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Original Article

Performance of two commercial multiplex polymerase chain reaction assays for the etiological diagnosis of sexually transmitted infections among men who have sex with men

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KEYWORDS
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Abstract *Background and purpose:* This study aimed to investigate the etiologies of sexually transmitted infections (STIs) among men who have sex with men (MSM) in Taiwan.
Methods: Two commercial assays, the BD MAX *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), and *Trichomonas vaginalis* (TV) panel and the Allplex™ STI Essential assay (CT, GC, *Mycoplasma genitalium* [MG], *Mycoplasma hominis* [MH], *Ureaplasma urealyticum* [UU], *Ureaplasma parvum* [UP], and TV) were evaluated. During the first stage, urine and rectal swab samples from 168 patients were evaluated using the BD MAX assay, and the multiplex RT-PCR Allplex™ STI Essential assay was applied only to the patients with positive results on the BD MAX asay (n = 49). During the second stage, urine and rectal swab samples from 90 patients were evaluated using the BD MAX assay and the Allplex™ qPCR.
Results: The Allplex qPCR identified all CT, missed one and additionally one TV from the positive samples (n = 49) by the BD MAX assay in the first stage. At the second stage, both commercial assays showed similar detection rate of CT, NG or CT/NG coinfection (11.1%, 1.1% and 4.4% by the BD MAX assay; 10.0%, 1.1% and 2.2% by the Allplex qPCR). The positivity rates of

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MG, MH, and UU by the Allplex qPCR were 4.4%, 2.2%, and 12.2%, respectively, for urine samples and 10%, 13.3%, and 22.2%, respectively, for anal swab samples.
Conclusions: High rates of STI-associated etiologies were observed in MSM. The positive rates were higher in rectal swabs than in urine samples.

Introduction

Sexually transmitted infections (STIs) are among the most common communicable diseases, affecting more than 1 million people worldwide.[1,2] Each year, there are an estimated 376 million new infections with one of the following four STIs: chlamydia, gonorrhea, syphilis, and trichomoniasis. The rates of STIs continue to rise in the United States, with 1.8 million cases of *Chlamydia trachomatis* (CT) infection and 616,000 cases of *Neisseria gonorrhoeae* (GC) infection reported in 2019.[3] Marginalized populations that have been historically disadvantaged, such as racial and ethnic minorities, and sexual minorities, including gay, bisexual, transgender, and other men who have sex with men (MSM), are disproportionately affected by CT and GC infections.

Anorectal STIs are common in MSM, and it is widely accepted that CT and GC are causative agents of proctitis.[4] It has also been reported that *Mycoplasma genitalium* (MG), one of the major etiologic agents of male urethritis, is associated with proctitis.[5] These microorganisms are important pathogens in anorectal STIs detected in MSM. There is little evidence that *M. hominis* (MH) and *Ureaplasma* spp. Are causative agents of urethritis in men. The genus *Ureaplasma* is divided into two groups, biovar 1 (*U. parvum* [UP]) and biovar 2 (*U.urealyticum* [UU]). Previous studies have reported that UU can cause urethritis in men, whereas no such relationship with UP was detected.[6] Although MH has not been identified as a causative agent of urethritis in men, both MH and UU have been associated with male infertility. However, the pathogenicity of MH and *Ureaplasma* spp. In the MSM population has not yet been recognized.

While other testing methods, such as microscopy, culture, and antigen detection, remain available, the lack of sensitivity combined with the need for well-controlled transportation conditions to maintain viable organisms for culture makes these tests less desirable than nucleic acid amplification tests (NAATs). There are several commercially available STI NAATs. Most patients underwent combined CT/GC testing with the option of re-testing the same samples for *Trichomonas vaginalis* (TV). The throughput and time-to-results for these systems varied from 1 to more than 200 samples per shift and 90 min to 6 h, respectively).

Previous studies have demonstrated a high prevalence of chlamydia, gonorrhea, and mycoplasma at different anatomic sites in MSM.[7,8] The prevalence rates of these microorganisms in the anus and urine of MSM have been previously reported in other countries; however, data on the etiologies associated with STIs in MSM in Taiwan are lacking. This study aimed to investigate the etiologies of STIs in both urine and rectal swab samples of MSM in Taiwan.

