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Original article

Clinical performance of four multiplex real-time PCR kits detecting urogenital and sexually transmitted pathogens

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ABSTRACT

Objectives: We evaluated the clinical performances of four multiplex real-time PCR commercial kits for the detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Trichomonas vaginalis*: the STI PLUS ELITe MGB kit (ELITechGroup), *N. gonorrhoeae*/*C. trachomatis*/*M. genitalium*/*T.vaginalis* Real-TM kit (Sacace Biotechnologies), Allplex STI Essential kit (Seegene), and FTD Urethritis Plus kit (Fast-Track Diagnostics).
Methods: The kit performance for *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis* detection was compared to that of the cobas CT/NG and TV/MG kits (Roche Diagnostics) using 425 samples, mainly urine and cervicovaginal, throat and rectal swabs. Detection of *Ureaplasma parvum*, *U. urealyticum* and *Mycoplasma hominis* were compared to that of in-house TaqMan PCRs.
Results: The four kits showed good performances for the detection of *C. trachomatis*. They all presented a low positive agreement for the detection of *M. genitalium* and *T. vaginalis* (ranges 63.3–74.1% and 51.2–68.4%, respectively) compared to the cobas MG/TV kit. The Seegene and Sacace kits showed additional low positive agreement for the detection of *N. gonorrhoeae* (71.2%, 95%CI 61.8–79.0 and 63.1%, 95%CI 53.5–71.8, respectively). We observed a slight but significant lower negative agreement for *N. gonorrhoeae* detection using the ELITechGroup kit (92.5%, 89.1–94.9) and for *M. genitalium* detection using the Fast-Track kit (93.2%, 89.6–95.7) compared to other kits.
Conclusion: Multiplex real-time PCR kits are convenient methods for the detection of several pathogens associated with sexually transmitted infections (STIs) in a single step, but colonizing *Ureaplasma* spp. and *M. hominis* species should not be included in these kits. Users should be aware of the weak performance of some kits for the detection of *M. genitalium* and *T. vaginalis*. **Sabine Pereyre, Clin Microbiol Infect 2022;28:733.e7–733.e13**

Introduction

Sexually transmitted infections (STIs) are a worldwide health problem [1]. Because of the involvement of fastidious pathogens, the high number of asymptomatic cases and the possibility of multiple infections, multiplex PCR assays are of interest for the diagnosis of these infections in a single step. Several commercially available multiplex real-time PCRs targeting microorganisms involved in non-viral STIs have recently been launched, but only a few of them have already been evaluated [2–4].

The objective of the present study was to evaluate the clinical performances of four multiplex real-time PCR commercial kits for the detection of non-viral STIs: the STI PLUS ELITe MGB Kit (ELITech kit) (ELITech Group, France), the Allplex STI Essential kit (Seegene kit) (Seegene, Republic of Korea), the *N. gonorrhoeae*/*C. trachomatis*/*M. genitalium*/*T. vaginalis* Real-TM kit (Sacace kit) (Sacace Biotechnologies, Italy) and the FTD Urethritis plus kit (Fast-Track kit)

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(Fast-Track Diagnostics, Luxembourg). The performance of the kits for the detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Trichomonas vaginalis* was compared to that of the cobas CT/NG and TV/MG kits (Roche Diagnostics, USA) [5–10]. For the two kits that also detect *Ureaplasma urealyticum*, *U. parvum* and *Mycoplasma hominis*, the detection performance was compared to that of published in-house TaqMan PCRs [11,12].

Materials and methods

Clinical samples

Between March and October 2019, remnants of clinical samples in cobas PCR medium (Roche Molecular Systems) received at the French National Reference Centre for Bacterial STIs in the Bacteriology Department of Saint-Louis Hospital (Paris, France) were prospectively and consecutively collected and stored at −80°C until testing with commercial kits. *T. vaginalis*-positive samples collected in the Bacteriology Department of Bordeaux University Hospital (France) during the same period were also included. The aim of the sample selection process was to systematically enrol (a) the first 100 samples detected as positive for *C. trachomatis* and the first 100 samples detected as positive for *N. gonorrhoeae* using the cobas CT/NG (Roche Diagnostics) detection kit on the cobas 6800 system, (b) the first 100 samples detected as positive for *M. genitalium* and as many samples as possible detected as positive for *T. vaginalis* using the cobas MG/TV (Roche Diagnostics) detection kit, and (c) 100 samples detected as negative for *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis* using both kits.

