

Isolation of individual colonies essay



**ASSIGN
BUSTER**

A. Define the following: Enriched Media: An enrichment medium contains some important growth factor (vitamin, amino acid, blood component, or carbon source) necessary for the growth of fastidious organisms. Selective Media: Selective media allow for the selection of particular microorganisms that may be present in a mixed culture. Selective media usually contain a component that enhances the growth of the desired organism or inhibits the growth of competing organisms.

Differential Media: Differential media allow for the separation of organisms based on some observable change in the appearance of the medium or by an observable effect on the microbe. Complex Media: A complex medium is composed of a mixture of proteins and extracts in which the exact amount of a particular amino acid, sugar, or other nutrient is not known. Synthetic Media: In a synthetic medium, the exact amount of pure chemicals used to formulate the medium is known.

B. Why is it necessary to use a solid agar medium to obtain a pure culture of *S. epidermidis*?

To obtain a pure culture, it is necessary to separate individual cells of a particular microbe. This requires the use of a solid medium that provides a surface for the individual cells to be separated and isolated from the other microbial cells that may be present in the original sample.

C. Compare your *L. acidophilus* pour plates and spread plate. Which method do you think worked better to isolate individual colonies? Why? I think spread plates worked best because a solid medium that provides a surface for the

individual cells to be separated and isolated from the other microbial cells that may be present in the original sample.

Also the pour is more likely to gain unwanted bacteria through the dilution process.

D. What are the six qualities included in a description of colony morphology?

1. Shape: What is the basic formation of the colony? Is it circular, irregular, or filamentous? 2. Elevation: What is the cross-sectional form of the colony when viewed from the side? Is it flat, raised, or convex? 3. Margin: How does the edge of the colony appear when magnified? Is it smooth, lobed, or curled? 4. Surface: What is the appearance of the surface of the colony? Is it glistening, rough, or dull? 5. Pigmentation: Is the colony colored?

Is it white, cream colored, pink, etc.? 6. Opacity: Is the colony transparent, opaque, translucent, or iridescent?

E. Describe the colony morphology seen on your *S. epidermidis* dish. Small,

White, Round, Raised, Opaque colony. F. What is the difference between a

viable and total count? What is the difference between direct and indirect counts? Several methods can be used to determine the number of microbes in a given sample. Viable counts include cells that can be cultured or are metabolically active. Total counts include all cells present, including dead or inactive cells.

Direct methods count actual cells or colonies; indirect methods estimate the number of cells present based on the measurement of an indicator such as light absorption. G. What is a spectrophotometer? How is it used to

enumerate microbes? A spectrophotometer is used to measure the amount of light at a wavelength of 686 nm that is transmitted through a bacterial culture. Because the bacteria absorb the light of that wavelength, the amount of light transmitted through the culture, rather than absorbed by it, is inversely proportional to the number of bacteria present in the sample. The more bacteria present, the less light that will transmit through the sample.

H. What is a hemocytometer? How is it used to enumerate microbes? The hemocytometer is a device used to count cells. It was originally designed for the counting of blood cells. It consists of a thick glass microscope slide with a rectangular indentation that creates a chamber. This chamber is engraved with a laser-etched grid of perpendicular lines. The device is carefully crafted so that the area bounded by the lines is known, and the depth of the chamber is also known.

It is therefore possible to count the number of cells or particles in a specific volume of fluid, and thereby calculate the concentration of cells in the fluid overall. I. Define the following acronyms: CFU- Colony Forming Units (CFUs) are divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of organisms per mL that were present in the original solution. TNTC- Dishes containing more than a few hundred colonies are considered Too Numerous To Count (TNTC) and are recorded as TNTC in data records.

TFTC- Dishes with only a few colonies are considered Too Few To Count (TFTC) and recorded as TFTC. OD- The measurements can also be converted

to Optical Density (OD) which is a quantitative method of describing the cellular mass of a culture. The measurements obtained through spectrophotometer readings are considered total count measurements because they include all cells present, both viable and nonviable. J. When serial dilution is used to enumerate microbes in a real life application, such as in a water quality study, each dilution is plated on a series of dishes.

The data from each dish (the number of CFUs) is pooled together and an average CFU per dish is generated for the dilution. It is this average, rather than the actual plate counts, that is used to calculate the final CFU/mL result. Why do you think an average is used rather than the actual plate counts? Why might there be differences in the number of CFUs on each dish when they are grown from the same dilution?

CFU is only an estimate of the number of cells present. It is a skewed estimate at best as the only cells able to form colonies are those that can grow under the conditions of the test (e. g. incubation media, temperature, time, oxygen conditions). the kind of bacteria in the material under examination will have an influence on the size of the colonies, and consequently, on the number that can develop on a plate. Colonies close to each other on the plate may merge, and neighbor colonies may inhibit growth or conversely stimulate growth. “ Because of these and other difficulties, certain plates in any series made for a given sample are more satisfactory for use in computing a total than are others. The matter of selecting plates to be used in computing a count becomes, therefore, a matter requiring considerable judgment”