Materials and methods

Patients

This study was conducted at the National Taiwan University Hospital, a tertiary center for HIV care in northern Taiwan. We included 258 consecutive participants (MSM, $n = 247$), including HIV-positive adults ($n = 246$) and HIV-negative adults undergoing pre-exposure prophylaxis (PrEP) for HIV infection ($n = 12$) (Table 1). All participants had a history of STI or symptoms suggestive of an STI from May 14, 2019, to October 4, 2019. During the first stage, urine and rectal swab samples from 168 patients were evaluated using the BD MAX CT/GC/TV assay, and the multiplex RT-PCR Allplex™ STI Essential assay (Seegene, Seoul, Korea) was applied only to patients (n = 49) with positive results on the initial BD MAX CT/GC/TV panel. During the second stage, urine and rectal swab samples from 90 patients were simultaneously evaluated using the BD MAX assay and the Allplex™ qPCR.

In the first stage, cultures for GC (Modified Thayer—Martin, MTM II Agar, BBL™ Becton Dickinson Microbiology Systems, Sparks, MD, USA) and antigen testings for CT (IMAGEN™ Immunofluorescence Test, OXOID, UK) of the clinical samples were performed based on the clinical consideration by the attending physicians. During the second stage, cultures for GC and antigen tests for CT were not performed.

Specimen collection

First-void urine (10 mL) and anal swab specimens were collected from the participants at the clinic. All participants were instructed to insert a swab approximately 3 cm above their anal verge. The swab was gently rotated for 10 s and withdrawn without touching the skin. The specimen was collected from the patient using the BD MAX UVE specimen collection kit and transported to the laboratory under time and temperature conditions determined to maintain the integrity of the target nucleic acids.

BD MAX CT/GC/TV assay

The BD MAX CT/GC/TV assay, which was performed using the BD MAX system, incorporates automated DNA extraction and real-time polymerase chain reaction (PCR) for the direct, qualitative detection of DNA from CT, GC, and TV.

Table 1 Demographic and epidemiological characteristics of the 258 patients included in this study.

Characteristics	No. (%) of patients		
	First stage (n = 168)	Second stage (n = 90)	All (n = 258)
Age, years (Mean ± SD)	38 (29–47)	38 (29–47)	38 (29–47)
21–30	34 (18.9)	20 (22.2)	54 (20.9)
31–40	71 (39.4)	33 (36.7)	104 (40.3)
41–50	49 (27.2)	30 (33.3)	79 (30.6)
51–60	13 (7.2)	7 (7.8)	20 (7.8)
61–70	1 (0.6)	0 (0)	1 (0.4)
Gender (M/F)	168/0	90/0	258/0
Route of transmission			
MSM	162 (90.0)	85 (94.4)	247 (95.7)
Heterosexual	3 (1.7)	1 (1.1)	4 (1.6)
Bisexual	3 (1.7)	4 (4.4)	7 (2.7)
Underlying disease			
HIV	163 (90.6)	83 (92.2)	246 (95.4)
HBsAg positivity	24 (13.3)	6 (6.7)	30 (11.6)
Anti-HCV positivity	40 (22.2)	12 (13.3)	52 (20.2)
Acute hepatitis A	10 (5.6)	2 (2.2)	12 (4.7)
Syphilis	121 (67.2)	41 (45.6)	162 (62.8)
Latent syphilis	88 (52.4)	14 (15.6)	102 (39.5)
Secondary syphilis	21 (12.5)	16 (17.8)	37 (14.3)
DM	4 (2.4)	0 (0)	4 (1.6)
Hypertension	11 (6.5)	3 (3.3)	14 (5.4)
CD4 count (Mean ± SD)	598 (318–878)	664 (328–1000)	625 (317–933)
CD4 (>350)	141 (83.9)	76 (84.4)	217 (84.1)
PVL <200 copies/mL at screening			
<200	160 (95.2)	88 (97.8)	248 (96.1)

HIV, human immunodeficiency virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; MSM, men who have sex with men; PVL, plasma HIV RNA load.

Real-time multiplex PCR assay

Real-time multiplex PCR was performed using the Allplex™ qPCR. This assay can simultaneously detect seven pathogens of STIs (CT, GC, TV, MG, MH, UU, and UP) in a single tube using dual priming oligonucleotide™ (DPO) and multiple detection temperature™ (MuDT) technologies, thereby providing individual cycle threshold values for multiple pathogens in a single channel. The DPO system structurally and functionally differs from the conventional primer system by including a polydeoxyinosine (poly) (I) linker between the two segments of the primer sequences. This poly (I) linker allows the DPO primer to be divided into two functional segments at different hybridization temperatures. Elongation occurs when the two segments hybridize correctly, giving rise to a high specificity between similar or related sequences. The previous nucleic acid residual DNA was extracted from the BD MAX system (BD Biosciences), and the nucleic acids were eluted to a final volume of 10 μL. Real-time PCR was performed in a CFX-96 real-time thermocycler (Bio-Rad, Hercules, CA, USA), according to the manufacturer's instructions.[9]

