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Performance of Three Commercial Molecular Diagnostic Assays for the Simultaneous Detection of *Mycoplasma genitalium* and Macrolide Resistance

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ABSTRACT The increasing frequency of macrolide resistance is an emerging issue in the treatment of *Mycoplasma genitalium* infection. Because evaluation of new commercial kits detecting *M. genitalium* and macrolide resistance is needed, we evaluated the performance and handling characteristics of the Allplex MG & AziR (Seegene), the Macrolide-R/MG ELITe MGB (ELITechGroup), and the ResistancePlus MG FleXible (SpeeDx-Cepheid) kits in comparison with those of an in-house real-time PCR and 23S rRNA gene sequencing used as the reference. A total of 239 urogenital specimens (135 *M. genitalium*-positive and 104 *M. genitalium*-negative specimens) collected between April and December 2019 at the French National Reference Center for Bacterial Sexually Transmitted Infections were assessed. The overall agreement for *M. genitalium* detection of the three commercial kits compared with the in-house real-time PCR was 94.6 to 97.6%, and there was no significant difference. A total of 97 specimens were found to be *M. genitalium* positive with the three kits and were used to assess macrolide resistance detection. The clinical sensitivities for resistance detection were 74.5% (95% confidence interval, 61.7 to 84.2%), 96.2% (87.2 to 99.0%), and 92.8% (82.7 to 97.1%) for the Allplex MG & AziR, Macrolide-R/MG ELITe MGB, and ResistancePlus MG FleXible kits, respectively. The sensitivity of the Macrolide-R/MG ELITe MGB kit was significantly higher than that of the Allplex MG & AziR kit. The clinical specificity for resistance detection of the three kits was 97.4 to 97.6%. The random-access possibility, input sample volume, and DNA extract availability for detecting resistance to other antibiotics may also influence the selection of a commercial kit by diagnostic laboratories.

KEYWORDS *Mycoplasma genitalium*, detection, commercial kits, antibiotic resistance, macrolides

Mycoplasma genitalium is responsible for sexually transmitted infections (STIs), for which macrolides are generally the first-line treatment. However, the frequency of macrolide-resistant *M. genitalium* is increasing worldwide (1, 2), compromising the effectiveness of azithromycin. As a consequence, detection of macrolide resistance-associated mutations is recommended for all *M. genitalium*-positive specimens, according to the European guidelines for *M. genitalium* infection (3), the BASHH United Kingdom national guidelines for the management of *M. genitalium* (4), and the Australian STI management guidelines for primary care (http://www.sti.guidelines.org.au/sexually-transmissible-infections/mycoplasma-genitalium). Also, kits that detect *M. genitalium* concurrently with macrolide resistance-associated mutations are needed to implement the resistance-guided sequential therapy recommended in Australia (http://www.sti.guidelines.org.au/sexually-transmissible-infections/mycoplasma-genitalium) and

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the United Kingdom (4). This sequential therapy relies on the initial use of doxycycline, immediately followed by the administration of azithromycin or moxifloxacin, depending on the macrolide susceptibility of the *M. genitalium* strain. Several such commercial kits have recently been launched. The first commercialized assay from SpeeDx (Australia) has been evaluated by several teams (5–9) and was adapted for the GeneXpert system (Cepheid, USA) (10–12). We also recently evaluated two other assays that detect *M. genitalium* concurrently with macrolide resistance developed by PathoFinder and Diagenode (9). Several other kits have subsequently been developed.

In the present study, we evaluated the performance of three commercial real-time PCR kits for the detection of *M. genitalium* and macrolide resistance-associated mutations: the Allplex MG & AziR kit (Seegene, Republic of Korea), the Macrolide-R/MG ELITe MGB kit (ELITechGroup, USA), and the ResistancePlus MG FleXible kit (SpeeDx, Australia). The *M. genitalium* detection performance of the three commercial kits was compared to that of an in-house TaqMan PCR assay targeting the MgPa adhesin gene (13). The clinical sensitivity and specificity for the detection of macrolide resistance-associated mutations were determined in comparison with 23S rRNA Sanger sequencing used as a reference method. Finally, the handling characteristics of the three commercial kits were compared to assist kit selection by diagnostic laboratories according to their routine need.

