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Editorial

# Gram Stain and Ziehl Neelsen Stain: Limited but Important, Specific Diagnostic Tool

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The service of a microbiologists in a laboratory are often limited to culture and sensitivity, and microscopy of the common inexpensive methods. Both bacterial and fungal infections can be diagnosed by microscopy (1). Presently, particularly during this COVID 19 era molecular techniques took the bulk of laboratorywork. Interpretation of microscopy findings poses a formidable challenge to the nonmicrobiologists or clinicians. Both Gram staining and Ziehl Neelsen staining are common and useful tests conducted by a microbiologist almost every day. It is mandatory to interpret the findings with the specific objective to clinch the diagnosis or to facilitate the process of diagnosis. The judgment on the smear often requires years of experience and patience with multiple observations. The presence of normal flora on certain specimens make the interpretation more challenging yet unconditionally important requiring in the immediate remedy to the condition of the patient.

Gram stain serves the purpose of Gold standard, since it is an easy procedure taking less time and equipment without any technical assistance, ensuring treatment with great relief at both patient's and doctor's end. It is very valuable in soft tissue, urinary tract infection, genital tract infection, lower respiratory tract infection (2). The fields containing neutrophils or pus cells provide the most useful information. Specimens like sputum, skin swab, genital secretions, throat swabs are difficult to interpret by Gram staining unless normal flora or contaminated/improperly sample are excluded with proper judgment. Neutrophils in presence of typical organism, especially intracellular give the report validity and reliability with 95% confirmation in certain cases (3). Mixed type of organisms or polymicrobial presence usually confers to normal flora unless it is actually a case of polymicrobial infection. In that case only one morphotype will be seen intracellularly. Regarding High Vaginal Swab (HVS), the predominance of Gram negative coccobacilli (typical morphotype of *Gardenella vaginalis*) in absence of inflammatory cells, and in decreased number of Gram positive lactobacilli signifies vaginosis rather than vaginitis in presence of "clue cells", a valuable aid for the specialist (4). Co infection also can be diagnosed by Gram staining of HVS (Fig.1

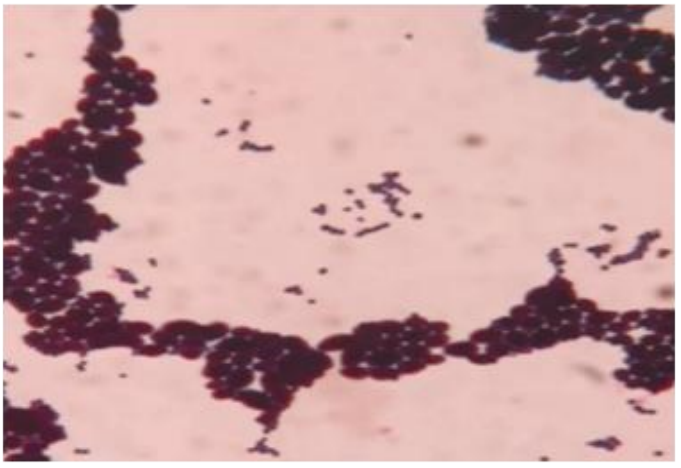

Figure 1: Budding yeasts and Gram positive cocci clusters in HVS (photo credit, Dr. Samira Afroz)

Rapid and precise detection of pathogens in tissue samples or body fluids like ascitic fluid, pleural fluid, bronchoalveolar lavage, gastrointestinal lavage are vital to predict an infectious process. Important issues such as cerebrospinal fluid and culture positive finding of pathogens on Gram reaction and morphology serve as best tool in rapid diagnosis (5). In association with other biochemical tests, Gram staining is considered as a cornerstone of a clinical laboratory for decades (6). It yields result much faster than culture and provides important data for the patient's treatment and diagnosis. Swiftness in Blood culture positive cases especially from NICU or ICU, contributes important guidance towards commencement

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of specific treatment for the intensivist. Finding of Gram positive or Gram negative cocci or rods in primary growth is a safety guide in the antibiotic selection. Initialcrucial decision allowing early therapy in Blood stream infection (BSI) by the physician depends on the report taken verbally a day prior to the expected date. This may save lives in threatened condition of patients, also gives a clue of the source whether community or (HAI) health care associated infection (7). At initial stage, cephalosporin can be started in *Enterobacteriaceae* (production of gas) or antipseudomonal (non fermentative) agents should be employed as empirical treatment, depending on biochemical finding with Gram stain which may shift the status of the patient from left to right (8). A simple gram stain from the post operative wound before dressing during cleaning or changing should permit an easy way to find out an infection or determines the type of organism present (Fig.2)

