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Simultaneous identification of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* – multicenter evaluation of the Alinity m STI assay

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Abstract

Objectives: Accurate and rapid diagnosis of sexually transmitted infections (STIs) is essential for timely administration of appropriate treatment and reducing the spread of the disease. We examined the performance of the new Alinity m STI assay, a qualitative real-time multiplex PCR test for simultaneous identification of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Mycoplasma genitalium* (MG), and *Trichomonas vaginalis* (TV) run on the fully automated Alinity m platform.

Methods: This international, multicenter study evaluated the accuracy, reproducibility, and clinical performance of the Alinity m STI assay compared to commonly used STI assays in a large series of patient samples encountered in clinical practice.

Results: The Alinity m STI assay identified accurately and precisely single and mixed pathogens from an analytical panel of specimens. The Alinity m STI assay demonstrated high overall agreement rates with comparator STI assays (99.6% for CT [n=2,127], 99.2% for NG [n=2,160], 97.1% for MG [n=491], and 99.4% for TV [n=313]).

Conclusions: The newly developed Alinity m STI assay accurately detects the 4 sexually transmitted target pathogens in various collection devices across clinically relevant specimen types, regardless of single or mixed infection status.

Keywords: molecular diagnostics; nucleic acid amplification test; screening; sexually transmitted infection.

Emily Goldstein and Laura Martínez-García contributed equally to this work.

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Introduction

The World Health Organization (WHO) estimates that more than 1 million individuals contract a sexually transmitted infection (STI) every day [1]. The most common STI pathogens are *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) [2, 3]. The medical community's understanding of diseases associated with STIs is rapidly advancing, as evidenced by the recent classification of *Mycoplasma genitalium* (MG) as an emerging STI pathogen by the Centers for Disease Control and Prevention (CDC) [4] and European guidelines on the diagnosis and treatment of MG infections [1, 5, 6]. Urogenital infections due to CT, NG, MG, or TV can have severe consequences for reproductive health and increase the risk of acquiring or transmitting HIV [6–10]. Extragenital CT and NG infections in the oropharynx and rectum in men

and women can serve as reservoirs, potentially leading to future infections and severe clinical complications [11–14].

Diagnosis of STIs can be challenging, as most of these infections in urogenital and extragenital sites are asymptomatic, and symptomatic presentations often overlap but can have varied etiology. Current empirical treatment paradigms may result in undiagnosed MG and NG infections, contributing to the rise of resistant strains [15]. Accurate and rapid diagnosis of STIs is essential for timely administration of pathogen-specific treatment and reduction in the spread of disease. Nucleic acid amplification tests (NAATs) for CT and NG infection are part of national screening programs [16, 17], and are recommended for the diagnosis of MG [6, 18] and TV [19] infections. Currently available commercial NAATs are single or multiplex assays, some of which utilize manufacturer-specific specimen collection devices. Laboratories may employ multiple tests, often sequentially, to identify the causative pathogen, which can prolong the time to diagnosis and delay initiation of treatment.

To address these challenges, Abbott Molecular recently introduced the Alinity m STI assay, a single-reaction qualitative multiplex polymerase-chain reaction (PCR) test for simultaneous detection and differentiation of nucleic acids from CT, NG, MG, and TV. The Alinity m is an automated, continuous, and random-access molecular diagnostic analyzer with a processing capacity of 300 samples per 8-h shift, reporting of results within less than 120 min following sample aspiration, and the ability to immediately process urgent (STAT) samples [20].

Here, we report results from the first international multicenter field study of the Alinity m STI assay in terms of analytical performance and comparison to commercially available molecular tests routinely used for the detection of CT, NG, MG, and TV in clinical specimens.

Materials and methods

Study design

In this international, multicenter study, the analytical performance of the Alinity m STI assay (Abbott Molecular, Des Plaines, IL, USA) was assessed by testing a contrived STI panel across the study sites. The clinical performance of the Alinity m STI assay was evaluated by testing remnant unselected de-identified patient samples obtained by the study sites for the presence of CT, NG, MG, or TV, and comparing results with those from the molecular assay platforms in routine use at the time of the study. All clinical specimens were sourced per site policies, procedures, and applicable local regulations, including approval by an Independent Ethics Committee or waiver.

