



BioCode[®]

STI + Resistance Panel (RUO)

Application Note

The BioCode[®] STI + Resistance Panel (RUO) and the BioCode[®] MDx-3000 System employs an advanced molecular technique to swiftly identify sexually transmitted infections and single nucleotide polymorphisms (SNPs) associated with antibiotic resistance profiles with minimal hands-on time.

Introduction

As the world recovered from the aftermath of the COVID-19 pandemic, an unexpected resurgence emerged, casting a shadow over public health: the rise of sexually transmitted infections (STIs) among individuals over the age of 14. Healthcare systems faced significant challenges, including overwhelming facilities, staff shortages, and redirection of resources to address COVID-19. As a result, routine healthcare services, including STI testing and screening programs, were often deprioritized or disrupted to accommodate pandemic-related demands.⁶ Every day, more than 1 million new STIs are acquired, posing a significant global health challenge. Macrolides and fluoroquinolones are commonly prescribed antibiotics for the management and treatment of certain STIs. Macrolide antibiotics such as azithromycin and erythromycin are commonly used to treat STIs like *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG). They work by inhibiting bacterial protein synthesis, effectively killing the bacteria responsible for the infection. Fluoroquinolone antibiotics like ciprofloxacin and levofloxacin are used to treat a variety of bacterial infections, including certain STIs like NG. Fluoroquinolones work by inhibiting bacterial DNA synthesis, leading to bacterial cell death.³

Emerging resistance among pathogens that cause STIs is a major public health concern. Overuse or inappropriate use of antibiotics can contribute to the development of antibiotic-resistant strains of bacteria. Therefore, healthcare providers need to be cautious when prescribing antibiotics for STIs and should consider factors such as antibiotic resistance patterns in the community and individual patient factors. The simultaneous detection of potential pathogens and their associated drug resistance markers directly from clinical specimens is the most rapid means to both diagnose the infection and direct effective therapy for improved patient outcomes. This approach also supports antimicrobial stewardship efforts to prevent the inappropriate use of antibiotics and control the spread of antibiotic-resistant infections. Moreover, since antimicrobial susceptibility testing on STI pathogens requires a clinical isolate, and culture is not routinely performed, especially from extragenital specimens, resistance marker detection is the major approach to detect and monitor drug resistance in tested populations.⁵

To address the pressing need for detecting the most prevalent STIs and their associated resistance profiles, Applied BioCode Inc. has developed the BioCode[®] STI + Resistance Panel (RUO) that can simultaneously detect nucleic acids from bacterial and protozoan pathogens, and SNP mutations associated with macrolide and fluoroquinolone resistance, directly from clinical specimens, empowering healthcare professionals to swiftly identify infections and tailor treatment strategies in research settings.



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In this application note, we show that the BioCode® STI + Resistance Panel (RUO):

- Is a highly multiplexed PCR-based qualitative assay capable of simultaneously detecting and identifying nucleic acids from CT, MG, NG, and TV as well as mutations associated with antimicrobial resistance for MG and NG.
- Workflow is optimized for the accurate performance for sensitivity and specificity utilizing end-point PCR.
- Is designed for medium to high throughput, enabling testing of up to 96 samples per run.
- Is part of a cost-effective system solution (BioCode® MDx-3000) with minimal hands-on time.
- Offers the flexibility to customize the panel by masking results.

Summary and Explanation of the Test

The BioCode® STI+ Panel is a multiplex PCR-based panel that can identify the presence of CT, MG, NG, and TV, as well as mutations associated with antimicrobial resistance (AMR) for MG (A2058C/G/T, A2059C/G/T and S83I), NG (S91F), NG WT (S91), Endogenous Internal Control (EIC), and Assay Process Control (APC), as shown in Table 1, from extracted DNA utilizing Barcoded Magnetic Beads (BMBs) and the BioCode® MDx-3000 with results in as little as 4 hours.²

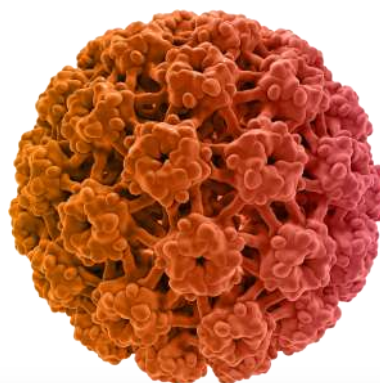
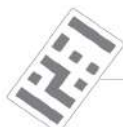


Table 1. STI+ Panel targets.

STI Organisms	
• <i>Chlamydia trachomatis</i> (CT)	• <i>Mycoplasma genitalium</i> (MG)
• <i>Neisseria gonorrhoeae</i> (NG)	• <i>Trichomonas vaginalis</i> (TV)
Macrolide Resistance	Fluoroquinolone Resistance
• MG A2058C/G/T	• MG S83I
• MG A2058C/G/T	• NG S91F*
Assay Controls	
• Endogenous Internal Control (EIC)	• Assay Process Control (APC)

* The STI+ Panel assay also identifies the NG wildtype allele.



