MEDICAL LAB REPORT WRITING AND EVALUATION

MICROBIOLOGY REPORTING

STAINING PROCEDURES

GRAM STAINING

GRAM STAINING Principle: Christian Gram originally described this stain in 1884. The mechanism of Gram staining is not fully understood. Gram-positive bacteria stain with crystal violet and are not decolourised with acetone iodine, while Gramnegative bacteria are decolourised with acetone iodine and hence take up the colour of the counter stain (carbol fuchsin). The difference in staining is due to the difference in the cell wall structure. Gram-positive bacteria have thick layer of peptidoglycan in their wall while Gram-negative bacteria have a thin layer.

- The original technique has undergone many modifications and the most widely used is the Preston and Morrell’s modification, which is described belo.

- Reagents 1. Ammonium oxalate crystal violet solution Crystal violet 20 g Methylated spirit 200 ml Ammonium oxalate 1% aqueous solution 800 ml 2. Iodine solution Iodine 10 g Potassium iodide 20 g Distilled water 1000 ml 3. Iodine acetone Liquor iodi fortis 35 ml Iodine 10g Potassium Iodide 6g Methylated spirit 90 ml Distilled water 10 ml Acetone 965 ml 4. Dilute carbol fuchsin Carbol fuchsin 50 ml Distilled water 950 ml. **Procedure**: Make a thin smear, dry in air and fix in flame. Cover with crystal violet for 30 seconds. Wash and apply iodine solution for 30 seconds. Wash and decolourise with acetone iodine until no further violet washes off. Wash and counterstain with dilute carbol fuchsin for 30 seconds. Wash with water, blot and dry.

- **Result**
- Gram-positive bacteria Dark purple Yeast cells Dark purple Gram-negative bacteria Pale to dark red

1. Nuclei of pus cells Red/pinkish Epithelial cells Pale red/pinkish **Interpretation**: The report should include the following information: 1. The number of bacteria (numerous, moderate, few or scanty) 2. The Gram reaction (Gram-positive or Gramnegative) 3. The morphology (cocci, intracellular or not) 4. The presence and number of pus cells 5. Presence of yeast or epithelial cells

Findings: **Gram stain of urethral smear** shows numerous pus cells and moderate number of Gram-negative diplococci, some of which are intracellular. Similarly, **Gram stain of sputum** may show numerous pus cells with a few epithelial cells and a moderate number of Grampositive cocci in chains and a few Gramnegative bacilli are present. **Variations**: Gram-positive organisms may lose their ability to retain crystal violet and stain Gram negatively for the following reasons: 1. Cell wall damage due to antibiotic therapy or excessive heat during fixation. 2. Over-decolourisation of the smear. 3. Use of an old Iodine solution (yellow instead of brown). It is to be stored in brown bottle. 4. Old culture Quality Control: Known Gram-positive (Staphylococcus aureus) and negative (Escherichia coli) organisms can act as controls on the same slide.

ZIEHL-NEELSEN STAINING Principle

- **ZIEHL-NEELSEN STAINING Principle:** The technique is used to stain Mycobacterium and Nocardia species. Mycobacteria when stained with carbol fuchsin resist decolourisation by acid while other bacteria get decolourised. Hence these are called Acid Fast Bacteria (AFB). Mycobacterium leprae is less acid fast and is decolourised with 5% acid. Similarly Nocardia species and Legionella species are even lesser acid fast and 1% acid is used for decolourisation.

- **Reagents** 1. Carbol fuchsin (mordant and dye): Basic fuchsin solution (10 g in 100 ml 95% ethyl alcohol) to be added to 900 ml 5% aqueous solution of crystalline phenol

- **2. Acid alcohol (decolourising agent)** a. Sulphuric acid 20 percent b. Ethyl alcohol 95% 3. **Counter stain, Loeffler's methylene blue** (saturated solution of methylene blue in alcohol 300 ml). Potassium hydroxide, 0.01% in water up to 1000 ml. **Procedure:** Fix smear by rapidly passing over flame. Cover with filtered carbol fuchsin and heat until steam rises. Allow staining for 5 min, heat being applied at intervals to keep the stain hot. Do not boil or allow to dry. Wash with water. Decolourise with acid alcohol for 2 min. The red colour of the preparation changes to yellowish brown. Wash with distilled water and counter-stain with Loeffler's methylene blue for 15-20 seconds. Wash in distilled water, dry and examine

- **Results**: Acid Fast Bacilli stain red against blue background. Interpretation: If bacilli are seen, report as "AFB positive" or "Acid Fast Bacilli seen". To report the result quantitatively as bacterial index the following chart is used: **No per entire smear Significance** 1-2 Doubtful (repeat) 3-9 Rare (1+) ≥10 Few (2+) ≥1 (per oil immersion field) Numerous (3+) Control: Two sputum smears of known high and low AFB positivity should be stained with the routine smears to check the procedure and interpretation at regular intervals and whenever a new batch of stain is introduced.

