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Review Article

Stains Employed in the Detection of Microorganisms

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Abstract

A clear diagnosis of infection can often be made based on the macroscopic appearance of tissue. Infection can be detected by frank pus, abscess formation, cavitation, hyperkeratosis, demyelination, pseudo-membrane formation, focal necrosis, and granulomas. Even if the precise nature of the suspect organism is never identified, some of these appearances may be sufficient to provide an initial, or at least provisional, diagnosis and allow treatment to begin, even if the precise nature of the suspect organism is never identified. It is important to remember that a well-executed hematoxylin and eosin method will stain a wide range of organisms. Romanowsky stains, such as Giemsa and Papanicolaou stains will also stain organisms and their cellular environment. Other infectious agents are difficult to detect using standard stains, necessitating the use of special techniques to demonstrate their presence. This review aims to highlight the commonly used stains for the detection of microorganisms in various histopathology and cytology material.

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Introduction

Microorganisms (also known as microbes) are organisms that can only be seen by microscopy. Many do not cause disease in humans and colonize human hosts normally. Clinical infections are the result of complex interactions between pathogens that can cause disease, the host, and the environment. The diagnosis of infectious disease illness usually begins with the patient's clinical presentation, and in most cases, a diagnosis is made without taking a tissue sample. Tissue diagnosis is used in a variety of situations, from autopsy specimens, where material is plentiful and sampling error is minimal, to cytology samples, where cellular material is often scarce and lesions are easily missed. A complete clinical history is always required, including information about the patient's ethnic origin, immune status, recent foreign travel history, and current medications. A clear diagnosis of infection can often be made based on the macroscopic appearance of tissue. Infection can be detected by frank pus, abscess formation, cavitation, hyperkeratosis, demyelination, pseudo-membrane formation, focal necrosis, and granulomas.

Neutrophil or lymphocytic infiltrates, granuloma formation, microabscesses, eosinophilic aggregates, Charcot-Leyden crystals, and caseous necrosis are all examples of indirect evidence of infection visible under low-power magnification on routine stained sections. Even if the precise nature of the suspect organism is never identified, some of these appearances may be sufficient to provide an initial, or at least provisional, diagnosis and allow treatment to begin, even if the precise nature of the suspect organism is never identified, particularly in the case of tuberculosis.

It's important to remember that a well-executed hematoxylin and eosin method will stain a wide range of organisms. Romanowsky stains, such as Giemsa, and Papanicolaou stains will also stain organisms and their cellular environment. Other infectious agents are difficult to detect using standard stains, necessitating the use of special techniques to demonstrate their presence. This could be due to the organism's small size, such as in the case of viruses, which necessitate electron microscopy. Alternatively, as with mycobacteria, spirochetes, and

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cryptococci, the organism may be hydrophobic or weakly charged, necessitating the use of specific histochemical methods for detection. Therefore, this review aims to highlight the commonly used stains for the detection of microorganisms in various histopathology and cytology material.

Gram Stain

One of the most important stains for bacteria is the Gram stain. The Gram stain, named after Hans Christian Gram, who invented the method in 1884, distinguishes Gram-positive and Gram-negative bacteria based on the composition of the cell wall and its permeability to dyes and stains. Peptidoglycan is found in both Gram-positive and Gram-negative bacteria's cell walls, but Gram-positive bacteria's cell walls are thicker, and Gram-negative bacteria have an additional layer of lipopolysaccharide outside the cell wall. The way these bacteria retain dye complexes is affected by these structural differences. All bacteria take up a crystal violet-iodine complex when stained with the Gram stain. Gram-positive bacteria, on the other hand, retain the dye complex after decolorization, whereas Gram-negative bacteria do not. Subsequent counterstaining allows visualization of Gram-negative organisms [Figures 1,2 and 3].[1]

The Gram stain is used to distinguish microorganisms that cause bacterial infections such as meningitis, pneumonia, bacteriuria, gonorrhea, and infections of the brain, lung, abdomen, pelvis, and wounds.

Examples of Gram-positive bacteria include *Clostridium botulinum, Clostridium tetani, Staphlococcus aureus,* and *Corynebacterium diphtheriae and they appear blue or violet*. Gram-negative bacteria include *Salmonella, Shigella dysenteriae, Escherichia coli,* and *Pseudomonas aeruginosa* and they appear red.