Participating study sites

Six independent International Standard Organization (ISO)-accredited clinical laboratories in Glasgow, United Kingdom (West of Scotland Specialist Virology Centre); Berlin, Germany (Medizinisches Infektiologiezentrum Berlin); Madrid, Spain (Hospital Universitario Ramón y Cajal); Padua, Italy (Azienda Ospedaliera di Padova); Johannesburg, South Africa (Lancet Laboratories); and Melbourne, Australia (Victorian Infectious Diseases Reference Laboratory) participated in the study.

Alinity m STI assay

The Alinity m STI assay detects ribosomal RNA sequences from CT, TV, and MG, and genomic DNA from NG. The assay utilizes an endogenous human DNA sequence as a validity control for sample adequacy, sample extraction, and amplification efficiency as well as an exogenous armored RNA internal control to confirm the absence of PCR inhibition during amplification. The Alinity m STI assay may be used to test urine, endocervical and vaginal swabs collected in Alinity m multi-Collect Specimen Collection tubes (referred to as Alinity mSC tubes), and gynecological specimens sampled in PreservCyt solution for the detection of CT, NG, and TV. For MG, the assay may be used to test endocervical swabs collected in Alinity mSC tubes. The Alinity m STI assay detects the 4 targeted organisms with the following limits of detection (LOD) per assay in specimen types validated by the manufacturer: CT, 0.5 Inclusion Forming Units; NG, 1.5 Colony Forming Units; TV, 0.02 Trophozoite; and MG, 33 genome equivalents. The Alinity m software allows selection of any single or combination of the 4 analytes for result reporting. Results not chosen for reporting upon initial testing by the laboratory can be "unmasked" at a later point in time, if required.

Analytical performance

The analytical performance of the Alinity m STI assay was assessed with a 5-member STI panel prepared from cultured microorganisms spiked into Alinity mSC tubes supplied to the 6 study sites by Abbott Molecular. One panel member per analyte and 1 panel member containing CT, NG, MG, and TV set at concentrations 2-times the claimed LOD each were stored at −20 or −80 °C from preparation to testing. A total of 6 replicates for each panel member were tested over 2 days (3 replicates per day) at each study site.

Clinical specimens and STI comparator tests

To compare the clinical performance of the Alinity m STI assay to other commercial molecular STI assays, remnant unselected urine, urogenital, and extragenital swab samples were used at 5 participating study centers. All patient specimens were collected, processed, and tested for the presence of CT, NG, MG, or TV with comparator tests (Table 1): (i) Aptima Combo 2® and (ii) Aptima® *M. genitalium* assays (Hologic, Inc., San Diego, CA, USA); (iii) RealTi*m*e CT/NG (Abbott Molecular; Inc., Des Plaines, IL, USA); (iv) Allplex™ STI Essential Assay and (v) Anyplex™ II STI 7 Detection V1.1 (Seegene Inc., Seoul, Korea); (vi) genesig® Advanced CT and NG kits, Primerdesign™ Ltd (Yorkhouse, United Kingdom); and (vii) Vaginitis and Vaginosis 8-well Panel (AusDiagnostics, Mascot NSW, Australia), following the

respective manufacturers' instructions or per the laboratory's standard operating procedures for specimen handling (Table 2).

Statistical analysis

Detection rates for each of the analytes, mean target cycle threshold numbers, and corresponding standard deviations observed with the analytical performance panel were assessed. Concordance for each of the 4 pathogens identified by Alinity m STI assay and comparator assays stratified by sample types was estimated by calculating positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA), with 95% confidence intervals (CI) and kappa values. All analyses were performed using PC SAS version 9.3 (SAS, Cary, NC, USA).

Results

Analytical performance of the Alinity m STI assay

The Alinity m STI assay accurately identified the analytes present in all 5 members of the analytical STI panel tested across study sites (Table 3). The difference in the mean Ct for each analyte in the presence of the other 3 analytes, compared to those from samples containing a single analyte, ranged between 0.2 and 0.5 cycles. The maximum standard deviations observed with single and multiple analyte panel members were 0.57 and 0.62 cycles, respectively.

Clinical performance of Alinity m STI compared to routine test methods

Chlamydia trachomatis

A total of 2,127 Alinity m STI CT results from 5 study sites were compared with those from either Aptima Combo 2 (AC2), RealTi*m*e CT/NG (RT-CTNG), Allplex, or genesig CT assays (Table 4). Overall, 2,118/2,127 samples showed concordant results with both Alinity m and the respective comparator assay, yielding an OPA of 99.6% (range 99–100% [95% CI 99.2–99.8%]; kappa 0.97 [range 0.93–1.00]) for the detection of CT in urine and urogenital or extragenital swab samples.