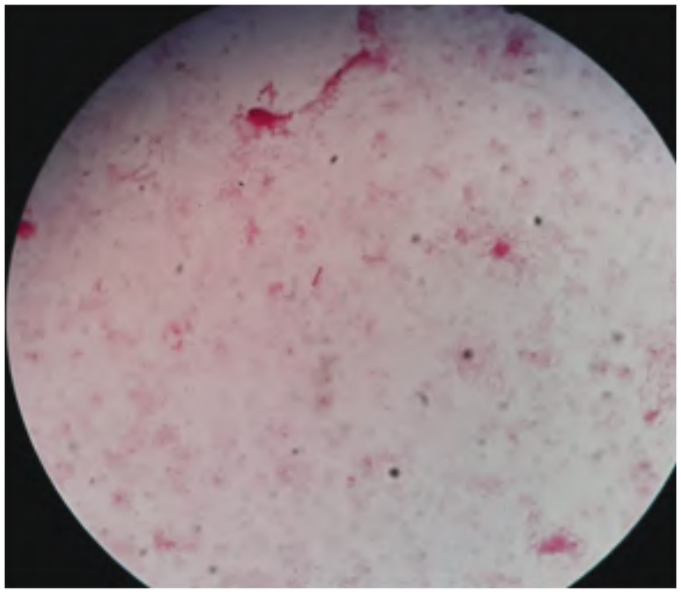
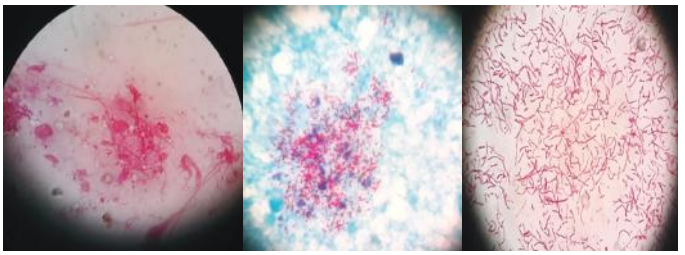


Figure: Gram negative rods in wound swab

It may even help in antibiotic stewardship by de-escalation of the prophylactic antibiotics used (9). In many conditions, HAI should be borne in mind before discarding Gram negative cocci like *Moraxella* or *Acinetobacters* pecies as contaminant which may often predicted as Gram positive (8).

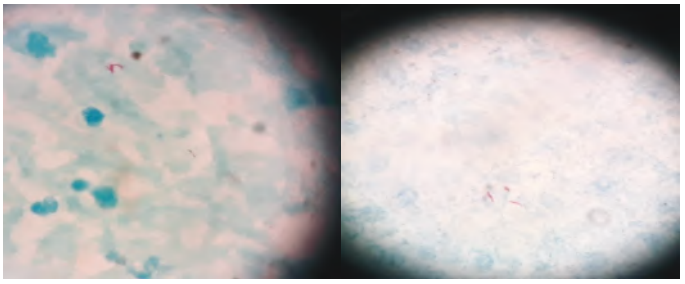
Among chronic cases, *Nocardiaspp*. can easily be identified by its typical Gram positive filamentous, coccobacillary form (Fig.3) and acid fastness (Fig.4)in the specimen as well as in the staining from the growth (Fig.5) as it yields similar dry aerial colony as *Bacillus subtilis* (10).



(3) (4) (5)

Fig 3,4,5 : (3) Filamentous Gram positive bacilli in pus from chronic discharging sinus (4) AFB in Modified Zeihl Neelsen Staining of pus (5) Gram staining of Nocardia spp. from susceptibility plate

Staining sometimes allows you to fit in the missing pieces of information required to obtain the conformity. Finding of *Mycobacterium tuberculosis* (MTB) or *atypical mycobacteria*/MOTT in pus or w/s (Fig.6) is of utmost importance since MOTT cannot be identified by Gene Xpert (11).