Ethics statement

Remnants of specimens were preserved under the collection number BB-0033-00094 and authorization AC-2014-2166 from the French Ministry of Higher Education and Research, with no information regarding the patient identity.

DNA extraction

Prior to DNA extraction, internal controls (ICs) provided in each kit were added to samples according to the manufacturer's instructions (Fig. 1). DNA was extracted using the DSP Virus/Pathogen Midi Kit (Qiagen, Germany) on a Qiasymphony instrument (Qiagen), yielding an elution volume of 110 µL.

Detection of Ureaplasma spp. and M. hominis

The detection of *U. parvum*, *U. urealyticum* and *M. hominis* species was performed on all enrolled samples using in-house TaqMan PCR assays, as previously reported [11,12]. PCR amplifications were performed on a Light Cycler 480 instrument (Roche Diagnostics).

Evaluated commercial kits

The four commercial kits are CE-IVD-marked and were run according to the manufacturer's instructions (Fig. 1, Table 1).

Data analysis

The cobas CT/NG and TV/MG kits were used as reference methods for *C. trachomatis*–*N. gonorrhoeae* detection and *M. genitalium*–*T. vaginalis* detection, respectively. The in-house PCRs were used as reference methods for the detection of *U. urealyticum*, *U. parvum* and *M. hominis*. Overall (OPA), positive (PPA) and negative (NPA) percentage agreements were calculated, along with the corresponding 95% confidence intervals (CIs) and Cohen's kappa value (κ) for the OPP. Statistical analyses were performed using the biostaTGV website (https://biostatgv.sentiweb.fr/).

Results

Characteristics of included samples

A total of 425 samples (403 from Saint-Louis Hospital and 22 from Bordeaux Hospital) collected from 323 patients were enrolled in the study (Table 2). Among them, 91 samples were collected from 85 women, and 333 samples were collected from 237 men. The mean age of patients from whom samples were collected was 32 years (27 years for women, 33 years for men). A total of 27.0% of patients (83/307) reported urogenital symptoms. Among the 425 samples included, 105 were positive for *C. trachomatis* and 104 were positive for *N. gonorrhoeae* using the cobas CT/NG kit, 109 were positive for *M. genitalium* and 41 were positive for *T. vaginalis* using

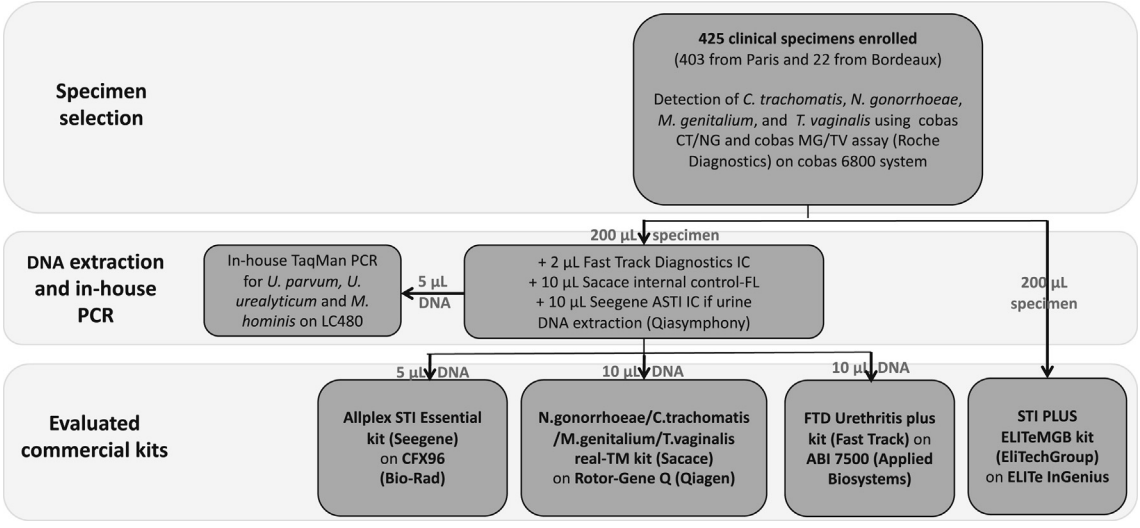

Fig. 1. Flowchart of the comparison study.