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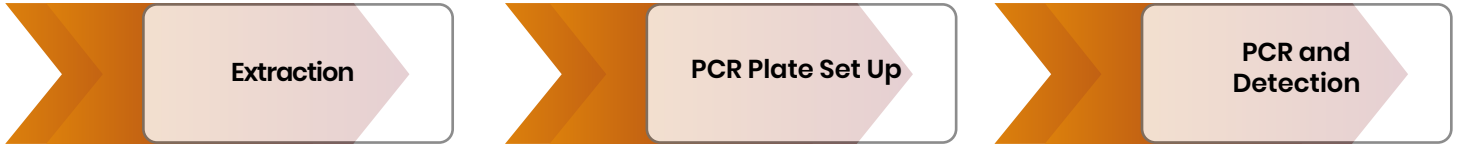
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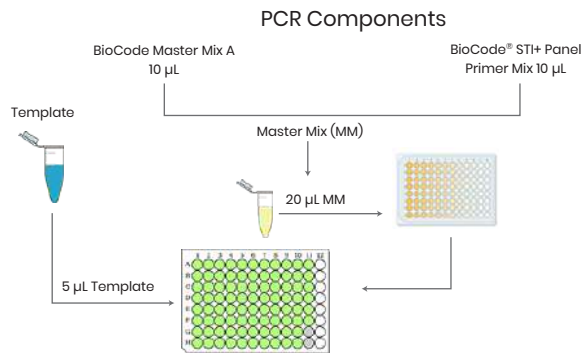


STI+ Panel Workflow

The STI+ Panel streamlines the process into three straightforward steps with minimal hands-on time:



Compatible with various extraction systems. See the BioCode® STI + Resistance Panel (RUO) Package Insert



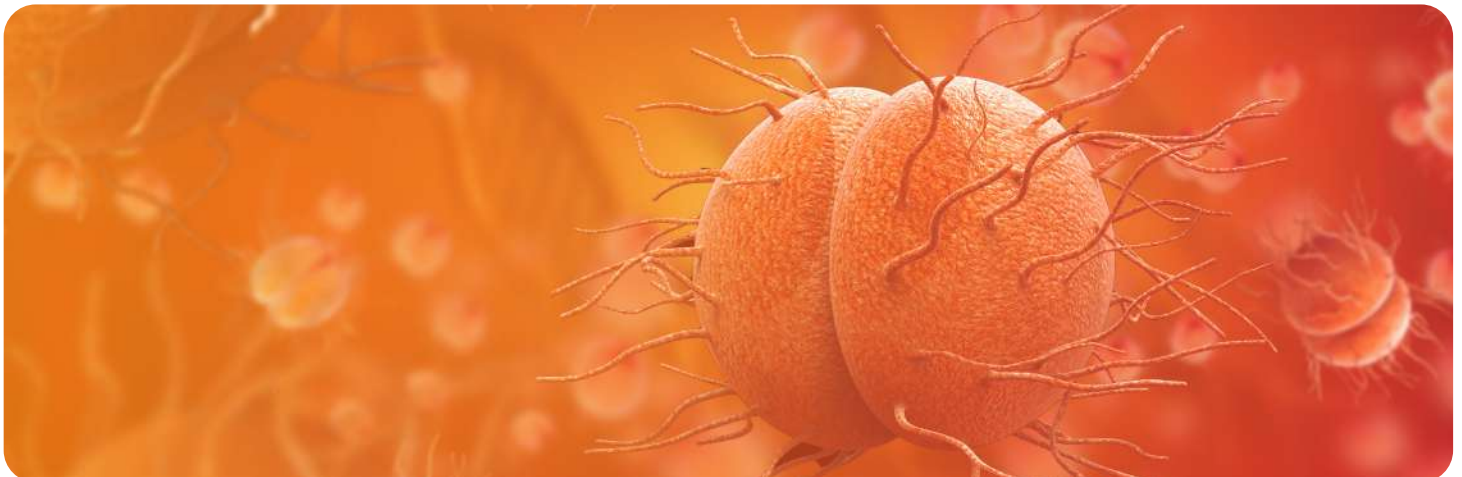
The workflow begins with the extraction of nucleic acids from samples utilizing an extraction instrument. Next, the PCR plate is prepared using the STI+ Panel reagents. Subsequently, the amplification and detection of target sequences occur in the BioCode® MDx-3000 instrument, with analysis facilitated through imaging techniques.

Materials and Methods

Please refer to the BioCode® STI + Resistance Panel (RUO) Instructions for Use.

Multiplex Detection Targets

The BioCode® STI+ Panel consists of 2 ID probes/STI target and 1 probe each for all AMR mutations, NG S91 WT, EIC and APC.