Table 1: STI assay platforms compared in the study.

Manufacturer	Assay	Analyzer	CT	NG	MG	TV	Process control	Cellular control
Abbott	Alinity m STI	Alinity m System	rRNA	Genomic DNA	rRNA	rRNA	x	x
Abbott	RealTi*m*e CT/NG	*m*2000 System (*m*2000sp/rt)	Cryptic plasmid	Genomic DNA: Opa gene			x	
Hologic	Aptima Combo 2	Panther System	23s rRNA	16s rRNA				
Hologic	Aptima *Mycoplasma genitalium* assay	Panther System			16s rRNA		x	
Seegene	Allplex STI Essential assay[a]	Extraction: StarMag 96 × 4/Hamilton MICROLAB STARlet PCR: CFX 96 RT System[c]	Genomic DNA	Genomic DNA	Genomic DNA	Genomic DNA	x	
Seegene	Anyplex II STI-7 Detection V1.1[a]	Extraction: Quick DNA/RNA Magbead kit/ Freedom EVO 1000 PCR: CFX 96 RT System[c]	Genomic DNA	Genomic DNA	Genomic DNA	Genomic DNA	x	
Primerdesign Ltd	genesig Advanced CT and NG Kits	Extraction: MagnaPure 96 System Plate set-up: Microlab NIMBUS PCR: LightCycler 480 II[c]	Cryptic Plasmid: Helicase (DnaB) gene	Genomic DNA: PorA gene			x	x
AusDiagnostics	Vaginitis and Vaginosis 8-well Panel[b]	High-Plex 24 System				Genomic DNA	x	x

CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; MG, *Mycoplasma genitalium*; TV, *Trichomonas vaginalis*; rRNA, ribosomal ribonucleic acid; DNA, deoxyribonucleic acid. [a]Detection of additional microorganisms: *Mycoplasma hominis* (MH), *Ureaplasma parvum* (UP), *Ureaplasma urealyticum* (UU). [b]Detection of additional microogranisms: *Candida albicans*, *glabrata*, *parapsilosis* (including *C. orthopsilosis* and *C. metapsilosis*), and *krusei*; *Gardnerella vaginalis*; *Atopobium vaginae*; *Lactobacillus iners* and *crispatus*. [c]Instrumentation used in this study.

Table 2: Molecular diagnostic analyzers and assays used at each study site for clinical specimens.

Study site	Assays	Specimen type[a]	Population	Specimen handling preceding testing with Alinity m STI assay
1	Allplex STI essential assay	Urine (neat)	Symptomatic patients	Transportation at room temperature. Vortexed upon arrival, followed by aliquot transfer into Alinity mSC tubes[e] and storage at −20 °C for up to 12 weeks.
2	Genesig advanced CT and NG Kits	Urine (neat)	STI screening	Transportation and storage at room temperature until completion of routine testing. Subsequent storage at 2–8 °C for up to 48 h prior to vortexing, aliquot transfer into Alinity mSC tubes, and immediate testing.
3	Aptima Combo 2 Aptima *Mycoplasma genitalium* assay	Urine (male); (Aptima Urine Collection Kit, Hologic) Swabs (Aptima Unisex Swab Specimen Collection Kit, Hologic)	HIV-1 infected MSM[d]	Subset (n=120): no storage prior to testing. Subset (n=46): storage at 2–8 °C for up to 25 days.
4	RealTi*m*e CT/NG	Urine (male) Swabs (female) (*m*2000 multi-Collect Specimen Collection Kit, Abbott)	STI screening	Transportation and storage at room temperature, followed by storage at −20 °C for up to 90 days.
5	RealTi*m*e CT/NG Anyplex II STI-7 Detection V1.1[b]	Urine (neat) Swabs (Amies charcoal; Copan)	STI screening	Urine aliquoted into *m*2000 mSC tubes[f] Swabs stored following manufacturer's instructions, swirled in *m*2000 mSC tubes. Storage at 4 °C for up to 7 days or at −70 °C for longer term.
	Vaginitis and Vaginosis 8-well Panel[c]	Swabs (Swab Elution tubes; AusDiagnostics)	STI screening	Storage at 4 °C for up to 7 days or at −70 °C for longer term.

