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ADVANCED MOLECULAR DIAGNOSTICS:

Addressing Complicated and Recurrent Urinary Tract Infections through Clinical Metagenomics

KEY TAKEAWAYS

- Urine culture has many limitations that are often overlooked by clinicians, leading to diagnostic uncertainty and unnecessary empiric use of antimicrobial therapy.
- The BIOTIA-ID Urine Test is backed by comprehensive analytical and clinical validation. Our end-to-end solution delivers accurate pathogen identification in patients with recurrent or complicated UTIs, immunocompromised patients, as well as patients for whom urine culture testing failed.
- With secure patient management guidelines, in addition to wet lab and bioinformatic optimizations, the BIOTIA-ID Urine Test overcomes common concerns in clinical metagenomics, such as overdiagnosis.

Urinary tract infections (UTIs) are among the most prevalent bacterial infections globally, responsible for an estimated 404 million cases annually worldwide and approximately 11 million cases in the United States alone (1). While many UTIs are self-limited or uncomplicated, a significant subset progresses to complicated or recurrent infections (cUTIs and rUTIs), especially in patients with urological abnormalities, immunosuppression, or catheterization. These high-risk populations face increased morbidity, elevated healthcare costs, and a heightened risk of progression to urosepsis, which carries substantial mortality (2).

The limitations of conventional urine culture, the current diagnostic gold standard, have been increasingly recognized in both the research and clinical communities. Culture can take from 24 to 72 hours for results, and lacks sensitivity in detecting fastidious organisms, mixed infections, or pathogens in patients who have received prior antimicrobial therapy. In such cases, false negatives range from 30-50%, leading to diagnostic uncertainty and empiric use of broad-spectrum antibiotics, which in turn drives antimicrobial resistance and poor outcomes (3-4).

Given these challenges, there is a growing demand for diagnostic tools that provide rapid, comprehensive, and reliable detection of urinary pathogens to support clinical decision-making (5). In the last decade, next-generation sequencing (NGS) has emerged as a transformative tool for infectious disease diagnostics. Unlike culture or PCR-based methods, NGS enables hypothesis-free pathogen detection by sequencing all nucleic acids in a clinical sample. Several new tests are entering the clinical market, aimed at identifying pathogens in blood, sterile fluid (cerebrospinal fluid and joint fluid), and urine. These metagenomic approaches provide broad-spectrum detection and hold promise for overcoming the limitations of traditional microbiological methods, especially in detecting unculturable or unexpected organisms.

To address these diagnostic gaps, the BIOTIA-ID Urine Test was developed as a next-generation sequencing (NGS)-based metagenomic assay that enables comprehensive, culture-independent identification of uropathogens directly from urine specimens. The test employs an end-to-end clinical workflow that includes genomic DNA extraction, DNA sequencing, and a proprietary bioinformatics pipeline (BIOTIA-DX) that leverages machine learning (ML) for highly accurate pathogen classification.

Unlike targeted molecular panels or 16S sequencing, BIOTIA-ID uses shotgun metagenomics to identify DNA from a wide range of organisms, including bacteria, fungi, viruses, and parasites. The BIOTIA-DX pipeline includes a rigorously curated microbial genome database and a ML-based classifier that distinguishes true infections from low-level contamination or commensal DNA, thereby enhancing specificity without sacrificing sensitivity. This technology also enables the characterization of antimicrobial resistance gene markers with one test. Clinical metagenomics has the power to transform how we diagnose and manage patients with complicated, recurrent, or culture-negative urinary tract infections.

