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Editorial

Staining Techniques: A Point-of-Care Approach to Microbiological Diagnosis in the Indian Clinical Setup

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INTRODUCTION

Staining techniques have been fundamental to clinical microbiology since the 19th century, serving as indispensable point-of-care tools for rapid pathogen detection and identification. In resource-limited settings like India, where access to advanced molecular diagnostics may be restricted, bedside staining techniques play a crucial role in early infection diagnosis and treatment initiation. These methods provide real-time, actionable insights that influence clinical decision-making, particularly in critical care and emergency settings.

Rapid staining techniques such as Gram stain, Ziehl-Neelsen (ZN) stain, India ink, and Giemsa stain allow for immediate pathogen identification at the bedside, guiding empirical therapy while awaiting culture results. Additionally, recognizing inflammatory patterns in tissue sections can provide essential diagnostic clues, further aiding in the selection of specialized stains for suspected infections. A thorough patient history and examination remains indispensable in correlating microbiological findings with clinical presentation, ensuring accurate interpretation.

In India, where presumptive diagnoses are often made before confirmatory tests become available, staining techniques continue to be a practical, cost-effective, and efficient diagnostic approach. This editorial explores the clinical utility of point-of-care/bedside staining techniques in routine practice, emphasizing their impact on timely diagnosis, antimicrobial stewardship, and improved patient outcomes.

STAINING IN BACTERIOLOGY

Gram stain is the most frequently used method in clinical microbiology. Although described by Hans Christian Gram more than a century ago, it remains foundational in clinical microbiology for its ability to detect and differentiate a broad array of pathogens. The Gram stain has been used for several purposes including:

- Preliminary pathogen identification: Direct examination of clinical specimens, such as body fluids or biopsy, is essential when infection is suspected. For instance, cerebrospinal fluid in suspected meningitis cases or synovial fluid in cases of septic arthritis.
- Specimen quality assessment: Respiratory samples are prone to contamination with normal flora. A sputum sample is considered adequate for processing if it meets the Murray and Washington criteria or the Bartlett criteria. The Bartlett criteria uses a score based on the number of neutrophils, squamous epithelial cells, and the presence of mucous strands per low-power field (LPF). As per the Murray and Washington criteria, a sample is considered adequate if it has fewer than 10 squamous epithelial cells per LPF.[1,2]

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- Antibiotic therapy guidance in ventilator-associated pneumonia (VAP): Gram staining of respiratory samples, a simple and cost-effective tool, may guide initial antibiotic therapy, particularly in resource-limited settings. In the GRACE-VAP trial Gram stain–guided therapy was noninferior to guideline-based treatment in clinical response (76.7% vs 71.8%) and significantly reduced the use of antipseudomonal and anti-MRSA agents.[3]
- Reducing turn-around time and mortality in bloodstream infections: Prompt Gram staining of positive blood cultures significantly improves therapeutic appropriateness and reduces mortality. In a study of 99 matched pairs, time-to-detection (TAT) <1 hour was associated with significantly lower mortality (10.1% vs 19.2%, $P = .0389$) compared to TAT ≥1 hour (3.3 hours; $P < .0001$).[4]
- Wound and abscess management: The value of Gram's stain in facilitating early and appropriate treatment of wound infections especially in cases of necrotising fasciitis and gas-gangrene remains pivotal. In clean surgical wounds, where the microbial etiology is often monomicrobial (e.g., clusters of Gram-positive cocci), Gram staining provides a reasonable diagnostic approach.
- Urinary tract infection: In patients with suspected urinary tract infection, Gram stain of uncentrifuged urine showing ≥2 bacteria per oil immersion field reflects approximately 10^5 CFU/mL i.e. significant bacteriuria.[5]

One notable challenge with Gram staining is its limited ability to distinguish between aerobic and anaerobic bacteria, especially among Gram-positive spore-forming anaerobes like *Clostridium perfringens*. This difficulty is further compounded by the tendency of many Gram-positive anaerobes to exhibit Gram-variable staining patterns upon exposure to oxygen.

Acid-fast stains or Ziehl-Neelsen (ZN) stain for *Mycobacterium tuberculosis* and non-tuberculous mycobacteria, is the second most commonly employed stain in clinical practice. Modified acid-fast staining extends this utility to other pathogens, such as those responsible for nocardiosis and Actinomycosis. The detection of *Nocardia* species, characterized by thin, filamentous, branching weakly acid-fast bacteria, highlight the utility of this stain in identifying rare and atypical pathogens.[6]

A microbiological smear of a suspected diphtheria pseudomembrane can reveal Gram-positive, club-shaped bacilli arranged in a "Chinese letter" pattern. Additionally, ***Albert staining*** demonstrates the presence of metachromatic granules, which appear as purple-black dots against a light green cytoplasm.