MATERIALS AND METHODS

Clinical specimens. Between April and December 2019, 250 remnants of clinically collected specimens (146 *M. genitalium*-positive and 104 *M. genitalium*-negative specimens) in universal transport medium (UTM) or in Cobas PCR medium (Roche Molecular Systems) received at the French National Reference Center for Bacterial Sexually Transmitted Infections in the Bacteriology Department of Bordeaux University Hospital (France) were systematically collected if the available volume was ≥1.5 ml. The specimens were stored at −80°C until testing. Eleven *M. genitalium*-positive specimens were excluded because the reference 23S rRNA sequencing assay did not yield results. Overall, 239 clinical specimens, 135 *M. genitalium* positive and 104 *M. genitalium* negative, were analyzed. They comprised 108 cervicovaginal swabs, 13 first-void urine samples, and 1 intrauterine device from 122 women and 98 first-void urine samples, 14 rectal swabs, and 5 urethral swabs from 117 men.

Detection of *M. genitalium* and macrolide resistance by in-house real-time PCR and 23S rRNA sequencing. DNA was extracted from 200 μl of the specimen using the MagNA Pure 96 DNA and viral NA small-volume kit on the MagNA Pure 96 instrument (Roche Diagnostics), yielding an elution volume of 100 μl. Prior to DNA extraction from urine specimens, 10 μl of ASTI IC(2) (Seegene) was spiked into 190 μl of the specimen according to the manufacturer's instructions. The in-house TaqMan real-time PCR targeting the *M. genitalium* MgPa adhesin gene was performed as described previously for a total of 40 cycles (9, 13). For *M. genitalium* 23S rRNA sequencing, a 266-bp fragment of domain V of the 23S rRNA gene was amplified from 5 μl of DNA as previously described (9). The PCR products were sent to Eurofins Genomics (Germany) for Sanger sequencing. Sequencing data were analyzed using BioEdit version 7.2.5 software (Isis Pharmaceuticals, USA) and were compared to the sequence of wild-type *M. genitalium* G37 (14).

Detection of *M. genitalium* and macrolide resistance using the commercial kits. (i) Allplex MG & AziR kit. The Allplex MG & AziR assay (Seegene, Republic of Korea) is a CE-IVD-marked multiplex real-time PCR assay that detects *M. genitalium* (undisclosed target) and six mutations in the 23S rRNA gene associated with macrolide resistance (A2058G, A2058C, A2058T, A2059G, A2059C, and A2059T [*Escherichia coli* numbering]) (Table 1). An endogenous human gene is detected as an internal control, except in urine specimens, to which the exogenous ASTI IC(2) internal control is added prior to DNA extraction. The Allplex MG & AziR kit was validated using urine, genital swabs, and liquid-based cytology. This assay was performed using 5 μl of DNA extract and a CFX96 real-time PCR system (Bio-Rad) according to the manufacturer's instructions. Data were analyzed using Seegene viewer software version 1.6.

(ii) Macrolide-R/MG ELITe MGB kit. The ELITe InGenius platform (ELITechGroup) is a fully automated sample-to-result PCR system that performs nucleic acid extraction and real-time PCR amplification directly from primary patient specimens. The Macrolide-R/MG ELITe MGB kit is a CE-IVD-marked assay that uses the ELITe InGenius platform. This assay targets the 23S rRNA gene to detect *M. genitalium* and five mutations associated with macrolide resistance (A2058G, A2058C, A2058T, A2059G, and A2059C) (Table 1). An endogenous human gene is detected as an internal control, and the exogenous CPE internal control is used for urine specimens. The Macrolide-R/MG ELITe MGB kit was validated on urine. Validation is ongoing on cervical and vaginal swabs. Primary specimens (200 μl) were loaded onto the ELITe InGenius platform according to the manufacturer's instructions, and data were analyzed using ELITe InGenius software version 1.3.0.12.

