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Edited by Rashmi Laddha

E-mail: [drashmirdaga@gmail.com](mailto:drashmirdaga@gmail.com)

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# Diagnostic value of gram staining in urinary tract infections: A review

Swati Narayan Thakare & Dhruva Chandi\*

Department of Microbiology, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi, Wardha, Maharashtra, India; \*Corresponding author

#### Affiliation URL:

<https://www.dmiher.edu.in/>

#### Author contact:

Swati Narayan Thakare - E-mail: [tswati24@gmail.com](mailto:tswati24@gmail.com)

Dhruva Chandi - E-mail: [dhruva.chandi@dmiher.edu.in](mailto:dhruva.chandi@dmiher.edu.in)

**Abstract:**

Urinary tract infections are common bacterial infections and rapid diagnostic information may support timely and appropriate antimicrobial treatment. Although urine culture remains the reference method for identifying uropathogens and determining antimicrobial susceptibility, Gram staining provides rapid information on bacteriuria and bacterial morphology. Therefore, it is of interest to summarize the evidence on urine Gram staining, including direct and centrifuged-sediment approaches, in comparison with urine culture. Available evidence indicates that diagnostic performance varies with bacterial burden, specimen processing, patient population and staining methodology, particularly in specimens with low bacterial counts or contamination. Gram staining may therefore serve as an inexpensive and rapid adjunct to urinalysis and culture, while standardized protocols and computer-assisted interpretation require further clinical validation.

**Keywords:** Urinary tract infection (UTI), gram stain; urine microscopy, bacteriuria, diagnostic accuracy

**Background:**

Urinary tract infections (UTIs) are among the most common bacterial infections encountered in community and healthcare settings and represent an important cause of morbidity and healthcare utilization. Although UTIs can occur across different age groups and in both sexes, women experience a substantially higher incidence because of anatomical and host-related factors [1, 2]. The diagnosis and management of UTI contribute substantially to antimicrobial use, making accurate and timely microbiological assessment important for antimicrobial stewardship [2, 3]. Rapid availability of microbiological information may assist clinical decision-making while reducing unnecessary exposure to broad-spectrum antimicrobial agents [3, 4]. Urine culture remains an essential reference method because it enables detection and identification of viable uropathogens and, when indicated, antimicrobial susceptibility testing. However, culture requires bacterial growth and therefore cannot always provide definitive microbiological information immediately. Rapid diagnostic approaches, including microscopy, Gram staining and automated urinalysis, can provide preliminary information before final culture results become available [4, 5]. Gram staining is particularly useful because it provides rapid information about bacterial morphology and Gram reaction, allowing preliminary differentiation between Gram-positive and Gram-negative organisms [6, 7]. The Gram-staining method, introduced by Hans Christian Gram in 1884, differentiates bacteria according to differences in their cell-envelope structures and staining characteristics [6]. In urine specimens, Gram staining can provide preliminary information regarding the presence and morphology of bacteria and may help classify organisms according to their Gram reaction [7, 9]. However, Gram-stain morphology alone does not establish species-level identification; for example, Gram-negative rods may represent several different bacterial taxa, while Gram-positive cocci may include organisms from multiple clinically relevant genera. Therefore, Gram-stain findings should be interpreted as preliminary microbiological information and correlated with clinical findings and urine culture results [4, 7]. The diagnostic performance of urine Gram staining is influenced by bacterial concentration, specimen quality, specimen processing, staining methodology and observer interpretation [7, 8]. Direct examination of uncentrifuged urine may have limited sensitivity when bacterial concentrations are low, whereas examination of centrifuged

urinary sediment can improve bacterial visualization in selected clinical settings [8]. Published studies have reported variable diagnostic performance, indicating that sensitivity and specificity should not be represented by a single universal estimate across different patient populations and methodologies [7–9]. Despite these limitations, Gram staining remains attractive as a rapid and relatively inexpensive adjunctive method because it requires comparatively limited laboratory resources and can provide preliminary microbiological information while culture is pending [5, 7]. Digital microscopy and computer-assisted interpretation are emerging approaches that may help reduce observer-dependent variability in Gram-stain interpretation [15, 17]. Recent studies have evaluated artificial intelligence-assisted interpretation of urinary Gram-stain images, demonstrating potential for standardized and reproducible classification while requiring further clinical validation. In the context of increasing antimicrobial resistance, rapid diagnostic methods such as Gram staining may complement urine culture and other laboratory investigations by providing preliminary information that can support more informed antimicrobial decision-making [3, 4 and 14]. Therefore, it is of interest to evaluate the contemporary diagnostic value, clinical utility, and limitations of urine Gram staining in managing urinary tract infections.

**Diagnostic performance of urine gram staining:**

The diagnostic performance of urine Gram staining varies according to bacterial burden, specimen processing, staining methodology and observer interpretation [7–9]. Early studies showed that Gram staining can provide useful preliminary information regarding bacteriuria, although reported sensitivity and specificity have varied across patient populations and study designs [7, 8]. Therefore, urine Gram staining should be considered a rapid adjunctive method rather than a definitive replacement for urine culture.