Table 1
Main characteristics of the four commercial kits

Kits	STI PLUS ELITe MGB (ELITechGroup)	Allplex STI Essential (Seegene)	NG/CT/MG/TV Real-TM (Sacace Biotechnologies)	FTD urethritis plus[a] (Fast Track Diagnostics)
Detected targets	CT: *dnaB* gene (endogenous plasmid) and *ompA* gene (chromosomal gene) NG: *pivNG* gene MG: 23S rRNA gene TV: L23861 repeated sequence	CT, NG, MG, TV, UU, UP and MH Undisclosed	CT, NG, MG and TV Undisclosed	CT, NG, MG, TV, UU, UP and MH Undisclosed
Validated specimen types	Cervicovaginal swabs, urine	Genital swabs, urine, liquid-based cytology	Cervical, urethral, conjunctival swabs, urine, prostatic liquid, semen	Genital and rectal swabs, urine
Input sample volume	200 µL primary specimen or 20 µL DNA	5 µL DNA	10 µL DNA	10 µL DNA
Internal control (IC) (extraction and amplification)	Endogenous IC and additional exogenous IC for urine	Endogenous IC and additional exogenous IC for urine	Exogenous IC	Exogenous IC
Automation	DNA extraction, PCR set up, amplification and analysis	Amplification and analysis	Amplification	Amplification
Number of test/kit	12	50	100	32 or 64
Number of reaction/run	Batches of 12 tests[b] (can run distinct molecular tests at the same time)	Batches; up to 94 samples per PCR run	Batches; up to 72 samples per PCR run	Batches; up to 94 samples per PCR run
Hands-on time (min)[c]	30	30–60, depending on the number of samples	80–240, depending on the number of samples	30–60, depending on the number of samples
Test turnaround time	3 h including DNA extraction	1.75 h, excluding DNA extraction	2 h excluding DNA extraction	1.5 h excluding DNA extraction
Thermal cycler used in this study	ELITe InGenious (EliTechGroup)	CFX96 (Bio-Rad)	Rotor-Gene Q (Qiagen)	ABI 7500 (Applied Biosystems)
Data analysis software	ELITe InGenius software version 1.3.0.12	Seegene Viewer version 1.6	Rotor-Gene Q software version 2.3.1.49	Applied Biosystems 7500 software version 2.3

CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; MG, *Mycoplasma genitalium*; TV, *Trichomonas vaginalis*; UP, *Ureaplasma parvum*; UU, *U. urealyticum*; MH, *Mycoplasma hominis*.
[a] The commercialization of this kit has been recently discontinued.
[b] Positive and negative controls had to be run for each lot number.
[c] Hands-on time includes time for specimen processing.

Table 2
Specimen types included in the study, according to sex

Specimen types	Women	Men	Unknown	Total
First void urines	8	140	1	149
Rectal swabs	4	114	0	118
Cervicovaginal swabs	77	0	0	77
Urethral swabs	0	11	0	11
Throat swabs	2	68	0	70
Total	91	333	1	425

the cobas MG/TV kit, including 35 samples positive for two distinct microorganisms. In total, 101 samples were negative for the four pathogens. Additionally, 64 samples were found to be positive for *U. parvum*, 53 for *U. urealyticum*, and 75 for *M. hominis* using in-house PCRs.