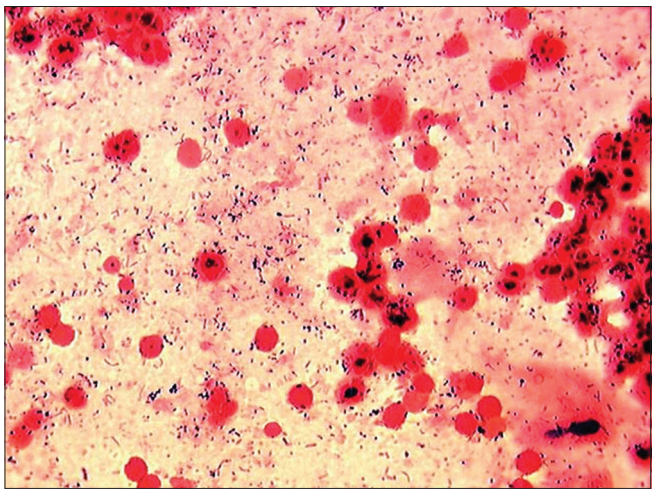

Figure 1: Gram stain highlighting the presence of thin gram negative rods and large clusters of gram positive cocci

Acid Fast Bacteria Stain

Mycobacteria infection is diagnosed using the acid-fast bacteria (AFB) stain. The Ziehl − Neelsen method is another name for it. Mycobacteria produce a waxy substance called mycolic acids, which forms a covalent bond with the peptidoglycan in the bacterial cell wall. Standard staining procedures are difficult for mycolic acids, but they can withstand alcohol decolorization after staining with hot carbol-fuchsin. Acid fast is an excellent diagnostic differentiator because of this property. In a light blue background, the AFB appear bright red or fuschia [Figures 3 and 4].[2]

Members of this family include *Mycobacterium tuberculosis* and *Mycobacterium leprae*, the causative agents of tuberculosis and leprosy, respectively.

Grocott's Methenamine Silver Stain

Aspergillus fumigatus, *Blastomyces dermatitidis*, *Candida albicans*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Nocardia asteroids*, *Pneumocystis carnii*, and *Sporothrix schenskii* are among the pathogenic fungi that can be identified using the Grocott's methenamine silver stain Methenamine silver methods rely on the oxidation of carbohydrates to form aldehyde groups within the tissue/fungus. These groups will act directly on silver ions in the methenamine silver solution, converting them to visible metallic silver. To remove any unreduced silver, sodium thiosulfate is used. Fungi usually appear black against a light green background [Figure 5].[3]

Warthin-Starry Stain

Spirochetes and other bacteria such as *Helicobacter pylori*, as well as two causative agents of cat scratch disease,

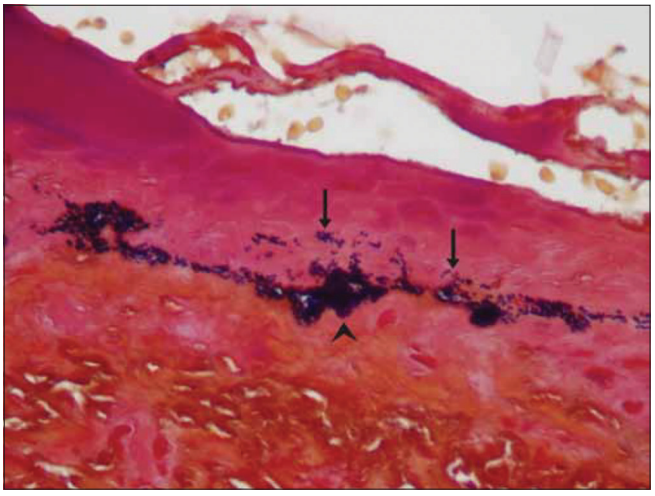

Figure 2: Photomicrograph of ulcerated skin stained with Gram's stain. The purple stain represents Gram-positive bacteria which are seen as clumps (arrowhead) or as separate clusters of cocci (arrows)

