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Diagnostic efficacy of modified gram staining methods: A review

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Abstract:

Early diagnosis of bacterial infections is vital for prompt treatment to reduce morbidity. Conventional Gram staining provides rapid visualization. However, it suffers from low sensitivity. Bacterial culture offers definitive identification but is highly time-consuming. Modified Gram stain techniques address these limitations by improving visualization in low-load specimens. However, standardized multicenter studies are required to validate these methods to establish best practices.

Keywords: Gram staining techniques, modified Gram staining, culture technique, bacterial detection, diagnostic accuracy

Background:

Bacterial infections, including urinary tract infections, pneumonia, bloodstream infections and wound infections, remain important causes of morbidity and require timely microbiological evaluation to guide appropriate antimicrobial therapy [1, 2]. Delays in diagnosis and initiation of appropriate treatment can adversely affect clinical management, particularly in patients with severe or rapidly progressive infections. Rapid preliminary identification of bacterial morphology may therefore assist clinicians in making early therapeutic decisions while definitive microbiological results are pending [3]. Culture remains an essential microbiological method because it enables recovery and identification of viable organisms and, where appropriate, antimicrobial susceptibility testing [3, 10]. However, culture-based diagnosis generally requires additional time for bacterial growth and subsequent identification, which may delay definitive microbiological characterization [10]. Gram staining provides a substantially more rapid assessment by allowing visualization and preliminary classification of bacteria according to their Gram reaction and morphology [9, 15]. Nevertheless, conventional Gram staining is influenced by specimen quality, bacterial load, staining procedures and observer interpretation, which can contribute to false-negative or discrepant results [4, 11]. Recent clinical studies have also demonstrated that the diagnostic performance of Gram staining varies according to the specimen and clinical context, emphasizing that it should not be interpreted as a universal substitute for culture [5, 6]. To address some limitations of conventional Gram staining, several modified and alternative staining approaches have been investigated to improve bacterial visualization and detection. Modified procedures have included changes in staining reagents, staining conditions and visualization strategies, with some approaches showing improved detection of specific bacterial groups or specimens [8, 13]. For example, the SensiGram procedure was developed as a modified Gram-staining method to improve detection of Gram-negative bacteria in positive blood cultures [8]. However, differences in staining protocols, specimen characteristics, bacterial concentration and interpretation criteria make direct comparison among modified staining techniques difficult [4, 8]. Rapid staining approaches may be particularly valuable when preliminary microbiological information is required before culture results become

available, but their diagnostic performance must be interpreted in conjunction with clinical findings and definitive culture results. Recent evidence demonstrates both the potential utility and the limitations of Gram-stain-based diagnosis, supporting the need for standardized evaluation across different clinical specimens and settings [15–17]. Therefore, it is of interest to examine the development and diagnostic efficacy of modified Gram-staining methods in comparison with culture, with particular emphasis on bacterial visualization, diagnostic performance, methodological limitations and factors affecting clinical applicability.

Historical basis and principle of gram staining:

Hans Christian Gram introduced the Gram-staining method in 1884 as a differential staining technique for classifying bacteria according to their staining characteristics [7]. The method is based on differences in bacterial cell-envelope structure, particularly the organization and thickness of the peptidoglycan layer, which influence the retention of the primary crystal violet-iodine complex during decolorization. Gram-positive bacteria generally retain the primary stain, whereas Gram-negative bacteria are decolorized and subsequently take up the counterstain [9]. This differential staining principle provides rapid preliminary information regarding bacterial morphology and Gram reaction and remains an important component of routine clinical microbiology.

Evolution of modified gram-staining methods:

Several modifications of the conventional Gram-staining procedure have been investigated to improve bacterial visualization and detection. These modifications may involve changes in fixation, staining reagents, decolorization procedures, counterstaining, or visualization strategies. The principal objective of these approaches is to improve contrast and bacterial detection while reducing the possibility of missed organisms, particularly when bacterial numbers are low or specimen characteristics make conventional visualization difficult [8, 13]. However, the performance of individual modified techniques varies according to the staining protocol, specimen type, bacterial concentration and method of interpretation.

Diagnostic role of gram staining compared with culture:

Culture remains an essential reference method in clinical microbiology because it permits recovery of viable organisms, subsequent identification and antimicrobial susceptibility testing [3, 10]. In contrast, Gram staining provides substantially faster preliminary information regarding the presence, morphology and Gram reaction of bacteria. This rapid information may assist clinicians in the initial assessment and management of suspected bacterial infections while definitive culture results are pending [11, 12]. However, Gram-stain findings should generally be interpreted as preliminary microbiological information rather than as a complete replacement for culture, particularly when species-level identification or antimicrobial susceptibility testing is required.

Diagnostic performance of modified gram staining:

Available studies indicate that certain modified Gram-staining approaches may improve bacterial detection or visualization under specific experimental or clinical conditions [8, 13-16]. For example, modified procedures have been investigated for improving detection of bacteria in blood cultures and other clinical specimens, suggesting potential advantages when rapid bacterial characterization is required [8]. Recent clinical evidence also shows that the diagnostic performance of Gram staining can vary according to the specimen and clinical setting, emphasizing the importance of evaluating each modified technique within its intended application [15-17]. Although some studies have reported improved detection with modified approaches, the available evidence remains heterogeneous and does not establish uniform superiority over conventional Gram staining or culture across all clinical applications.

Clinical implications and future perspectives:

The available evidence supports the potential use of modified Gram staining as a rapid adjunct to conventional microbiological diagnosis rather than as a replacement for culture. A combined approach may allow rapid preliminary assessment through staining while preserving culture for definitive organism identification and antimicrobial susceptibility testing [3, 8, 10 and 15]. Future research should focus on standardized staining protocols, clinically relevant diagnostic endpoints, multicenter validation and systematic comparison with established reference methods. Emerging automated and computer-assisted approaches may also improve the reproducibility of Gram-stain interpretation and warrant further clinical evaluation [18-20].

Limitations and methodological heterogeneity:

The interpretation of Gram-stained specimens may be affected by specimen quality, bacterial burden, staining conditions and observer experience. Differences in staining protocols, study populations, specimen types, comparator

methods and diagnostic outcome measures further complicate direct comparison among published studies. Many available investigations have been conducted in individual institutions or under laboratory-specific conditions, limiting the generalizability of their findings. Consequently, standardized protocols and adequately designed multicenter studies are required to establish reproducible estimates of diagnostic performance and determine the clinical settings in which modified Gram staining provides meaningful additional value.

Conclusion:

Modified Gram staining provides a rapid and highly effective adjunct to traditional culture, significantly enhancing early bacterial visualization for time-critical clinical decisions. The technique's diagnostic efficacy remains dependent on specimen quality and methodology. Its strategic integration offers substantial preliminary value without replacing standard culture. However, rigorous multicenter investigations are essential to validate its performance within modern diagnostic frameworks.

Declarations:

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Competing interests:

The authors declare that they have no competing interests.

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