Results

During the first stage, 168 participants were enrolled in the study. Ninety participants were tested using both the methods during the second phase. The clinical characteristics of the study participants are presented in Table 1. The median age of the participants was 38 years, of which 31–40 years old accounted for 40%. Most participants were MSM (95.7%), with a median CD4 cell count of 625 cells/mm^3, and 96.1% had achieved a plasma viral load (PVL) of <200 copies/mL after undergoing antiretroviral therapy. Among the 258 participants, 30 (11.6%) were positive for hepatitis B surface antigen, 52 (20.2%) were positive for anti-HCV antibody, 12 (4.7%) had acute hepatitis A, and 162 (62.8%) were co-infected with syphilis.

In the 168 participants in the first study stage, after the detection by BD MAX CT/GC/TV, the positivity rates of CT, GC, and TV in the urine samples were 4.2%, 0.6%, and 0%, respectively, and the positivity rates of CT, GC, and TV in the anal samples were 22.0%, 9.5%, and 0%, respectively. When the participants' urine or anal swab samples were positive, we retested the samples using the Allplex™ STI Essential assay, and a total of 49 participants were further tested. The positivity rates of CT, GC, TV, MG, MH, UU, and UP in the urine samples of the 49 participants were 12.2%, 2.0%, 0%, 6.1%, 4.0%, 26.5%, and 0%, respectively, whereas those of CT, GC, TV, MG, MH, UU, and UP in the anal samples of these 49 participants were 75.5%, 30.6%, 2.0%, 10.2%, 26.5%, 36.7%, and 4.1%, respectively. In the second phase, the specimens from 90 participants were simultaneously tested using both methods. The positivity rates for

Table 2 Prevalence of the STI pathogens detected by BD MAX CT/GC/TV and Allplex™ STI Essential assay from urine and anal swab samples.

First stage								
BD MAX (n = 168)								
Sampling site		No. (%) of samples positive for the indicated pathogens tested						
C. trachomatis	*N. gonorrhoeae*	*T. vaginals*	*M. genitalium*	*M. hominis*	*U. urealyticum*	*U. parvum*		
	Urine	7 (4.2)	1 (0.6)	0	—	—	—	—
	Anal	37 (22.0)	16 (9.5)	0	—	—	—	—
	Both[a]	1 (0.6)	1 (0.6)	0	—	—	—	—
Allplex (n = 49)								
	Urine	6 (12.2)	1 (2.0)	0	3 (6.1)	2 (4.0)	13 (26.5)	0
	Anal	37 (75.5)	15 (30.6)	1 (2.0)	5 (10.2)	13 (26.5)	18 (36.7)	2 (4.1)
	Both[a]	1 (2.0)	1 (2.0)	0	0	1 (2.0)	5 (10.2)	0
Second stage								
BD MAX (n = 90)								
	Urine	4 (4.4)	0	0	—	—	—	—
	Anal	15 (16.7)	6 (6.7)	0	—	—	—	—
	Both[a]	1 (1.1)	0	0	—	—	—	—
Allplex (n = 90)								
	Urine	4 (4.4)	0	0	4 (4.4)	2 (2.2)	11 (12.2)	0
	Anal	15 (16.7)	6 (6.7)	0	9 (10)	12 (13.3)	20 (22.2)	0
	Both[a]	1 (1.1)	0	0	1 (1.1)	0	2 (2.2)	0

[a] Both, urine and anal swab samples.
- Not included in the assay.

CT, GC, MG, MH, and UU in the urine samples (n = 90) were 4.4%, 0%, 4.4%, 2.2%, and 12.2%, respectively, and those in the anal swab samples (n = 90) were 16.7%, 6.7%, 10%, 13.3%, and 22.2%, respectively. TV and UP were not detected in any of the samples.

Table 2 shows the prevalence rates of the STI microorganisms detected by the BD MAX CT/GC/TV and Allplex™ STI Essential assays in the urine and anal swab samples. In the first stage, the urine samples and anal swabs from 167 participants were cultured for GC, and nine samples were positive for anal swab cultures, with a positivity rate of 5.4%. CT antigen testing was performed on 107 of the anal samples, nine specimens from which were positive, with a positivity rate of 8.4%.