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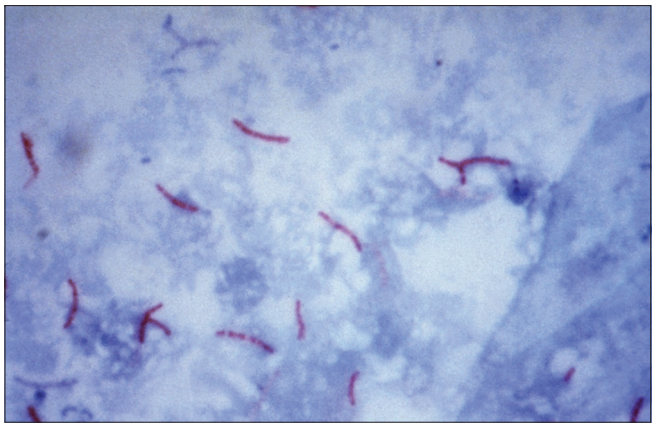

Figure 3: *Mycobacterium tuberculosis* on an oil-immersion smear slide stained with Ziehl-Neelsen or Acid-fast staining

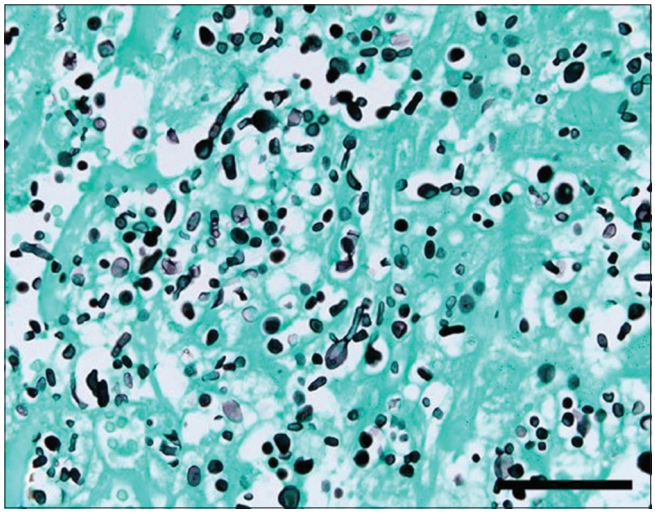

Figure 5: With the modified Grocott's methanamine silver method, fungus stains black, while other tissue elements are bluish green

Bartonella henselae and *Afipia felis*, can be identified using the Warthin-Starry stain. The ability of certain bacteria to bind silver ions from solution is used in the Warthin-Starry stain. After that, a reducing agent is added to convert the bound silver to visible metallic silver [Figure 6].[4]

The tissue is sensitised before the silver complex is applied in the Warthin-Starry stain. A silver diamine complex is formed by combining an aqueous silver nitrate solution with the reducing agent hydroquinone. Microorganisms appear black against a yellow to light brown background as a result of this.[5]

Alcian Yellow-Toluidine Blue Stain

The alcian yellow/toluidine blue (AY/TB) stain is a commonly used method for detecting *H. pylori* in patient specimens. *H. pylori* is a spiral-shaped bacterium that has been linked to gastric inflammation, ulcers, and cancer. Because *H. pylori* is usually embedded in the mucus lining the stomach, it can be difficult to

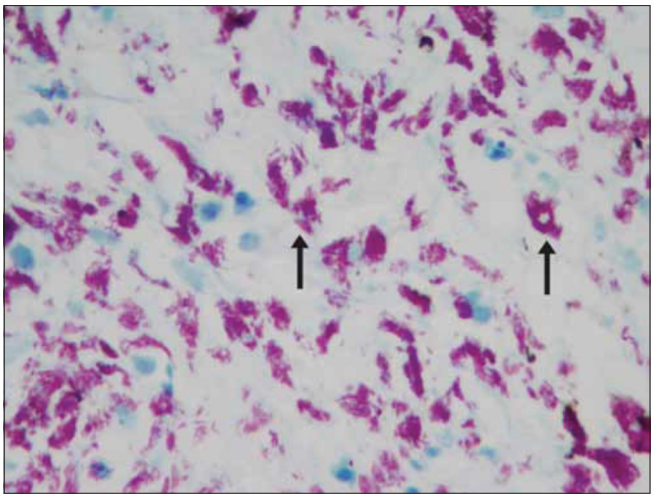

Figure 4: Ziehl-Neelsen stained section of lymph node. The pink color demonstrates clusters of mycobacteria stained with carbol-fuschin (arrows). The stain has resisted decolorisation by acid-alcohol. Other cells in the background are stained light blue by the methylene blue counterstain