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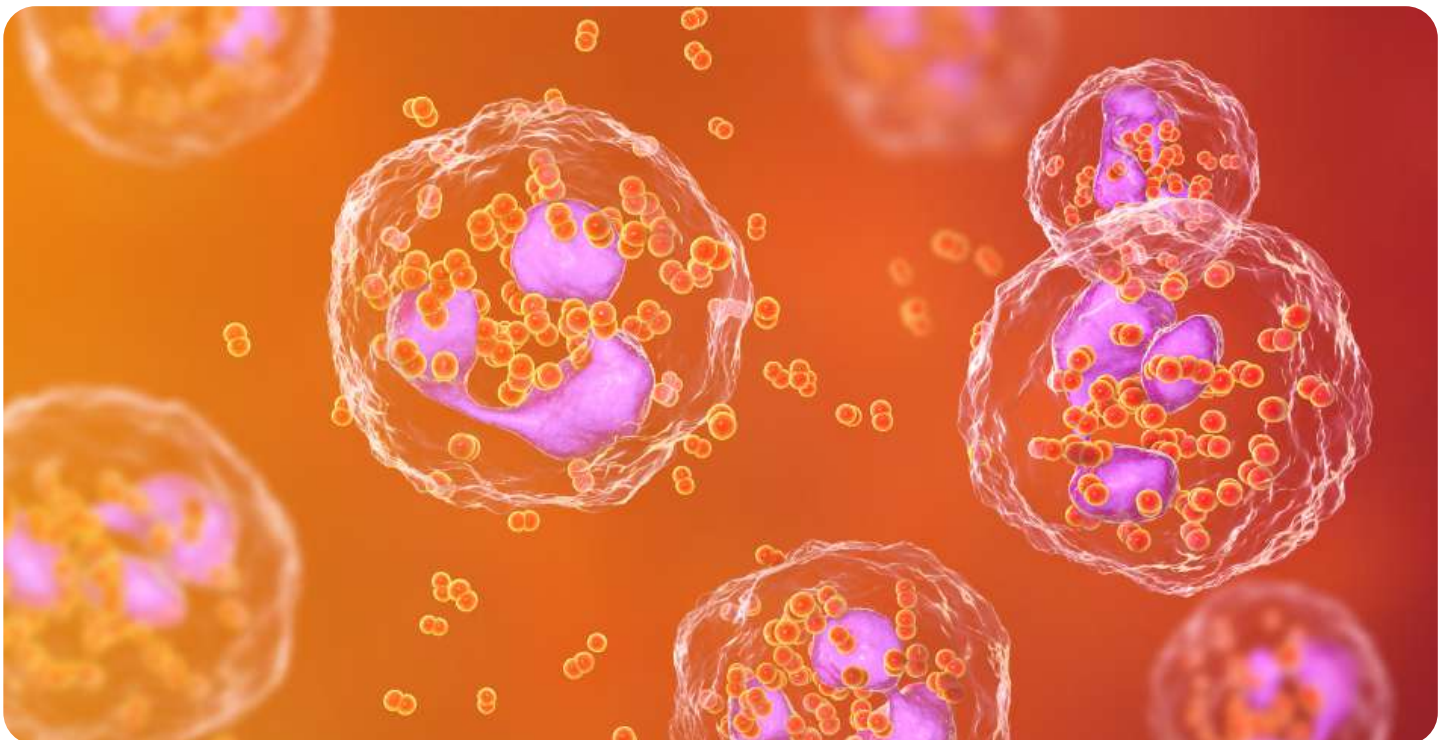
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Table 2. STI+ Panel detection probes.

Probe	ID Plex				EIC	APC	Antibiotic Microbial Resistance									
	<i>N. gonorrhoeae</i>	<i>C. trachomatis</i>	<i>M. genitalium</i>	<i>T. vaginalis</i>			<i>M. genitalium</i>									
					MG A2058G	MG A2058C	MG A2058T	MG A2059G	MG A2059C	MG A2059T	MG ParC S83I	NG S91WT	NG S91F			
NG ID 1	Yes	No	No	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	No	No
CT ID 1	No	Yes	No	No	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	No	No
MG ID 1	No	No	Yes	No	No	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	No	No
TV ID 1	No	No	No	Yes	No	No	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	No	No
EIC	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No
DNA IC	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No
MG A2058G	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No
MG A2059G	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No
MG A2058C	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No
MG A2059C	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No
MG A2058T	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No
MG A2059T	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No
ParC S83I	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No
NG S91 WT	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No
NG S91F MUT	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes

Note: MDx-3000 software has masking capabilities to assign custom testing requirements per test sample.