After the urine samples were analyzed by the BD MAX system in the first stage, 7 (4.2%) samples were positive for one microorganism; among the anal swab samples, 23 (13.8%) were positive for at least one microorganism, 13 (7.8%) of which were positive for one microorganism and 10 (6.0) of which were positive for two microorganisms. After the preliminary screening, 49 of the positive samples were retested using the Allplex™ qPCR assay. Of the urine samples retested, 15 were positive for one microorganism and 5 were positive for two microorganisms. The number of positive samples increased to 46 for the anal specimens, 17 being positive for one microorganism, 16 for two microorganisms, 10 for three microorganisms, and 3 for four microorganisms.

During the second stage, 90 participants were analyzed, and 18 of the 90 urine specimens (20%) were positive for the DNA from at least one microorganism, 15 (16.7%) of which were positive for one microorganism and 3 (3.3%) of which were positive for two microorganisms. For the anal swab samples, 42 (46.7%) samples were positive for at least one microorganism, 26 (28.9%) of which were positive for one microorganism, 12 (13.3%) of which were positive for two microorganisms, 2 (2.2%) of which were positive for three microorganisms, and 2 (2.2%) of which were positive for four microorganisms. Table 3 shows the single and multiple infections detected in the urine and anal swab samples.

Table 4 shows the samples collected from the urine and anal swab samples: the evaluation of single infections, co-infections with other microorganisms, and co-infections with more than one microorganism, expressed as the counts. During the first evaluation period, the rates of single infection for CT, GC, and UU were 3.6%, 0.6%, and 5.4%, respectively, and the rates of co-infection for CT, MG, MH, and UU were 0.6%, 1.8%, 1.2%, and 2.4%, respectively. For the anal swab samples, the rates of single infection for CT, GC, MH, and UU were 6.5%, 1.2%, 0.6%, and 1.2%, respectively, whereas the rates of co-infection rates for CT, GC, MG, MH, UU, and UP were 15.5%, 8.3%, 3.0%, 7.1%, 9.5%, and 1.2%, respectively. Owing to the experimental design, the rates of single infection for MG, MH, and UU increased significantly in the second stage, and the positivity rates for MG, MH, and UU in the urine samples were 3.3%, 0%, and 8.9%, and those for MG, MH, and UU in the anal swab samples were 3.3%, 6.7%, and 11.1%, respectively.

Discussion

The relatively high prevalence of STIs and the need for a rapid and accurate diagnostic tool to detect them justify the need for a thorough evaluation of any new methodology

Table 3 Single and multiple infections detected in urine and anal swab samples.

	No. (%) of patients with pathogens detected in the indicated specimen type			
	First stage		Second stage	
Specimen type	Urine	Anal swab	Urine	Anal swab
BD MAX, No. (%)	(n = 168)		(n = 90)	
Single pathogen detected	7 (4.2)	13 (7.8)	4 (4.4)	7 (7.8)
CT	6	11	4	6
GC	1	2	0	1
TV	0	0	0	0
Two pathogens detected	0	10 (6.0)	0	4 (4.4)
CT + GC	0	10	0	4
Allplex, No. (%)	(n = 49)		(n = 90)	
Single pathogen detected	15 (30.6)	17 (34.7)	15 (16.7)	26 (28.9)
CT	5	11	4	5
GC	1	2	0	1
TV	0	0	0	0
MG	0	1	3	3
MH	0	1	0	7
UU	9	2	8	10
UP	0	0	0	0
Two pathogens detected	5 (10.2)	16 (32.7)	3 (3.3)	12 (13.3)
CT + GC	0	3	0	2
CT + MG	1	4	0	2
CT + MH	0	1	0	1
CT + UU	0	6	0	2
GC + UU	0	2	0	1
MG + MH	0	0	0	0
MG + UU	2	0	1	2
MH + UU	2	0	2	2
Three pathogens detected	0	10 (20.4)	0	2 (2.2)
CT + GC + MH	0	3	0	0
CT + GC + UU	0	1	0	1
CT + MG + MH	0	0	0	1
CT + MH + UU	0	4	0	0
CT + MH + UP	0	1	0	0
GC + MH + UU	0	1	0	0
Four pathogens detected	0	3 (6.1)	0	2 (2.2)
CT + GC + MH + UU	0	1	0	1
CT + GC + MH + TV	0	1	0	0
CT + GC + UU + UP	0	1	0	0
CT + MG + MH + UU	0	0	0	1

CT, *Chlamydia trachomatis*; GC, *Neisseria gonorrhoeae*; TV, *Trichomonas vaginalis*; MG, *Mycoplasma genitalium*; MH, *Mycoplasma hominis*; UU, *Ureaplasma urealyticum*; UP, *Ureaplasma parvum*.

