Biotia

BIOTIA TEST REPORT
BIOTIA-ID URINE NGS ASSAY (LDT)

ORDERING	SAMPLE	PATIENT
Institution: Biotia	ID: 100725-7846	Name: CIT Patient
Name: Biotia Clinician	Specimen Type: Urine	DOB: 11-21-2002
Address: 3935 AVIATION WAY LOS ANGELES CA 90071	Collection Date: 02-02-2026	Sex: Male
Phone Number: 2138841147	Received By Lab: 02-03-2026	ID#/HN/MRN: N/A
Email: clinician@biotia.io	Report Date: 02-04-2026	

DISCLAIMER: The urine NGS Assay is a qualitative next-generation sequencing-based *in-vitro* diagnostic test powered by Biotia-DX software. This test was developed and its performance characteristics determined by Biotia Inc. It has not been cleared or approved by the U.S. Food and Drug Administration. The Biotia Laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) and is accredited to perform high-complexity clinical laboratory testing. The reported microbial organisms may or may not be the cause of symptoms or disease. The report should be interpreted within the context of clinical information, medical history, epidemiological findings, and other laboratory results.

RESULTS SUMMARY

Detected: *Enterococcus faecalis, Klebsiella pneumoniae complex*	**Controls:** VALID

Species Detected: ⊕ *; Species Not Detected:* ⊖

Reference Value: Species Not Detected ⊖

KEY UROGENITAL PATHOGENS

Gram-Negative Enterobacteriales					
⊖	*Citrobacter species*	⊖	*Enterobacter cloacae complex*	⊖	*Escherichia coli*
⊖	*Klebsiella (Enterobacter) aerogenes*	⊖	*Klebsiella oxytoca*	⊕	*Klebsiella pneumoniae complex*
⊖	*Klebsiella variicola*	⊖	*Morganella morganii*	⊖	*Proteus mirabilis*
⊖	*Proteus vulgaris*	⊖	*Providencia rettgeri*	⊖	*Providencia stuartii*
⊖	*Raoultella ornithinolytica*	⊖	*Serratia marcescens*		

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Gram-Negative Non-Enterobacteriales								
−	*Acinetobacter calcoaceticus baumannii complex*		−	*Acinetobacter lwoffii*		−	*Pseudomonas aeruginosa*
−	*Stenotrophomonas maltophilia*							

Gram-Positive Bacteria								
−	*Aerococcus species*		−	*Anginosus Group Streptococci*		−	*Corynebacterium urealyticum*
+	*Enterococcus faecalis*		−	*Enterococcus faecium*		−	*Mitis Group Streptococci*
−	*Other Staphylococcus species*		−	*Staphylococcus aureus*		−	*Staphylococcus epidermidis*
−	*Staphylococcus lugdunensis*		−	*Staphylococcus saprophyticus*		−	*Streptococcus agalactiae*

Anaerobic Bacteria								
−	*Anaerococcus vaginalis*		−	*Bacteroides fragilis*		−	*Prevotella species*

Other Bacteria								
−	*Gardnerella vaginalis*							

Fungi								
−	*Candida albicans*		−	*Candida auris*		−	*Candida dubliniensis*
−	*Candida glabrata*		−	*Candida guilliermondii*		−	*Candida kefyr*
−	*Candida krusei*		−	*Candida lusitaniae*		−	*Candida parapsilosis*
−	*Candida tropicalis*							

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AMR RESULTS SUMMARY

Detected: *blaSHV, dfrA, dfrG, sul1*	**Controls:** VALID

Antimicrobial Resistance (AMR) Genes detected: ⊕ *; AMR Genes Not Detected:* ⊖
When all applicable bacteria are Not Detected, regardless of the result for the antimicrobial resistance gene assay(s): ***N/A***
Reference Value: AMR Genes Not Detected ⊖

ANTIMICROBIAL RESISTANCE GENES

	Resistance mechanisms to drug classes	**Gene detected**
⊕	*ESBLs*	blaSHV
⊖	*Carbapenemases*	
⊖	*AmpC beta-lactamase*	
⊕	*Folic Acid Synthesis*	dfrA, dfrG, sul1
N/A	*Methicillin*	
⊖	*Vancomycin-Glycopeptide*	

Test Description: The BIOTIA-ID Urine NGS Assay includes the detection of genetic determinants associated with resistance to ESBLs (*blaCTX-M, blaSHV, blaTEM*), carbapenemases (*blaKPC, blaNDM, blaOXA, blaVIM*), AmpC beta-lactamases (*blaACT, blaADC, blaCMY, blaPDC, CMY2-MIR-ACT-EC*), folic acid synthesis (*dfrA/B/G, sul1-2*), methicillin (*mecA/C*), and vancomycin-glycopeptide (*vanA/B*). Detection of these genetic determinants may aid in the identification of antimicrobial resistant organisms in urine specimens. The antimicrobial resistance genes detected may or may not be associated with the pathogen responsible for disease. Positive results for these select antimicrobial resistance genes may indicate a high likelihood of resistance to the indicated drug class. Negative results for these select antimicrobial resistance genes do not indicate susceptibility, as multiple mechanisms of resistance exist.

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METHODS AND LIMITATIONS

METHODS

Methods - Clean-catch midstream urine specimens were preserved in UTT. Genomic DNA was isolated from clinical and contrived specimens using a QIAcube-MDx extraction and were quantified with Qubit-Flex. Metagenomic libraries were prepared using Illumina DNA Prep Library preparation kit. Libraries were quality checked for size and concentration using Tapestation 4200 and Qubit-Flex, respectively. Libraries were pooled with a maximum of 24-plex reactions and sequenced on an Illumina NextSeq 550 platform using a NextSeq 500/550 Mid-Output kit set to 150bp single-end reads with i5 and i7 indexes.

