WHITE PAPER

THE ROLE OF NEXT-GENERATION SEQUENCING IN RECURRENT, COMPLICATED, AND CULTURE-NEGATIVE UTI

URINE DIAGNOSTIC STEWARDSHIP

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Disclaimer: This white paper is intended for clinicians and laboratory and diagnostics professionals. It is provided for educational and informational purposes only, has not been peer-reviewed, and does not constitute medical advice or replace professional clinical judgment. It may also be shared with patients as educational material relating to this diagnostic. BIOTIA-ID Urine is a laboratory-developed test; results should be interpreted in the context of each patient's clinical presentation.

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Urine diagnostic stewardship centers on using laboratory testing thoughtfully to improve patient outcomes while minimizing unnecessary antibiotics and misdiagnosis. Clinical guidance from organizations such as the Infectious Diseases Society of America emphasizes that urine testing should be driven by symptoms and clinical context, not performed routinely in asymptomatic individuals[1], and always interpreted with an understanding that not all detected microbes represent true infection. A key principle is distinguishing infection from colonization and contamination, particularly in a biological environment like the urinary tract where low levels of microbes may be present without causing disease.

Traditional urine culture remains the standard diagnostic method, but it has important limitations that affect patient care. It may fail to detect fastidious or slow-growing organisms, underestimate polymicrobial infections, and produce false negatives in patients who have already received antibiotics or who have recurrent or complicated infections. These gaps can lead to repeated empiric treatment, persistent symptoms, and frustration for both patients and clinicians.

Next-generation sequencing (NGS), also called clinical metagenomics in our field, introduces a complementary, culture-independent approach that can identify a broader spectrum of bacterial and fungal organisms, as well as antimicrobial resistance markers, directly from urine samples. When applied appropriately, NGS has particular value in complex clinical scenarios such as recurrent and complicated urinary tract infections, persistent symptoms despite treatment, or culture-negative cases where suspicion for infection remains high. However, antibiotic stewardship principles remain essential: detection alone does not equal disease, and results must be interpreted in the context of symptoms, organism burden (or presence/absence based on a clinical threshold), and clinical history. Advances such as machine learning–based classifiers can improve specificity by helping distinguish likely pathogens from background microbial signals.

Integrating BIOTIA-ID Urine into Clinical Practice

BIOTIA-ID Urine is an NGS test designed to bring this advanced diagnostic capability into patient care by combining metagenomic sequencing with analytical methods that improve clinical relevance. The assay is analytically validated as a laboratory-developed test[2]; it enables detection of bacteria and fungi that may be missed by culture, supports identification of polymicrobial infections, and provides antimicrobial resistance gene insights that can guide therapy selection.

A key component of this approach is the integration of machine learning within the NGS workflow. By training on large clinical datasets, Biotia's classifier (BIOTIA-DX) helps establish clinically meaningful thresholds that differentiate true infection from background microbiome, colonization, or contamination. Rather than relying solely on presence or absence of organisms, the model evaluates patterns such as relative abundance, co-detection signatures, and known pathogenicity to prioritize organisms most likely driving disease. This added layer of interpretation improves diagnostic accuracy, reduces false positives, and supports more confident clinical decision-making, particularly in complex or polymicrobial samples where conventional methods often fall short.

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In practice, a stewardship-aligned approach begins with standard evaluation for patients presenting with typical, uncomplicated urinary symptoms. Initial management may still rely on urinalysis and culture where appropriate. However, when patients experience recurrent infections, fail to respond to treatment, have an infection that occurs alongside other health issues, physical blockages, or medical devices which indicates a potential complicated UTI, or have negative cultures despite ongoing symptoms, incorporating an NGS-based test such as BIOTIA-ID Urine can provide additional resolution. The results are then interpreted alongside the patient's symptoms and history, using classifier-informed outputs to prioritize clinically meaningful organisms. This approach supports more targeted therapy, helps avoid unnecessary antibiotics directed at colonizing organisms, and can reduce the cycle of repeated empiric treatments.

