Mixture). PCR was conducted using the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The mixture was incubated at 50°C for 3 minutes to activate the uracil-DNA glycosylase (UDG) system, followed by pre-denaturation at 95°C for 15 minutes and 45 cycles of PCR (20 seconds at 95°C and 40 seconds at 60°C). A positive test result was defined as a cycle threshold (Ct) ≤43 for individual targets.

The procedures for the GeneFinder assay were performed according to the manufacturer's instructions. The GeneFinder assay is a Korean MFDS-approved multiplex real-time assay for the detection of STD pathogens. The GeneFinder assay targets six STD pathogens: STD I detects CT, NG, and UU; STD II detects MG, MH, and TV. PCR was performed in a total volume of 20 µL (10 µL PCR reaction mixture, 5 µL probe mixture, and 5 µL DNA) using the 7500 Fast Real-Time PCR System. A positive test result was defined as a cycle threshold (Ct) ≤40 for individual targets.

Comparison of diagnostic performance between the DiaPlexQ and GeneFinder assays was assessed by measuring positive/negative percent agreement values and kappa coefficients using the VassarStats website (<https://vassarstats.net/>). Specimens with discordant results between the DiaPlexQ assay and the GeneFinder assay were further confirmed by monoplex PCR and sequencing. The PCR protocols and primers used are listed in Table S1. After

detection of PCR products by gel electrophoresis and purification, sequencing was performed on an automated ABI Prism 3730 instrument using a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Positive and negative controls were included in each run.

Analytical sensitivity was determined by directly comparing dilutions of six type strains in cell culture and in the DiaPlex assay. Serial dilutions of quantified target organism were made from 10⁰ to 10⁴ copies/reaction by spiking negative genital swabs/urine samples. Serial dilutions were analyzed with 24 replicates per dilution. The lower detection limit was determined via Probit analysis. Analytical specificity of the DiaPlexQ assay was evaluated using 41 different microorganisms (Table 1).

3 | RESULTS

At least one microorganism was detected in each of 877 specimens (433 genital swabs and 444 urine samples) by at least one of the two assays (the DiaPlexQ or the GeneFinder assay) after discrepancy resolution. A total of 643 specimens were positive for a single microorganism, while 234 specimens were positive for multiple microorganisms (184 with two microorganisms, and 50 with ≥3 microorganisms).

A total of 885 (80.0%) and 884 (80.0%) samples were DiaPlexQ- and GeneFinder-positive, respectively. The positive percent agreement between the DiaPlexQ and GeneFinder assays ranged from 96.6% to 99.4% (Table 2). The negative percent agreement for the two assays ranged from 98.2% to 99.8%. The kappa value range was 0.93-0.99. When stratified by specimen type, the range of kappa values was 0.93-1.00 in genital swabs and 0.93-0.99 in urine samples.

A total of 6.8% (75/1106) samples yielded discrepant results. Among those, three samples had two discordant targets; thus, 78 discrepancies were produced. Fifty-nine results were DiaPlexQ-positive and GeneFinder-negative. As shown in Table 3, two were confirmed to be positive on monoplex PCR and sequencing. Likewise, among 19 DiaPlexQ-negative and GeneFinder-positive results, one was confirmed to be positive by the monoplex PCR and sequencing.

The detection limits of the DiaPlexQ assay for CT and NG were one copy/reaction, whereas those for the other microorganisms (MH, TV, UU, and MG) were 10 copies/reaction. On the analytical specificity test, no false-positive results were obtained for any of the 41 microorganisms (Table 1).

4 | DISCUSSION

Recent advances in molecular testing for the detection of STD pathogens have led to changes in the diagnosis of STIs. NAATs have increasingly replaced conventional diagnostics due to their superior sensitivity and speed.^{2,4,5,14} Variable molecular technologies for the detection of STD pathogens from clinical specimens