The BIOTIA-DX pipeline (**software version 1.0**) included removal of low quality reads and human reads. The remaining reads were pseudo-aligned to a large database of microbial genomes in a coarse classification step (the database was not reviewed by New York State). Organisms identified from coarse classification were filtered for identification quality and the remaining candidates were sent to a fine classification step. Reads were aligned to curated pangenomes for each organism and summary statistics were generated. These statistics were fed into a multiple decision tree which assigned a confidence score for whether the organism was present or absent.

Results Interpretation -
POSITIVE REPORT: Key urogenital pathogen was detected.
NEGATIVE REPORT: Key urogenital pathogen was NOT detected.
INVALID REPORT: One or more Quality Controls did not pass filter such as (1) human reads are too low (<10,000), (2) sequencing complexity is too low after human reads removal (microbial reads <10), (3) internal positive control (IPC) is not in target (<1-5%) and/or (4) external controls (PC, NEC, NTC) are invalid.

PERFORMANCE CHARACTERISTICS

Bacterial and Fungal Validation Data Summary - BIOTIA-ID Urine NGS Assay was tested using a combination of urine clinical specimens (n=143) and contrived samples (n=811) spiked in with whole organism microbial reference strains and clinical isolates for bacterial species detection. The validation yielded an overall assay performance of 99.89% sensitivity and 99.94% specificity when evaluating the entire validation dataset. The assay was also tested on clinical specimens (n=35) and contrived samples (n=493) with an overall assay performance of 99.98% sensitivity and 100% specificity for fungal species detection.

Accuracy - Urine clinical specimens were collected based on the pathogens diagnosed by culture and were comprised of five of the most common uropathogens (*E. coli, E. faecalis, K. pneumoniae, S. aureus and P. mirabilis*). We collected at least 30 specimens per analyte and used contrived specimens when clinical specimens were not available. **The clinical accuracy yielded an overall performance of 96.77% sensitivity and 99.58% specificity.**Testing the six most common fungal uropathogens (*Candida albicans, Candida auris, Candida glabrata, Candida krusei, Candida parapsilosis, and Candida tropicalis*), **the clinical accuracy yielded an overall performance of 99.58% sensitivity and 100% specificity.**

Specificity - A total of 119 microbial species and strains (bacteria, fungi, viruses, and parasites; key urogenital pathogens and genetically related organisms that may present in urine) were evaluated in the laboratory. Whole organisms were spiked into negative urine matrix, processed, and resulted in an overall sensitivity and specificity of 100% and 99.95%. In silico analysis to further evaluate the assay specificity was performed on a total of 8,266 simulated samples and yielded an overall sensitivity of 99.96% and specificity of 99.95%. As of the fungal validation, an additional 37 microbial strains were evaluated in the laboratory and a total of 4,142 simulated samples in silico resulted in an overall sensitivity and specificity of 100%.

Sensitivity - The limit of detection (LoD) was assessed on ten of the most prevalent uropathogens using whole organisms spiked into negative urine matrix at different CFU/mL concentrations and 6 total replicates were tested per concentration (n=495). The LoD was reproducibly verified and determined based on a 100% positivity rate. The overall LoD was <25,000 CFU/mL. Specifically, a LoDs of 7,500 CFU/ mL for *E. coli, G. vaginalis, K. pneumoniae* and *P. mirabilis*; 10,000 CFU/mL for *E. faecalis*; 12,500 CFU/mL for *Prevotella spp.*; 15,000 CFU/mL for *A. baumannii* and *S. aureus*; 25,000 CFU/mL for *P. aeruginosa* and *B. fragilis* was determined. The LoD was also assessed on six of the most prevalent fungal uropathogens (n=214) with an overall LoD <5,000 CFU/mL. Specifically, a LoD of 1,000 CFU/mL for *C. glabrata, C. krusei, C. parapsilosis, C. tropicalis* and 5,000 CFU/mL for *C. albicans* and *C. auris* was determined.

Antimicrobial Resistance Genes Validation Data Summary - BIOTIA-ID Urine NGS Assay was tested for the detection of 15 antimicrobial resistance (AMR) gene markers belonging to 6 different resistance mechanisms. This validation included a combination of urine clinical specimens (n=371) and contrived samples (n=226). A total of 1,977 AMR genes were tested in this analytical validation that yielded an overall assay performance of 99.50% sensitivity and 99.92% specificity when evaluating the entire validation dataset(n= 2,395 analytes).

AMR Accuracy – Specificity – Sensitivity - The accuracy verification study consistedof 429 total specimens (371 clinical, 58 contrived). This study was a randomized, double blinded study. **The clinical validation yielded an overall performance of 99.26% sensitivity and 99.80% specificity.** A total of 18 bacterial species, 59 strains and 140 of AMR genes were evaluated for the specificity study. Whole organisms were spiked into negative urine matrix at a concentration of 25,000 CFU/mL and processed with the assay yielding an overall sensitivity and specificity of 100%. The sensitivity (limit of detection, LoD) was assessed on two bacterial pathogens containing 8 AMR genes(*Klebsiellapneumoniae: blaKPC, blaTEM, blaCTXM, blaOXA, blaSHV, sul, dfr; Enterococcusfaecalis: van*). Whole organisms were spiked together into negative urine matrix at different CFU/mL concentrations and 6 total replicates were tested per concentration. The overall AMR LoD was <12,500 CFU/mL, which is closely aligned with our LoD for bacterial taxa identification.

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