**Effect of bacterial burden and specimen processing:**

Bacterial concentration and specimen preparation are important determinants of Gram-stain performance. Low-count bacteriuria and dilute urine may reduce the probability of detecting organisms by direct microscopy, whereas centrifugation and examination of the urinary sediment may improve bacterial visualization in selected populations [8, 9]. The study by McNair *et al.* showed the potential utility of centrifuged and Gram-stained urine specimens in obstetric patients, although the

diagnostic performance should be interpreted in the context of the specific population and methodology used [8]. These findings highlight the importance of standardized specimen collection and processing when evaluating urine Gram staining.

#### **Comparison with urine culture:**

Urine culture remains essential for definitive microbiological diagnosis because it permits recovery and identification of viable uropathogens and facilitates antimicrobial susceptibility testing [4]. Gram staining, in contrast, provides rapid information regarding the presence, morphology and Gram reaction of bacteria while culture results are pending [7, 9]. A negative Gram stain does not, however, exclude urinary infection, particularly when bacterial concentrations are low or when organisms are poorly visualized by conventional staining [9]. Consequently, Gram-stain findings should be interpreted together with clinical findings, urinalysis and culture results.

#### **Clinical utility and antimicrobial stewardship:**

The principal advantage of urine Gram staining is the rapid availability of preliminary microbiological information [7, 10]. In selected clinical settings, this information may assist clinicians in assessing the likely bacterial group while awaiting definitive culture and susceptibility results. A 2024 study evaluating point-of-care Gram staining in patients with obstructive pyelonephritis demonstrated useful agreement between Gram-stain findings and urine culture for Gram-negative rods, supporting its potential role in selected time-sensitive situations [10]. However, Gram-stain morphology alone should not be used to prescribe a specific antimicrobial regimen because treatment decisions also depend on clinical severity, patient characteristics, local resistance patterns and susceptibility results.

#### **Evidence from recent clinical studies:**

Recent studies show that the diagnostic performance of urine Gram staining is not uniform across clinical populations. A 2024 cohort study in young febrile infants with suspected UTI reported good diagnostic performance for predicting a positive urine culture, whereas another 2024 study conducted in an emergency-department population reported more limited overall clinical usefulness [11, 12]. A 2024 study investigating Sternheimer staining also explored the potential diagnostic value of alternative urine-staining approaches in emergency-department patients [13]. These findings indicate that the clinical value of staining methods depends substantially on the population, specimen characteristics and diagnostic objective.

#### **Integration with urinalysis and other diagnostic methods:**

Combining Gram staining with other rapid laboratory parameters may provide more comprehensive preliminary information than relying on staining alone. Recent diagnostic-accuracy research has evaluated Gram staining together with leukocyte esterase and nitrite testing for predicting urinary tract infection, supporting further investigation of integrated diagnostic approaches. Such approaches may be particularly useful when rapid information is required, but culture remains

necessary when definitive organism identification and antimicrobial susceptibility testing are required [14].

#### **Digital and artificial intelligence-assisted interpretation:**

Observer-dependent interpretation is an important limitation of conventional Gram staining, creating an opportunity for automated and computer-assisted analysis. Recent studies have evaluated artificial intelligence-based interpretation of urinary Gram-stain images. A 2025 study comparing a computer-aided diagnosis application with microbiology specialists demonstrated promising classification performance, while a 2026 study further evaluated an artificial intelligence-powered smartphone-based approach for Gram-stain interpretation [15, 17]. Although these findings are encouraging, larger prospective studies across different laboratories, imaging systems, bacterial morphologies and patient populations are required before such technologies can be routinely implemented in clinical practice.

#### **Future perspectives:**

Future research should focus on standardized urine collection, specimen-processing and staining protocols, clearly defined diagnostic thresholds and prospective multicenter validation [8, 9]. Studies should also determine whether combining Gram staining with urinalysis, molecular methods, or computer-assisted interpretation improves clinically meaningful outcomes rather than only analytical performance [14-17]. Such evidence will help define the clinical settings in which urine Gram staining provides meaningful additional value while maintaining urine culture as the reference method for definitive microbiological characterization.

#### **Limitations of gram staining:**

Despite its speed and low cost, urine Gram staining has several limitations. Diagnostic accuracy can be affected by low bacterial burden, dilute or contaminated specimens, specimen-processing procedures, staining quality and observer experience. Gram staining also provides limited information compared with culture because it does not independently establish species-level identification or antimicrobial susceptibility. These limitations are particularly important when mixed bacterial morphologies are observed or when clinical suspicion remains high despite a negative Gram-stain result.

#### **Conclusion:**

Urine Gram staining is a rapid and inexpensive adjunct to urine culture that can provide preliminary information on bacteriuria and bacterial morphology, although its diagnostic performance varies with bacterial burden, specimen processing and clinical setting. Centrifuged-sediment and other modified approaches may improve bacterial detection in selected settings, but limitations in sensitivity, interpretation and standardization prevent Gram staining from replacing culture for definitive identification and antimicrobial susceptibility testing. Standardized protocols, prospective multicenter validation and evaluation of automated or computer-assisted interpretation are

needed to establish its optimal role in rapid UTI diagnosis and antimicrobial stewardship.

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