Simple differentiate nearly all types of bacteria. these

Simple staining is used to study the size, shape, motility and other morphological characteristics of micro-organisms. In this type of staining, the simple stain is applied to the heat fixed film and allowed to react for 30 seconds to 3 minutes (depending on the type of stain used).

Then the smear is washed with water and dried. Bacterial cells will take up the colour of the dye which will make the identification easier. Examine the slide under oil emersion lens of the microscope either directly or after mounting in glycerin.

B. Differential Staining Methods:

In differential staining methods more than one dye is used which when properly employed will differentiate nearly all types of bacteria. These methods are also used to study the morphological characteristics of bacterial cells, spores and capsules.

The various stains used for differential staining are Gram, crystal violet, methyl violet, fuchsine and Ziehl-Neelsen. The various staining methods used for differential staining include: (i) Gram’s staining method (ii) Acid fast staining technique (iii) Ziehl-Neelsen method (iv) Staining of spores (v) Staining of capsules (i) Gram’s Staining Method: This is the most commonly used method for differential staining of bacteria. It is a very simple and useful method which was first used in 1884 by Gram and till now it has not lost its practical significance. All bacteria stained by Gram method can be grouped according to colour as gram positive and gram negative. The procedure for staining is as follows: Reagents Used in Gram’s Staining: (a)

Gentian violet 0. 5 gm. Distilled water up to 100 ml. Dissolve in distilled water.

(b) Iodine I. O gm. Potassium iodide 2. 0 gm. Distilled water up to I00 ml. Dissolve potassium iodide in water and to this dissolve iodine.

Add sufficient water to make up the volume to 100 ml. Store the solution in amber coloured glass bottles. (c) Basic fuchsine 0. 1 gm. Alcohol 10. 0 ml. Distilled water up to I00 mi.

Dissolve basic fuchsine in alcohol and allow to stand for 24 hours. Add sufficient distilled water to make up the volume to 100 ml. Procedure: (a) As described earlier prepare a thin film or smear of a test bacterium on a clean slide using aseptic precautions. (b) Heat fixes the film by passing through Bunsen’s flame 2-3 times.

If heat fixation is contraindicated then dip the film in alcohol for fixation. Heat fixation coagulates the proteins of bacteria which disturbs the morphological characters of micro-organisms. (c) Cover the fixed smear with gentian violet (crystal violet or methyl violet) stain and allow the stain to act for about one minute. (d) Remove the excess stain and wash the slide with excess of Gram’s iodine solution thoroughly. (e) Cover the whole slide with fresh Gram’s iodine solution and leave it as such for one minute. During this time compounds are formed in the cytoplasm of the bacterial cell, which are retained by some bacterial species during deceleration with alcohol. (f) Wash the slide with alcohol or acetone in order to decolorise the slide. Go on washing the slide till no colour comes out.

This process is very rapid and completes in 2-3 seconds. (g) After this process wash the slide under running tap water and counter stain it with an aqueous solution of fuchsine for 30 seconds. (h) Wash the slide with tap water, dry it and examine the slide under oil immersion lens without mounting. Those bacteria which cannot be decolorised with alcohol or acetone and retain violet colour are known as gram positive bacteria and those bacteria which are decolorised by alcohol or acetone and stains red due to fuchsine solution are known as gram negative bacteria.

The examples of gram positive bacteria are staphylococci, streptococci, pneumococci, C. diphtheria, B. anthrasis, subtilus, CI. tetani, CI.

welchi etc. The examples of gram negative bacteria are gonococci, meningococci, E. coli, S.

typhi, Cholera vibrio etc. The Gram staining method is commonly used for the identification of mycobacterium, streptococci, staphylococci, gonococci, E. coli etc. but this method cannot be applied to capsules, spores, flagella, fungi and protozoa. For this purpose other techniques are used which are described under respective categories. (ii) Acid Fast Staining Technique: Acid fast staining technique was first developed by Paul Ehrlich in 1882 for differential staining of microorganisms. In this method dyes like malachite green and methylene blue are used. When the smears are treated with these dyes and washed with acids and alcohols they are not decolorised and retain the stain of the dye.

Such bacteria which are not decolorised are known as acid fast bacteria but the bacteria which lose the stain and get decolorised are known as non-acid

fast bacteria. (iii) Ziehl-Neelsen Method: The Ziehl-Neelson method is used for differentiating acid fast bacteria (mycobacterium tuberculosis, a causative organism of tuberculosis and mycobacterium leprae, a causative organism of leprosy) which stain with difficulty due to their structure and hence steaming concentrated carbol fuchsine is used to stain them. The reagents used for Ziehl- Neelsen staining are: A. Ziehl-Neelsen’s (strong) carbol fuchsin solution : Basic fuchsine 10 gm. Absolute alcohol 100 ml. 5% solution of phenol in water up to 1000 ml.

Dissolve basic fuchsine in alcohol and add to the phenol solution. B. Sulphuric acid 20% solution. C. Alcohol 95% D. Counterstain methylene blue or malachite green.

Procedure: (a) As described earlier prepare a smear of the sputum on a slide and fix it by passing through Bunsen’s flame. (b) Cover the slide with strong carbon fuchsine solution and heat until steam rises. Allow the stain to remain in contact for 5 minutes, heat being applied at intervals to keep the stain hot but the stain must not be allowed to evaporate to dryness. (c) Wash the smear with water. (d) Cover the slide with 20% sulphuric acid for one minute and remove excess of the acid. (e) Wash the slide with water till the colour of the smear ceases to come out. (f) Counter stain the slide with methylene blue or dilute malachite green for 30 seconds. (g) Wash the slide thoroughly with water, dry it and see under oil immersion lens.

The slide will appear pink coloured and rod shaped tubercle bacilli will be seen scattered in the film. The acid fast microorganisms are stained pink or bright red whereas the background tissues, cells and other non-acid fast

bacilli are stained blue or green. (iv) Staining of Spores: Bacterial spores are highly resistant to high temperature, radiation, dessication and chemical agents. The ordinary staining dyes do not penetrate the spore walls therefore a specialised technique is used for staining the spores which is described as follows: (a) As discussed earlier prepare a thin smear on glass slide and heat fix it by passing through Bunsen’s flame. (b) Apply the primary stain, malachite green to the heat fixed smear and gently heat to steaming to enhance penetration of the dye into the spores. Continue steaming for about ten minutes. During steaming care must be taken that the stain should not evaporate to dryness.

(c) Wash the slide under running tap water to remove malachite green from cellular parts other than spores. (d) Counterstain the smear with safranin for 40 second. (e) Wash the slide under running tap water, dry the slide and examine under oil immersion lens. The spores are stained green in colour and vegetative portion of the cell is stained red or pink in colour. Spore producing bacteria like bacillus anthracia, the causative agent of anthrax and Clostridium tetani, the causative agent of tetanus are identified by this technique. (v) Staining of Capsules: The bacterial capsules are often clearly stained when treated by common stains like crystal violet or methylene blue. The procedure is as follows: (a) As described earlier prepare a thin film of bacterial culture and air dry.

(b) Stain the slide with 1% crystal violet solution for one minute. (c) Wash the slide gently with 20% copper sulphate solution to remove excess dye in the capsules. (d) Examine the slide under oil immersion lens. Staining contrast will be seen between the organism and the capsule.

Bacteria is seen as a highly refractile outline against dark background of the dye. The capsular zone is seen as a clear space between the refractile portion and dark background of the dye.