Invalid results

Three kits—namely the ELITech, the Seegene, and the Sacace kits—yielded only four invalid samples (0.9%) due to an absence of detection or a delayed detection of the IC (Supplementary Material Tables S1–S7). The four invalid samples were not the same for the three kits. Regarding the Fast-Track kit, 23 samples could not be evaluated due to the lack of availability of reagents. Among the 402 samples evaluated, 37 (9.2%) yielded invalid results. While the IC was not detected for two samples, 35 samples presented a cycle threshold value > 33 with no other positive results, and were interpreted as invalid according to the manufacturer's instructions.

Performance for C. trachomatis detection

Good detection performances were observed for the four kits for *C. trachomatis* detection (Table 3, Supplementary Material Tables S1 and S8), with no statistically significant difference in OPA, PPA and NPA between the kits. The κ coefficient ranged between 0.84 and 0.92 (Table 3, Supplementary Material Table S1).

Performance for N. gonorrhoeae detection

Regarding *N. gonorrhoeae* detection, the Seegene and the Sacace kits showed a significantly lower PPA (71.2% and 63.1%) than the ELITech and Fast-Track kits (88.1% and 90.6%), respectively (Table 3, Supplementary Material Tables S2 and S8). The ELITech kit presented a significantly lower NPA (92.5%) than the other three kits (all >97.4%), with 24/320 samples (7.5%) yielding false-positive results. All 24 false-positive samples were invalid or negative for *N. gonorrhoeae* using the other evaluated kits. These samples included 12 rectal and five genital swabs, four urines and three throat swabs.

Performance for M. genitalium detection

For *M. genitalium,* quite low PPAs, ranging between 63.3% and 74.1%, were found for the four kits, with no significant difference between the kits (Table 3, Supplementary Material Tables S3 and S8). In addition, a significantly lower NPA was observed for the Fast-Track kit (93.2%) compared to the other three kits (all >98.1%).

Table 3
Summary of the performances of the four commercial kits by microorganisms in comparison to that of the cobas CT/NG and TV/MG kits (Roche Diagnostics)

Pathogens	Evaluated kit	No. of valid samples	Detected positive with reference	Detected negative with reference	Detected positive with reference	Detected negative with reference	Overall % Agreement (95%CI)	Positive % Agreement (95%CI)	Negative % Agreement (95%CI)	κ
			Detected **positive** with the evaluated kit		Detected **negative** with the evaluated kit					
CT	STI PLUS ELITeMGB	421	94	2	11	314	96.9 (94.8–98.2)	89.5 (82.2–94.1)	99.4 (97.7–99.8)	0.92
	AllplexTM STI Essential	421	84	5	19	313	94.3 (91.7–96.1)	81.6 (73.0–87.9)	98.4 (96.4–99.3)	0.84
	NG/CT/MG/TV Real-TM	421	89	8	16	308	94.3 (91.7–96.2)	84.8 (76.7–90.4)	97.5 (95.1–98.7)	0.84
	FTD Urethritis plus	365	83	4	11	267	95.9 (93.3–97.5)	88.3 (80.3–93.3)	98.5 (96.3–99.4)	0.89
NG	STI PLUS ELITeMGB	421	89	24	12	296	91.4 (88.4–93.8)	88.1 (80.4–93.1)	**92.5 (89.1–94.9)**	0.77
	AllplexTM STI Essential	421	74	1	30	316	92.6 (89.7–94.8)	**71.2 (61.8–79.0)**	99.7 (98.2–99.9)	0.78
	NG/CT/MG/TV Real-TM	421	65	7	38	311	89.3 (86.0–91.9)	**63.1 (53.5–71.8)**	97.8 (95.5–98.9)	0.68
	FTD Urethritis plus	365	87	7	9	262	95.6 (93.0–97.3)	90.6 (83.1–95.0)	97.4 (94.7–98.7)	0.89
MG	STI PLUS ELITeMGB	421	80	1	28	312	93.1 (90.3–95.2)	74.1 (65.1–81.4)	99.7 (98.2–99.9)	0.80
	AllplexTM STI Essential	421	69	3	40	309	89.8 (86.5–92.3)	63.3 (54.0–71.8)	99.0 (97.2–99.7)	0.70
	NG/CT/MG/TV Real-TM	421	74	6	34	307	90.5 (87.3–92.9)	68.5 (59.3–76.5)	98.1 (95.9–99.1)	0.73
	FTD Urethritis plus	365	72	18	27	248	87.7 (83.9–90.7)	72.7 (63.2–80.5)	**93.2 (89.6–95.7)**	0.68
TV	STI PLUS ELITeMGB	421	25	0	16	380	96.2 (93.9–97.7)	61.0 (45.7–74.3)	100 (99.0–100)	0.74
	AllplexTM STI Essential	421	21	0	20	380	95.2 (92.8–96.9)	51.2 (36.5–65.8)	100 (99.0–100)	0.65
	NG/CT/MG/TV Real-TM	421	26	5	15	375	95.2 (92.8–96.9)	63.4 (48.1–76.4)	98.7 (97.0–99.4)	0.70
	FTD Urethritis plus	365	13	15	6	331	94.2 (91.4–96.2)	68.4 (46.0–84.6)	95.7 (93.0–97.4)	0.52
UU	AllplexTM STI Essential	421	53	37	0	331	91.2 (88.1–93.6)	100 (93.2–100)	89.9 (86.5–92.6)	0.69
	FTD Urethritis plus	365	50	43	1	271	87.9 (84.2–90.9)	98.0 (89.7–99.7)	86.3 (82.1–89.7)	0.63
UP	AllplexTM STI Essential	421	62	5	2	352	98.3 (96.6–99.2)	96.9 (89.3–99.1)	98.6 (96.8–99.4)	0.94
	FTD Urethritis plus	365	31	3	21	310	**93.4 (90.4–95.5)**	**59.6 (46.1–71.8)**	99.0 (97.2–99.7)	0.69
MH	AllplexTM STI Essential	421	70	1	5	345	98.6 (96.9–99.4)	93.3 (85.3–97.1)	99.7 (98.4–100)	0.95
	FTD Urethritis plus	365	56	11	4	294	95.9 (93.3–97.5)	93.3 (84.1–97.4)	**96.4 (93.7–98.0)**	0.86

CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; MG, *Mycoplasma genitalium*; TV, *Trichomonas vaginalis*; UU, *Ureaplasma urealyticum*; UP, *U. parvum*; MH, *Mycoplasma hominis*.
For a given microorganism, the overall, positive and negative % agreements that are significantly different from agreement with the other evaluated kits are in bold.

Performance for T. vaginalis detection

Regarding *T. vaginalis* detection, a very low PPA, ranging between 51.2% and 68.4%, was observed for the four kits, with no significant difference between them (Table 3, Supplementary Material Tables S4 and S8). The κ coefficient between the four kits and the MG/TV cobas kit ranged between 0.52 and 0.74 only (Table 3, Supplementary Material Table S4). The Seegene kit missed half of the *T. vaginalis*-positive samples (21/41). The Fast-Track kit had a significantly lower NPA (95.7%) than the ELITech and Seegene kits, which both had an NPA of 100%.

Performance for U. parvum, U. urealyticum and M. hominis detection

Two commercial kits also detect *U. parvum*, *U. urealyticum* and *M. hominis*: the Seegene and the Fast-Track kits. There were no significant differences between the kits for *U. urealyticum* detection. However, they both showed a moderate NPA (89.9% and 86.3%, respectively) with the in-house real-time PCR, with 37 and 43 samples detected positive by the Seegene and Fast-Track kits, respectively, but found negative by the in-house PCR (Table 3, Supplementary Material Tables S5 and S8). Among the latter 37 and 43 samples, 30 samples were detected by both commercial kits; thus, a lack of sensitivity of the in-house method for detection of *U. urealyticum* cannot be excluded. For *U. parvum* detection, a significantly lower PPA was observed with the Fast-Track kit (59.6%) compared to the Seegene kit (96.9%) (Table 3, Supplementary Material Tables S6 and S8). Regarding *M. hominis* detection, good clinical performances were observed for both kits (Table 3, Supplementary Material Tables S7 and S8).