[a]Specimen type (collection device) delivered to the laboratory. [b]Used for MG detection. [c]Used for TV detection. [d]Men who have sex with men. [e]Alinity m multi-Collect Specimen Collection tubes (Abbott). [f]*m*2000 multi-Collect Specimen Collection tubes (Abbott).

Table 3: Analytical performance of the Alinity m STI assay tested with STI panels across study sites.

STI panel member	Alinity m STI (Valid replicates)									CT+		NG+		MG+		TV+	
	CT +	CT −	NG +	NG −	MG +	MG −	TV +	TV −	Total	Ct mean	Ct SD	Ct mean	Ct SD	Ct mean	Ct SD	Ct mean	Ct SD
CT+ single analyte	36	0	0	36	0	36	0	36	144	29.2	0.25						
NG+ single analyte[a]	0	35	35	0	0	35	0	35	140			31.1	0.54				
MG+ single analyte	0	36	0	36	36	0	0	36	144					32.4	0.43		
TV+ single analyte	0	36	0	36	0	36	36	0	144							27.3	0.57
CT+, NG+, TV+, MG+ multi-analyte[b]	35	0	35	0	35	0	35	0	140	29.0	0.28	30.7	0.62	31.9	0.49	27.1	0.61
Total	71	107	70	108	71	107	71	107	712								

CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; MG, *Mycoplasma genitalium*; TV, *Trichomonas vaginalis*; Ct, cycle threshold; SD, standard deviation; STI, sexually transmitted infection. Five panel members for the 4 analytes were run in triplicate over 2 days at 6 study sites. [a]One result not reported due to pipettor aspiration error. [b]One invalid sample.

PPA and NPA were 96.5% (95% CI 92.6–98.4%) and 99.8% (95% CI 99.5–99.9%), respectively.

Neisseria gonorrhoeae

A total of 2,160 Alinity m STI NG results from 5 study sites were compared to results from either AC2, RT-CTNG, Allplex, or genesig NG assays (Table 5). Overall, there were 2,143/2,160 concordant samples with the Alinity m STI and comparator assays, with an OPA of 99.2% (range 99.1–100% [95% CI 98.7–99.5%]; kappa: 0.91 [range 0.88–0.93]) for the detection of NG in urine and urogenital or extragenital swab samples. PPA and NPA were 93.9% (95% CI 87.4–97.2%) and 99.5% (95% CI 99.0–99.7%), respectively.

Table 4: Performance of the Alinity m STI assay and comparator assays for *C. trachomatis*.

Assay	Specimen type	n	Comparator + Alinity m +	Comparator + Alinity m −	Comparator − Alinity m +	Comparator − Alinity m −	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)	Kappa
Aptima Combo 2	Urine	166	15	1	0	150	93.8 (71.7, 98.8)	100.0 (97.5, 100.0)	99.4 (96.7, 99.9)	0.96
	Swabs[a]	98	11	1	0[e]	86[f]	91.7 (64.6, 98.5)	100.0 (95.7,100.0)	99.0 (94.4,99.8)	0.95
RealTi*m*e CT/NG	Urine	426	39	2	0	385	95.1 (83.9, 98.7)	100.0 (99.0, 100.0)	99.5 (98.3, 99.9)	0.97
	Urogenital swabs[b]	679	48	0	0	631	100.0 (92.6, 100.0)	100.0 (99.4, 100.0)	100.0 (99.4, 100.0)	1
	Extragenital swabs[c]	360	25	2[g]	0	333	92.6 (76.6, 97.9)	100.0 (98.9, 100.0)	99.4 (98.0, 99.8)	0.96
	Swabs[a]	3	1	0	0	2	100.0 (20.7, 100.0)	100.0 (34.2, 100.0)	100.0 (43.9, 100.0)	1
Allplex STI essential assay	Urine[d]	198	12	0	1	185	100.0 (75.8, 100.0)	99.5 (97.0, 99.9)	99.5 (97.2, 99.9)	0.96
Genesig advanced CT kit	Urine[d]	197	15	0	2	180	100.0 (79.6, 100.0)	98.9 (96.1, 99.7)	99.0 (96.4, 99.7)	0.93
Total		2,127	166	6	3	1952	96.5% (92.6, 98.4)	99.8% (99.5, 99.9)	99.6% (99.2, 99.8)	0.97

PPA, positive percent agreement; NPA, negative percent agreement; OPA, overall percent agreement. [a]Anatomic site not specified. [b]Endocervical, cervical, genital, vaginal, vulvovaginal, penile, urethral. [c]Rectal, oropharyngeal. [d]Urine samples transferred into Abbott Alinity m multi-Collect Specimen Collection tubes prior to testing with Alinity m STI assay. [e]Two Aptima Combo 2 *C. trachomatis* gray zone results with Alinity m CT positive results were excluded from the analysis. [f]One Aptima Combo 2 *C. trachomatis* gray zone results with Alinity m CT negative result was excluded from the analysis. [g]Rectal.