Patient Selection and Eligibility

Because a comprehensive, culture-independent assay detects organisms across the full spectrum from established urogenital pathogens to commensal and contaminating flora, its value depends on selecting the right patients. BIOTIA-ID Urine is intended as a problem-solving test for patients for whom standard culture is most likely to fail or to mislead, not as a screening test for every urinary complaint. Eligibility is established at the point of order through a structured intake that captures the patient's current symptoms and their onset, risk factors, prior UTI history and previous response to treatments, culture and/or PCR results, comorbidities, and any urinary devices or procedures. These inputs map to three groups of patients in whom NGS-based testing adds the most clinical value: those with recurrent or complicated UTI[3]; those who remain symptomatic despite negative or non-diagnostic cultures; and those at high risk for urosepsis or other poor outcomes because they are immunocompromised or medically complex. Figure 1 shows how these groups fit within a stewardship-aligned pathway, and Table 1 details the eligibility criteria for each.

The three indicated groups overlap in practice — a transplant recipient with recurrent, culture-negative symptoms may satisfy all three — and it is precisely these patients, who accumulate multiple risk factors, for whom conventional workup most often falls short. Conversely, the test is not indicated for asymptomatic bacteriuria or for routine, uncomplicated infections expected to respond to first-line therapy, where standard culture remains appropriate and additional testing would run counter to stewardship goals.

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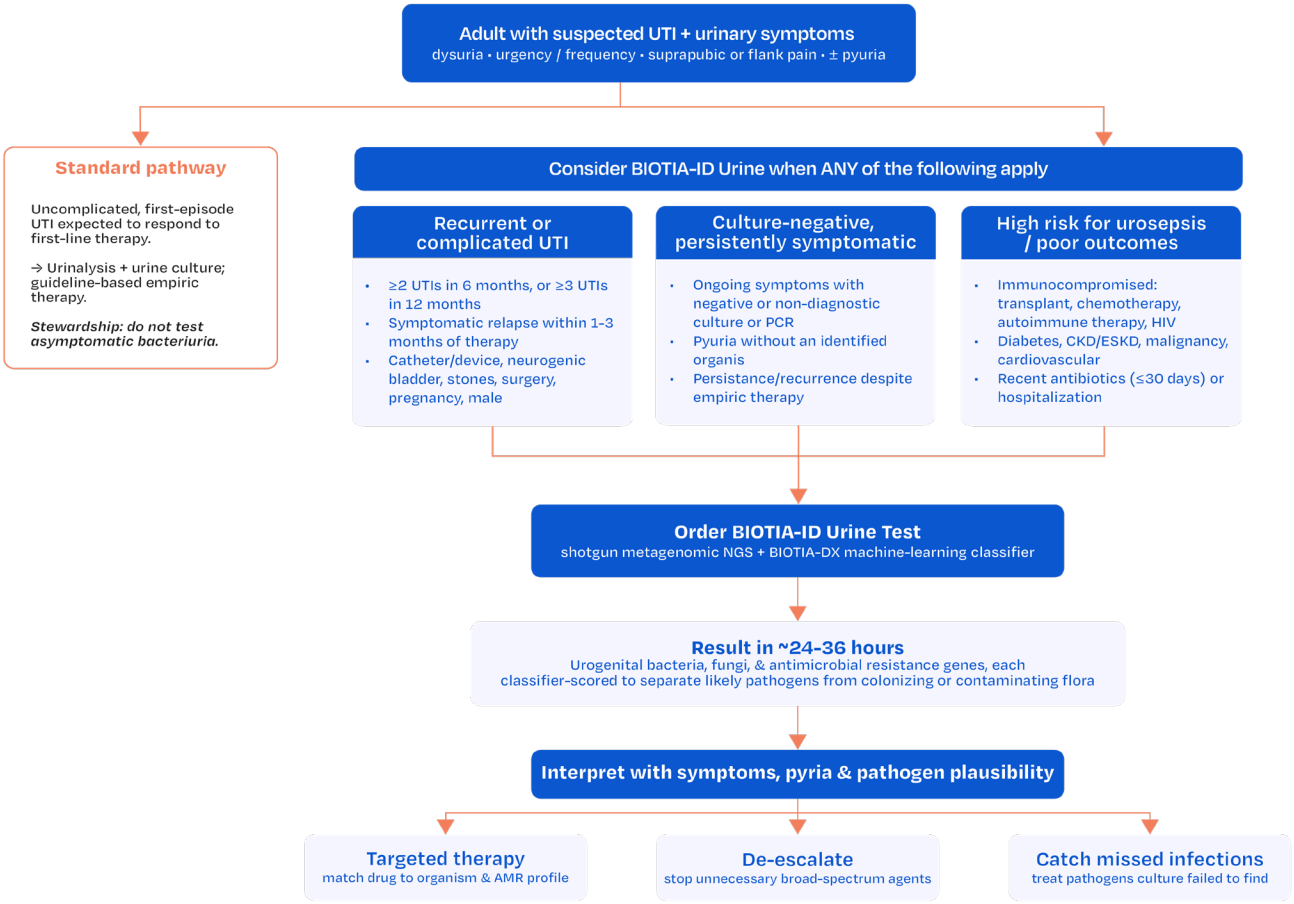