TABLE 1 Analytic specificity of the DiaPlexQ™ STI6 Detection Kit

No.	Microbial species	Strain	Result	
			Set 1	Set 2
1	<i>Gardnerella vaginalis</i>	ATCC-49145D-5	Negative	Negative
2	<i>Treponema pallidum</i>	ATCC-BAA-2642SD	Negative	Negative
3	<i>Haemophilus ducreyi</i>	ATCC-51620	Negative	Negative
4	<i>Candida albicans</i>	KCTC-7678	Negative	Negative
5	<i>Ureaplasma parvum</i>	ATCC-27815	Negative	Negative
6	<i>Neisseria meningitidis</i>	ATCC-700532DQ	Negative	Negative
7	<i>Proteus mirabilis</i>	KCTC-2510	Negative	Negative
8	<i>Corynebacterium accolens</i>	KCTC-3431	Negative	Negative
9	<i>Corynebacterium ammoniagenes</i>	KCTC-1838	Negative	Negative
10	<i>Corynebacterium diphtheriae</i>	KCTC-3075	Negative	Negative
11	<i>Corynebacterium glutamicum</i>	KCTC-3017	Negative	Negative
12	<i>Enterobacter aerogenes</i>	KCTC-2190	Negative	Negative
13	<i>Enterobacter cloacae</i>	KCTC-2361	Negative	Negative
14	<i>Enterococcus faecalis</i>	KCTC-3206	Negative	Negative
15	<i>Klebsiella oxytoca</i>	KCTC-1686	Negative	Negative
16	<i>Klebsiella planticola</i>	KCTC-1687	Negative	Negative
17	<i>Klebsiella pneumoniae</i>	KCTC-2242	Negative	Negative
18	<i>Pseudomonas aeruginosa</i>	KCTC-2004	Negative	Negative
19	<i>Sphingomonas paucimobilis</i>	ATCC-29837	Negative	Negative
20	<i>Staphylococcus aureus</i>	ATCC-49444	Negative	Negative
21	<i>Streptococcus alactolyticus</i>	KCTC-3644	Negative	Negative
22	<i>Streptococcus criceti</i>	KCTC-3292	Negative	Negative
23	<i>Streptococcus downei</i>	KCTC-3634	Negative	Negative
24	<i>Streptococcus ferus</i>	KCTC-3637	Negative	Negative
25	<i>Streptococcus gallinaceus</i>	KCTC-3876	Negative	Negative
26	<i>Streptococcus hyointestinalis</i>	KCTC-3660	Negative	Negative
27	<i>Streptococcus intermedius</i>	KCTC-3268	Negative	Negative
28	<i>Streptococcus lutetiensis</i>	KCTC-3877	Negative	Negative
29	<i>Streptococcus oralis</i>	KCTC-13048	Negative	Negative
30	<i>Streptococcus parasanguinis</i>	KCTC-13046	Negative	Negative
31	<i>Streptococcus vestibularis</i>	KCTC-3650	Negative	Negative
32	<i>Streptococcus anginosus</i>	KCTC-3983	Negative	Negative
33	<i>Streptococcus macacae</i>	KCTC-3659	Negative	Negative
34	<i>Streptococcus mitis</i>	KCTC-5650	Negative	Negative
35	<i>Streptococcus parauberis</i>	KCTC-3651	Negative	Negative
36	<i>Streptococcus pneumoniae</i>	KCCM-40410	Negative	Negative
37	<i>Streptococcus pyogenes</i>	KCCM-11817	Negative	Negative
38	<i>Streptococcus suis</i>	KCTC-3557	Negative	Negative
39	<i>Escherichia coli</i>	KCTC-2593	Negative	Negative
40	Herpes simplex virus 1	ATCC-VR-260D	Negative	Negative
41	Herpes simplex virus 2	ATCC-VR-734D	Negative	Negative

ATCC, American type culture collection; KCCM, Korean culture center of microorganisms; KCTC, Korean collection for type cultures.

TABLE 2 Comparison of the DiaPlexQ™ STI6 Detection Kit and the GeneFinder™ STD I (CT/NG/UU) and STD II (MG/MH/TV) Multiplex Real-time PCR Kit for the detection of STD pathogens

Virus	Specimen type	Percent agreement						Kappa value	
		Positive			Negative			Observed kappa	95% CI
		%		95% CI	%		95% CI		
<i>Chlamydia trachomatis</i>	Total	98.7	(234/237)	96.0-99.7	99.5	(865/869)	98.7-99.9	0.98	0.96-0.99
	Genital swab	98.3	(117/119)	93.5-99.7	99.5	(419/423)	98.1-99.9	0.97	0.94-0.99
	Urine	99.2	(117/118)	94.7-99.9	100	(446/446)	98.9-100	0.99	0.98-1.00
<i>Neisseria gonorrhoeae</i>	Total	99.4	(162/163)	96.1-99.9	99.0	(934/943)	98.1-99.5	0.96	0.94-0.99
	Genital swab	100	(75/75)	93.9-100	99.1	(463/467)	97.7-99.7	0.97	0.94-0.99
	Urine	98.9	(87/88)	92.9-99.9	98.9	(471/476)	97.4-99.6	0.96	0.93-0.99
<i>Mycoplasma hominis</i>	Total	98.3	(229/233)	95.4-99.4	98.7	(862/873)	97.7-99.3	0.96	0.94-0.98
	Genital swab	99.2	(126/127)	95.0-99.9	99.8	(407/415)	98.4-99.9	0.95	0.93-0.98
	Urine	97.2	(103/106)	91.3-99.3	99.3	(455/458)	97.9-99.8	0.97	0.94-0.99
<i>Trichomonas vaginalis</i>	Total	99.4	(154/155)	95.9-99.9	99.8	(949/951)	99.2-99.9	0.99	0.98-1.00
	Genital swab	100	(79/79)	94.2-100	100	(463/463)	99.0-100	1.00	–
	Urine	98.7	(75/76)	91.9-99.9	99.6	(486/488)	98.4-99.9	0.98	0.95-1.00
<i>Ureaplasma urealyticum</i>	Total	96.6	(230/238)	93.2 – 98.4	98.2	(852/868)	97.0 – 98.9	0.94	0.91 – 0.96
	Genital swab	97.4	(111/114)	91.9-99.3	97.7	(418/428)	95.6-98.8	0.93	0.89-0.97
	Urine	96.0	(119/124)	90.4-98.5	98.9	(434/440)	97.2-99.6	0.94	0.91-0.98
<i>Mycoplasma genitalium</i>	Total	98.7	(155/157)	95.0-99.8	98.2	(932/949)	97.1-98.9	0.93	0.90-0.96
	Genital swab	98.7	(76/77)	92.0-99.9	98.3	(457/465)	96.5-99.2	0.93	0.89-0.98
	Urine	98.8	(79/80)	92.3-99.9	98.1	(475/484)	96.4-99.1	0.93	0.89-0.97