(6) (7)

Figure : (6) AFB in ZN staining (7) AFB in urine

ZiehlNeelsen stain or MZN stain negativity also enables *Actinomycesspp*. exclusion in absence of culture as it's an anaerobe (10). Infrequent finding of Lepra bacilli from nasal scraping or from slit ear smear by MZN staining is invaluable as there is no other direct method of diagnosis in early leprosy. Finding of AFB in urine sample is undoubtedly rare and difficult but when found it spares the dilemma in diagnosis as well as painstaking physical condition of the patient and indecisiveness of both doctor and patient (Fig.7).

In case of outbreaks of food borne infections and a possible bioterrorism event, microbiology lab services act as the first line defense in detection of pathogens (12).

Though staining requires high level of observational quality, but the visual acuity with experience permits the authenticity of what you are seeing. Requiring a little training it allows observing living organismin the sample withminor maintenances cost adjustable to comfort level.

Disadvantages of both Gram and ZN staining are welldocumented. Light microscopes do not magnify the same level as other options. Most are resolution up to 200nm and thereabouts (1). There may be lack of consistency in the quality and quantity of information which depends on experience and knowledge. There is a risk of misdiagnosis, under diagnosis on the information. There may even be change in bacterial morphology (Fig.8) due to antimicrobial therapy such as Gram negative rods becoming filamentous and Pleomorphic (1).

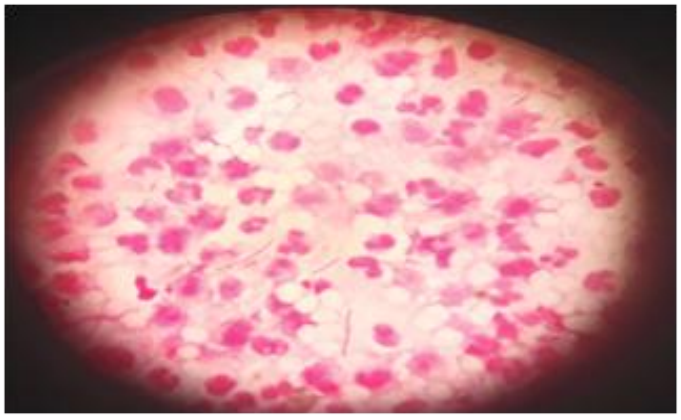


Figure 8: Pus showing long filaments of Gram negativerods later confirmed as Klebsiella spp. in culture

Acid fastness is not inevitable in most *Nocardia spp*. where diagnosis should be withheld for organism to grow in extended culture for confirmation (10). In old culture Gram positive often may turn into Gram negative, which is not uncommon.

Specimens of sputum should always be screened by Gram staining before culture, it increases acceptability by finding neutrophils and the organisms associated (13). At the same time, properly expectorate sputum or induced sputum be requested when only presence of epithelial cells indicates saliva. To avoid missing out nosocomial pneumonia, specific morphotypes of the bacteria should be reported. *Acinetobacter bowmanni* resembling as *Moraxella (Branhamella) catarrahlis; Pasturellamultocida* or Prevotellaintermedia resembling as *H.influenzae* are not uncommon as they are short rods (4). *Enterococcus faecalis* or *VRE*, a notorious cause of HAI can often be overlooked as it resembles *S. pneumoniae* (1).

Among the rare findings, *Clostridium* organism with or without spores may often appear as gram negative in clinical specimens as observed by certain scientists. It may also be in absence of neutrophils as they have enzymes that lyse the host's cell (4).

Infectious diseases practice has changed dramatically by placing the microbiologists in the key role of Infection Prevention Control (IPC) process. Maintaining a high quality clinical microbiology laboratory of an institution can serve as the best approach for managing today's problem of HCI and emerging infectious diseases. Reporting can be more elaborative by suggestions or by mentioning, whetherbacteria are in moderate number, in presence of neutrophils or not, if intracellular or if more than 2 or 3 yeasts are seen (7). If the intention is to diagnose a case or to help a patient, it is better to give the finding in own words instead of a prototype comment. If the interpretation does not correlate with the expected finding or inconsistent with the guidelines, the laboratorian should reconsider the interpretation and another opinion from other colleagues should be sought. A consultation will improve the quality of the report as well as the confidence level will be established in the laboratory by precise reporting and effective communication with the clinicians as well as with other microbiologists.

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