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Preparation of selective and differential media

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Aim: To learn how to prepare the selective and differential media.

Theory: A culture medium is a solid on liquid preparation used to grow, transport and store microorganisms. To be effective, the media must contain all the nutrients the microorganisms require for growth. Specialized media are essential in the isolation and identification of microorganisms, the testing of antibiotic sensitivities, water and food analysis, industrial microbiology, and other activities. In addition to nutrients, special purpose media contain one or more chemical compounds. These are: Selective media, Differential media, enriched media.

Selective media: This allows certain types of organisms to grow, inhibit the growth of other organisms. It can be achieved by adding dyes, antibiotics, salts or specific inhibitors which affect the metabolism or enzyme systems of the organisms.
Example: Eosine Methylene Blue (EMB) agar, Mannitol salt agar, Hektoen enteric (HE) agar, Phenylethyl alcohol agar,

Differential media: This used to differentiate closely related organisms or group of organisms. Example: MacConkey agar, EMB agar,

Required materials: Bacterial cultures, inoculating loop, differential and selective media's: mannitol salt agar, MacConkey agar, Eosine Methylene Blue (EMB) agar, Hektoen entric (HE) agar.

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Procedure: ① Arrange all the cultures and media in the Laminar air flow.

② Flame the inoculating loop and transfer a loopful of culture from the tube labelled E.coli on to an EMB plate and perform a continuous streak. Similarly to a second plate of EMB, do the steps for Enterobacter aerogenes. Similar steps were repeated in all selective and differential media for differentiating their growth characteristics of the organisms.

③ All the plates were incubated aerobically at 37°c for 24 hrs.

Results: Mannitol salt agar is used for the isolation of pathogenic Staphylococci from mixed cultures. Staphylococcus aureus was grown in one plate of MSA and into a second plate Staphylococcus epidermidis was grown. The first one ferments mannitol so the colour changes from red to yellow. The latter one produce colonies with red or purple zones without mannitol fermentation.

EMB agar is both a selective & differential medium used for the detection and isolation of gram negative intestinal pathogen Here E.coli produce dark blue black colonies with metallic green sheen indicating vigorous fermentation of lactose & acid production.

Enterobacteria aerogenes produce blue, dark centered mucoid colonies indicating lactose fermentation & acid production.

MacConkey agar is selective for Gram negative bacteria and can differentiate those bacterias that are able to ferment lacto.

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E.coli are lactose fermenters and thereby appear pink while pseudomonas aeruginosa are non lactose fermenters and appear colourless or golden coloured.
HE agar is a moderately selective medium used for the isolation and cultivation of gram negative enteric micro-organisms, eg. Shigella & Salmonella.
On HEA agar, Shigella species develop into green coloured colonies with darker blue green centers. Salmonella species appear as blue green colonies with or without black centers

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Gram Staining

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Aim: To learn how to perform gram staining.

Requirements: Wax pencil, slide, microscope, inoculation loop, bacteria colony, crystal violet, grams Iodine, Decolorizer, Safranin, burner.

Procedures: ① Using a wax pencil, label a microscope slide with your initials and then draw a circle about 1cm in diameter on the slide.

② Sterilise the inoculation loop. Using aseptic technique, add a drop of sterile water so that a film appears across the loop.

③ Transfer the water into the circle. Flame the metal loop to sterilize again.

④ After cooling, use the loop to transfer a colony of bacteria growing on an auger plate. Touch lightly to avoid transferring too many bacteria.

⑤ Sterilize the loop again after finishing. Let the liquid evaporate completely leaving behind a dryspot on the slide. This will take atleast 10 minutes.

⑥ Once the liquid has dried completely, heat fix the bacteria to the slide by passing the slide quickly through the flame three times. The flame should touch the slide for about one second during each pass. Turn off all the bunsen burners

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before obtaining the set of gram stains.

⑦ Add a few drops of crystal violet stain to the bacteria until the circle is filled with stain. Let it stand for 1 min.

⑧ Dip the slide in a beaker of water two to three times until most of the stains has washed off.

⑨ Add a few drops of grams Iodine to cover the bacteria. Let this stand for 1 minute. Repeat the steps in a second beaker of water.

⑩ Add a few drops of decolonizer. Let it flow over the bacteria at a 45° angle until the flow is clear. Then finish by rinsing the slide in a beaker of water

⑪ Counterstain by adding Safranin stain to cover the bacteria and let it stand for 1 minute. After standing, completely rinse the slide in water.

⑫ Allow the slide to dry completely. After drying completely, observe the sample under a microscope.