(iii) ResistancePlus MG FleXible kit. The ResistancePlus MG FleXible kit (SpeeDx, Australia) utilizes single-use FleXible cartridges. The kit was adapted from the ResistancePlus MG kit (SpeeDx) for the GeneXpert system (Cepheid), a fully automated sample-to-result PCR system that performs nucleic acid extraction and real-time PCR amplification directly from primary patient specimens. The ResistancePlus

TABLE 1 Main characteristics of the three commercial assays

Parameter	Allplex MG & AziR (Seegene)	Macrolide-R/MG ELITe MGB (ELITech)	ResistancePlus MG FleXible (SpeeDx)
M. genitalium detection target	Undisclosed	23S rRNA gene	MgPa adhesin gene
23S rRNA macrolide resistance-associated mutations detected	6 mutations, A2058G/C/T and A2059G/C/T	Wild type and 5 mutations, A2058G/C/T and A2059G/C	4 mutations, A2058G/C/T and A2059G
Input sample vol (μl)	200	200	1,000
DNA extract available for other purposes	Yes	Yes	No
IC[a]	Yes	Yes	Yes
Automation steps	Amplification and analysis	Nucleic acid extraction, PCR setup, amplification, and analysis	Cartridge (DNA extraction and amplification) and analysis
Type and no. of reactions/run[b]	Batches; up to 94 samples/PCR run	Batches, 12 tests (can run distinct molecular tests)	Random access; up to 16 depending on the apparatus (can run distinct molecular tests)
Hands-on time (min)[c]	30–60, depending on the no. of samples	30	20
Test turnaround time	1 h 45 min, excluding DNA extraction	3 h, including DNA extraction	2 h, including DNA extraction
Instrument	Bio-Rad CFX 96	ELITe InGenius	Cepheid GeneXpert
Consumable(s) not included	DNA extraction PCR plate and caps (Bio-Rad)	ELITe InGenius consumables	None
Data analysis software	Seegene Viewer	ELITe InGenius	GeneXpert Dx system
List price/reaction (€), excluding taxes[d]	53	40	30

[a]Seegene and ELITech use an endogenous internal control (IC), but an additional exogenous IC is added for urine samples. SpeeDx uses only an exogenous internal control.
[b]Seegene and ELITech assays include positive and negative controls for the PCR step. Positive and negative controls have to be run for each lot number for the ELITech assay.
[c]Hands-on time includes time for specimen processing.
[d]Including DNA extraction and consumables.

MG FleXible assay is a CE-IVD multiplex PCR assay that targets the MgPa adhesin gene for the detection of *M. genitalium* and four 23S rRNA mutations associated with macrolide resistance (A2058G, A2058C, A2058T, and A2059G) (Table 1). The kit was validated on urine, vaginal, cervical, urethral, and rectal swabs. Prior to loading onto the GeneXpert system, the reagent chamber of the FleXible cartridge was filled with 44 μl of MG FleXible reaction mix, and 1 ml of the specimen plus 10 μl of internal control cells were added to the sample chamber, according to the manufacturer's instructions. Data were analyzed using GeneXpert Dx system version 5.1 software.

Data analysis. Because of the limited available volume of specimens, no retesting was performed in cases of invalid or system error results. Overall, positive, and negative agreements for *M. genitalium* detection were calculated for the kits using the in-house real-time PCR assay (13) as a reference. The kappa (κ) value was determined as a measure of the overall agreement. Sanger sequencing of 23S rRNA PCR products was considered the reference standard for detecting macrolide resistance-associated mutations. The clinical sensitivity and specificity for macrolide resistance-associated mutation detection were calculated together with the corresponding 95% confidence intervals (CIs). Statistical analysis was performed on the biostaTGV website (https://biostatgv.sentiweb.fr/).

RESULTS

Detection of *M. genitalium* by commercial assays. The 239 clinical specimens were concurrently tested using the three commercial assays and the in-house real-time PCR (13), used as a reference. There was no significant difference in overall agreements (Table 2), which were 94.6% (95% CI, 90.9 to 96.9%) for the Allplex MG & AziR assay, 95.7% (95% CI, 92.3 to 97.7%) for the Macrolide-R/MG ELITe MGB assay, and 97.6% (95% CI, 94.6 to 99.0%) for the ResistancePlus MG FleXible assay, yielding κ values of 0.89, 0.91, and 0.95, respectively.