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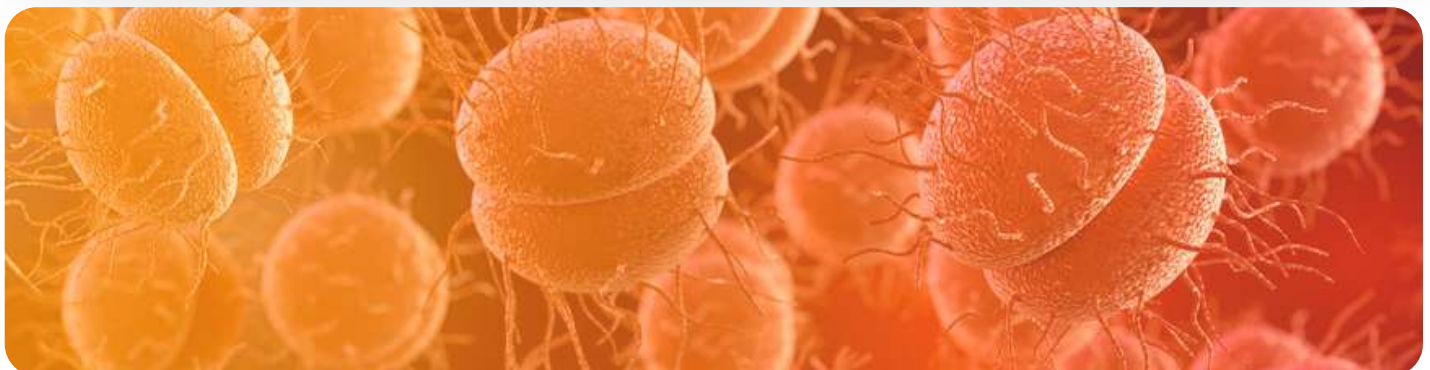
Analytical Reactivity

Analytical reactivity wet bench testing (1 extraction/2 duplicates) was conducted on 12 strains of *Chlamydia trachomatis*, 5 strains of *Mycoplasma genitalium*, 30 strains of *Neisseria gonorrhoeae* (including some fluoroquinolone-resistant strains with S91F mutation), and 17 strains of *Trichomonas vaginalis*, each at concentrations $\geq 10X$ above the estimated limit of detection (LoD), with the higher estimated LoD (eLoD) concentration contrived in negative urine matrix.

Table 3. Analytical reactivity organisms tested.

<i>Chlamydia trachomatis</i> Strains (n=12)	Concentration	<i>Neisseria gonorrhoeae</i> Strains (n=30)	Concentration
<i>Chlamydia trachomatis</i> , Serovar A	5.00E+02 IFU/mL	<i>Neisseria gonorrhoeae</i> FAI090	1.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> , Serovar B	5.00E+02 IFU/mL	<i>Neisseria gonorrhoeae</i> NHI 1	>2.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> , Serovar Ba	5.00E+02 IFU/mL	<i>Neisseria gonorrhoeae</i> C-58	>2.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> , Serovar C	5.00E+02 IFU/mL	<i>Neisseria gonorrhoeae</i> PID24-1 C	>2.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> , Serovar E	5.00E+02 IFU/mL	<i>Neisseria gonorrhoeae</i> 1291	>2.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> , Serovar F	5.00E+02 IFU/mL	<i>Neisseria gonorrhoeae</i> DGI18	>2.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> , Serovar H	5.00E+02 IFU/mL	<i>Neisseria gonorrhoeae</i> SK-93-1035	>2.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> , Serovar I	5.00E+02 IFU/mL	<i>Neisseria gonorrhoeae</i> CDC Ng-98	>2.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> , Serovar J	5.00E+02 IFU/mL	<i>Neisseria gonorrhoeae</i> GC/CB/001	>2.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> , Serovar K	5.00E+02 IFU/mL	<i>Neisseria gonorrhoeae</i> F62	>2.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> LGV I strain 440 ^a	5.00E+01 copies/ μ L	<i>Neisseria gonorrhoeae</i> FA19	>2.00E+04 CFU/mL
<i>Chlamydia trachomatis</i> LGV II strain 434 ^a	5.00E+01 copies/ μ L	WHO gonococcal reference strain F ^b	1.00E+04 CFU/mL
<i>Mycoplasma genitalium</i> Strains (n=5)	Concentration	WHO gonococcal reference strain G ^b	1.00E+04 CFU/mL
<i>Mycoplasma genitalium</i> strain TW 10-5G	1.00E+03 CCU/vial	WHO gonococcal reference strain K	1.00E+04 CFU/mL
<i>Mycoplasma genitalium</i> strain TW48-5G	1.00E+03 CCU/vial	WHO gonococcal reference strain L	1.00E+04 CFU/mL
<i>Mycoplasma genitalium</i> strain [UMTB-10G]	1.00E+03 CCU/vial	WHO gonococcal reference strain M	1.00E+04 CFU/mL
<i>Mycoplasma genitalium</i> strain TW10-6G	1.00E+03 CCU/vial	WHO gonococcal reference strain N	4.86E-02 CFU/mL
<i>Mycoplasma genitalium</i> strain R32G [R32]	1.00E+03 CCU/vial	WHO gonococcal reference strain O	4.86E-02 CFU/mL
<i>Trichomonas vaginalis</i> Strains (n=17)	Concentration	WHO gonococcal reference strain P	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> JH 31A # 4	5.00E+03 cells/mL	WHO gonococcal reference strain U ^b	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> CDC 085	5.00E+03 cells/mL	WHO gonococcal reference strain V	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> 123414	5.00E+03 cells/mL	WHO gonococcal reference strain W	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> G3	5.00E+03 cells/mL	WHO gonococcal reference strain X ^b	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> 11769	5.00E+03 cells/mL	WHO gonococcal reference strain Y ^b	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> JH 32A #4	5.00E+03 cells/mL	WHO gonococcal reference strain Z ^b	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> B7RC2	5.00E+03 cells/mL	CDC culture AR Bank # 963 ^b	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> 123413	5.00E+03 cells/mL	CDC culture AR Bank # 964 ^b	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> 165307-1	5.00E+03 cells/mL	CDC culture AR Bank # 971 ^b	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> JRS-TV-141	5.00E+03 cells/mL	<i>Neisseria gonorrhoeae</i> Z017	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> MT87	5.00E+03 cells/mL	<i>Neisseria gonorrhoeae</i> Z001	1.00E+04 CFU/mL
<i>Trichomonas vaginalis</i> 1 29155-8	5.00E+03 cells/mL	Synthetic genes (gBlocks or plasmids)	Concentration
<i>Trichomonas vaginalis</i> RP	5.00E+03 cells/mL	A2058C/G/T gBlocks and plasmids	1.00E+03 copies/mL
<i>Trichomonas vaginalis</i> IR78	5.00E+03 cells/mL	A2059C/G/T gBlocks and plasmids	1.00E+03 copies/mL
<i>Trichomonas vaginalis</i> Z070	5.00E+03 trophozoites/mL	MG parC S831 gBlock and plasmid	1.00E+03 copies/mL
<i>Trichomonas vaginalis</i> Z158	5.00E+03 trophozoites/mL	NG S91F gBlock and plasmid	1.00E+03 copies/mL
<i>Trichomonas vaginalis</i> Z159	5.00E+03 trophozoites/mL	N/A	