The Standard of Care is Not Enough

Urine culture remains the standard diagnostic tool for UTI detection. It involves growing bacteria on selective media and interpreting colony counts using thresholds originally defined for healthy outpatient women. However, this method is inherently limited by critical limitations:

- **Sensitivity:** Culture can fail to detect organisms such as anaerobes, fungi, and fastidious bacteria that do not grow well on standard media. *Candida* species, for example, are often missed or simply reported as "yeast" without speciation.
- **Prior Antibiotic Use:** Culture yield is significantly reduced in patients who have received antibiotics before urine collection, leading to false-negative results despite ongoing infection.
- **Polymicrobial Infections:** Mixed cultures are frequently dismissed as contamination, despite evidence that polymicrobial UTIs are clinically significant, particularly in cUTI and catheter-associated UTI cases.
- **Diagnostic Turnaround Time:** Delays in pathogen identification slow appropriate treatment decisions, especially critical in sepsis or immunocompromised patients.
- **Interpretive Ambiguity:** Culture thresholds (e.g., 10^5 CFU/mL) were developed for healthy outpatient women and may not be appropriate in hospitalized or catheterized patients.

Polymerase-chain reaction (PCR) is another technology that has gained popularity in addressing cUTI/rUTIs. PCR amplifies fragments of the genomes of specific pathogens. PCR-based assays represent a step forward, offering faster turnaround and improved sensitivity over culture. However, they are inherently limited by their targeted nature. PCR can only detect organisms explicitly included in the assay design and thus may miss rare or unexpected pathogens. Additionally, many PCR panels fail to account for microbial load or the context of polymicrobial infections. Furthermore, PCR is susceptible to amplification bias, where certain sequences are preferentially amplified over others, potentially leading to false positive results.

Clinical metagenomic next-generation sequencing (mNGS) assays allow for the testing of patient urine samples without the need for culturing. By sequencing genetic material and comparing detected sequences to a comprehensive microbial genome database, these tests can accurately and rapidly identify pathogens in cUTI/rUTI patient samples, which may have atypical pathogens, multiple co-infecting organisms, and complex drug resistance profiles. The BIOTIA-ID Urine Test is the first test using mNGS approaches on urine samples to receive approval across all 50 U.S. states as a laboratory-developed test, demonstrating its extensive analytical and clinical rigor and marking a huge step forward in bringing this technology to patients.

Clinical metagenomics has the power to transform how we diagnose and manage patients with complicated, recurrent, or culture-negative UTIs.

That's why we created the BIOTIA-ID Urine Test.

The BIOTIA-ID Urine Test

In a comprehensive analytical and clinical validation study, BIOTIA-ID was tested on 1,470 urine specimens across 65 sequencing runs (6). The validation followed NY State Department of Health guidelines and included both clinical and contrived specimens, orthogonally validated with culture, qPCR, and Sanger sequencing.

- **Clinical Accuracy:** A total of 335 clinical urine specimens were tested with 97.2% sensitivity and 99.6% specificity. Across >14,500 analytes in contrived and clinical samples, the BIOTIA-ID Urine Test achieved >99.9% overall sensitivity and specificity, underscoring its high precision and clinical reliability.
- **Specificity:** BIOTIA-ID demonstrated 100% sensitivity and 99.96% specificity for bacterial targets, and 100% sensitivity and specificity for fungal targets evaluating a total of 69 microbial species and 154 strains.

- **Analytical Sensitivity (Limit of Detection):** The test was assessed in the 16 most frequently found uropathogens to reliably detect pathogens at a low, but clinically-relevant concentration.
- **Reproducibility:** In reproducibility studies using 19 diverse microbial pools over multiple days, BIOTIA-ID maintained 100% sensitivity, specificity, and qualitative reproducibility, even when tested across genetically variable strains.
- **In Silico Testing:** In silico analysis revealed 99.99% sensitivity and specificity, with a low 1.7% rate of initial cross-reactivity—mostly among non-urogenital or closely related species. Bioinformatic refinements reduced false positives to 0.3%, demonstrating high accuracy in distinguishing clinically relevant pathogens.
- **AMR Validation:** 14 antimicrobial resistance gene markers related to 6 different resistance mechanism classes were validated evaluating 597 specimens and 2,395 total analytes achieving an overall clinical accuracy of 100% sensitivity and 99.6% specificity. 34.8% of clinical specimens were positive for AMR with 271 analytes detected.

