WHITE PAPER

PATIENT EDUCATION FOR RECURRENT, CHRONIC, OR COMPLICATED URINARY TRACT INFECTIONS

NEGATIVE CULTURES

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What is Culture-Based Testing?

If you've ever had a urine sample sent to a lab, you may have heard your provider say they were "sending it for culture." It's a phrase that gets used often, but rarely explained. So what does it actually mean? Culture-based testing is a laboratory method that works by growing microorganisms — like bacteria and fungi — in a controlled environment, typically in a petri dish containing nutrients that allow those organisms to multiply. If bacteria are present in your sample, they grow into visible colonies that lab professionals can identify and study. From there, providers can determine what organism may be causing an infection and, in many cases, which antibiotics it responds to. This method has been a cornerstone of clinical microbiology since the 1800s, and for good reason. It helped transform how infectious diseases — from strep throat to fungal infections to urinary tract infections — are diagnosed and treated. For many straightforward infections, it works well.

For patients with recurrent, complicated, or persistent urinary symptoms, however, culture-based testing has recognized limitations. Understanding how culture-based testing works — and where its limitations lie — is an important first step in understanding why some patients continue to experience symptoms without a clear diagnosis, and why newer testing approaches are being explored to help fill those gaps.

Why Standard Cultures Can Come Back Negative Despite Symptoms

Standard urine and vaginal cultures are designed to detect common pathogens by growing bacteria and fungi from the specimen(s) collected from the patient. Patients with urinary tract infection (UTI) symptoms or vaginal discomfort often face frustration when lab cultures come back negative or unchanged despite ongoing symptoms. It is not unusual for women to report classic UTI symptoms (burning, urgency, and frequency, among others) even when urine cultures show no significant bacterial or fungal growth. In fact, as many as 1 in 3 urine cultures will come back negative even though the patient has a UTI [1]. As with all laboratory testing methods, urine and vaginal culture have important limitations.

Some of these include: using one-size fits all thresholds across species and/or clinical cases, pathogens needing different growth requirements, slow growth rate of the microorganism, complications due to trying to culture and identify polymicrobial infections, low microbial burden, timing of collection, among other factors.

THRESHOLDING CHALLENGES:
Traditional urine culture techniques use a specific threshold to define a "positive" infection, which was chosen based on studies of kidney infections in the 1950s. However, more recent research shows that in symptomatic bladder infections, bacterial counts below current threshold guidance (especially for E. coli) can indicate infection. Many true infections may be missed if this threshold is not reached through culture-based testing. High fluid intake or frequent urination can dilute pathogens in the urine, making the pathogens less likely to successfully grow in the laboratory. This, in turn, can cause the pathogen to be less likely to be detected. Similarly, testing urine during or directly following treatment may increase the risk of false-negative results as there will be fewer viable pathogens to grow, despite the infection persisting.

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Guidelines advise that in patients who still have UTI symptoms after treatment, a repeat urine culture should be obtained (rather than assuming the infection is gone). On the other hand, routine "test of cure" cultures are not recommended in patients who have no symptoms, as residual bacteria might be harmless, and over-treatment should be avoided.

GROWTH CHALLENGES:
Standard culture media and techniques are optimized for common bacteria, like E. coli. If an infection is caused by less common organisms or organisms that do not grow under standard culture conditions, the tests may not capture them. For example, certain bacteria such as Ureaplasma urealyticum, Mycoplasma species, or Chlamydia trachomatis do not grow on routine culture plates. These organisms can infect the urinary or genital tract, but specialized tests are needed to detect them. In women, some sexually transmitted infections can cause urethral or vaginal symptoms that mimic UTIs or vaginitis, yet they will not appear on a standard urine culture or basic vaginal swab. Trichomonas vaginalis, a protozoan parasite, can cause vaginal burning or urethritis, but would be missed by routine bacterial cultures. Fungal infections of the urinary tract or vagina might also be overlooked if the lab is only looking for bacteria. In short, a "negative" culture could simply mean the test was not suited to find the actual culprit.