The three commercial kits yielded several false-negative *M. genitalium* detection results compared with the in-house assay (Table 2; discrepant and concordant results are presented in Tables S1 and S2, respectively, in the supplemental material). Although not statistically significant, the Allplex MG & AziR kit showed a marginally lower positive agreement than the two other kits (91.0% compared to 94.8% and 96.0% for the Macrolide-R/MG ELITe MGB and ResistancePlus MG FleXible kits, respectively) (Table 2). The negative agreement was 97.0 to 100% for all three kits. The three commercial kits yielded invalid results or system errors (Table 2). The frequency of

TABLE 2 Evaluation of *M. genitalium* detection by the commercial kits and comparison with the in-house TaqMan PCR[a]

Commercial assay (manufacturer)	*M. genitalium* detection result	No. of samples with in-house TaqMan PCR result for *M. genitalium* detection			Overall % agreement (95% CI), κ value	Positive % agreement (95% CI)	Negative % agreement (95% CI)
		Positive	Negative	Total			
Allplex MG & AziR (Seegene)	Detected	111	1	112	94.6 (90.9–96.9), κ = 0.89	91.0 (84.6–94.9)	99.0 (94.7–99.8)
	Not detected	11	101	112			
	Total	122	102	224			
	Invalid	13	2	15			
Macrolide-R/MG ELITe MGB (ELITech)	Detected	128	3	131	95.7 (92.3–97.7), κ = 0.91	94.8 (89.7–97.5)	97.0 (91.5–99.0)
	Not detected	7	96	103			
	Total	135	99	234			
	Invalid	0	5	5			
ResistancePlus MG FleXible (SpeeDx)	Detected	121	0	121	97.6 (94.6–99.0), κ = 0.95	96.0 (91.1–98.3)	100 (95.6–100)
	Not detected	5	84	89			
	Total	126	84	210			
	Invalid	2	16	18			
	System error	7	4	11			

[a]CI, confidence interval.

specimens with no *M. genitalium* detection results was significantly lower for the Macrolide-R/MG ELITe MGB kit at 2.1% (5/239), compared to 6.3% (15/239) for the Allplex MG & AziR kit ($P = 0.02$ by a chi-squared test) and 12.1% (29/239) for the ResistancePlus MG FleXible kit ($P < 0.001$). Regarding the 11 specimens that had a system error result using the ResistancePlus MG FleXible kit on the GeneXpert system, 7 errors corresponded to a probe check failure for the *M. genitalium* or the internal control probe, 2 errors were associated with an anomaly in syringe pressure, and 2 errors were associated with technical issues of the GeneXpert instrument.

Detection of macrolide-resistant *M. genitalium* by commercial assays. Among the 135 *M. genitalium*-positive specimens used in this study, 97 were found to be *M. genitalium* positive by the three commercial kits and were thus used to assess their performance for macrolide resistance detection on the same set of specimens. Among the 97 specimens, 23S rRNA Sanger sequencing showed no macrolide resistance-associated mutation in 42 specimens (43.3%), whereas 55 (56.7%) harbored at least one mutation (Table 3). The A2059G mutation (*E. coli* numbering) was the most frequent, being present in 52.7% (29/55) of mutated specimens, followed by A2058G (13.4%; 13/55), A2058T (11.3%; 11/55), and A2062T (7.3%; 4/55). A total of 11 (20%) mutated specimens harbored a mixture of one or two mutations and a wild-type 23S rRNA sequence (Table 3), as determined by the presence of double peaks on sequencing chromatograms. Regarding the Macrolide-R/MG ELITe MGB kit, the software interprets each specimen for which the cycle threshold (C_T) for *M. genitalium* detection is over 37 as typing nonfeasible. However, according to the instructions for use, the experimenter can deduce a macrolide resistance result from the curve if the melting temperature (T_m) is <63°C. Among the 97 specimens, seven initially yielded a typing-nonfeasible result. The macrolide resistance status of five specimens could not be determined (Table 4), and two specimens were accurately determined to be macrolide resistant.