Figure 1. *Stewardship-aligned pathway for BIOTIA-ID Urine. Patients with uncomplicated, first-episode infection follow the standard culture pathway, while three higher-complexity groups are directed to NGS-based testing. Classifier-scored results are interpreted alongside the clinical picture to enable targeted therapy, de-escalation, and detection of infections that culture misses.*

Table 1. Eligibility criteria for BIOTIA-ID Urine by indicated patient group.

Indicated group	Key eligibility criteria (captured at order)	Why NGS adds value
Recurrent or complicated UTI	≥ 2 UTIs in 6 months or ≥ 3 in 12 months; symptomatic relapse within 1–3 months of completing therapy; culture-confirmed prior infections; complicating factors such as an indwelling catheter or urinary device, neurogenic bladder, urolithiasis, recent urologic procedure, pregnancy, or male anatomy.	Polymicrobial and resistant organisms are common in this group and are frequently under-reported by culture; NGS resolves mixed communities and detects resistance markers to guide targeted therapy.

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Indicated group	Key eligibility criteria (captured at order)	Why NGS adds value
Culture-negative but persistently symptomatic	Ongoing UTI symptoms (dysuria, urgency or frequency, suprapubic or flank pain) with a urine culture or PCR that is negative, non-diagnostic, or below the reporting threshold; pyuria without an identified organism; symptoms persisting or recurring despite empiric treatment.	Culture-independent detection identifies fastidious, anaerobic, slow-growing, and atypical organisms missed by culture, converting a "no-growth" result into an actionable diagnosis.
High risk for urosepsis or poor outcomes	Immunocompromised patients — solid-organ transplant, active chemotherapy, autoimmune or immunosuppressive therapy, HIV/AIDS; medically complex patients — diabetes, chronic or end-stage kidney disease, malignancy, cardiovascular disease; recent antibiotic exposure (≤ 30 days) or hospitalization.	A missed or mistreated infection carries disproportionate risk; earlier organism-and-resistance identification supports prompt, appropriate therapy and reduces the chance of progression to sepsis.
Not indicated (stewardship)	Asymptomatic bacteriuria should not be tested. Routine, uncomplicated, first-episode UTI expected to respond to first-line therapy is managed with standard urinalysis and culture.	

How to Understand This as a Clinician

For clinicians, urine diagnostic stewardship is fundamentally about decision-making under uncertainty. The goal is not to replace existing tools but to layer diagnostic methods in a way that increases confidence and precision. Standard culture remains appropriate for many first-line scenarios, but its limitations should be recognized, particularly in patients with complex or persistent symptoms. NGS-based diagnostics provide an opportunity to resolve ambiguity, but only when used selectively and interpreted carefully.

