Chapter 2
Tools of the Laboratory: The Methods for Studying Microorganisms

In culturing microorganisms, many can be cultured on artificial media, but some can be cultured only in living tissue or in cells such as with viruses. Artificial media are classified by their *physical state* as either liquid, semisolid, liquefiable solid, or nonliquefiable solid. Artificial media are classified by their *chemical composition* as either *defined* or *complex,* depending on whether the exact chemical composition is known. Enriched, selective, differential, transport, assay, and enumerating media are all examples of media designed for specific purposes.

Microbiologists use five basic techniques to manipulate, grow, examine, and characterize microorganisms in the laboratory. These are called the Five I's: inoculation, incubation, isolation, inspection, and identification which summarize the kinds of laboratory procedures used in microbiology. Following *inoculation,* cultures are *incubated* at a specified temperature to encourage growth. *Isolated* colonies that originate from single cells are composed of large numbers of cells piled up together. A culture may be *pure,* containing only one species or type of microorganism; *mixed,* containing two or more known species; or *contaminated,* containing both known and unknown (unwanted) microorganisms. During *inspection,* the cultures are examined and evaluated macroscopically and microscopically. Microorganisms are *identified* in terms of their macroscopic or immunologic morphology, their microscopic morphology, their biochemical reactions, and their genetic characteristics.

Magnification, resolving power, and contrast all influence the clarity of specimens viewed through the optical microscope. The maximum resolving power of the optical microscope is 200 nm, or 0.2 µm. This is sufficient to see the internal structures of eukaryotes and the morphology of most bacteria. There are six types of optical microscopes. Four types use visible light for illumination: bright-field, dark-field, phase-contrast, and interference microscopes. The fluorescence microscope uses UV light for illumination. The confocal microscope can use UV light or visible light reflected from specimens. Electron microscopes (EM) use electrons, not light waves, as an illumination source to provide high magnification (5,000× to1,000,000×) and high resolution (0.5 nm). Specimens viewed through optical microscopes can be either alive or dead, depending on the type of specimen preparation, but all EM specimens are dead because they must be viewed in a vacuum.

The Gram stain is an immensely useful differential stain that divides bacteria into two main groups, gram-positive and gram-negative. Some bacteria do not fall in either of these categories such as the tuberculosis bacterium. Stains increase the contrast of specimens and they can be designed to differentiate cell shape, structure, and biochemical composition of the specimens being viewed.

Learning Objectives

2.1 How to Culture Microorganisms

1. Explain what the Five I's mean and what each step entails.
2. Name and define the three ways to categorize media.
3. Provide examples for each of the three categories of media.

2.2 The Microscope

4. Convert among different lengths within the metric system.
5. List and describe the three elements of good microscopy.
6. Differentiate between the principles of light and electron microscopy.
7. Name the two main categories of stains.
8. Give examples of a simple, differential, and special stain.

Key Terms and Phrases

Medium
Culture
Inoculation
Incubation
Pure culture
Mixed culture
Contaminated culture
Streak plate
Pour plate
Colonies
Subculture
Liquid media
Semisolid media
Solid media
Agar
Synthetic media
Non-synthetic media
General-purpose medium
Fastidious
Enriched medium
Selective medium
Differential medium
Reducing medium
Carbohydrate fermentation medium
Transport media
Assay media
Enumeration media
Simple microscope
Compound microscope
Ocular lens
Objective lens
Total magnification
Resolution
Bright-field microscopy
Dark-field microscopy
Phase-contrast microscopy
Interference microscopy
Fluorescence microscopy
Transmission electron microscope
Scanning electron microscope
Wet or hanging drop mounts
Stained smear
Heat-fixed
Basic dye
Negative staining
Simple stain
Differential stain
Positive stain
Gram stain
Acid-fast stain
Endospore stain
Spore stain
Capsule stain
Flagella stain
Gram-positive
Gram-negative
Growth factors
Isolation techniques
Real image
Virtual image
Resolving power
Oil immersion lens
Vegetative cell

Topics for Discussion

Did you know?

- The most common microscope used in microbiology is the compound light microscope.
- Immersion oil is used with the oil immersion lens to reduce light loss between the slide and the lens, and therefore making the specimen as large as it can be seen with the microscope.
- A beam of electrons, instead of light, is used with an electron microscope.
- Probe microscopes have a magnification greater than 100,000,000x with resolving power greater than electron microscopes!
- Differential stains such as the acid-fast stain and Gram stain will differentiate bacteria according to their reaction to the stains.
- The high lipid content of acid-fast cell walls such as the tuberculosis bacterium makes them impermeable to most stains.
- In a Gram stain, the mordant combines with the basic dye to form a complex that will not wash out of gram-positive cells.
- In the Gram stain, the decolorizer removes the color from gram-negative cells.
- If one does a smear and stain of *Neisseria gonorrhoeae*, one will find gram-negative (red) diplococci in much larger phagocytic white blood cells.
- In 1884, Hans Christian Gram discovered a staining technique that could be used to make bacteria in infectious specimens more visible and we are still using this basic technique today!
- Gram staining a fresh urine or throat specimen can help focus on the possible cause of infection and in guiding drug treatment.
- A special concern in culturing is possible contamination, so sterile techniques such as media and transfer equipment help ensure that only microbes that came from the sample are present.
- Microbiologists at Stanford University discovered 31 new species of bacteria that thrive between the teeth and gums that could not be grown in their laboratories; these microbes are called viable but nonculturable (VBNC).