SAMPLING CHALLENGES:
Researchers have shown that women with classic UTI symptoms can have an underlying infection that standard tests miss: in one study, 90.5% of women with UTI symptoms but a negative culture were found to have E. coli DNA in their urine by molecular testing [1]. This means that a negative culture did not always equal "no infection", but rather meant that the bacteria were just at low levels or did not successfully grow in the laboratory. Some bacteria can persist inside the bladder walls, evading antibiotics and the immune system, to later reactivate and cause UTI symptoms to return.

VAGINAL MICROBIOME CHALLENGES:
Patients experiencing urinary symptoms may benefit from evaluation of the vaginal environment. Because the urethra and vagina are anatomically adjacent, certain vaginal conditions can contribute to symptoms that overlap with urinary tract infection. It is important to recognize that first-line vaginal testing is typically culture-based and shares many of the same limitations as urine culture. Results can be influenced by the anatomical site sampled (e.g., mid-vagina vs. fornix), hormonal status, recent sexual activity, menstruation, and contraceptive use. Additionally, vaginal cultures rely on predefined laboratory thresholds and may not detect slow-growing or fastidious organisms. As with urine culture, false-negative results can occur, and traditional culture techniques may not fully represent the complexity of the vaginal microbiome.

POLYMICROBIAL CHALLENGES:
Another consequence is unchanged colony counts on culture: a patient's urine culture might repeatedly grow a small amount of the same bacteria (e.g., a low-grade colony count of E. coli or another species) that neither increases nor is eliminated. Standard lab definitions might label such low counts as "contamination" or insignificant, even though the patient's symptoms suggest otherwise. Emerging research suggests that for patients with persistent symptoms, laboratories should report all growth regardless of quantity, and consider lowering the threshold for a positive result.

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The limitations of these diagnostic approaches for urinary and vaginal symptoms are well-documented in clinical research. In response, new diagnostic approaches have been developed to improve the consistency and accuracy of detection of both harmful and nonharmful microbes in the urinary and vaginal microbiomes. These techniques are called molecular diagnostics.

Molecular Approaches to Diagnosing Culture-Negative UTIs

Where culture-based methods attempt to grow any pathogens that may be in a urine or vaginal sample, new laboratory methods have been developed that look for the presence and/or sequence of pathogen DNA, called molecular diagnostics. These approaches have significantly improved sensitivity and specificity over traditional culture-based methods and are being increasingly adopted.

The most common molecular method, which may be familiar due to the COVID-19 pandemic, is a technique called polymerase-chain reaction (PCR). PCR tests for urinary tract infections work by making millions of copies of a unique piece of a pathogen's DNA, which is tracked by the PCR machine in the lab. If there is no pathogen DNA in the sample, no copies are made, and no signal is generated for the PCR machine to read and report on. PCR tests can cover several different pathogens or antimicrobial resistance genes from a single test in "panels."

As with culture-based methods, PCR tests have limitations as well. Importantly, PCR tests are pre-designed to test for the most common UTI-causing pathogens; if a pathogen is not included on the test panel, it will not be reported as present, even if the patient's infection is caused by that pathogen (a false negative). Moreover, there are limits to the total number of targets you can include in a PCR test, making it inherently limited to a predetermined list of usually between 4-20 pathogens. If a provider sends a sample for a PCR test, patients should feel empowered to ask which pathogens are included in the panel.

An emerging method called clinical metagenomics, which uses next-generation sequencing, is rapidly changing how providers diagnose and treat urinary tract infections. Rather than growing the pathogens (culture) or looking for specific pathogen DNA fragments (PCR), clinical metagenomic tests make copies of all DNA present in the urine or vaginal swab sample. The data generated is then analyzed by software that compares the DNA that was found to a curated reference database of different microorganisms. This approach is "pathogen-agnostic," meaning any pathogen may be identified via this method; the test doesn't need to be pre-designed, and so doesn't face the same limitations that PCR faces. Some clinical metagenomics tests are also able to provide insight into antimicrobial resistance by identifying the genes that enable pathogens to evade different treatments. This helps providers understand which treatment may work best to eliminate the pathogen(s) found.