*a = genomic DNA, b = NG fluoroquinolone resistant strain, S91F



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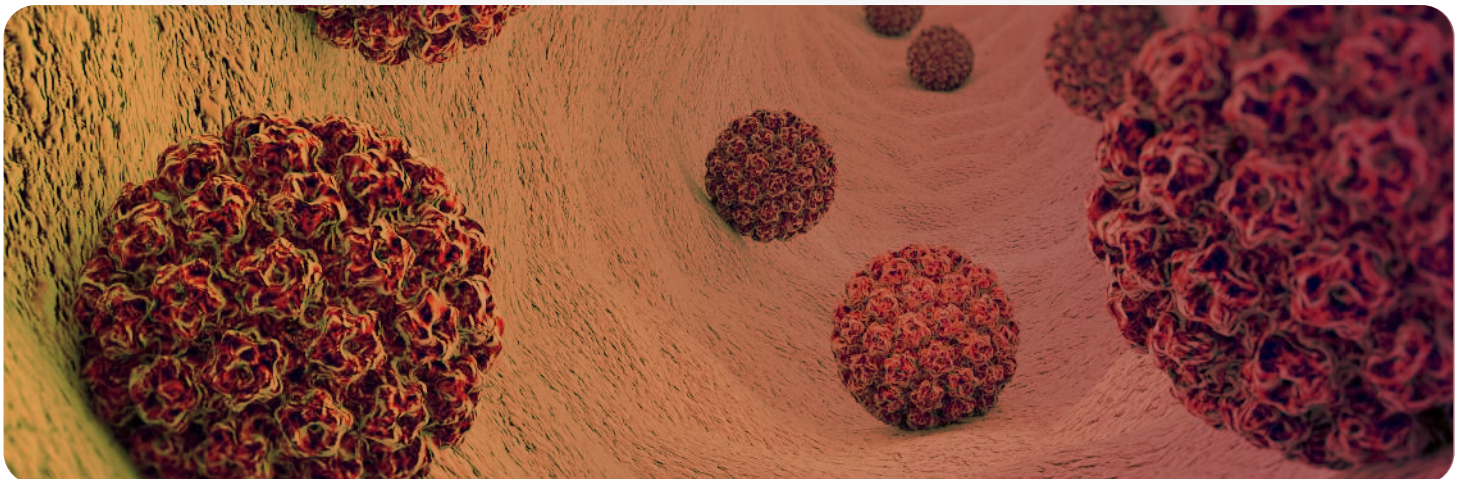
Cross-Reactivity

Specificity was determined by testing 18 closely-related organisms contrived in negative urine matrix and evaluating 179 additional organisms not targeted by the panel via *in silico* analysis. No cross-reactivity was observed for the closely related species tested on the bench (Table 4) nor for the other organisms (via *in silico* analysis) that may be present in the same anatomical area (Table 5). Closely related organisms tested on the bench (1 extraction/1 replicate) were evaluated at $\geq 10^6$ cells/mL, IFU/mL, CFU/mL or CFU/vial when available.