Table 5: Performance of the Alinity m STI assay and comparator assays for *N. gonorrhoeae*.

Assay	Specimen type	n	Comparator + Alinity m +	Comparator + Alinity m −	Comparator − Alinity m +	Comparator − Alinity m −	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)	Kappa
Aptima Combo 2	Urine	158	5	1	0	152	83.3 (43.6, 97.0)	100.0 (97.5, 100.0)	99.4 (96.5, 99.9)	0.91
	Swabs[a]	100	29	0	3	68[e]	100.0 (88.3, 100.0)	95.8 (88.3, 98.6)	97.0 (91.5, 99.0)	0.93
RealTime CT/NG	Urine	430	16	1	3	410	94.1 (73.0, 99.0)	99.3 (97.9, 99.8)	99.1 (97.6, 99.6)	0.88
	Urogenital swabs[b]	714	9	1[f]	1[f]	703	90.0 (59.6, 98.2)	99.9 (99.2, 100.0)	99.7 (99.0, 99.9)	0.90
	Extragenital swabs[c]	360	25	2[g]	3[h]	330	92.6 (76.6, 97.9)	99.1 (97.4, 99.7)	98.6 (96.8, 99.4)	0.90
	Swabs[a]	3	0	0	0	3	–	100.0 (43.9, 100.0)	100.0 (43.9, 100.0)	–
Allplex STI essential assay	Urine[d]	198	5	0	1	192	100.0 (56.6, 100.0)	99.5 (97.1, 99.9)	99.5 (97.2, 99.9)	0.91
Genesig advanced NG kit	Urine[d]	197	4	1	0	192	80.0 (37.6, 96.4)	100.0 (98.0, 100.0)	99.0 (97.2, 99.9)	0.89
Total		2,160	93	6	11	2,050	93.9% (87.4, 97.2)	99.5% (99.0, 99.7)	99.2% (98.7, 99.5)	0.91

PPA, positive percent agreement; NPA, negative percent agreement; OPA, overall percent agreement. [a]Anatomic site not specified. [b]Endocervical, cervical, genital, vaginal, vulvovaginal, penile, urethral. [c]Rectal, oropharyngeal. [d]Urine samples transferred into Abbott Alinity m multi-Collect Specimen Collection tubes prior to testing with Alinity m STI assay. [e]One Aptima Combo 2 *N. gonorrhoeae* gray zone result with Alinity m NG negative result was excluded from the analysis. [f]Vaginal. [g]One rectal and 1 oropharyngeal. [h]One rectal and 2 oropharyngeal.

Mycoplasma genitalium

A total of 491 Alinity m STI MG assay results reported from 3 study sites were compared against results from Aptima *M. genitalium* (Aptima MG), Allplex, and Anyplex assays (Table 6). Overall, 477/491 samples showed concordant results with both Alinity m and the comparator assay, yielding an OPA of 97.1% (range 88.2–100% [95% CI 95.3–98.3%]; kappa 0.90 [range 0.77–1.00]) for the detection of MG in urine and urogenital or extragenital swab samples. PPA and NPA were 90.1% (95% CI 81.7–94.9%) and 98.5% (95% CI 96.8–99.3%), respectively.

Trichomonas vaginalis

For detection of TV, 313 Alinity m STI assay results reported from 3 study sites were compared to results from Allplex and Anyplex assays, and the Vaginitis and Vaginosis 8-well Panel (Table 7). 311/313 samples showed concordant results with both Alinity m STI and comparator assays, with an OPA of 99.4% (range 90–100%; [95% CI 97.7–99.8%]; kappa 0.96 [range 0.96–1.00]) for the detection of TV in urine and urogenital or extragenital swab samples. PPA and NPA were 96.3% (95% CI 81.7–99.3%) and 99.7% (95% CI 98.0–99.9%), respectively.