- Modifications: Modifications of Ziehl-Neelsen staining method for other acid-fast organisms are: 1. For Mycobacterium leprae decolourisation is done with 5% sulphuric acid (or 3% HCl in alcohol). 2. Sections of tissue containing 'clubs' of Nocardia and Legionella are decolourised with 1% sulphuric acid. 3. Cultures of some specimens of Nocardia are decolourised with 0.5% sulphuric acid. 4. Stool specimen for Cryptosporidium parvum are decolourised with 3% hydrochloric acid or 10% sulphuric acid. 5. Auramine phenol method has the advantage that large areas of film can be stained in a short time.

ALBERT'S STAINING

- **ALBERT'S STAINING** This stain is used to identify Corynebacterium diphtheriae and to stain the volutin (metachromatic) granules. **Reagents** 1. **Solution 1 (Albert's stain):** Dissolve 0.15 g Toluidine blue and 0.2 g Malachite green in 2 ml 95% ethyl alcohol, add 100 distilled water and 1 ml glacial acetic acid. Mix well, let stand for 24 hours and filter before use. 2. **Solution 2 (Albert's iodide):** Grind 2 g Iodine crystals and 3 g Potassium iodine in about 10 ml distilled water and make volume to 300 ml with distilled water. **Procedure:** Allow smear to dry and fix by heat. Stain with solution 1 for 1-5 min. Rinse with water and blot dry. Apply solution 2 for 1 min. Rinse with saline, blot dry and examine**. Result:** Bacterial cells stain green and volutin granules stain green black. Control: Smear of a positive control and negative control on the same slide are made for comparison

SPORE STAINING Principle

- SPORE STAINING Principle: The wall of spores is relatively impermeable, but dyes can be made to penetrate it by heating, once stained, these resist decolourisation. Reagents 1. Ziehl Neelsen's carbol fuchsin. 2. Sulphuric acid 0.5% 3. 1% aqueous methylene blue or 5% aqueous malachite green. 4. 5% safranin or 0.05% basic fuchsin. Acid Fast Stain for Spores Procedure: Make film, fix, dry and stain with for 3-5 min with heat. Wash in water and decolourise with 0.5% sulphuric acid. Wash with water and counterstain with 1% aqueous solution of methylene blue for 3 min. Rinse in water, blot and dry, and examine.

Malachite Green Staining Procedure

- Malachite Green Staining Procedure: Make smear, fix and dry. Place slide over a beaker of boiling water. When large drops of water condenses on the under side of slide, flood it with malachite green and leave for 1 min. Wash in cold water. Counterstain with 0.5% safranin or 0.05% basic fuchsin for 30 seconds. Wash, dry and mount.

- Interpretation: By Ziehl-Neelsen stain spores appear bright red while protoplasm stains blue. With malachite green spores stain green while vegetative bacilli stain red. Result: Report as presence or absence of "spore forming bacteria". Specify position of spores (terminal, sub-terminal or central) and also number of spores per bacteria. Control: Control should be included in the smear to assess reagent’s efficacy.

GIEMSA STAINING

- GIEMSA STAINING It is one of the Romanowsky stains described in haematology (page 258). These stain cytoplasm as blue and nuclei as red. Reagents: Stock Giemsa stain: Grind 3.8 g Giemsa stain powder in 200 ml glycerine. Place it at 60°C for 2 hours. Cool and add 312 ml methanol. Procedure: Prepare smear and dry. Fix in methanol for 3-5 min and dry in air. Place in 1:10 diluted Giemsa stain1 for 30-45 min. Sodium carbonate (1%) can be used as diluent instead of distilled water. Spirochetes may require up to 4 hours. Wash with water, blot dry and examine

INDIA INK STAINING OF BLOOD AND CSF

- INDIA INK STAINING OF BLOOD AND CSF India ink is useful for visualisation of capsulated organism such as Cryptococcus neoformans (page 197) or B. anthracis in clinical samples or from fungal cultures. Procedure: Take 100 µl of sample (blood, CSF, suspension of fungus culture) or control to a slide, place a cover glass and add 5-10 µl
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- ink to the edge of the cover glass. The preparation is ready for examination. Control: Klebsiella pneumoniae on SBA or equivalent or known C.neoformans are used as positive control. E. coli ATCC 25922 or equivalent act as negative control. Transfer a small amount of growth (1 mm diameter) from each control SBA plate into 0.5 ml whole EDTAtreated blood or serum and mix. Control strains are assayed on each day of testing. Interpretation: The capsule will appear as a well-defined clear zone around the cells for the positive control. No zone should be present in the negative control.

MCFADYEAN'S STAIN

- MCFADYEAN'S STAIN This is a special stain for the capsule of Bacillus anthracis. Procedure: Make a thick smear of blood, exudate or tissue fluid, dry in air and fix imperfectly by passing quickly three times through a flame. Stain with polychrome methylene blue for 30 seconds. Wash and dry. Interpretation: Irregular pink-purple capsular material, both surrounding the bacilli and chains of bacilli and some detached from them, is indicative of anthrax bacilli.

PHENOL-AURAMINE STAIN

- PHENOL-AURAMINE STAIN This stain is used for detection of cryptosporidium oocyst. It gives consistent results with few false positives. The oocyst shows a bright centre with inclusions and a pale halo, when examined with blue light under a fluorescent microscope.