Table 4. Closely related cross-reactivity organisms tested.

Organism	Cross-Reactivity Organism (n=18)	Source	Concentration
Protozoan	<i>Trichomonas tenax</i> (Muller) Dobell	ATCC 30207	3.40E+06 cells/mL
	<i>Pentatrichomonas hominis</i> R51	ATCC 30098	1.07E+06 cells/mL
	<i>Pentatrichomonas hominis</i> Hs-3:NIH	ATCC 30000	5.80E+06 cells/mL
Bacteria	<i>Chlamydomphila pneumoniae</i>	ATCC 53592	>5.00E+02 IFU/mL*
	<i>Neisseria flava</i> Z119	Zepto 801887	3.27E+08 CFU/mL
	<i>Neisseria meningitidis</i> - A	Zepto 801511	2.55E+07 CFU/mL
	<i>Neisseria meningitidis</i> - B	ATCC 13090	8.30E+04 CFU/mL*
	<i>Neisseria meningitidis</i> - C	ATCC 13102	8.40E+06 CFU/mL
	<i>Neisseria meningitidis</i> - Y	ATCC 35561	1.61E+08 CFU/vial
	<i>Neisseria perflava</i>	ATCC 10555	>1.00E+03 CFU/mL
	<i>Neisseria cinerea</i>	ATCC 14685	1.99E+07 CFU/mL
	<i>Neisseria dentrificans</i>	ATCC 14686	>1.00E+03 CFU/mL
	<i>Neisseria mucosa</i>	ATCC 49233	2.40E+07 CFU/mL
	<i>Neisseria subflava</i>	ATCC 14221	>1.00E+03 CFU/mL*
	<i>Neisseria elongata</i> (Strain Z071)	Zepto 801510	1.74E+08 CFU/mL
	<i>Neisseria sicca</i> (Strain Z043)	Zepto 801754	1.02E+07 CFU/mL
	<i>Mycoplasma hominis</i>	ATCC 23114	2.70E+07 CFU/vial
	<i>Mycoplasma pneumoniae</i>	ATCC 29085	1.00E+05 CFU/vial*

*Highest titer available at time of testing



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Table 5. Cross-reactivity organisms evaluated via *in silico* analysis.