In our clinical accuracy study, BIOTIA-ID detected a broader spectrum of pathogens compared to urine culture. These pathogens were primarily key uropathogens (71.8%) but also organisms not usually detectable by standard urine culture methods. Due to limitations of the culture and the high sensitivity of NGS, apparent false positives and false negatives were tested using qPCR and Sanger sequencing as an additional comparator to confirm the results. 87% of the NGS results were concordant with the comparator. The combined results from these studies have yielded a novel advanced diagnostic for urinary tract infections, confirmed to outperform traditional urine culture in the detection of key pathogens.

Demonstrated Clinical Utility

We conducted a clinical study in partnership with State University of New York (SUNY) Downstate to assess the performance of the BIOTIA-ID Urine Test on 200 urine specimens from patients with UTI symptoms but negative culture results (7). The BIOTIA-ID Urine Test was able to detect known uropathogens in 63% of culture-negative specimens, 50% of which were single organism infections and 50% of which were polymicrobial infections. Retrospective evaluation of the patient records found that in 70% of cases the prescribed antimicrobial would have had no effect on the pathogen detected, and in 18% it could have been de-escalated. These findings have immense implications for improving health outcomes of patients with negative culture and supporting clinicians to provide targeted antimicrobial therapy when standard of care diagnostics fail.

In addition, we partnered with a private hospital in Southeast Asia to evaluate the BIOTIA-ID Urine Test's applicability to populations outside of the United States. 382 clinical specimens were included in this study, 228 of which were positive and 154 negative by urine culture (8). BIOTIA-ID identified at least one organism in 97.1% of culture-positive samples, with an overall organism-level sensitivity of 94.6%. Notably, BIOTIA-ID detected co-infecting organisms in 18.4% of culture-positive samples. BIOTIA-ID identified organisms in 74.0% of culture-negative samples, the majority of which were anaerobic or fastidious. Among detections not captured by standard culture, 75.2% were anaerobic or fastidious bacteria, and more than half of the remaining organisms were found in polymicrobial infections. This data demonstrates that our test had enhanced detection of key urogenital pathogens that may be missed by the traditional standard of care. In addition, these findings underscore the BIOTIA-ID Urine Test's ability to identify anaerobic, fastidious, and polymicrobial infections more effectively than standard urine culture, supporting its clinical accuracy and global applicability.

Laboratory

- **Validated on 950+ clinical specimens in over 1500+ reactions**
- **Optimized sample processing for fastidious bacteria and fungi**
- **Quality controls provide rigorous and standardized clinical-grade results**

Bioinformatics

- **Over 7,000+ curated microbes in reference database for analysis**
- **High accuracy in prediction of antimicrobial resistance**
- **Proprietary machine learning classification step**

Results

- **97% sensitivity and 99% specificity**
- **40+ key uropathogens and resistance genes for 6 resistance mechanisms**
- **Implemented clinical thresholds calibrated to colony-forming units (CFUs) to eliminate overdiagnosis**

Guidelines for Clinical Implementation

We built out a secure patient management guideline to achieve accurate diagnostic stewardship, identify the right patients for the test, and potentially improve patient outcomes. The BIOTIA-ID Urine Test is intended to be used as a reflex, supplemental, or replacement diagnostic in specific clinical contexts where culture is known to underperform and advanced diagnostic tests are needed. Recommended use cases include:

- **Recurrent UTIs (rUTIs):** To identify persistent or previously undetected pathogens in patients with ≥3 UTIs per year or ≥2 in the past six months.
- **Complicated UTIs (cUTIs):** Including those with urinary tract abnormalities, catheter-associated infections, or pyelonephritis.
- **Culture-Negative Symptomatic Patients:** Persistent UTI symptoms with negative or inconclusive culture results.
- **Immunocompromised Patients:** Elderly with co-morbidities (diabetes, dementia, catheterized patients), transplant and oncology patients, or those on immunosuppressive therapy.
- **Sepsis of Unknown Origin:** When a urinary source of sepsis is suspected but not confirmed by culture.

