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Performance Characterization of the BioCode® STI + Resistance Panel (RUO)

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Introduction

Early detection and identification of pathogens involved in sexually transmitted infections (STIs) is critical for effective treatment. While single and multi-target FDA cleared polymerase chain reaction (PCR) based kits for STIs exist, these kits are not able to simultaneously detect nucleic acids of multiple STI pathogen targets and gene mutations associated with antimicrobial resistance (AMR). In April 2024, Applied BioCode launched the BioCode® STI + Resistance Panel (RUO) that is capable of simultaneously detecting *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV), as well as single nucleic polymorphism (SNP) mutations associated with antimicrobial resistance for MG (23S rRNA and ParC) and NG (gyrase A) from extracted DNA utilizing Barcoded Magnetic Beads (BMBs) and the BioCode® MDx-3000 with results in 4 hours.

Table 1: ID/AMR targets detected by the BioCode® STI + Resistance Panel (RUO)

STI Organisms	
• *Chlamydia trachomatis* (CT)	• *Mycoplasma genitalium* (MG)
• *Neisseria gonorrhoeae* (NG)	• *Trichomonas vaginalis* (TV)
Macrolide Resistance	**Fluoroquinolone Resistance**
• MG 23S rRNA A2058C/G/T	• MG ParC S83I
• MG 23S rRNA A2059C/G/T	• NG gyrase A S91F*
Assay Controls	
• T4 Phage as DNA Internal Control (DNA-IC) for Extraction	

*The STI + AMR assay also identifies the NG gyrase A S91 (WT) allele.

Methods

- Contrived and native urine samples for all studies were extracted with the Roche MagNA Pure 96 System prior to testing with the BioCode® MDx-3000.
- To assess analytical performance, several studies were executed. The limit of detection (LoD) for each target plus AMRs were determined and analytical reactivity testing were performed on at least 11 strains per target. Potential competitive inhibition for all 4 ID targets were evaluated. Over 200 cross-reactivity organisms were tested *in vitro* or analyzed *in silico* to confirm specificity. The robustness of the assay was challenged with potentially interfering substances in urine samples.
- To assess clinical performance and potentially discover AMR strains, previously characterized patient urine samples (n=20/target) positive for each STI target were tested and discordants were confirmed by sequencing.
- Oral and rectal swabs for CT and NG were tested to confirm assay performance with alternative sample types.

Multiplex Detection Targets

The BioCode® STI + Resistance Panel (RUO) consists of 2 identification (ID) probes for each STI target organism and 1 probe each for all SNP mutations and DNA-IC. A sample will be called positive if at least one of the ID probes is detected. Note: BioCode® MDx-3000 software has masking capabilities to assign custom testing requirements per test sample.

Table 2: BioCode® STI + Resistance Panel (RUO) Detection Probes

Probe	ID Plex				DNA-IC	MG AMR							NG AMR	
	N. gonorrhoeae	*C. trachomatis*	*M. genitalium*	*T. vaginalis*	T4 Phage	A2058G	A2058C	A2058T	A2059G	A2059C	A2059T	ParC S83I	S91 WT	S91F MUT
NG ID 1	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No
NG ID 2	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No
CT ID 1	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No
CT ID 2	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No
MG ID 1	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No
MG ID 2	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No
TV ID 1	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No
TV ID 2	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No
DNA IC	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No
MG A2058G	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No
MG A2059G	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No
MG A2058C	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No
MG A2059C	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No
MG A2058T	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No
MG A2059T	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No
ParC S83I	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No
NG S91 WT	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No
NG S81F MUT	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes

Assay Workflow

MagNA Pure 96 Extraction (96 samples) → PCR Setup → BioCode® MDx-3000 (PCR and Detection)

	MagNA Pure 96 Extraction (96 samples)	PCR Setup	BioCode® MDx-3000 (PCR and Detection)
TAT	~1 hr	~30 min	~3 hrs
HOT	~30 min	~30 min	~10 min

Figure 1: Workflow for BioCode® STI + Resistance Panel (RUO). Samples were extracted on the Roche MagNA Pure 96 System followed by manual PCR plate setup, then automated PCR and detection on the BioCode® MDx-3000 system.