Cross-Reactivity Organisms Evaluated via <i>in silico</i> Analysis (n=179)		
<i>Acinetobacter baumannii</i>	<i>Fusobacterium nucleatum</i>	<i>Pantoea agglomerans</i>
<i>Acinetobacter calcoaceticus</i>	<i>Gardnerella vaginalis</i>	<i>Paracoccus denitrificans</i>
<i>Acinetobacter Iwoffii</i>	<i>Gemella haemolysans</i>	<i>Parvimonas micra</i>
<i>Actinomyces israelii</i>	<i>Giardia lamblia</i>	<i>Peptostreptococcus anaerobius</i>
<i>Actinomyces pyogenes</i>	<i>Haemophilus ducreyi</i>	<i>Peptostreptococcus asaccharolyticus</i>
<i>Aerococcus viridans</i>	<i>Haemophilus influenzae</i>	<i>Peptostreptococcus magnus</i>
<i>Aeromonas hydrophila</i>	<i>Helicobacter pylori</i>	<i>Peptostreptococcus productus</i>
<i>Aggregatibacter actinomycetemcomitans</i>	Herpes simplex virus I	<i>Plesiomonas shigelloides</i>
<i>Agrobacterium radiobacter</i>	Herpes simplex virus II	<i>Porphyromonas gingivalis</i>
<i>Alcaligenes faecalis</i>	HIV-1	<i>Prevotella bivia</i>
<i>Anaerococcus prevotti</i>	Human Coronavirus 229E	<i>Prevotella oralis</i>
<i>Arcanobacterium haemolyticum</i>	Human Immunodeficiency virus I	<i>Propionibacterium acnes</i>
<i>Atopobium vaginae</i>	Human metapneumovirus	<i>Proteus mirabilis</i>
<i>Bacillus subtilis</i>	Human papilloma virus 16	<i>Proteus penneri</i>
<i>Bacteroides fragilis</i>	<i>Kingella dentrificans</i>	<i>Proteus vulgaris</i>
<i>Bacteroides ureolyticus</i>	<i>Kingella kingae</i>	<i>Providencia rettgeri</i>
<i>Bifidobacterium adolescentis</i>	<i>Klebsiella oxytoca</i>	<i>Providencia stuartii</i>
<i>Bifidobacterium breve</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
<i>Bifidobacterium longum</i>	<i>Lactobacillus acidophilus</i>	<i>Pseudomonas fluorescens</i>
<i>Bordetella pertussis</i>	<i>Lactobacillus brevis</i>	<i>Pseudomonas putida</i>
<i>Branhamella catarrhalis</i>	<i>Lactobacillus crispatus</i>	<i>Rahnella aquatilis</i>
<i>Brevibacterium linens</i>	<i>Lactobacillus jensenii</i>	Respiratory syncytial virus
<i>Campylobacter coli</i>	<i>Lactobacillus lactis</i>	Rhinovirus
<i>Campylobacter jejuni</i>	<i>Lactobacillus leichmannii</i>	<i>Rhodospirillum rubrum</i>
<i>Campylobacter rectus</i>	<i>Lactobacillus oris</i>	<i>Saccharomyces cerevisiae</i>
<i>Candida albicans</i>	<i>Lactobacillus reuteri</i>	<i>Salmonella choleraesuis</i>
<i>Candida glabrata</i>	<i>Lactobacillus vaginalis</i>	<i>Salmonella enterica</i>
<i>Candida parapsilosis</i>	<i>Legionella pneumophila</i>	<i>Salmonella minnesota</i>
<i>Candida tropicalis</i>	<i>Leuconostoc paramensenteroides</i>	<i>Salmonella typhimurium</i>
<i>Chlamydia muridarum</i>	<i>Listeria monocytogenes</i>	<i>Serratia marcescens</i>
<i>Chlamydia pneumoniae</i>	<i>Micrococcus luteus</i>	<i>Shigella dysenteriae</i>
<i>Chlamydia psittaci</i>	<i>Mobiluncus curtisii</i>	<i>Shigella flexneri</i>
<i>Chlamydia suis</i>	<i>Mobiluncus mulieris</i>	<i>Shigella sonnei</i>
<i>Chromobacterium violaceum</i>	<i>Moraxella catarrhalis</i>	<i>Staphylococcus aureus</i>
<i>Citrobacter freundii</i>	<i>Moraxella lacunata</i>	<i>Staphylococcus epidermidis</i>
<i>Clostridium difficile</i>	<i>Moraxella osloensis</i>	<i>Staphylococcus saprophyticus</i>
<i>Clostridium perfringens</i>	<i>Morganella morganii</i>	<i>Streptococcus agalactiae</i>
Coronavirus	<i>Mycobacterium smegmatis</i>	<i>Streptococcus anginosus</i>
<i>Corynebacterium diphtheriae</i>	<i>Mycoplasma hominis</i>	<i>Streptococcus bovis</i>
<i>Corynebacterium genitalium</i>	<i>Mycoplasma pneumoniae</i>	<i>Streptococcus dysgalactiae</i>
<i>Corynebacterium xerosis</i>	<i>Neisseria animalis</i>	<i>Streptococcus equinus</i>
<i>Cryptococcus neoformans</i>	<i>Neisseria bergeri</i>	<i>Streptococcus mitis</i>
Cytomegalovirus	<i>Neisseria cinerea</i>	<i>Streptococcus mutans</i>
<i>Deinococcus radiodurans</i>	<i>Neisseria dentrificans</i>	<i>Streptococcus pneumoniae</i>
<i>Dermia gummosa</i>	<i>Neisseria elongata</i>	<i>Streptococcus pyogenes</i>
<i>Dientamoeba fragilis</i>	<i>Neisseria flavescens</i>	<i>Streptococcus salivarius</i>
<i>Eikenella corrodens</i>	<i>Neisseria lactamica</i>	<i>Streptococcus sanguis</i>
<i>Entamoeba histolytica</i>	<i>Neisseria macacae</i>	<i>Streptomyces griseinus</i>
<i>Enterococcus avium</i>	<i>Neisseria meningitidis</i> Serogroup A	<i>Tannerella forsythia</i>
<i>Enterococcus faecalis</i>	<i>Neisseria meningitidis</i> Serogroup B	<i>Treponema denticola</i>
<i>Enterococcus faecium</i>	<i>Neisseria meningitidis</i> Serogroup C	<i>Trichomonas gallinae</i>
<i>Enterobacter aerogenes</i>	<i>Neisseria meningitidis</i> Serogroup D	<i>Trichomonas stableri</i>
<i>Enterobacter cloacae</i>	<i>Neisseria meningitidis</i> Serogroup W135	<i>Trueperella pyogenes</i>
Enterovirus	<i>Neisseria mucosa</i>	<i>Ureaplasma parvum</i>
<i>Erwinia herbicola</i>	<i>Neisseria oralis</i>	<i>Ureaplasma urealyticum</i>
<i>Erysipelothrix rhusiopathiae</i>	<i>Neisseria perflava</i>	<i>Veillonella parvula</i>
<i>Escherichia coli</i>	<i>Neisseria polysaccharea</i>	<i>Vibrio cholerae</i>
<i>Escherichia fergusonii</i>	<i>Neisseria sicca</i>	<i>Vibrio parahaemolyticus</i>
<i>Flavobacterium meningosepticum</i>	<i>Neisseria subflava</i>	<i>Yersinia enterocolitica</i>
<i>Fusobacterium necrophorum</i>	Norovirus	N/A

Orange = Virus, Yellow = Protozoan



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Antimicrobial Resistance Detection

To assess the assay's ability to detect mutations associated with AMR for both MG and NG, previously characterized clinical urine samples, NG strains from the CDC and WHO reference panels, and synthetic genes were evaluated. The BioCode® STI+ Panel was able to accurately detect SNP mutations associated with macrolide and fluoroquinolone resistance for MG and NG in clinical urine samples (Table 6a; previously characterized and/or confirmed by DNA bi-directional sequencing), CDC and WHO NG strains (Table 6b) and synthetic AMR targets (Table 6c).