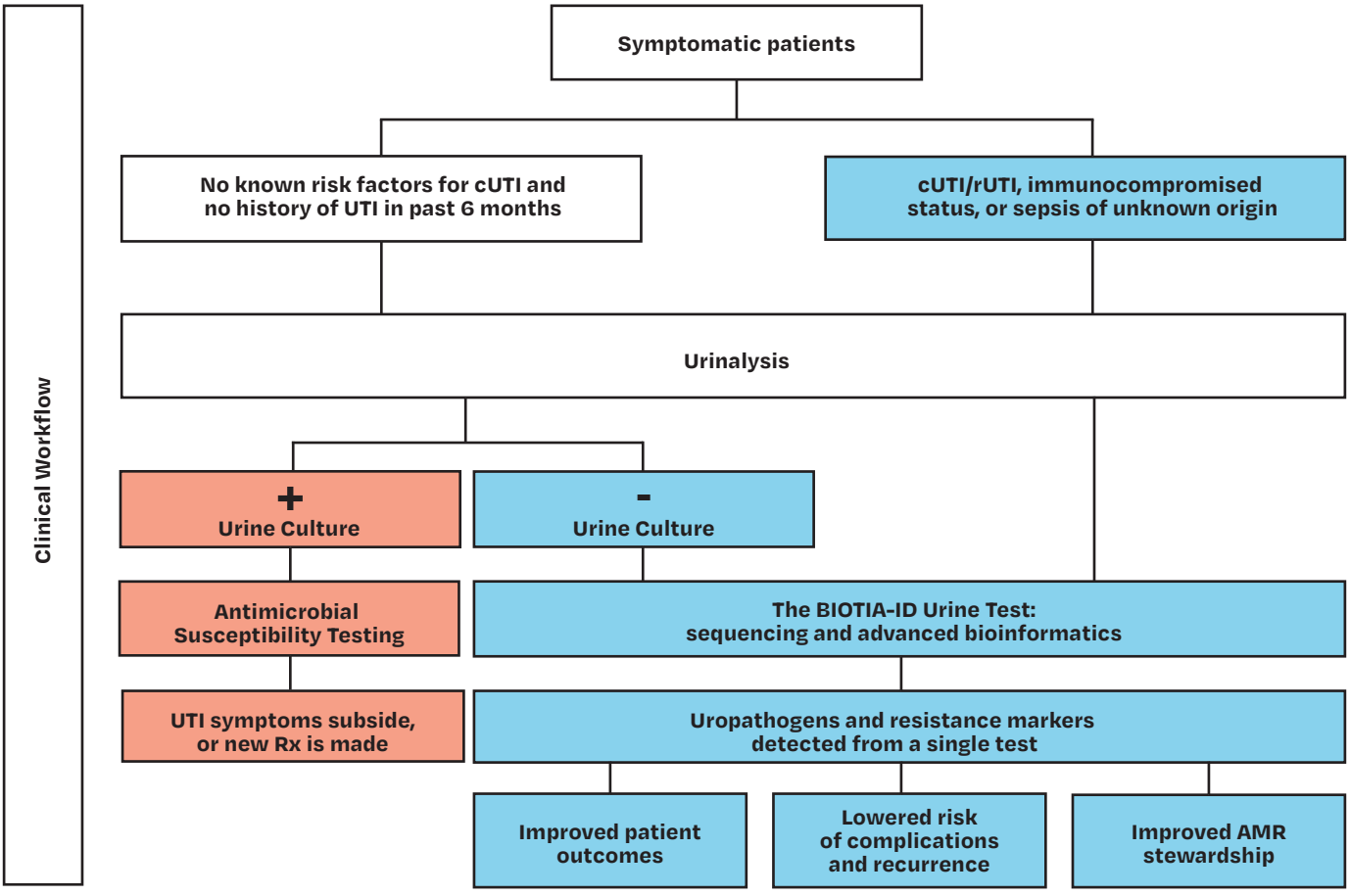
Integration into Clinical Workflow

UTI diagnosis is a clinical process that combines patient history, presenting symptoms, physical examination, and laboratory findings to confirm the presence and type of infection. The BIOTIA-ID Urine Test can be seamlessly integrated at multiple points in the patient care pathway to enhance clinical decision-making and support value-based care:

- **Initial Diagnostic Workup:** Provides rapid, comprehensive results for high-risk patients or those with prior antibiotic exposure, enabling early targeted therapy.
- **Post-Culture Follow-Up (Reflex test):** Delivers actionable insights for unresolved infections or cases with negative culture results, reducing diagnostic uncertainty.
- **Antimicrobial Stewardship:** Supports precision prescribing through accurate pathogen identification and resistance profiling, reducing unnecessary antibiotic use and improving outcomes.

By embedding advanced diagnostics into routine clinical workflows, providers can improve diagnostic accuracy, accelerate appropriate treatment, and optimize resource utilization.

Urinary Tract Infection Clinical Workflow Diagram



Challenges in Metagenomic Approaches to UTI

The landscape for clinical metagenomic tests for complicated/recurrent UTI is rapidly evolving. Several companies are marketing their laboratory-developed tests (LDTs) directly to consumers, posing numerous challenges for clinicians and patients alike that the BIOTIA-ID Urine Test overcomes.

With the ability to get a snapshot of the entire urinary microbiome from a single test, there are many questions about what to do with the information following results reporting. One key area of concern for clinicians is the potential for overdiagnosis due to the high sensitivity of mNGS. There is concern that reporting both pathogenic and commensal microbes, as many tests on the market do, will lead to overtreatment of nonpathogenic microbes. From the patient perspective, receiving a report of both pathogenic and commensal microbes may cause undue stress without appropriate clinical context being provided. While it may look attractive to patients to get tested for thousands of microbes, there is little understanding of the clinical significance of the presence or relative abundance of these species, ultimately obfuscating clinical decision-making.

With subject matter expert consultation, a total of 44 known uropathogens were validated to be reported by the BIOTIA-ID Urine Test. The bioinformatics pipeline, which includes a proprietary machine learning (ML) step, was trained on real patient specimens with substantial metadata, ensuring reliability in the reporting of detection. Additionally, the bioinformatics pipeline has been set to filter out commensal microflora and species related to contamination – simplifying clinical-decision making for the providers and preventing unnecessary worry for the patients. Clinical reports are provided with organism-specific data, including classification confidence. Polymicrobial detections are included when clinically relevant, enabling informed decisions on targeted antimicrobial therapy.

Additionally, many tests on the market purport to offer insight into antimicrobial resistance, posing another challenge. The detection of antibiotic resistance genes (ARGs) alone is insufficient to determine resistance. Through mNGS approaches it is quite possible to identify a wide number of ARGs in a patient sample, however, the presence of these genes is not enough evidence to rule out a particular therapy. ARGs may: (1) be present in commensal microbial species and not the pathogenic species detected; (2) work in tandem with one another to produce different levels of susceptibility/resistance; and (3) be present in a given uropathogen but not necessarily confer functional resistance to a given therapy. Thus, reporting of all ARGs in a patient sample paradoxically poses a threat to appropriate antimicrobial stewardship. Great care must be taken bioinformatically to reference curated, clinical databases with appropriate clinical thresholds for susceptibility.

In a study using both culture-positive and culture-negative patient samples, we assessed the performance of the BIOTIA-ID Urine Test's ability to detect ARGs (9). We found that our assay's ability to detect ARGs had a corresponding accuracy between 96-100% for all examined genes as compared to traditional PCR assays. Through this research, we additionally demonstrated that our test can reliably predict common antimicrobial resistance profiles by leveraging our clinically curated database of over 7,000 microbes, addressing a key challenge in applying clinical metagenomics to cUTI/rUTI cases. In fact, the BIOTIA-DX software ability to predict antimicrobial resistance was awarded "Best Prediction Accuracy" from the Critical Assessment of Massive Data Analytics conference in 2025, highlighting the rigor of the bioinformatics pipeline.