The BIOTIA-ID Urine Test

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In a study of 200 negative culture patients at SUNY Downstate Hospital, the BIOTIA-ID Urine Test found key urogenital pathogens in 63% of the samples, with 50% of these positives containing multiple pathogens, indicating a polymicrobial infection. Many of these pathogens would be difficult to grow or would not have grown at all via traditional urine culture. When looking back at what the providers had prescribed to treat the patient's symptoms, it was found that in 70% of the cases the prescribed drug would have had no effect on the pathogen detected [2]. These findings highlight the importance of pursuing advanced molecular diagnostic testing in the face of recurrent or persistent UTI symptoms to truly rule out infectious causes and guide next-steps.

Key differentiators between the BIOTIA-ID Urine Test and other tests that leverage next-generation sequencing approaches for UTIs are its untargeted approach and clinical rigor. Next-generation sequencing approaches may be targeted or untargeted – for targeted sequencing, the test looks at a small part of pathogen genomes, and for untargeted, the test looks at all pathogen genomes. When you only look at a small section of the genome, it is harder to identify the species or characterize it. Furthermore, these tests struggle to identify pathogens at a strain-level (important for clinical decision-making), may miss pathogens that rapidly evolve, and will not detect antimicrobial resistance genes unless specifically designed to do so.

Clinical metagenomic tests overcome the limitations of targeted sequencing approaches, but still have limitations to consider. The first consideration is overdiagnosis. Many microbes may not be clinically relevant or understood, causing unnecessary stress to patients and little guidance for providers on next steps when they are included in clinical reports. Rather than reporting every organism detected, the BIOTIA-ID Urine Test was validated to detect 44 key urogenital pathogens and 15 different antibiotic resistance markers.

The second limitation is cost. Providers should consider whether the information generated will truly be actionable and if the test has undergone thorough clinical evaluation. This is especially important given that few mNGS tests are eligible for insurance reimbursement, necessitating patients or providers to pay for the test themselves. Payors, regulators, and diagnostics companies like Biotia are increasingly working together to get these tests covered by insurance.

Post-UTI Hypersensitivity Syndrome & Non-Infectious Causes of UTI-like Symptoms

Not all persistent urinary or vaginal symptoms are related to an ongoing infection. Repeated infections or inflammation can lead to lasting changes in the bladder and nervous system, a phenomenon sometimes called post-UTI hypersensitivity syndrome. After a particularly severe or prolonged UTI, some patients continue to experience bladder irritation (urgency, pain, frequent urination) even after the infection has been cleared. The bladder wall may remain inflamed, and the sensory nerves within the bladder can become sensitized, leading to persistent urgency, frequency, and pelvic discomfort even after bacterial clearance. Ongoing inflammation and neural sensitization can also affect the detrusor muscle, resulting in increased detrusor overactivity or altered contractile signaling. This may manifest as heightened bladder pressure, urgency with small volumes, or bladder spasms. Over time, this neuro-inflammatory cycle can contribute to post-UTI hypersensitivity and functional bladder symptoms independent of active infection.