Table 6a. Clinical urine samples.

Sample	Genotype
CUS-MG-1	A2059G
CUS-MG-2	A2059G
CUS-MG-3	A2059G
CUS-MG-4	A2059G
CUS-MG-5	A2058C
CUS-MG-6	A2058G
CUS-MG-7	A2058G
CUS-MG-8	A2058G
CUS-MG-9	A2058G
CUS-MG-10	A2058G
CUS-NG-1	NG S91F
CUS-NG-2	NG S91F
CUS-NG-3	NG S91F
CUS-NG-4	NG S91F
CUS-NG-5	NG S91F

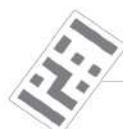
CUS = Clinical urine sample positive for AMR (Confirmed by DNA bi-directional sequencing)

Table 6b. NG reference strains.

NG Strain	Concentration	Genotype
WHO gonococcal reference strain F	1.00E+04 CFU/mL	NG S91F
WHO gonococcal reference strain G	1.00E+04 CFU/mL	NG S91F
WHO gonococcal reference strain K	1.00E+04 CFU/mL	NG S91 WT
WHO gonococcal reference strain L	1.00E+04 CFU/mL	NG S91 WT
WHO gonococcal reference strain M	1.00E+04 CFU/mL	NG S91 WT
WHO gonococcal reference strain N	4.86E-02 CFU/mL	NG S91 WT
WHO gonococcal reference strain O	4.86E-02 CFU/mL	NG S91 WT
WHO gonococcal reference strain P	1.00E+04 CFU/mL	NG S91 WT
WHO gonococcal reference strain U	1.00E+04 CFU/mL	NG S91F
WHO gonococcal reference strain V	1.00E+04 CFU/mL	NG S91 WT
WHO gonococcal reference strain W	1.00E+04 CFU/mL	NG S91 WT
WHO gonococcal reference strain X	1.00E+04 CFU/mL	NG S91F
WHO gonococcal reference strain Y	1.00E+04 CFU/mL	NG S91F
WHO gonococcal reference strain Z	1.00E+04 CFU/mL	NG S91F
CDC culture AR Bank # 963	1.00E+04 CFU/mL	NG S91F
CDC culture AR Bank # 964	1.00E+04 CFU/mL	NG S91F
CDC culture AR Bank # 971	1.00E+04 CFU/mL	NG S91F

Table 6c. Synthetic gene targets.

Synthetic Gene	Concentration	Genotype
MG A2058C gBlock	1.00E+03 copies/mL	MG A2058C
MG A2058G gBlock	1.00E+03 copies/mL	MG A2058G
MG A2058T gBlock	1.00E+03 copies/mL	MG A2058T
MG A2058C/G/T Plasmid	1.00E+03 copies/mL	MG A2058C/G/T
MG A2059C gBlock	1.00E+03 copies/mL	MG A2059C
MG A2059G gBlock	1.00E+03 copies/mL	MG A2059G
MG A2059T gBlock	1.00E+03 copies/mL	MG A2059T
MG A2058C/G/T Plasmid	1.00E+03 copies/mL	MG A2059C/G/T
MG parC S83I gBlock & Plasmid	1.00E+03 copies/mL	MG parC S83I
NG S91F gBlock & Plasmid	1.00E+03 copies/mL	NG S91F



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Conclusion

The BioCode® STI + Resistance Panel (RUO) offers a robust solution for multiplexed PCR-based detection of *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) nucleic acids directly from clinical samples, along with mutations linked to antimicrobial resistance in MG and NG. With the capacity to simultaneously test up to 96 samples per run, this assay demonstrates high specificity, comprehensive inclusivity, clinically relevant sensitivity, and no competitive inhibition as tested. This assay is for Research Use Only and not for use in diagnostic procedures. Information presented in this document was from the RUO assay. Additionally, the BioCode® MDx-3000 software provides customizable testing options for individual sample requirements, enhancing flexibility and efficiency in diagnostic workflows.

References:

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5. WHO releases new guidance to improve testing and diagnosis of sexually transmitted infections- <https://www.who.int/news/item/24-07-2023-who-releases-new-guidance-to-improve-testing-and-diagnosis-of-sexually-transmitted-infections>
6. Center for Disease Control and Prevention, Sexually Transmitted Diseases (STDs)- <https://www.cdc.gov/StD/>



BioCode® STI + Resistance Panel (RUO) Application Note

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