Clinicians should view tests like BIOTIA-ID Urine as problem-solving tools rather than screening tools. The added sensitivity of NGS requires a shift in interpretation, where clinical correlation becomes even more important to avoid overcalling infection. When used within a structured framework, these tools can improve diagnostic clarity, enable more precise antimicrobial selection, and support broader stewardship goals.

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How to Understand This as a Patient

For patients, this approach reflects a shift toward more personalized and precise care. Not every urinary symptom requires extensive testing, and not every detected microbe requires antibiotic treatment; initial testing may still use traditional methods for straightforward infections that respond quickly to therapy. When symptoms persist, recur, or are not explained by standard tests, a more advanced diagnostic like BIOTIA-ID Urine can look deeper — identifying which organisms are truly driving symptoms and which are simply present without harm, so treatment can be more targeted and unnecessary antibiotics avoided. Ultimately, this reduces trial-and-error treatment and provides clearer answers, especially in complex or long-standing cases.

Evidence of Clinical Utility: The SUNY Downstate Study

In a study conducted with SUNY Downstate Health Sciences University[4], BIOTIA-ID Urine was applied to 201 culture-negative urine specimens from symptomatic, high-risk adults — median age 64, with diabetes in 24%, immunosuppression in 12%, an indwelling catheter in 19%, and 35% requiring hospitalization — whose standard cultures showed no growth, mixed or contaminated flora, or growth below the diagnostic threshold. The assay identified a urogenital pathogen in 61.5% of these culture-negative specimens (including one-third of true no-growth cultures) and detected antimicrobial-resistance gene markers in 37.5%, with 100% agreement against confirmatory qPCR. When the results were compared retrospectively with the antibiotics actually prescribed — blinded to the NGS result — empiric therapy was fully appropriate in only 15% of treated patients, while NGS findings indicated a potential change in management in 85%, including de-escalation of unnecessary broad-spectrum therapy in 56% of those on active treatment and detection of a treatable pathogen in 54% of patients who had received no antibiotics at all. As a single-center, retrospective analysis, these treatment implications reflect modeled concordance rather than prospectively measured outcomes; prospective, outcomes-linked studies are planned.

Illustrative case: where standard culture failed and NGS found the pathogen

Consider a representative patient from this population: a symptomatic adult with pyuria whose urine culture is reported as "mixed flora, no predominant organism" — a non-diagnostic result — and who is started empirically on ceftriaxone, the cohort's most common empiric agent. BIOTIA-ID Urine resolves the specimen into a defined polymicrobial infection (for example, Escherichia coli together with Enterococcus faecalis) and detects a trimethoprim resistance marker (dfrA).

A cephalosporin does not reliably cover enterococci, so the empiric regimen would have left part of the infection untreated, while the resistance marker signals that trimethoprim-sulfamethoxazole — a common oral fallback — would likely fail as well. Had the NGS profile been available up front (within ~24–36 hours), it could have guided selection of an agent active against both organisms from the start and avoided a second course of treatment. Across the cohort, exactly this kind of mismatch is why empiric therapy proved appropriate in only 15% of treated patients.

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The culture-negative population is also resource-intensive. In hospitalized patients, inappropriate empiric therapy has been associated with roughly 5.2 additional hospital days and $10,312 in excess cost per patient[5], and a single complicated-UTI hospitalization averages about $15,000. By converting a culture-negative result into a specific pathogen-and-resistance profile within approximately 24–36 hours, BIOTIA-ID Urine can shorten time to appropriate therapy and support de-escalation — precisely where empiric management most often fails.

A Step Forward in Diagnostic Innovation

Urine diagnostic stewardship, combined with advances in metagenomic sequencing, represents a meaningful evolution in how urinary tract infections are evaluated and managed. By integrating established guidelines with innovative tools like BIOTIA-ID Urine — and by directing those tools to the patients most likely to benefit — healthcare providers can move toward more accurate diagnoses, better-targeted treatments, and improved patient outcomes while maintaining a strong commitment to responsible antibiotic use.

References

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