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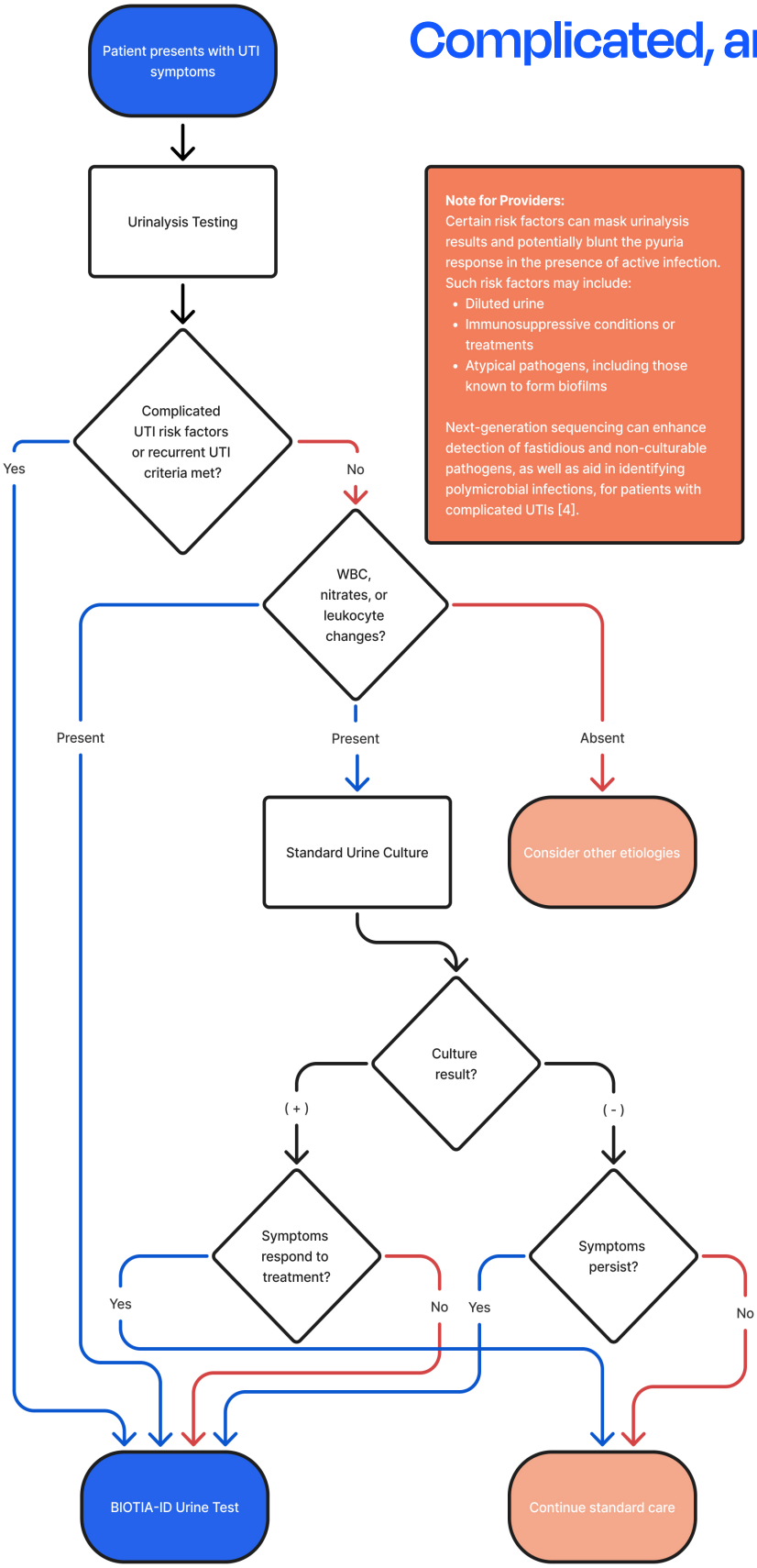
Diagnostic Workflow for Recurrent, Complicated, and Culture-Negative UTI



To help both patients and providers interpret complex clinical and laboratory information, Biotia and Clinova.Solutions has proposed a diagnostic algorithm that balances clinical presentation, diagnostic findings from urine culture and urinalysis testing, as well as treatment response to determine when the use of advanced molecular diagnostics may be warranted. Through this, providers can make better decisions as to when more comprehensive information is necessary to inform patient care, or whether testing options currently covered by insurance may be sufficient, saving their patients unnecessary medical costs.

Patients are also able to better understand how their providers are approaching clinical decision-making, and use this tool to advocate for advanced testing when other routes have been exhausted.

The advice outlined in this flowchart is not intended to replace guidance from a licensed healthcare provider. Please consult your provider or access a telehealth appointment with Clinova.Solutions.