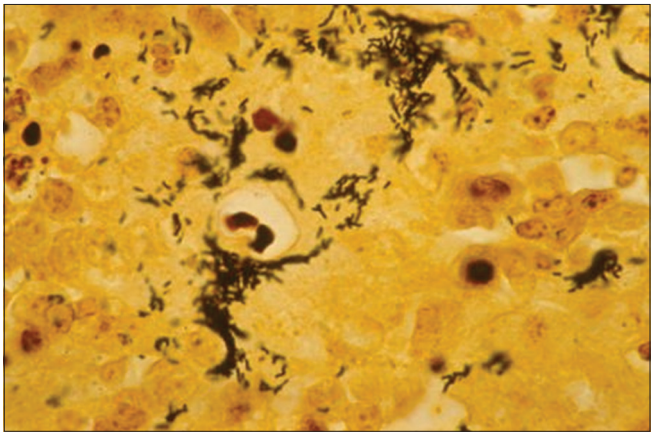

Figure 6: The Warthin-Starry stain is used for the visualization of Spirochetes

detect. Mucus is a neutral substance that will not stain when exposed to basic dyes. The mucus is treated with periodic acid to oxidize the hydroxyls to aldehydes with the Alcian yellow/toluidine blue stain.[6] When natural alcian yellow dye is used, the tissue is treated with sodium metabisulfite to yield highly acidic mucins, which then stain with alcian yellow. When synthetic alcian yellow dye is used, the mucins stain readily after periodic acid oxidation with the dye. Washing with acetic acid removes excess dye. In both cases, toluidine blue stains the bacteria, so that they stand out clearly against the yellow mucin. Normally, the *H. pylori* is stained as blue and mucin as yellow in color [Figure 7].

Giemsa Stain

The Romanowsky stains, which include the Giemsa stain, are a group of stains. Giemsa is a versatile polychromatic stain that can be used to stain a wide

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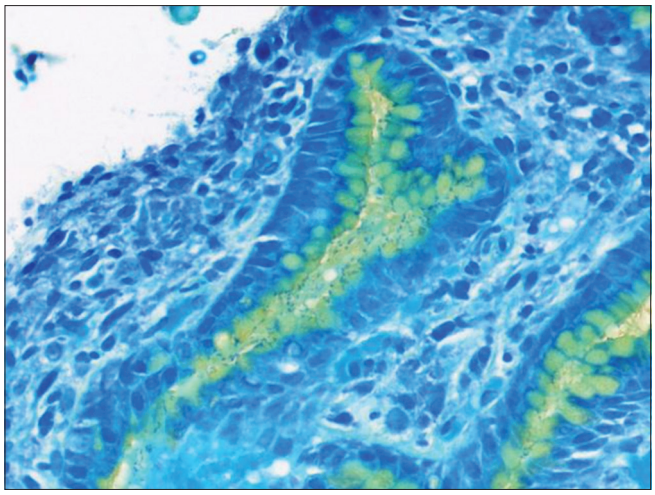

Figure 7: Alcian yellow-Toluidine blue stain used here to visualize *Helicobacter pylori* in a gastric biopsy

