Smear preparation and microbial staining

Smear preparation and microbial staining were conducted following methods as indicated on Ex. 10-13 of the Laboratory Manual. Smear preparation was prepared using two types of media: solid (E. coli) and liquid media (B. megaterium). Two loopfuls of liquid inoculum was transferred to the marked circumference on the slide. For the solid media, only a small amount of inoculum was transferred and mixed to the pre-deposited drop of water to the slide. Slides were air-dried and then heat-fixed.

For simple staining, the bacterial smears prepared were flooded with Crsytal Violet for one minute, followed by washing. For negative staining, Gram positive S. aureus and B. megaterium were separately introduced to a small amount of Nigrosin Black pre-placed on the slides. A spreader slide is placed on top, pressed upwards until adequate spread has been achieved as evident by the spread of the stain to the slide edge.

This technique for negative staining allows for easier drying. For capsular staining, two loopfuls of K. pneumoniae was introduced to the Nigrosin dye pre-placed on the slide and followed by immediate spreading and heat fixing. Smear is then flooded with basic stain Crystal Violet, washed and then blotted of with the tissue. All slides prepared were observed under HPO and OIO of the microscope and observations were drawn and noted in data blanks in pg 97 of the lab manual. It is also noted that all procedures in smear preparation and staining follow strict implementation of aseptic techniques to prevent cross contamination.

III. Data/Results

Aside from cultural techniques, microscopic examination can also be use in identifying, classifying and observing characteristics of microorganisms.

Smear preparation and staining procedures are basic techniques for microbial examination. Aseptic techniques were followed (e. g. cleaning slides and flaming) to prevent cross-/contamination from possible sources (e. g. slide, loop tip, handler). Flaming was conducted to allow adherence of cells to slide and coagulation of the protoplasm. Stains were used to make cells more easily seen, to show certain cell structure and reveal their chemical structure. Microorganisms used for the experiment were all rod-shaped Gram-Negative bacteria except for Staphylocccus aureus which is spherical in shape and is Gram positive.

The S. aureus bacteria is clustered (grape-like) and is usually in chains and E. coli may be in cluster or solitary whereas B. megaterium is single or solitary. Smear preparation or the preparation of microbial slides for microscopic observation can come from solid or liquid media although there are marked differences between the two. To differentiate and identify variances in appearances and morphology under OIO, cationic Methylene Blue was used. MB dye carries a positive charge and thus stains acidic cell components making the microbial cell more visible and colored ‘ blue’ under OIO. Rod shaped E. coli obtained from the liquid medium appear less numerous under the microscope compared to round S. aureus and rod-shaped B. megaterium. Inocula control is facilitated easier using liquid since there is dilution of cells in the suspension compared to the solid media. To prevent cell cumping and to observed individual cells dilution of inocula is a must.

Negative staining using acidic Nigrosin Ink on S. aureus allows black or dark coloration or distinct halo formation around the colorless spherical S. aureus which may be similarly attributed to the repulsion of charges. Structural identification and size estimation using negative staining is only possible without heat fixation so as prevent cell shrinkage. Capsule is a layer of gelatinous material or viscous substance produced or secreted by many bacterial cells which accumulates around the cell or coats the cell wall. Nigrosin dye used is deposited outside the capsule of the rod shaped K. pneumonia due to repulsion of charges whereas the cell is stained blue or violet due to primary stain Crystal Violet. Capsule appears colorless.

Summary/Conclusion

Steps involved prior to microscopic examination are smear preparation, fixation, and staining. Staining of different microorgansim—B. megaterium, S. aureus, and K. pneumoniae—allows increased cell visibility, accentuate specific structure, most specifically capsule for the conducted exercise and reveal chemical nature of the cell. Answers to Short Questions 1. Smear preparation from a liquid medium and a solid medium differ since the inocula in the former was pre-diluted diluted using a known amount of liquid medium whereas the 2. Quantity of cells must be de-limited so that clumping of cells is avoided and individual cells can be examined more thoroughly during microscopic examination. 3. Heat fixation confers cell death, coagulation of the protoplasm, and allows the cells to adhere to the slide.

4. Stains have constituent colored ionic chemicals which allow them to adhere to the charged sites on the cells. Not all colored dyes can be used

because their chemical components are complicated meaning their ions are many and may have multiple charges and their color reaction to cell sites may be difficult to distinguished. 5. Dark Field Microscopy 6. Fluorescence microscopy makes use of fluorescent-tagged materials/reagents (e. g. isothiocyanates) to stain the microorganisms. 7. Capsules (thick material deposition) confer protection during phagocyte reaction of the host making them more difficult to digest (phagocyte ingestion: immune system reaction).