Conclusion

The BIOTIA-ID Urine Test demonstrates a marked improvement over standard culture methods in sensitivity, specificity, and pathogen breadth, including accurate identification of fastidious, fungal, and polymicrobial infections. Its robust validation and high diagnostic accuracy, as described by Couto-Rodriguez et al., support its clinical utility in managing complicated, recurrent, and high-risk UTI cases. As UTI diagnostics continue to evolve, the BIOTIA-ID Urine Test offers a precision medicine approach that aligns with the goals of antimicrobial stewardship and improved patient outcomes, addressing key challenges in leveraging this technology for the clinic.

Biotia remains dedicated to fighting infectious diseases through pathogen genomics and artificial intelligence. Together with our collaborators, we are changing the way we diagnose complicated and recurrent urinary tract infections for the better.

References:

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Get Better Answers with the BIOTIA-ID Urine Test Today

The BIOTIA-ID Urine Test is a qualitative next-generation sequencing (NGS)-based in vitro diagnostic test powered by BIOTIA-DX software. The assay detects nucleic acid of a panel of common, clinically-relevant uropathogens in urine. The identification of specific pathogen DNA in samples from patients with signs and symptoms of urinary tract infection caused by bacteria helps to diagnose these infections in conjunction with other clinical or laboratory findings. Detection of the reported microbial organisms should be interpreted within the context of clinical information, medical history, epidemiological findings, and other laboratory results.

Species and Resistance Classes Reported

Species:

- Gram-Negative Enterobacteriales
- Gram-Negative Non-Enterobacteriales
- Gram-Positive Bacteria
- Anaerobic Bacteria
- Other Bacteria
- Fungal Species

Resistance classes (RUO):

- Extended-spectrum beta-lactamases (ESBLs)
- Carbapenemases
- AmpC beta-lactamases
- Folic acid synthesis
- Methicillin
- Vancomycin-glycopeptide

How to Order

The BIOTIA-ID Urine Test is available for ordering across all 50 U.S. states. To order, clinicians may register for an account on the Biotia Portal using their email address and NPI number. On the portal, clinicians are able to complete an order request, print a requisition form, and receive test results. Results are typically returned in three business days and are easily integrated into electronic health record (EHR) systems.

Additionally, this test will soon be available for at-home collection. This will allow patients who receive an order for this test from a clinician to acquire a test kit by mail and collect their sample in the privacy of their home. Patients will be required to ship the specimen to the Biotia Laboratory using a pre-paid shipping label included in the test kit.

**The BIOTIA-ID Urine NGS Assay has been approved by New York State Department of Health's Clinical Laboratory Evaluation Program as an in vitro diagnostic laboratory developed test (LDT). This assay was developed and its performance characteristics determined by Biotia. The assay has not been cleared or approved by the FDA, nor is it required to be. 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.*

Specimen Requirements/Transport

Samples should be collected as clean catch midstream urine specimen collected in a sterile urine cup and transferred into a urine culture transport tube (UTT). Invasively collected specimens, such as those collected by a suprapubic catheter or from the renal pelvis, are also acceptable, and should be transported in a UTT. Specimens in UTT are stable for up to 48 hours at room temperature or for up to 1 week when stored at 2°C to 8°C.

After collection in the clinic, UTTs should be placed in a biohazard bag with absorbent paper and shipped to the Biotia Laboratory in a UN3373 Category B Pack. Transportation of patient samples must comply with all applicable governing regulations for the transport of etiologic agents. Specimens must be received within 48 hours of collection if shipped at room temperature or within a week if maintained and shipped on ice packs (2°C-8°C). If interested, Biotia will assist in setting up a courier for your clinic. Reach out to our business development team to get started.

If you would like additional support in onboarding this test, you may reach out to:

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Or visit us at **www.biotia.io**.

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Answers start here.