STAINING IN MYCOLOGY

Microscopy with ***10% potassium hydroxide (KOH) wet mounts***, remains the cornerstone for the diagnosis of fungal infections. Optical brighteners, such as calcofluor white, are frequently utilized in wet mount preparations to enhance the visualization of fungal structures. In histopathology, stains like ***periodic acid-Schiff (PAS)*** and ***Grocott-Gomori's methenamine silver (GMS)*** are preferred, as they highlight the fungal hyphae effectively, facilitating accurate identification. The characteristic fungal morphology in mucormycosis includes wide (5 to 20 µm), ribbon-like, thin-walled, pauciseptate hyphae with crinkled or folded appearance with branching at right-angles.[11] This microscopic pattern, when clinically correlated, supports the diagnosis of mucormycosis and in guiding with appropriate treatment. In contrast, the presence of narrow, acute-angle branched septate hyphae most commonly indicate *Aspergillus* spp. or other septate fungal species. Diagnosis of cryptococcal infection by ***Indian Ink*** revealing the presence of round budding yeast cells with a halo is a very rapid, simple, and inexpensive method.[7]

STAINING IN PARASITOLOGY

A ***wet mount*** examination of an aspirate from liver abscess with a classic "anchovy sauce" appearance, is highly suggestive of an amoebic abscess and often identifies *Entamoeba histolytica* trophozoites. Early diagnosis and treatment with metronidazole can lead to a favourable outcome, underscoring the importance of a skilled microbiologist's contribution to differential diagnosis. Diagnosis of intestinal parasitic infections primarily relies on the microscopic detection of various life stages—eggs, larvae, trophozoites, cysts, and/or oocysts—present in human fecal samples. However, the sensitivity of stool microscopy is generally low, necessitating the use of appropriate microscopic techniques to enhance diagnostic accuracy.[8]

Acid fast staining enables rapid differentiation and the initiation of presumptive therapy of intestinal protozoans such as *Cyclospora, Cryptosporidium,* and *Isospora*. This method is particularly useful, as oocyst size can distinguish these pathogens, facilitating early diagnostic accuracy and treatment. One key method is the ***modified Acid-Fast staining*** procedure, which is particularly useful for identifying oocysts of coccidian species such as *Cryptosporidium* (3-6 µm),

Cystoisospora (25-30 µm*10-15 µm), and *Cyclospora* (8-10 µm), organisms that may not be readily detected with routine stains like trichrome. Unlike the Ziehl-Neelsen stain, this technique does not require heating reagents.[8] The ***trichrome staining*** procedure is another widely recognized method for detecting intestinal protozoa in stool samples. Smaller protozoa that may be missed on wet mount preparations, whether unconcentrated or concentrated, are often clearly visible on stained smears.[8]

The ***calcofluor white staining*** procedure, a chemofluorescent technique, is particularly valuable for detecting *Acanthamoeba* spp., *Microsporidia*, and *Dirofilaria* spp. Optical brightening agents, such as Calcofluor, or Uvitex 2B, are used in this method. These reagents are rapid, inexpensive, and sensitive, though nonspecific, as many non-parasitic organisms can also fluoresce. Therefore, while useful as a screening tool, this technique is not suitable for species-level identification.

In malaria, species identification and quantification of parasitemia are essential for guiding effective therapy. In the preparation of blood smears for parasitological examination, it is recommended to stain only one set of smears, while leaving duplicates unstained. The unstained smears serve as a valuable backup, should issues arise during the staining process or if the sample needs to be sent to a reference laboratory for further analysis.[9] The ***Wright stain (or Wright-Giemsa)*** is commonly used in hematology for routine blood smear preparation. Although it is not the optimal stain for detecting blood parasites, it may be employed when rapid results are required. However, due to its limitations in visualizing certain parasitic features, such as Schüffner's dots, Wright stain should be followed by a ***Giemsa stain*** for definitive confirmation, particularly in the diagnosis of *Plasmodium* species and other blood-borne parasites. The Giemsa stain, is highly recommended for the detection and accurate identification of blood parasites. This stain allows for the detailed visualization of the morphological features of parasitic organisms, including the distinctive characteristics of *Plasmodium* species, such as the presence of Schüffner's dots in infected erythrocytes, thereby facilitating reliable diagnosis and species identification.[9]