Performance according to sample types

A similar analysis was separately performed on the 149 first-void urines, 77 cervicovaginal swabs, 118 rectal swabs and 77 throat swabs (Supplementary Material Tables S9–S12). Very small variations in the percentage of agreement were observed for first-void urines and cervicovaginal swabs compared to the global analysis (Table 3). Of note, the PPA for *M. genitalium* detection notably increased by 17.2% and 20.2% using the Sacace and Fast-Track kits with cervicovaginal swabs, respectively, although the increase was not significant (Supplementary Material Table S10). Regarding rectal swabs (Supplementary Material Table S11), a slight decrease in OPA and PPA for *C. trachomatis* detection was noted for all kits except the ELITech kit compared to the global analysis. The lower percentage of NPA for *N. gonorrhoeae* detection reported for the ELITech kit in the global analysis (92.5%) was worse at 85.2% when considering only rectal swabs. Regarding throat swabs, a decrease of 6% to 23% of OPA and PPA was noted for *N. gonorrhoae* detection using all the kits except the Fast-Track kit (Supplementary Material Table S12).

Discussion

Multiplex PCRs appear to be convenient methods for the detection of several microorganisms involved in STIs in a single step because they allow improvement in time commitments and may detect a pathogen not initially suspected by the clinician.

In comparative evaluations, the choice of the reference is of importance for the data interpretation. In the present study, the cobas CT/NG and MG/TV kits were chosen as reference assays since good performances have been reported in the literature for the detection of *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, and *T. vaginalis* [5–10,13,14]. Notably, a study on more than 5000 patients and 16 000 urogenital samples found a sensitivity/specificity for the detection of *C. trachomatis* and *N. gonorrhoeae* of 95.5%/99.5% and 97.2%/99.9%, respectively, in comparison to a patient infected status composed of a combination of FDA-approved NAATs [7]. Regarding the detection of *M. genitalium*, the sensitivity of the cobas MG/TV kit was 96.6% in female vaginal swabs and 100% in male urine samples, while the specificity was 97.0% and 97.6%, respectively [9]. In addition, a study on 1648 female samples reported an overall sensitivity of 100% and a specificity of 99.5% for the detection of *T. vaginalis* [10].

In the present study we found that the four kits gave good performances for *C. trachomatis* detection. However, they all presented a low PPA for *T. vaginalis* detection (range 51.2–68.4%) and for *M. genitalium* detection (range 63.3–74.1%) compared to the cobas MG/TV kit. The 100-fold lower bacterial load reported in specimens positive for *M. genitalium* compared to those positive for *C. trachomatis* may be involved in this lack of sensitivity [15]. The Seegene kit was previously compared to the TMA-based Aptima assay (Hologic) [2]. The sensitivity for *M. genitalium* detection was reported to be lower than the PPA measured (41.7% versus 63.3% in the present study), suggesting that this kit would miss a high number of samples positive for *M. genitalium* that would have been detected using the Aptima or cobas kits. Moreover, the Seegene and Sacace kits showed additional low PPA (71.2% and 63.1%, respectively) for *N. gonorrhoeae* detection compared to the cobas CT/NG kit. Accordingly, the sensitivity for *N. gonorrhoeae* detection using the Seegene kit was previously measured at 72.4% compared to the Aptima assay [2]. It could be suggested that multiplexing PCRs may affect the sensitivity of detection. However, no significant difference in PPA was observed in this study when detection performances were compared separately on specimens positive for one, two or three microorganisms (Supplementary Material Table S13).

An additional analysis per sample type was performed and showed good performance for *C. trachomatis* and *N. gonorrhoeae* detection in first-void urine and cervicovaginal swabs for the four kits, which were all validated for both types of sample. The four kits were not validated either on throat swabs or on rectal swabs except the Fast-Track kit, which had been validated on rectal swabs. Although some data are hardly interpretable owing to a low number of positive samples included in the comparison, lower PPA noted for *C. trachomatis* detection on rectal swabs and for *N. gonorrhoeae* detection on throat swabs using some kits require caution for their use in these types of samples.