Detection of mixed infections

The Alinity m STI assay identified more than 1 of the 4 targeted pathogens in 32 specimens in the study population (Table 8). Among these, the Alinity m STI assay found 19 CT/NG dual infections (59.4%), 7 CT/MG dual infections (21.9%), 2 NG/MG dual infections (6.3%), 2 TV/MG dual infections (6.3%), and 2 triple infections (CT/NG/TV and CT/MG/TV). In contrast, 23 samples with multiple infections were identified by the comparator tests.

Table 6: Performance of the Alinity m STI assay and comparator assays for *M. genitalium*.

Assay	Specimen type	n	Comparator + Alinity m +	Comparator + Alinity m −	Comparator − Alinity m +	Comparator − Alinity m −	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)	Kappa
Aptima MG	Urine	60	8	0	0	52	100.0 (67.6, 100.0)	100.0 (93.1, 100.0)	100.0 (94.0, 100.0)	1
	Swabs[a]	101	20	7	0	74	74.1 (55.3, 86.8)	100.0 (95.1, 100.0)	93.1 (86.4, 96.6)	0.81
Allplex STI essential assay	Urine[b]	198	6	0	2	190	100.0 (61.0, 100.0)	99.0 (96.3, 99.7)	99.0 (96.4, 99.7)	0.85
Anyplex II STI-7 detection V1.1	Urine	55	26	1	1	27	96.3 (81.7, 99.3)	96.4 (82.3, 99.4)	96.4 (87.7, 99.0)	0.93
	Urogenital swabs[c]	60	6	0	1[e]	53	100.0 (61.0, 100.0)	98.1 (90.2, 99.7)	98.3 (91.1, 99.7)	0.91
	Extragenital swabs[d]	17	7	0	2[f]	8	100.0 (64.6, 100.0)	80.0 (49.0, 94.3)	88.2 (65.7, 96.7)	0.77
Total		491	73	8	6	404	90.1% (81.7, 94.9)	98.5% (96.8, 99.3)	97.1% (95.3, 98.3)	0.90

NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement. [a]Anatomic site not specified. [b]Urine samples transferred into Abbott Alinity m multi-Collect Specimen Collection tubes prior to testing with Alinity m STI assay. [c]Endocervical, cervical, vaginal, vulvovaginal, genital, penile, urethral. [d]Rectal, oropharyngeal. [e]Vaginal. [f]Rectal.

Table 7: Performance of the Alinity m STI assay and comparator assays for *T. vaginalis*.

Assay	Specimen type	n	Comparator + Alinity m +	Comparator + Alinity m −	Comparator − Alinity m +	Comparator − Alinity m −	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)	Kappa
Allplex STI essential assay	Urine[a]	198	0	0	0	198	–	100.0 (98.1, 100.0)	100.0 (98.1, 100.0)	–
Anyplex II STI-7 detection V1.1	Urine	42	6	0	0	36	100.0 (61.0, 100.0)	100.0 (90.4, 100.0)	100.0 (91.6, 100.0)	1
	Urogenital swabs[b]	49	17	1[d]	0	31	94.4 (74.2, 99.0)	100.0 (89.0, 100.0)	98.0 (89.3, 99.6)	0.96
	Extragenital swabs[c]	10	0	0	1[e]	9	–	90.0 (59.6, 98.2)	90.0 (59.6, 98.2)	–
Vaginitis and vaginosis 8-well Panel	Urogenital swabs	14	3	0	0	11	100.0 (43.9, 100.0)	100.0 (74.1, 100.0)	100.0 (78.5, 100.0)	1
Total		313	26	1	1	285	96.3% (81.7, 99.3)	99.7% (98.0, 99.9)	99.4% (97.7, 99.8)	0.96

NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement. [a]Urine samples transferred into Abbott Alinity m multi-Collect Specimen Collection tubes prior to testing with Alinity m STI assay. [b]Endocervical, cervical, vaginal, vulvovaginal, genital, penile, urethral. [c]Rectal, oropharyngeal. [d]Vaginal. [e]Rectal.

Table 8: Detection of mixed infections: agreement between Alinity m STI and comparator assays.