The overall agreements with 23S rRNA Sanger sequencing were 84.5% (95% CI, 76.0 to 90.4%) for the Allplex MG & AziR kit, 96.7% (95% CI, 90.8 to 98.8%) for the Macrolide-R/MG ELITe MGB kit, and 94.9% (95% CI, 88.5 to 97.8%) for the ResistancePlus MG FleXible kit (Table 4). The overall agreement of the Allplex MG & AziR kit was significantly lower than that of the Macrolide-R/MG ELITe MGB kit (κ value of 0.70, compared to 0.93 for the Macrolide-R/MG ELITe MGB kit). The clinical sensitivities for macrolide resistance detection were 74.5% (95% CI, 61.7 to 84.2%) for the Allplex MG & AziR kit, 96.2% (95% CI, 87.2 to 99.0%) for the Macrolide-R/MG ELITe MGB kit, and 92.8% (95% CI, 82.7 to 97.1%) for the ResistancePlus MG Flexible kit (Table 4). The sensitivities of the Macrolide-

TABLE 3 Macrolide resistance detection by the commercial kits compared with 23S rRNA Sanger sequencing[a]

Commercial assay (manufacturer)	23S rRNA mutation detection result	No. of specimens with 23S rRNA Sanger sequencing result(s)										
		A2058G	A2058G, WT	A2058G, A2062T, WT	A2058T	A2058T, A2062T, WT	A2059G	A2059G, WT	A2062T	A2062T, WT	WT	Total
Allplex MG & AziR (Seegene)	A2058G	7	3									10
	A2058T				9							9
	A2059G						18	4			1	23
	Not detected	2		1	1	1	6	1	1	1	41	55
Macrolide-R/MG ELITe MGB (ELITech)	Detected	9	3		10	1	23	5			1	52
	Not detected			1						1	38	40
	Typing not feasible						1		1		3	5
ResistancePlus MG FleXible (SpeeDx)	Detected	8	3		10	1	24	5			1	52
	Not detected	1		1					1	1	41	45
Total		9	3	1	10	1	24	5	1	1	42	97

[a]WT, wild type.

TABLE 4 Macrolide resistance detection performance of the three commercial kits[a]

Commercial assay (manufacturer)	23S rRNA mutation detection result	23S rRNA sequencing result			Overall % agreement (95% CI), κ value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
		Mutated	WT	Total			
Allplex MG & AziR (Seegene)	Detected	41	1	42	84.5 (76.0–90.4), κ = 0.70	74.5 (61.7–84.2)	97.6 (87.7–99.6)
	Not detected	14	41	55			
Macrolide-R/MG ELITe MGB (ELITech)	Detected	51	1	52	96.7 (90.8–98.9), κ = 0.93	96.2 (87.2–99.0)	97.4 (86.8–99.5)
	Not detected	2	38	40			
	Typing not feasible	2	3	5			
ResistancePlus MG FleXible (SpeeDx)	Detected	51	1	52	94.9 (88.5–97.8), κ = 0.90	92.8 (82.7–97.1)	97.6 (87.7–99.6)
	Not detected	4	41	45			

[a]WT, wild type; CI, confidence interval.

R/MG ELITe MGB and ResistancePlus MG Flexible kits were not significantly different, as indicated by overlapping CIs. In contrast, the sensitivity of the Allplex MG & AziR kit was significantly lower than that of the Macrolide-R/MG ELITe MGB kit. The Allplex MG & AziR kit missed 14.4% (14/97) of specimens containing macrolide-resistant *M. genitalium*. None of the three commercial kits detected macrolide resistance in the three specimens harboring either A2062T only, A2062T and a wild-type population, or A2062T, A2058G, and a wild-type population (Table 3). In contrast, the specimen harboring A2062T, A2058T, and a wild-type population was detected as mutated by the Macrolide-R/MG ELITe MGB kit and the ResistancePlus MG Flexible kit (Table 3).