Detection of all the 'true' STI agents in a single step is of interest for patients, especially for patients infected with *C. trachomatis*. Indeed, in patients infected with *C. trachomatis* in whom *M. genitalium* is also present but would not have been detected without a multiplex test, the use of azithromycin 1 g for *C. trachomatis* treatment—still a recommended treatment in some countries—would likely select for *M. genitalium* macrolide-resistant strains [16]. The use of multiplex PCRs would allow the diagnosis of such double infections, and a 5-day extended azithromycin treatment would simultaneously eradicate both pathogens more safely in the case of a macrolide-susceptible *M. genitalium* strain. This is an advantage of multiplex PCRs for STI detection over a two-step detection strategy composed of a first detection of *C. trachomatis*/*N. gonorrhoeae* followed by a search for *M. genitalium* in case of negative results. On the other hand,

multiplex PCR may increase the detection of asymptomatic *M. genitalium* single infections, which in the Australian and United Kingdom guidelines are not recommended to be detected and treated to limit the rise of antibiotic resistance [17] (http://www.sti.guidelines.org.au/sexually-transmissible-infections/mycoplasma-genitalium). Additionally, the lack of detection of macrolide-resistance-associated mutations in *M. genitalium* is also a limitation of the usefulness of these STI multiplex assays.

The Seegene and the Fast-Track kits also detected *U. parvum*, *U. urealyticum* and *M. hominis*. However, asymptomatic carriage of these species is common, and the great majority of colonized individuals do not develop any disease [18–20]. Only high *U. urealyticum* loads might be responsible for a few cases of male non-gonococcal urethritis [18]. Thus, according to the European branch of the International Union against Sexually Transmitted Infections (IUSTI), routine screening of asymptomatic individuals or routine testing of symptomatic patients for *M. hominis*, *U. parvum* and *U. urealyticum*—as well as subsequent antimicrobial treatment—is not recommended [18]. *U. urealyticum* may be sought in a second step only after the 'true' bacterial STI pathogens, namely, *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis*, have been excluded [18]. Although it may sound better for patients to detect as many microorganisms as possible at the same time, the use of multiplex PCR assays simultaneously detecting 'true' STI agents and colonizing bacteria is not accurate. With the frequent cases of detection of colonizing *U. parvum*, *U. urealyticum* or *M. hominis*, there is a risk of unnecessary antibiotic treatments resulting in an increase in antibiotic selective pressure on the general microbiota. In the context of increasing antimicrobial resistance, especially for *N. gonorrhoeae* and *M. genitalium* [21–23], the use of kits detecting these colonizing bacteria should be avoided.

This study has some limitations. First, cobas 6800 assays were used as reference assay. Although very good performances have been reported for these kits, they are not reference standards. Indeed, if the reference assay presents a lack of sensitivity, the NPA would possibly be underestimated. On the other hand, if the reference assay presents a lack of specificity, there is a risk of underestimation of the PPA. Whereas a lack of specificity was reported using cobas 4800 for *N. gonorrhoeae* in oropharyngeal specimens [24], performance seemed better using cobas 6800 [13] but might remain insufficient. A third assay and/or Sanger DNA sequencing would have helped to resolve discordant samples, but none of the original sample was left. Accordingly, we did not talk about sensitivity and specificity in this study but only of PPA and NPA. Additionally, this study was designed to compare the performance of the kits but did not intend to determine the prevalence of pathogens, as the specimen selection included approximately 100 samples positive for each main STI pathogen and resulted in 78.4% of specimens from men (333/425). The small number of *T. vaginalis*-positive specimens included is also a limit to accurately evaluating the performance of detection of this microorganism.

In conclusion, multiplex real-time PCR kits are convenient methods for the detection of several STI-associated pathogens at the same time, but the colonizing *U. parvum*, *U. urealyticum* and *M. hominis* species should not be included in these kits. Importantly, users should be aware of the lack of sensitivity for the detection of certain organisms, especially *M. genitalium* and *T. vaginalis*.

Author contributions

SP, BB and CB conceived and designed the study. FC and NH performed the research. SP, FC and NH analysed the data. SP wrote the manuscript. FC, BB and CB critically revised the manuscript.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.09.028.

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