University of Mosul
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Lecture title: BACTERIOLOGICAL MEDIA

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Summary:

4-Giemsa stain

Disease	Samples	Etiology	Appearance in stained smears
Anthrax in cattle, sheep and pigs	Blood smear from ear vein	*Bacillus anthracis*	Purplish, square-ended rods in short chains surrounded by a reddish-mauve capsule

5-Polychrome methylene blue(M'Fadyean's Reaction)

Disease	Samples	Etiology	Appearance in stained smears
Anthrax in cattle, sheep and pigs	Blood smear from ear vein	*Bacillus anthracis*	Blue, square-ended rods in short chains surrounded by a pinkish-red capsule

6- Wet preparations Leptospirosis in many animal species

Disease	Samples	Etiology	Appearance in stained smears
Leptospirosis in many animal species	Centrifuged deposit of urine or kidney tissue	*Leptospira serovars*	Helical, approx.15 µm long and 0.15 µm wide. Appears beaded with hooked ends. Dark field microscopy.

7-Methylene blue stains : The used of single stain to color the organism is commonly referred to as simple .This stain is used specially for *Pasteurella spp*. which characterized **bipolarity** .

8-Malachite green stain:(structural stain- endospore stain) species of Bacillus and Clostridium

spp. The the endospore stains green but the remainder of the cell stains light red.

9- Negative staining technique

1-Indian ink Or Capsule stain

2- Nigrosin stain

Some bacterial cell and yeasts are surrounding by an extracellular slime layer called a capsule (.protective outer structure)The capsule can be demonstration by combining the methods for the simple stain and negative stain.

Capsules do not absorb most basic dyes; therefore, a negative staining technique (staining around the cells) is typically used for capsule staining. The dye stains the background but does not penetrate the capsules, which appear like halos around the borders of the cell.

BACTERIOLOGICAL MEDIA

Culture medium A culture medium is nutrient material prepared in the laboratory for the growth of bacteria, molds, and other microorganisms.

Types of culture media

A- According to physical conditions

1. Liquid media, broth.

2. Solid media, Agar.

3. Semi solid media, Gelatin.

B- According to the purpose of application

1- Simple media Contains the essential nutrients as source of nitrogen and carbon such as nutrient broth, peptone water, nutrient agar.

2- Enriched media Certain types of bacteria need special nutrients in order to growth, simple media can be enriched by adding vitamins, yeast and salt

such as Blood agar, Heated Blood agar (Chocolate agar): For Hemophilus spp. , very nutritive media Hemolyzed RBC's with growth factors has increased Carbon dioxide , Brain heart infusion agar, Tissue and body fluid's extract,and Serum agar.

3- - Transport media Simple media used for transport samples from different regions to the lab. e.g. Stuart transport medium

4- Differential media are media that contain substances that cause some bacteria to take on a different appearance from other species, (allowing one to differentiate one species from another) .

Such as MacConkey agar and Eosin Methylene blue agar (contain lactose, which differentiate between lactose fermenting and non-lactose fermenting bacteria :Lactose fermentors (E. coli, Enterobacter, and Klebsiella) produce acid from lactose and grow as pinkish-red colonies while Lactose non-fermentors- produce colorless colonies), mannitol salt agar (for isolation of staphylococci), , blood agar is Differentiate between hemolytic and nonhemolytic bacteria(different species of Streptococcus).

Four types of hemolysis:

 Alpha hemolysis- partial hemolysis Narrow band of greenish slimy

discoloration around the colonies.

 Beta hemolysis- complete hemolysis Clear zone around the bacterial colony

 Gamma hemolysis- no hemolysis Delta hemolysis- Double zone hemolysis

5- Selective media are media that allow only certain types of organisms to grow in or on them due to the presence of inhibitory substances or the absence of certain critical nutrients that make it unfavorable for most, but not all, organisms.

The inhibitory substance may be salt (NaCl), acid, a toxic chemical (crystal violet), an antibiotic (streptomycin).

In some cases, media have been formulated that are both selective and differential.

Such as MacConkey agar and Mannitol salt agar.

 MacConkey agar contains bile salts and crystal violet, which inhibit the growth of gram-positive bacteria.

 Eosin Methylene blue agar for isolation of E. coli. Contain eosin,

methylene blue.

 Salmonella Shigella agar (selective) for isolation of Salmonella and Shigella.

Colony characteristics on culture media:

 Size- pin point, medium, large

 Color- yellow, white, gray, cream, etc.

 Density- opaque, transparent

 Elevation-raised, flat, convex, droplike

 Form-circular, irregular

 Consistency- buttery, brittle, sticky

 Odor: Decayed apple odor produced by Pseudomonas and Putrid fish odor produced by Proteus

Phenomena Some bacteria have the ability to produce phenomena :

a. Swarming(Proteus on nutrient agar)

b. Medusa head(Bacillus colony appears as curled hair).

Pigmentation Many bacteria produce pigments which are of two types.

a. Exopigment Some bacteria produce pigments which are excreted to the outside of the bacterial cell changing the color of the medium e.g. Pseudomonas aeruginosa which produce two colors, pyocyanin_ (bluish green) and fluorescein (greenish yellow).

b. Endopigment Some bacteria produce pigments which remain inside the bacterial cell. e.g. 1. Golden pigment: produce by Staphylococcus aureus. 2. Yellow pigment: produced by Staphylococcus citrus. 3. White pigment: produced by Staphylococcus albus