RAPID IDENTIFICATION USING DIRECT MICROSCOPY

Cholera, caused by *Vibrio cholerae*, is characterized by rapid onset of profuse watery diarrhea and can lead to severe dehydration. In an outbreak setting, the direct microscopic examination of stool samples can reveal the classic darting motility of *V. cholerae*, i.e. "shooting star" motility. This rapid motility is a unique diagnostic feature that can be observed under a wet mount preparation, providing immediate clues for diagnosis. Such rapid identification is crucial for outbreak control, allowing for the swift administration of antibiotics and rehydration therapy, preventing further spread and saving lives. Similarly, the diagnostic clues in Albert's stain coupled with the clinical presentation, allow rapid identification of *Corynebacterium diphtheriae*, enabling prompt initiation of diphtheria antitoxin and appropriate antibiotic therapy. This case exemplifies the pivotal role of traditional microbiological techniques, such as staining, in identifying pathogens in a timely manner during outbreaks.

CONCLUSIONS

Staining techniques, both nonspecific and specific, remain integral to clinical microbiology, enabling the rapid identification and characterization of infectious agents. Their point-of-care/bedside applicability is particularly crucial in resource-limited settings, where early diagnosis can significantly impact patient management (Box 1). However, optimal diagnostic accuracy requires combining staining techniques with patient history, clinical examination, and molecular/culture-based methods. The judicious use of stains at the point of care supports better infection control strategies and antimicrobial stewardship, reinforcing their continued relevance in modern clinical practice.

Box 1: Bedside Guide to Staining Techniques in Clinical Microbiology

Stain Name	Bedside Indications/ Possible Diagnosis	Important Points to Remember
1. Gram Stain	- Preliminary identification of bacterial pathogens in infections. - Respiratory infections (e.g., pneumonia, VAP). - Urinary tract infections (UTI).	- Rapid, cost-effective tool for initial bacterial differentiation. - Limited in polymicrobial infections and anaerobic differentiation. - Helps guide empirical antibiotic therapy.
2. Ziehl-Neelsen (ZN) Stain	- Diagnosis of tuberculosis (TB) and Nocardia infections. - Non-tuberculous mycobacteria (NTM) infections.	- Critical for TB diagnosis in high-prevalence areas. - Useful in immunocompromised patients.

3. Albert Stain	- Diphtheria diagnosis (pseudomembrane formation).	- Identifies *Corynebacterium diphtheriae* (metachromatic granules appear as purple-black dots).
4. Wet Mount	- Stool examination for parasites (*Giardia, E. histolytica*). - Cholera diagnosis (*Vibrio cholerae* motility). - Urine examination for pyuria or contamination assessment.	- Detects ova, cysts, and motile organisms. - Quick identification of *Vibrio cholerae* (darting motility).
5. Potassium Hydroxide (KOH) Wet Mount	- Fungal infections (*Candida, dermatophytes, subcutaneous mycoses*).	- Detects fungal elements (septate vs. aseptate hyphae, pigmented vs. hyaline fungi).
6. Periodic Acid-Schiff (PAS) Stain	- Deep fungal infections (*mucormycosis, aspergillosis*).	- Stains fungal cell walls in tissue biopsies. - Simple, rapid, and cost-effective.
7.Grocott-Gomori's Methenamine Silver (GMS) Stain	- Systemic fungal infections (*mucormycosis, aspergillosis*).	- Highlights fungal elements in tissue, useful in immunocompromised patients.
8. Trichrome Stain	- Intestinal protozoa (*E. histolytica, Giardia*).	- Enhances visualization of protozoa in stool samples.
9. Modified Acid-Fast Stain	- Coccidian parasites (*Cryptosporidium, Cyclospora, Isospora*).	- Essential for detecting small parasitic oocysts.
10. Modified Safranin Stain	- Cyclospora, Cryptosporidium, Isospora (stool samples).	- Offers better consistency than traditional acid-fast methods.
11. Calcofluor White Stain	- Fungal and parasitic detection (*Acanthamoeba, Microsporidia, Dirofilaria*).	- Fluorescent stain for fungal and parasitic elements. - Avoid misinterpretation of artifacts.
12. India Ink Stain	- Cryptococcus species (cryptococcal meningitis).	- Detects the capsular halo of *Cryptococcus* in CSF.
13. Giemsa Stain	- Malaria diagnosis (*Plasmodium* spp.). - Bloodborne parasites.	- Essential for Plasmodium species identification and parasitemia quantification. - In *P. vivax*, trophozoites appear amoeboid; in *P. falciparum*, gametocytes are crescent-shaped.
14. Wright-Giemsa Stain	- Bloodborne parasites (*Plasmodium, Leishmania*).	- Used for blood smear examinations, requires Giemsa stain confirmation for malaria.

CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest.

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None

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