There was no significant difference in clinical specificity among the three commercial kits, with clinical specificity ranging from 97.4 to 97.6% (Table 4). Each of the commercial kits detected only one wild-type specimen as mutated, which was not the same one for the three kits.

DISCUSSION

We assessed the clinical performance of three new commercial kits for the simultaneous detection of *M. genitalium* and macrolide resistance. Regarding *M. genitalium* detection, although the ResistancePlus MG FleXible kit had the highest overall agreement (97.6%; κ, 0.95), there was no significant difference among the three kits. The sensitivity for *M. genitalium* detection with the ResistancePlus MG FleXible kit is in agreement with a previous study reporting a sensitivity of 96.1% in urine and genital swabs (12). Regarding macrolide resistance detection, our results are in accordance with previous reports. A concordance of 99.5% with 23S rRNA sequencing was reported for the Macrolide-R/MG ELITe MGB assay during its development (15). The 92.8% sensitivity for macrolide resistance detection of the ResistancePlus MG FleXible kit is similar to a recent report of 94.1% (11) and a positive agreement with sequencing of 93% (12). Likewise, the specificity of 97.6% is in accordance with a previous report of 96% in comparison with sequencing (11). The lack of sensitivity of the Allplex MG & AziR assay is concerning; 14.4% (14/97) of the specimens yielded a false-susceptible result, suggesting a risk of treatment failure if azithromycin is subsequently administered.

A high percentage (12.1%) of specimens yielded invalid internal control or system error results using the ResistancePlus MG FleXible kit, consistent with a previous report of 11% (15/137) of specimens with invalid internal control or probe check failure results (12) but not with two other studies reporting <1.1% of specimens with invalid results (10, 11). A recent study also reported 7.8% (23/293) of specimens with failed analysis using the ResistancePlus MG FleXible kit (16). In the present study, among the 18 specimens with an invalid internal control result, amplification of the internal control was accurate for 7 (39%). The results for these seven specimens could thus have been interpreted as negative, in accordance with the reference in-house PCR assay results. However, these seven results were not considered in the agreement analysis because the instructions do not recommend interpreting the internal control curve.

Further development of the software may decrease the number of invalid results and reduce the number of specimens needing to be retested, thus reducing specimen wastage in routine diagnostic settings. Regarding the Macrolide-R/MG ELITe MGB kit, only 2.1% (5/239) of specimens reported invalid results for *M. genitalium* detection, but a further 5.2% (5/97) of specimens yielded a typing-nonfeasible result for macrolide resistance detection. Among the latter five specimens, three yielded proper amplification curves with a T_m of 65°C and could thus have been reported as wild-type *M. genitalium*. Revision of the instructions for use of the Macrolide-R/MG ELITe MGB kit may reduce the number of typing-nonfeasible results.

The influence of the specimen type (female urine, cervicovaginal swab, male urine, and male rectal swab) on *M. genitalium* detection and macrolide resistance detection was also analyzed (see Tables S3 and S4, respectively, in the supplemental material). In women, although the percentage of overall agreement appears lower in urine than in cervicovaginal swabs for both *M. genitalium* detection and macrolide resistance detection using the three commercial kits, the difference was not significant, possibly due to the small number of female urine samples included in this study. Nevertheless, it was previously shown that first-void urine specimens from women should be avoided for *M. genitalium* detection, notably because of lower *M. genitalium* loads than in cervicovaginal swabs (17, 18). Regarding male rectal swabs, which were validated only with the ResistancePlus MG FleXible kit, and male urine, there was no significant difference in performance for *M. genitalium* detection and macrolide resistance detection among the three commercial kits.

