Gram Stain versus Culture for Diagnosis of Pyogenic Infections

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Abstract

Out of the total fifty pus samples/swabs processed in the Department of Microbiology, RMC, Loni, during a three months period from November 2008 to January 2009, 37 (74 %) samples were positive by direct smear examination using Gram's staining and 32 (64 %) samples were positive by culture.18 cultures were sterile. Hence in this study Gram's staining was found to be better than culture.

Key words: *Purulence, Gram stain, direct microscopic examination, rapid presumptive diagnosis.*

Introduction

Direct microscopy for visualization of microorganisms has been possible for just more than 200 years, but it was not a practical reality until Koch established the germ theory of disease in the 1880's.[1] By 1880 a Scottish surgeon had published his direct observations of cluster forming cocci in purulence from human disease. He named these cocci Staphylococcus. In 1884 a Danish histologist Christian Gram developed the Gram Stain, which today allows us to examine the specimen directly for the microorganisms,only important stain in bacteriology. Gram staining is a simple and rapid diagnostic tool for the presumptive identification of pathogens and may the oldest and most entrenched technique still in use in the microbiology laboratory.[2]

The Gram stain is used routinely and as requested in the clinical specimens submitted for smear and culture. It may be used to characterize any specimen. Cerebrospinal fluid, sterile fluids ,expectorated sputum or bronchoalveolar lavages and wounds and exudates are routinely stained directly in which bacterial infections are strongly suspected.[3]

Streptococcus pneumoniae in pneumonia, Staphylococcal abscesses or pyodermas, Haemophilus influenzae tracheobronchitis or meningitis in infants, Clostridium perfringens in gas gangrene,Nocardia species in lung abscesses and Gonococcal urethritis are the infections caused by common single species or by classic infectious agents.

Polymicrobial presentations in smear require more interpretation and must take into account smear background, the morphology of the organisms and the anatomic location of the suspected infection as well as accompanying clinical symptoms.

Besides demonstrating the morphology of bacteria, Gram stain distinguishes two categories of genera:- the Gram positive, which stain dark purple, and the Gram negative which stain light red.[4] Many studies regarding utilization of Gram staining for the diagnosis of cystic fibrosis[5,6], pneumonia[7,8], bacteremia[9,10] has been done but no study regarding use of Gram staining in pyogenic infections is available to our knowledge.

This study was planned with an aim to assess the usefulness of Gram staining for the diagnosis of pyogenic infections.

Materials and Methods

A three months study (from November 2008 to January 2009) was carried out in the department of microbiology, RMC, Loni. Fifty pus samples / swabs from various clinical department were collected and processed in the microbiology laboratory. Samples included pus from ulcers at different sites, abscesses

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(Parotid, Breast, Cervical, Knee, Perineal), post operative wound gape, burns, Osteomylitis and fracture sites. Pus swabs were taken in Brain Heart Infusion broth.

Direct smears from all the samples/ swabs were stained using Jensen's modification of Gram staining method.[2] They were observed under oil immersion of microscope. Culture of all the samples/ swabs was done on blood agar and MacConkey's agar using standard techniques. Culture plates were incubated at 37^0C overnight. Next day, colonies obtained on the culture plates were further studied on the basis of colony morphology, colony smear and biochemical reactions.

Table 1:Showing results of pus samples

Departments	Total no. of pus samples / swabs processed (50)	Gram's staining Positive (37)	Culture positive (32)
Surgery	19	12	11
Burns	09	04	04
Orthopedics	09	08	05
Obstetrics & Gynae	08	08	08
Paediatrics	03	03	03
ENT	02	02	01

Results

The results after processing fifty pus samples collected from patients from various clinical departments are shown in table 1.

Maximum number of pus samples were received from the Department of Surgery followed by Burns and Orthopedics. Direct smear examination by Gram staining was found to be better than culture in 5 pus samples received from various departments. Out of the total fifty samples /swabs processed ,74% were positive by Gram's staining whereas only 64% were positive by culture.

Discussion

The Gram stain, a method for staining bacteria developed and described by the Danish pathologist and bacteriologist Hans Christian Gram in 1884,is still one of the most important and useful clinical laboratory tests. This simple, rapid stain separates most clinically significant bacteria into 2 groups: gram positive organisms, which appear blue or purple when observed under light microscope, gram negative bacteria, which appear red or pink. The gram stain reactions, in conjunction with the morphologic types of bacteria (cocci Vs bacilli) and arrangement of the bacteria,can be used to make presumptive identifications.[9]

In the study, Gram's staining was found to be better than culture. Differential staining and microscopy underpin the laboratory diagnosis of infectious diseases. This underpinning in the diagnostic microbiology laboratory, is the ability to combine the rapid response of direct specimen examination with culture isolation and antibiotic susceptibility testing to achieve the following :

1. Confirm that the material submitted for study is representative.
2. Identify the cellular components and debris of inflammation and thereby establish the probability of infection.
3. Identify specific infectious agents using direct visual detection of characteristic shape, size and Gram Stain reaction.
4. Provide antibiotic susceptibilities of isolated pathogens to guide treatment.

Direct viewing of pathogens becomes primary or direct evidence to confirm or refute the physicians initial clinical impression .Culture results usually are too late to alter presumptive therapy.[1]

Microbiologists are encouraged to perform direct microscopic examination on specimens submitted for culture. Not only may it be possible to provide the physician with a rapid presumptive diagnosis, but also the detection of specific microorganisms may serve as a guide for selecting appropriate culture media and to provide a valuable quality control comparison with isolates recovered.[3]

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