Limit of Detection

The Limit of Detection (LoD) for each strain was determined by testing 4 extractions x 1 replicate (n=4) in serial dilutions then confirmed with 10 extractions x 2 replicates (n=20). Synthetic gene fragments used for AMR LoD were run with 20 replicates. The LoD for each strain or gene fragment was the lowest concentration with ≥95% replicates detected.

Table 3: Limit of Detection (LoD)

LoD Strain	Source	LoD
Chlamydia trachomatis, Serovar D	ATCC VR-885	1.00E+01 IFU/mL
Chlamydia trachomatis, Serovar G	ATCC VR-878	3.33E+01 IFU/mL
Trichomonas vaginalis, Donne	ATCC 30001	3.00E+00 cells/mL
Trichomonas vaginalis, Donne (MTZ resistant)	ATCC 50143	1.00E+00 cells/mL
Neisseria gonorrhoeae, F-18 (WT gyrase A S91)	ATCC 49226	1.00E+02 CFU/mL
Neisseria gonorrhoeae, NCTC 8375 (WT gyrase A S91)	ATCC 19424	1.67E-01 CFU/mL
Neisseria gonorrhoeae, WHO reference strain G (gyrase A S91F)	CDC	1.00E+01 CFU/mL
Mycoplasma genitalium, G37 (WT)	ATCC 33530	1.00E-02 bacteria/mL
Mycoplasma genitalium, M30 (WT)	ATCC 49895	9.00E+00 CFU/mL
ParC S83I gene fragment	IDT	1.67E+03 copies/mL
23S rRNA A2058G gene fragment	IDT	2.25E+03 copies/mL
23S rRNA A2059G gene fragment	IDT	4.50E+03 copies/mL
23S rRNA A2058C gene fragment	IDT	1.50E+03 copies/mL
23S rRNA A2059C gene fragment	IDT	1.50E+03 copies/mL
23S rRNA A2058T gene fragment	IDT	4.50E+03 copies/mL
23S rRNA A2059T gene fragment	IDT	4.50E+03 copies/mL

Analytical Reactivity

To confirm the analytical reactivity of the BioCode® STI + Resistance Panel (RUO), commercially available strains for each of the four organisms were extracted once at 3x LoD of the strain associated with weaker sensitivity in the LoD table above. The extractions were run in triplicate and retested at higher concentrations if any 3x LoD replicates were missed.

Table 4: Analytical Reactivity Results

Target Organism	Number of Strains Tested	Number of Strains Detected at 3x LoD	Repeat Testing
Chlamydia trachomatis	12	12	
Trichomonas vaginalis	17	16	Strain 165307-1 detected at > 3x LoD
Neisseria gonorrhoeae (WT for gyrase A at S91)	15	15	
Neisseria gonorrhoeae (gyrase A S91F)	11	10	WHO reference strain Z detected at > 3x LoD
Mycoplasma genitalium (WT for 23S rRNA A2058/A2059)	5	5	
Mycoplasma genitalium (23S rRNA A2058 or A2059 mutants)	6	3	MEGA strains 1261, 1493, 292 AMR mutations detected at >3x LoD

Cross-Reactivity

***In vitro* Cross-Reactivity**
No cross-reactivity was observed *in vitro* with 55 closely-related species or other organisms/viruses found in the urogenital tract using the STI + Resistance Panel. Cross-reactivity was evaluated at ≥10^6 units/mL for organisms or 10^5 units/mL for viruses.