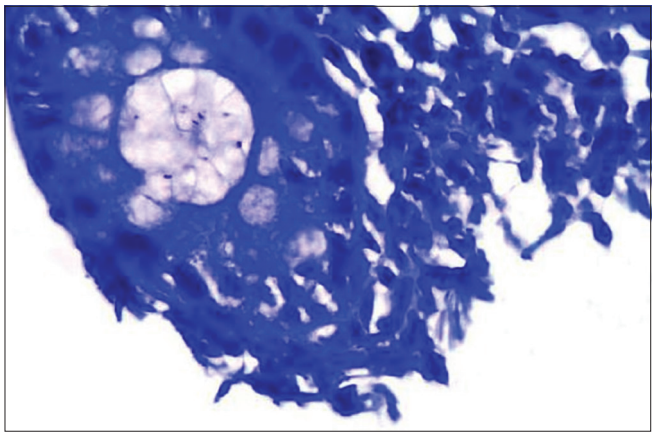

Figure 8: Gastric biopsy stained with Giemsa demonstrates *Helicobacter pylori*

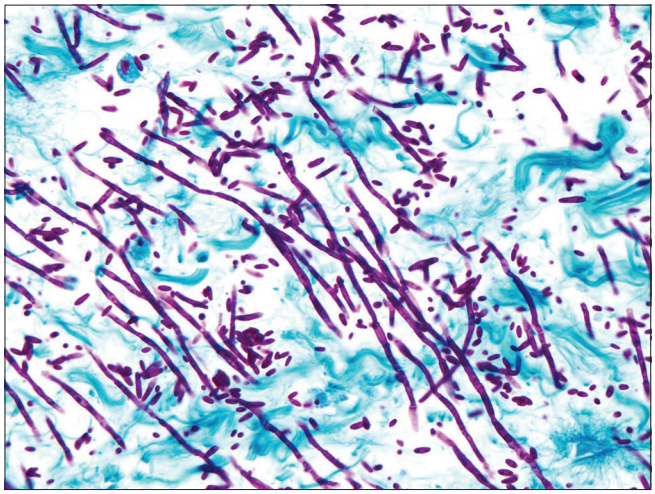

Figure 9: The periodic acid-Schiff-green stain, shown here on an esophagus specimen, is most commonly used for visualization of fungi

variety of specimens. 50 Gustav Giemsa created the Giemsa stain in the early 1900s to detect parasites like malaria and *Treponema pallidum* in blood smears. He devised a "secret" oxidation method that included a special blend of methylene azure, methylene blue, and eosin, as well as glycerol as a stabilizing agent. These stains combine a basic dye, such as methylene blue, with an acidic dye, such as eosin, to create neutral dyes. It is necessary to differentiate with a weak acid solution [Figure 8].[7]

Because of its unique staining of chromatin, nuclear membranes, and cytoplasmic elements, the Giemsa stain was adapted to histology. Because of the different steps required, the staining obtained in tissue sections is more variable than in smears (differentiation, dehydration, clearing). Pretreatment of the specimen material affects the color of the various cellular components. The Giemsa stain is also used to start clinical cytological material such as urine sediment, sputum, smears from fine needle aspiration biopsies, rinses, and touch preps.[8]

Giemsa can also detect microorganisms such as Histoplasma, Leishmania, Toxoplasma, and Pneumocystis, and *H. pylori* appears thin and distinctly blue in gastric tissues. The Giemsa stain, like the Gram stain, can be used to identify the morphological characteristics of bacteria. It does not, however, aid in the classification of bacteria as Gram-negative or Gram-positive. In hematology, Giemsa's stain is frequently used to distinguish the nuclear and cytoplasmic morphology of platelets, red blood cells, white blood cells, and parasites. May-Grünwald's solution for Pappenheim (MGG) and Wright-Giemsa are two dye solutions that are frequently used together. Fixation, staining times, and the pH values of the solutions or buffers can all have an impact on the final stain.[9]

The pH level has a big impact on Giemsa staining. Erythrocytes appear red at low pH levels, and they appear blue-gray to deep violet at higher pH levels. More chromatin staining and less cytoplasmic staining are associated with more acidic pH levels, whereas more alkaline pH levels promote the visibility of denser nuclei and increased cytoplasmic staining. Cell nuclei can be deep purple to dark blue in histological sections, collagen pale blue, acidic mucopolysaccharides reddish-violet, other acidic cellular materials orange-red, and in the case of *H. pylori* blue to dark blue.

Periodic Acid Schiff-Green Stain

The periodic acid-Schiff-Green stain, which has a wide range of applications, can be used to aid in diagnosis of fungal infections. Periodic acid oxidizes polysaccharides in the fungal cell wall to aldehydes, which in turn react with Schiff reagent to result in visualization. With fungal infection, a light green counterstain is typically

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used. Fungi appears as pink to fuschia in a light green background [Figure 9].[10]

Conclusion

Staining of microorganisms plays a pivotal role in identifying the underlying pathogenesis and the mechanism of action of that particular organism causing the infection. There are only handful number of staining methods employed for detecting the pathogenic organisms, hence this calls upon further research on discovering newer staining methods which aids in diagnosis of microbial infestations.

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Conflicts of interest

There are no conflicts of interest.

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