Alinity m STI assay					Comparator assays							Agreement of Alinity m STI with comparator test[b] for detection of mixed infections
Number of samples with multiple infections identified by Alinity m STI	Targeted pathogens				Assay	Number of samples	Targeted pathogens[a]				Number of samples with multiple infections identified by comparator tests	
	CT	NG	MG	TV			CT	NG	MG	TV		
19	x	x			Allplex STI Essential assay	2	x	x			18	Yes
	x	x			Genesig advanced CT & NG	1	x	x				Yes
	x	x			RealTime CT/NG	11	x	x				Yes
	x	x			Aptima Combo 2 & Aptima MG	4	x	x				Yes
	x	x			Aptima Combo 2 & Aptima MG	1	x				0	CT only
7	x		x		Aptima Combo 2 & Aptima MG	3	x		x		3	Yes
	x		x		RealTime CT/NG	4	x				0	Yes
2		x	x		Aptima Combo 2 & Aptima MG	2		x	x		2	Yes
2			x	x	RealTime CT/NG	1	x				0	–
			x	x	Genesig advanced CT & NG	1					0	Yes
1	x	x		x	RealTime CT/NG	1	x				0	CT only
1	x		x	x	Genesig advanced CT & NG	1		x			0	–

[a]Identical to pathogens detected with the Alinity m STI assay. [b]Capable of detecting pathogens detected with the Alinity m STI assay.

Discussion

In this international multicenter study evaluating a large number of clinical specimens, including urine, urogenital, and extragenital swabs collected in a variety of sample collection devices, we showed that the new Alinity m STI assay accurately and precisely detects CT, NG, MG, and TV simultaneously.

When testing an analytical 5-member STI panel, we observed consistent Ct values with the panel member containing all 4 pathogens compared to those containing only 1 of the pathogens at 2-times the claimed LOD. The Alinity m STI assay demonstrated equivalent analytical performance in single and mixed infections, independent of microorganism load.

In our study, the detection of CT and NG in clinical samples by the Alinity m STI assay was highly concordant with several commercial molecular STI tests, including AC2, RT-CTNG, and Allplex STI Essential assays. Previous studies have compared these assays, which were found to reliably detect CT and NG in clinical practice [21–23]. A recently published study [24] compared Alinity m STI and RT-CTNG results from 347 matched clinical specimens collected in assay-specific collection devices with positivity rates of 6.9% for CT and 4.5% for NG and found high agreement for detection of CT (98.8% [95% CI: 97.1–99.7]) and NG (98.3% [95% CI: 96.3–99.4]) between the tests. The same study also investigated an additional series of 67 clinical samples collected in Alinity mSC tubes, which were positive for NG with the Alinity m STI assay. Of these Alinity STI NG-positive samples, 51 were confirmed positive by in-house duplex PCR. Of the remaining 16 samples, 13 were interpreted as representing true NG infections either based on NG positivity of specimens from other anatomic sites from the same patients (n=4) or residual low pathogen load after treatment (n=9). The remaining 3 samples had very low NG pathogen load, which could have contributed to the discrepancy of results between the two tests.

In our evaluation, the overall agreement for the detection of MG was 97.1%. The MG detection rates of the Alinity m STI assay were higher than those of Allplex and Aptima MG in urine specimens, whereas Aptima MG identified 7 infections in swabs of unknown anatomic origin from HIV-1-infected MSM that were reported MG-negative by the Alinity m STI assay. A previous study found a slightly lower sensitivity of Anyplex for MG compared to Aptima MG [21]. Herrmann and Malm [24] also found a high concordance between Alinity m STI MG

results and an in-house PCR targeting a genomic sequence of MG (97.8% [95% CI: 95.4–99.2]) in 279 matched clinical specimens (6.5% MG prevalence determined with the in-house MG PCR). Five samples positive for MG with the Alinity m STI assay and negative on the in-house PCR had very low pathogen load. Another series of 169 Alinity m STI MG-positive samples were retested with an additional PCR (Diagenode) confirming the presence of MG in 159 samples, while eight samples were found to have very low pathogen load, which could have contributed to the discrepancy of results between the two tests.

For the detection of TV in our study, we also observed excellent overall agreement between the Alinity m STI and comparator tests, including Anyplex, Allplex, and the Vaginitis and Vaginosis 8-well Panel, suggesting comparable sensitivity for TV with these tests.

