Problem Set #6

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Techniques and Methods

Question 1: What is the difference between a laminar flow hood and a biosafety cabinet?

Answer: A laminar flow hood protects the sample from contamination by providing a sterile, HEPA-filtered airflow but does not protect the user or the environment. In contrast, a biosafety cabinet (BSC) is designed to protect the user, the environment, and the sample when working with hazardous biological materials by using HEPA filtration and controlled airflow to contain potential biohazards.

Question 2: How many biosafety levels are there and which one is the highest risk level?

Answer: There are 4 biosafety levels, BSL 1 – 4, with BSL 4 being the highest risk level designated for microbes that represent often fatal infections for which no treatment exists.

Question 3: What method can be used to sterilize both liquid medium as well as solid objects?

Answer: Autoclaving is a sterilization method that uses pressurized steam (typically at 121°C and 15 psi for 15–30 minutes) to effectively kill microorganisms, including bacteria, viruses, and spores. It can be used to sterilize both liquid media (such as culture broth) and solid objects (such as glassware, surgical instruments, and lab tools).

Question 4: Why are antibiotics often added to microbial culture media?

Answer: Antibiotics are added to microbial culture media to selectively inhibit the growth of unwanted or contaminating bacteria. In recombinant DNA experiments, antibiotics are used to ensure that only bacteria containing a specific plasmid (which carries an antibiotic resistance gene) can survive. Bacteria that have lost the plasmid or do not contain it will be sensitive to the antibiotic and will not grow, allowing for selective cultivation of the desired bacterial population.

Question 5: What is the purpose of streak plating?

Answer: The purpose of streak plating is to isolate individual bacterial colonies by progressively diluting the bacterial sample across the surface of an agar plate. This technique allows for the growth of discrete colonies, each derived from a single bacterial cell, which helps in pure culture isolation, identification, and further study of specific microorganisms.

Question 6: What is the most accurate method to determine the number of live bacteria in a liquid sample?

Answer: The most accurate method to determine the number of live (viable) bacteria in a liquid sample is the colony-forming unit (CFU) count, also known as colony counting. This is typically done using the spread plate or pour plate method, where a diluted sample is plated on agar and incubated. Each viable bacterial cell grows into a visible colony, allowing for an estimation of the original bacterial concentration in CFU per milliliter (CFU/mL).

Question 7: What is the main difference between a batch culture and a chemostat culture?

Answer:
The main difference between a batch culture and a chemostat culture is how nutrients and bacterial growth are managed:

- In a batch culture, bacteria grow in a fixed volume of nutrient medium with no additional input after inoculation. Growth follows distinct phases (lag, exponential, stationary, and death), with cells eventually depleting nutrients and accumulating waste, leading to a decline in growth.
- In a chemostat culture (a type of continuous culture), fresh medium is continuously added at a controlled rate, while an equal volume of culture is removed. This maintains a constant growth rate and steady-state cell density, preventing nutrient depletion and waste buildup, allowing prolonged bacterial growth.

Question 8: What are the 3 main functional components of a fluorescent filter cube and what do they do?

Answer:
A fluorescent filter cube is an essential component of a fluorescence microscope that helps isolate and direct specific wavelengths of light for fluorescence imaging. It consists of three main functional components:

Excitation Filter

- Function: Selects a specific wavelength of light from the illumination source that excites the fluorophore in the sample.
- How It Works: It blocks all unwanted wavelengths, allowing only the desired excitation wavelength to pass through to the specimen.

Dichroic Mirror (Beamsplitter)

- Function: Directs excitation light toward the sample while allowing emitted fluorescence to pass through toward the detector (eyepiece or camera).
- How It Works: It reflects shorter excitation wavelengths and transmits longer emission wavelengths, efficiently separating excitation and emission light paths.

Emission Filter

- Function: Blocks any remaining excitation light and selects the specific fluorescence emission wavelength to be detected.
- How It Works: It ensures that only the emitted fluorescence from the sample reaches the detector, improving image clarity and reducing background noise.

Question 9: How does a confocal microscope achieve a higher resolution than standard fluorescence microscopy?

Answer: A confocal microscope achieves higher resolution than standard fluorescence microscopy by using a pinhole aperture to block out-of-focus light, allowing only light from the focal plane to reach the detector. It also scans the sample point-by-point with a laser, improving optical sectioning and contrast, resulting in sharper, more detailed images.

Question 10: How does super resolution microscopy beat the diffraction limit?

Answer:
Super-resolution microscopy beats the diffraction limit by using advanced techniques that allow imaging at resolutions beyond the conventional diffraction limit of light (about 200 nm). Here are two key methods:

Stochastic Optical Reconstruction Microscopy (STORM) and Photo-Activated Localization Microscopy (PALM):
- These methods rely on the sequential activation and precise localization of individual fluorescent molecules.
- By imaging only a few molecules at a time and using their precise positions, they reconstruct high-resolution images with resolution down to 20-50 nm, well below the diffraction limit.

SIM (Structured Illumination Microscopy):
- This technique illuminates the sample with patterned light, capturing multiple images with different patterns.
- By mathematically reconstructing the images, SIM can achieve resolutions of around 100 nm, surpassing the diffraction limit.

Question 11: What are the two main uses for FACS?

Answer:
The two main uses for Fluorescence-Activated Cell Sorting (FACS) are:

Cell Sorting:
FACS is used to separate and isolate specific cell populations based on fluorescence markers or other characteristics (e.g., size, granularity). This allows researchers to isolate particular cell types from a heterogeneous sample for further analysis or experimentation.

Cell Analysis:

FACS enables the quantitative analysis of individual cells in a population, measuring parameters such as fluorescence intensity, cell surface markers, and other characteristics like DNA content or protein expression, often used in flow cytometry applications.