Get Tested

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In other words, the infection "wound up" the bladder's pain and urgency signals, and those signals don't immediately calm down once the microbe is gone. Crucially, this is a scenario where giving more antibiotics won't help, since the symptoms are due to residual inflammation and nerve upregulation rather than an ongoing infection.

Other non-infectious conditions can produce symptoms very similar to UTIs, and these should be considered by clinicians, especially when cultures are repeatedly normal. These conditions include:

- **Interstitial cystitis (IC)**: This is a chronic condition characterized by bladder pain, urinary frequency, and urgency, with urine cultures typically returning negative. The cause is not fully understood (it may involve a defective bladder lining, immune reactions, or nerve dysfunction), but it is not an infection. Patients with IC often have UTI-like symptoms for months or years and are commonly misdiagnosed or given many antibiotics before the true diagnosis is recognized.
- **Overactive bladder (OAB)**: In OAB, the bladder muscle contracts inappropriately, causing sudden urgency and frequency; there is no infection, and urinalysis is normal.
- **Pelvic floor muscle dysfunction**: Sometimes pelvic floor muscle tightness or spasms can cause urinary frequency, urgency, and burning (for example, a tight pelvic floor can irritate the urethra). This can coexist with prior UTIs or occur on its own, and it won't show up on a lab test.
- **Urethral syndrome**: an older term that described UTI-like symptoms (especially urethral burning and urgency) without infection, often linked to irritants or muscle issues.
- **Post-menopausal atrophic vaginitis**: Low estrogen levels after menopause can lead to vaginal and urethral tissue thinning and dryness, causing burning, irritation, and a feeling of needing to urinate often, all without an infection. In such cases, a urine or vaginal culture may show only normal flora.
- **Chemical Cystitis**: This refers to bladder irritation caused by chemical exposures rather than infection. Potential triggers include hygiene products, spermicides, douches, fragranced soaps, bubble baths, certain lubricants, chemotherapy agents, or even concentrated urine from dehydration. Symptoms can include burning, urgency, frequency, and pelvic discomfort with sterile urine cultures
- **Incomplete emptying**: Incomplete bladder emptying occurs when the bladder does not fully evacuate urine during voiding, leading to residual urine remaining in the bladder. This residual volume can cause urinary frequency, urgency, weak stream, hesitancy, straining, or a sensation of incomplete voiding. Patients may also experience suprapubic discomfort or recurrent "UTI-like" symptoms with negative cultures.
- **Nephrolithiasis (Kidney or Bladder Stones)**: Urinary stones can irritate the lining of the urinary tract and cause urgency, frequency, dysuria, hematuria, flank pain, or pelvic discomfort, even in the absence of infection. Small distal ureteral stones can present primarily with bladder symptoms rather than classic flank pain. Urinalysis may show blood, but sterile culture.
- **Poor Glycemic Control**: Elevated blood glucose levels increase glucose in the urine, which can promote urinary frequency, irritation, and increased risk of bacterial growth. Chronic hyperglycemia can also affect bladder innervation, characterized by altered bladder sensation, incomplete emptying, urgency, or overflow incontinence.

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- **Chronic Nonbacterial Prostatitis/Chronic Pelvic Pain Syndrome**: In men, chronic pelvic pain syndrome presents with urinary frequency, urgency, dysuria, perineal discomfort, pelvic pressure, or pain with ejaculation without evidence of active infection on culture. The condition is thought to involve pelvic floor dysfunction, neurogenic inflammation, immune dysregulation, or central pain sensitization rather than bacterial infection. Symptoms may wax and wane over months.