Amongst the noted limitations for the study was the small proportion of positive clinical samples tested, reflecting the positivity rates of the participating laboratories, in particular for TV. Only 3 of the 6 participating laboratories tested for TV as part of their clinical routine at the time of the study, with low positivity rates at each site. There is a lack of basic epidemiological data describing TV incidence and prevalence in the general population. Multiplex STI testing that includes TV as a target pathogen could provide greater insight into TV prevalence and improve future STI control programs.

Due to limited sample volumes, no discordant resolution testing was performed to assess the true infection status of the patients who provided clinical specimens for this study. In addition, as a result of the de-identification of remnant specimen sample volume prior to starting the study, there was no access to additional laboratory findings (i.e., culture, treatment history), demographic information, or sexual behavior data from the subjects tested. These limitations also prevented us from determining the true patient infection status. Although the Alinity m STI assay showed high negative and positive agreements relative to the respective comparator assays, the results should be carefully interpreted due to the low prevalence of the 4 pathogens targeted by the Alinity m STI assay among the patient populations tested at the different study sites and the lack of confirmatory testing [25]. In addition, the majority of specimens tested in the study were collected in sample media not validated for use with the Alinity m STI assay. The detection of target pathogens by the Alinity m STI assay but not with comparator assays and *vice versa* could also be due to sample pre-testing conditions, differences in analytical sensitivity between these tests or underlying assay principles, such as target-capture based transcription-mediated amplification (AC2, AMG) and PCR following nucleic acid extraction. For some of the discrepant samples, a more detailed review of test results revealed that these likely had very low pathogen loads, which would be expected to give inconsistent results since they were near the LODs for the assays used.

This study compared Alinity m STI with very well-characterized tests such as the RealTi*m*e CT/NG and AC2 and AMG assays, as well as with tests that lack published evidence of performance, such as the research-use-only genesig CT and NG assays and the Vaginitis and Vaginosis 8-well Panel. In addition, peer-reviewed data on the performance of Allplex [21, 23] and Anyplex [26] assays remain scarce. Thus, comparison of Alinity m STI results with these tests should be interpreted with caution. Future studies comparing the Alinity m STI assay with other highly sensitive tests, including discordant resolution in populations at high risk of infection with multiple STIs, would be helpful to further characterize the assay's ability to identify mixed infections.

There are several technical achievements related to the Alinity m STI assay: (i) The ability of the random continuous access Alinity m system to report results from 300 samples within an 8-h shift and provide results from STAT samples in less than 120 min without disrupting routine sample processing [20], combined with the flexibility of the Alinity m platform for customized reporting of results, may reduce the time to diagnosis and treatment initiation. The total turn-around time from sample arrival in the laboratory until reporting of STI results with Alinity m was recently reported by Obermeier et al. [20], who compared the operational characteristics of the Alinity m instrument to the comparator platforms used in our study. For STI samples tested in parallel to a variety of other assays, the total turnaround time ranged from 2 to 10 h. This study also documented a processing turnaround time (from sample aspiration to result reporting) for STI tests ordered on the Alinity m within a range from 113 to 117 min and reported a hands-on time during routine use of the Alinity m instrument and the Panther system ranging between 6 and 30 min, while semi-automated methods, such as Allplex (Seegene Inc, Seoul), required a hands-on time of approximately 60 min. (ii) Results not chosen for reporting upon initial testing by the laboratory can be "unmasked" within 14 days, if required. This avoids the need for requesting an additional patient sample for testing for the presence of pathogens not requested with the initial order and may help to reduce the time to diagnosis and treatment initiation in NAAT-based algorithms for STI diagnosis. (iii) The use of a single collection device for the Alinity m STI assay may facilitate collection of multiple sample types and improve material management at the collection sites.

(iv) The cellular control assessing sample adequacy and the internal process control of the Alinity m STI assay (Table 1) ensure confidence in negative test results.

Conclusions

In conclusion, the Alinity m STI assay accurately and precisely identified the presence of CT, NG, and MG or TV in an analytical panel of contrived specimens with 1 or all 4 target pathogens present. This observational field evaluation of the Alinity m STI assay against other frequently used molecular STI tests demonstrated high overall percent agreement with high kappa values for the 4 targeted pathogens. The Alinity m STI is a reliable assay for the sensitive and specific detection of 4 major sexually transmitted pathogens across a wide range of clinical sample types, including urine and urogenital and extragenital swabs, collected in a variety of sample collection devices.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: All clinical specimens were sourced per site policies, procedures, and applicable local regulations, including approval by an Independent Ethics Committee or waiver.

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