before it can be implemented in routine laboratory practice. This study evaluated the performance of the BD MAX CT/GC/TV assay on a platform for the simultaneous detection of CT, GC, and TV in comparison with a multiplex RT-PCR assay (Allplex™ STI Essential, Seegene). Although the BD MAX assay has previously demonstrated good performance in the diagnosis of these pathogens,[10–12] and the effectiveness of the Allplex™ qPCR has been previously proven,[13–15] we used nucleic acids extracted by the BD MAX system for further detection. In the first stage of detection, the number of positive samples for CT in the urine samples detected by the Allplex™ qPCR assay was 6, and the number of positive samples for GC in the anal swab samples detected by the Allplex™ qPCR assay was 5, which was 1 less than that for the samples detected by the BD MAX system. This may be because of the use of residual nucleic acids. We could not resolve all discordant results with a third test because our sample volume was low and we did not want to dilute the samples to prevent a loss of sensitivity.

Table 4 Samples collected from urine and anal swab samples: evaluation of single infections and co-infections.

No. (%)	CT		GC		MG		MH		UU		UP/TV	
	Urine	Anal swab	Urine	Anal swab	Urine	Anal swab	Urine	Anal swab	Urine	Anal swab	Urine	Anal swab
Single infection	6 (3.6)	11 (6.5)	1 (0.6)	2 (1.2)	0	0	0	1 (0.6)	9 (5.4)	2 (1.2)	0	0
Co-infection (≥1 pathogen detected)	1 (0.6)	26 (15.5)	0	14 (8.3)	3 (1.8)	5 (3.0)	2 (1.2)	12 (7.1)	4 (2.4)	16 (9.5)	0	1 (1.2)
Single infection	4 (4.4)	5 (5.6)	0	1 (1.1)	3 (3.3)	3 (3.3)	0	6 (6.7)	8 (8.9)	10 (11.1)	0	0
Co-infection (≥1 pathogen detected)	0	11 (12.2)	0	5 (5.6)	1 (1.1)	6 (6.7)	2 (2.2)	6 (6.7)	3 (3.3)	10 (11.1)	0	0

CT, *Chlamydia trachomatis*; GC, *Neisseria gonorrhoeae*; TV, *Trichomonas vaginalis*; MG, *Mycoplasma genitalium*; MH, *Mycoplasma hominis*; UU, *Ureaplasma urealyticum*; UP, *Ureaplasma parvum*.

Among the participants in this study, 95.7% were MSM, and the positivity rate of the anal samples was higher than that of the urine samples. For example, in the second stage, the positivity rates of CT, GC, MG, MH, and UU in the anal swab samples were 16.7%, 6.7%, 10%, 13.3%, and 22.2%, respectively, which were higher than those of the urine samples, which were 4.4%, 0, 4.4%, 2.2%, and 12.2%, respectively. A previous study conducted in Europe in 2017 demonstrated a low prevalence of specimen collection from the anus in the MSM population.[16] In the 2021 European guidelines for the management of proctitis, proctocolitis and enteritis are caused by sexually transmissible pathogens.[17] Increased rectal swab screening recommended. The Centers for Disease Control and Prevention guidelines recommend screening for rectal CT and GC annually and every 3 months for those with an increased risk for these infections and those undergoing PrEP for HIV infection.[18] Although the positivity rate of anal specimens is higher than that of urine specimens, the rate of positive specimens was very low. It thus was necessary to collect the two specimens simultaneously.

Furthermore, this study evaluated co-infections caused by different pathogens. If the participants were CT-positive in the anal swab specimen, the rates of co-infection with other pathogens were 15.5% and 12.2%, respectively, which were the highest co-infection rates. In the second phase of the experiment, it was demonstrated that there were many single infections of MG, MH, and UU, with rates of single infection of 3.3%, 6.7%, and 11.1%, respectively. This result indicates that if BD MAX is used alone as a screening test, individuals with a single infection would be undetected. Previous research demonstrated that one in seven MSM treated for rectal CT or rectal GC will have undiagnosed MG.[19] In addition, these MSM with MG pathogens will lead to MG mutations if undergoing treatment with azithromycin for CT or GC.[20,21] Therefore, it is important to be able to detect MG.

In the first phase, we also performed GC and CT antigen testing. However, if the laboratory only used traditional methods for STI testing, there would be seven fewer positives for GC cultures and 16 fewer positives for CT Ag. The responsibility of the laboratory was to select a suitable platform. The BD MAX system allows for the automatic processing of samples to avoid this task for the operator, which saves time and minimizes the risk of contamination in the laboratory. However, the Allplex™ qPCR assay provides clinicians with various pathogen detection results.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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