Table 5: Cross-reactivity Organisms Tested *in vitro*

Acinetobacter baumannii	*Cryptococcus neoformans*	*Klebsiella pneumoniae*	*Neisseria elongata* strain Z071	*Peptostreptococcus anaerobius*
Acinetobacter lwoffii	*Enterobacter aerogenes*	*Lactobacillus acidophilus*	*Neisseria flava* Z119	*Prevotella bivia*
Bacteroides fragilis	*Enterobacter cloacae*	*Lactobacillus jensenii*	*Neisseria meningitidis-A*	*Proteus mirabilis*
Bifidobacterium longum	*Enterococcus faecalis*	*Lactobacillus lactis*	*Neisseria meningitidis-B*	*Pseudomonas aeruginosa*
Campylobacter coli	*Escherichia coli*	*Lactobacillus vaginalis*	*Neisseria meningitidis-C*	*Staphylococcus aureus*
Candida albicans	*Fusobacterium necrophorum*	*Listeria monocytogenes*	*Neisseria meningitidis-Y*	*Staphylococcus epidermidis*
Candida glabrata	*Gardnerella vaginalis*	*Mobiluncus curtisii*	*Neisseria mucosa*	*Streptococcus agalactiae*
Candida parapsilosis	*Haemophilus influenzae*	*Mycoplasma hominis*	*Neisseria perflava*	*Streptococcus pyogenes*
Candida tropicalis	Herpes simplex virus I	*Mycoplasma pneumoniae*	*Neisseria sicca* strain Z043	*Streptococcus salivarius*
Chlamydophila pneumoniae	Herpes simplex virus II	*Neisseria cinerea*	*Neisseria subflava*	*Trichomonas tenax* (Muller) Dobell
Clostridium difficile	*Klebsiella oxytoca*	*Neisseria denitrificans*	*Pentatrichomonas hominis* R51	*Ureaplasma urealyticum*

***In silico* Cross-Reactivity**
An additional 191 organisms/viruses that may be found in urine, oral, or rectal samples were tested *in silico* using NCBI BLAST. Although some organisms closely related to NG showed >80% homology with NG AMR primers/probes, they had low homology with the NG ID primers/probes; the samples containing these organisms would not be identified as NG according to the software algorithm.

Competitive Inhibition

Competitive inhibition (CI) was not observed for CT, NG, TV or MG when each target was tested at medium concentration in the presence of the other 3 STI targets at high concentrations when contrived together in negative urine. Each CI pool was extracted once and tested in triplicate.

Table 6: Competitive Inhibition Pools

Pool Member	CT	NG	TV	MG
Pool 1	3.00E+01 IFU/mL	2.00E+05 CFU/mL	6.00E+04 cells/mL	1.00E+06 CFU/mL
Pool 2	3.00E+05 IFU/mL	5.00E+03 CFU/mL	6.00E+04 cells/mL	1.00E+06 CFU/mL
Pool 3	3.00E+05 IFU/mL	2.00E+05 CFU/mL	5.00E+01 cells/mL	1.00E+06 CFU/mL
Pool 4	3.00E+05 IFU/mL	2.00E+05 CFU/mL	6.00E+04 cells/mL	4.50E+02 CFU/mL

Interfering Substances

To determine if substances that may be present in clinical urine samples would affect the performance of the BioCode® STI + Resistance Panel (RUO), all four targets were pooled at 3x LoD in negative urine with and without the presence of potentially interfering substances. Samples were extracted and run in triplicate. All targets were detected with and without the potential inhibitors.