TABLE 3 Resolution of discordant results in the DiaPlexQ™ STI6 Detection Kit and the GeneFinder™ STD I (CT/NG/UU) and STD II (MG/MH/TV) Multiplex Real-time PCR Kit

Virus	Discordant results		
	DiaPlexQ assay/GeneFinder assay	No. of samples (genital swab/urine)	No. of positive results on monoplex PCR and sequencing (genital swab/urine)
<i>Chlamydia trachomatis</i>	±	4 (4/0)	0 (0/0)
	–/+	3 (2/1)	0 (0/0)
<i>Neisseria gonorrhoeae</i>	±	9 (4/5)	1 (0/1)
	–/+	1 (0/1)	0 (0/0)
<i>Mycoplasma hominis</i>	±	11 (8/3)	0 (0/0)
	–/+	4 (1/3)	0 (0/0)
<i>Trichomonas vaginalis</i>	±	2 (0/2)	0 (0/0)
	–/+	1 (0/1)	0 (0/0)
<i>Ureaplasma urealyticum</i>	±	16 (10/6)	1 (1/0)
	–/+	8 (3/5)	0 (0/0)
<i>Mycoplasma genitalium</i>	±	17 (8/9)	0 (0/0)
	–/+	2 (1/1)	1 (1/0)

have been introduced including strand displacement amplification, transcription-mediated amplification, and real-time PCR.^{4,15,16} Some of these technologies will allow for rapid near-patient testing at the point of care and others have potential for high-throughput batch testing.^{5–8,11,17} Rapid testing using random-access

platforms such as the GeneXpert system (Cepheid, Sunnyvale, CA) may be suitable for in-clinic testing handling of a low number of specimens, whereas high-throughput batch testing including the DiaPlexQ assay is useful for laboratories performing a large number of assays.^{12,17–19}

Numerous studies have shown that multiplex real-time PCR assays were sensitive and useful modality for the diagnosis of STIs.^{5,8,9,11,13} Chung et al recently evaluated the performance of two commercial multiplex real-time PCR assays including the GeneFinder assay for six STD pathogens and reported excellent agreement between the two assays ($\kappa = 0.87$).¹³ In the present study, there was excellent concordance between the results of the DiaPlexQ assay and the GeneFinder assay for each microorganism regardless of specimen type. Discordant results were observed in 3-24 of 1106 specimens for each microorganism. The vast majority of the discordant results were associated with a high Ct value, indicating low viral loads (data not shown). Among the discordant results, the majority (54/73) were DiaPlexQ-positive and GeneFinder-negative. This finding may suggest the DiaPlexQ assay detected more positive samples with low viral loads, considering the analytical sensitivity of the assays: the detection limit of the DiaPlexQ assay was 1-10 copies/reaction in the present study, whereas that of the GeneFinder assay was 50 copies/reaction according to the package insert. However, almost all discordant cases yielded negative results in discrepancy resolution with monoplex PCR and sequencing. Although false-positive reactions in real-time PCR assays could lead to these discrepancies, we cannot exclude the possibility that differences in analytical sensitivity between real-time PCR assays and monoplex PCR were the cause, since real-time PCR is generally more sensitive than conventional PCR.

The main limitation of this study was the lack of a reference standard and the retrospective nature of the study. We only resolved discrepancies for specimens with discordant results between the DiaPlexQ assay and the GeneFinder assay using monoplex PCR and sequencing. Therefore, we could not assess the diagnostic accuracy, including the sensitivity and specificity, of the real-time PCR assays. Furthermore, we resolved the discordant results with conventional PCR and sequencing, which is generally less sensitive than real-time PCR. In addition, it was not possible to estimate the clinical incidence of the six STIs and positive/negative predictive values due to the retrospective nature of our study.

In conclusion, the DiaPlexQ assay produces results comparable to those of the GeneFinder assay. The DiaPlexQ assay will be a useful tool for STD pathogen detection in clinical laboratories.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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