It is important for patients and providers to recognize when symptoms might be due to these non-infectious causes. If multiple reliable cultures are negative despite symptoms, clinicians are advised to pause and reconsider the diagnosis. Red flags like blood in the urine, severe pain, or other abnormalities might prompt further investigations (imaging or cystoscopy) to rule out other pathologies. For example, a patient with ongoing post-UTI bladder hypersensitivity should focus on calming the bladder and nerves: avoiding bladder irritants (caffeine, alcohol, spicy foods), using medications to reduce nerve pain or bladder spasm, and possibly bladder training exercises. Pelvic floor physical therapy can help if muscle tension is a contributing factor. The emphasis is on symptom management and restoring quality of life, rather than eradicating an infection. Patients often find relief when this approach is taken, and importantly, it spares them unnecessary antibiotics. When urine cultures remain negative, it is crucial to avoid repeated antibiotic courses and instead treat the bladder's hypersensitivity and any functional issues.

Colonization vs. Infection: Understanding Asymptomatic Bacteriuria

When interpreting results, it's essential to distinguish true infection from colonization. Colonization means bacteria are present in the body (for instance, in the urine or vagina) but are not causing harm or symptoms. An example is asymptomatic bacteriuria (ASB), when a urine culture finds a significant amount of bacteria in the urine in the absence of UTI symptoms. ASB is surprisingly common, especially in older adults, people with diabetes, or those with urinary catheters. For instance, an older woman might consistently test positive for E. coli by urine culture, but report minimal or absent symptoms. This is not an infection; it's colonization. The bacteria might simply be part of a stable microbial community in the bladder that isn't invading tissue or causing inflammation. Medical guidelines emphasize that asymptomatic bacteriuria should not be treated with antibiotics in most cases [3]. The reasoning is that treating a colonization does not confer benefit and can actually cause harm. The Infectious Diseases Society of America recommends screening and treating ASB only in very specific situations: pregnant women or patients about to undergo invasive urinary procedures. In almost all other scenarios, including the elderly in nursing homes, diabetics, or people with catheters, finding bacteria in the urine without symptoms should be managed with watchful observation, not antibiotics.

Why is Asymptomatic Bacteriuria Important?

It underscores that a positive culture alone isn't always cause for alarm if symptoms are absent, and conversely, a negative culture does not guarantee absence of an issue if

symptoms are present. Sometimes patients have both: a baseline colonization (e.g., always carries a bit of Enterococcus in the bladder) and intermittent true UTIs on top of that. Doctors sort this out by correlating cultures with symptoms and inflammatory markers. If the lab results remain completely normal apart from some bacterial growth, doctors consider whether it's just colonization or a contaminant from the skin/vagina. As a patient, understanding this distinction can prevent confusion. For example, some patients get repeated urine cultures "just to check" and are alarmed when they see bacteria listed on a lab report, but if they felt well when the urine was taken, that result may just be colonization that doesn't need treatment. Conversely, if you feel like you have a UTI but your culture is "no growth" or shows only tiny amounts of bacteria, that should prompt the provider to try a different diagnostic testing method as well as look for alternate explanations for your symptoms.

Conclusion

In scenarios of persistent symptoms with negative cultures, understanding the limitations of urine and vaginal cultures is key to addressing the root cause of your symptoms. Less common pathogens or improper specimen collection may explain why culture results are returning negative despite the presence of symptoms. Advanced diagnostic testing can play an important role in improving health outcomes, particularly for patients with persistent, recurrent, or culture-negative urinary symptoms. Traditional culture methods were developed decades ago and are limited by predefined growth thresholds, selective media conditions, and the inability to detect slow-growing, fastidious, or biofilm-associated organisms. As a result, clinically relevant pathogens may go undetected, potentially delaying appropriate treatment and prolonging patient suffering.

Improved diagnostic precision can translate into better symptom resolution, fewer recurrent episodes, reduced antibiotic resistance pressure, lower healthcare utilization, and improved quality of life. Importantly, advanced testing should not replace clinical judgment but rather serve as an adjunct that enhances individualized care, supports antimicrobial stewardship, and helps differentiate infectious processes from noninfectious causes of lower urinary tract symptoms. In these instances, asking your provider for a molecular diagnostic test may improve pathogen detection while non-infectious causes of your symptoms are investigated.

References

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