All assays detected a defined set of four to six macrolide resistance-associated mutations at positions A2058 and A2059 (Table 1), but none were designed to detect mutations at position A2062. As expected, no kit detected as mutated the two specimens harboring an A2062T mutation that was not associated with an additional mutation at position A2058. In this study, the A2062T mutation was present in 7.3% (4/55) of specimens harboring mutated *M. genitalium*. This suggests that up to 7.3% of *M. genitalium*-positive specimens could be falsely categorized as 23S rRNA wild type. To date, the A2062T and A2062C mutations have been reported in *M. genitalium* 23S rRNA with a frequency ranging between 2.3% and 7.7% of mutated *M. genitalium* strains (8, 19, 20), and the A2062T mutation has recently been associated with a case of pristinamycin treatment failure (21). In addition, the A2062G substitution has been associated with josamycin treatment failure (22) and might be associated with an increased pristinamycin MIC, as this is the case in *Mycoplasma pneumoniae*, the *Mycoplasma* species phylogenetically closest to *M. genitalium* (23). Considering the difficulty in growing *M. genitalium*, further studies of treatment failure using sequencing instead of real-time-PCR-based commercial kits to detect 23S rRNA mutations are required.

Importantly, the Allplex MG & AziR kit and the ResistancePlus MG FleXible kit target either an undisclosed target or the MgPa adhesin gene to detect *M. genitalium* and do not include a 23S rRNA amplification control. Because the amplification efficacy may differ between the MgPa and the 23S rRNA genes, the ResistancePlus MG FleXible kit may not be able to distinguish wild-type and mutated *M. genitalium* strains for which 23S rRNA amplification failed. This limitation has previously been reported for this kit (12) and others (8, 9). In contrast, the Macrolide-R/MG ELITe MGB kit targets 23S rRNA for the detection of *M. genitalium* and macrolide resistance-associated mutations. This eliminates the risk of false-macrolide-susceptible results caused by a lack of amplification of 23S rRNA.

We also evaluated the handling characteristics of the kits. The input sample volume varies from 200 μl for the Allplex MG & AziR kit and the Macrolide-R/MG ELITe MGB kit to 1,000 μl for the ResistancePlus MG FleXible kit. Such a large sample volume may be a limiting factor for some types of specimens. It also hampers retesting in cases of invalid results and limits the possibility of the addition of other molecular diagnostic tests on the same sample. It should be noted that the ResistancePlus MG FleXible kit is

the only kit validated on rectal swabs. The three commercial kits have a short workflow sequence, requiring the mixing of only two to four reagents. Unlike Sanger sequencing, the commercial kits provide *M. genitalium* and macrolide resistance-associated mutation detection results within 2 to 3.5 h (Table 1). One convenient feature of the ResistancePlus MG FleXible kit is the possibility of random access; in contrast, the Allplex MG & AziR and Macrolide-R/MG ELITe MGB kits are restricted to batch testing of 94 and 12 specimens, respectively. However, the ELITe InGenius instrument, like the GeneXpert instrument, can run several molecular diagnostic tests simultaneously. Regarding data analysis, the manufacturers of the three kits provide proprietary software for interpreting the results. Using the ResistancePlus MG FleXible and the Macrolide-R/MG ELITe MGB kits, the experimenter can check the amplification curves to validate a result, which is not possible with the Allplex MG & AziR kit. Importantly, DNA extracts can be collected and reused for other purposes with the Allplex MG & AziR and the Macrolide-R/MG ELITe MGB kits but not with the ResistancePlus MG FleXible kit. Detection of fluoroquinolone resistance-associated mutations, for example, is frequently requested for *M. genitalium*-positive specimens and requires a DNA sample for Sanger sequencing pending the availability of commercial kits (24, 25).

In conclusion, the three kits showed good performance for the detection of *M. genitalium*, but the Allplex MG & AziR kit showed lower sensitivity for detecting macrolide resistance-associated mutations. The handling characteristics of the kits, notably random access, input sample volume, number of samples per run, and availability of DNA samples for the detection of fluoroquinolone resistance-associated mutations or for detection of other microorganisms by molecular methods, will guide kit selection by diagnostic laboratories.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

SUPPLEMENTAL FILE 2, PDF file, 0.05 MB.

SUPPLEMENTAL FILE 3, PDF file, 0.1 MB.

SUPPLEMENTAL FILE 4, PDF file, 0.1 MB.

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SpeeDx provided the ResistancePlus MG FleXible assay. All instruments were made available by the manufacturers, who had no role in data collection or interpretation.

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