Table 7: Potentially Interfering Substances

Description		Concentration Tested
Endogenous Substances	Blood	5.0% v/v
	Leukocytes	1.00E+06 cells/mL
	Mucus/Mucin	0.2% v/v
	Seminal Fluid	5.0% v/v
	Albumin	60 mg/mL
	γ-globulin	60 mg/mL
	Glucose	10 mg/mL
	Bilirubin	0.4 mg/mL
Exogenous Substances	Aspirin	30 µg/mL
	Azithromycin	12 µg/mL
	Doxycycline	18 µg/mL
	Acetaminophen	156 µg/mL
	Acidic urine	pH 4.0
	Alkaline urine	pH 9.0

Clinical Sample Testing

The BioCode® STI + Resistance Panel (RUO) was able to accurately detect ≥ 90% of target organisms in previously characterized clinical urine samples. Discordant results were confirmed by DNA bi-directional Sanger sequencing) except for one sample that was unable to be confirmed due to low volume of the remnant clinical sample.

Table 8: Clinical Sample Testing Results

Target	No. of Characterized Positive Urine Samples Tested	Samples Confirmed by STI + Panel	No. of Discordant Samples by STI + Panel	Discordants Confirmed by Sequencing in Agreement with STI + Panel
Chlamydia trachomatis	20	90% (18/20)	2	1/2*
Neisseria gonorrhoeae	20	90% (18/20)	2	2/2
Trichomonas vaginalis	20	95% (19/20)	1	1/1
Mycoplasma genitalium	20	90% (18/20)	2	2/2

*Unable to test one sample due to low volume of remnant sample

Additional targets from the remnant clinical urine samples received were not called out by the vendor but were detected by the BioCode® STI + Resistance Panel (RUO). These additional calls were confirmed by Sanger bi-directional sequencing.

Table 9: Additional Targets Detected by the BioCode® STI + Resistance Panel (RUO)

Clinical Specimen	Vendor Result	STI + Panel Result	Additional STI + Panel Result	Sequencing Result
CT-814139	CT positive	CT positive	MG positive	MG positive
CT-814149	CT positive	CT positive	MG positive, A2059G	MG positive, A2059G
CT-807040	CT positive	CT positive	MG positive, A2059G	MG positive, A2059G
NG-823042	NG positive	NG positive	CT positive	CT positive
TV-2505RBY1004	TV positive	TV positive	MG positive, A2059G	MG positive, A2059G

Oral and rectal swabs previously characterized as positive for either *Chlamydia trachomatis* or *Neisseria gonorrhoeae* were evaluated with the BioCode® STI + Resistance Panel (RUO). Each sample was extracted once and run in duplicate and were determined to be positive for either *Chlamydia trachomatis* or *Neisseria gonorrhoeae* if both duplicates were detected.

Table 10: Oral and Rectal Swab Sample Results

Target	Swab Type	No. of Characterized Positive Swab Samples Tested	No. of Tested Swab Samples Detected by STI + Panel
Chlamydia trachomatis	Oral	18	18/18 (100%)
	Rectal	25	24/25 (96%)
Neisseria gonorrhoeae	Oral	14	12/14 (86%)
	Rectal	16	15/16 (94%)

Summary/Conclusions

- ✓ The LoD for each assay target was determined to have good sensitivity. All inclusivity strains tested for all 4 targets were detected. None of the cross-reactivity organisms were detected. There was no inhibition by other assay targets or interfering substances tested that could potentially be present in urine samples.
- ✓ Clinical sample testing resulted in ≥90% agreement, but discordant testing resulted in 100% agreement (1 sample was not retested due to insufficient remnant volume).
- ✓ Five of the clinical samples tested showed coinfection with another assay target and some AMR detection; results were confirmed via DNA sequencing.
- ✓ Limited testing with the BioCode ® STI + Resistance Panel (RUO) on rectal and oropharyngeal swab samples positive for CT or NG showed promising results.

The BioCode® STI + Resistance Panel (RUO) is a sensitive and specific PCR-based assay capable of detecting and simultaneously identifying CT, MG, NG, and TV, and SNPs associated with MG AMR and NG AMR in each sample. The assay provides a powerful tool to support better informed decisions regarding antibiotic treatments by combining high sensitivity, fast results, and scalable throughput (≤96 samples/run).

Note: The abstract has been revised since submission.