**Cowan Microbiology Fundamentals, 3/e, Critical Thinking Answers**

**Chapter 2**

1. Identification of a microbial agent is the final “I” of the five I’s of microbiology. Thus, to identify the possible pathogen causing the patient’s skin lesion, it is necessary to work through the stages leading to identification. First, a sample is obtained from the patient’s wound, using a sterile swab. Once a sample is obtained, the first of the five I’s, inoculation, is carried out. Inoculation occurs when the sample is placed into a container of sterile medium to encourage the growth of microorganisms.

The inoculated medium is then incubated, which is the placement of the medium in a temperature and gas-controlled environment (an incubator) designed to encourage growth of microorganisms. During incubation, the microorganisms grow to a point that they can be observed macroscopically. For example, on solid media, the appearance of bacteria and fungi is in the form of colonies.

Once growth is observed, the next stage is to isolate the organism of interest. Samples obtained from a patient are often mixed samples containing many organisms. In this case, you are most interested in the organism causing the skin lesion. Isolation of an organism can involve several techniques and tools. Techniques include the streak plate, loop dilution, and spread plate. Selective media can also be used to encourage growth of the organism of interest.

As the organism of interest is isolated from others in the original sample, the final two “I’s”, inspection and identification, can be instituted. Inspection can involve a variety of means. Examining the culture with one’s own eyes is often the first step. Microscopic examination can be utilized, as can biochemical tests. Immunological and genetic protocols can also be used to aid in the specific identification of microorganisms.

1. a. Blood agar can help identify *Streptococcus* species based on hemolytic abilities.

b. MacConkey agar could be used to isolate and identify pathogens present in a urine sample, as the agar differentiates between lactose-fermenters such as *E. coli* and other bacteria.

c. *Enteroccoccus faecalis* broth can be used to isolate gram-positive enteric organisms while MacConkeys agar can be used to isolate gram-negative Enterobacteriaceae.

d. Transport media is required to maintain the nasal swab specimen for further analysis.

1. a. Figure 2.9 shows methods for isolating organisms, through use of the streak plate, loop dilution, or spread plate methods. These methods are successful in separating samples so that individual colonies can be identified, and thus can also be used to determine the total number of cells present in a patient’s sample. A viable plate count uses loop dilutions combined with spread plate or pour plate methods to estimate the number of viable cells in a sample.

b. The fact that growth was noticed in the first quadrant of the streak plate indicates that the bacterial sample is capable of growing on this medium, and that bacterial organisms were present in the original sample.

The fact that growth was not observed in the remaining quadrants may have resulted from several possible errors. First, an inadequate amount of the sample from quadrant 1 may have been streaked into quadrant 2 and beyond, preventing observable amounts of growth from appearing. If an inoculating loop was used to streak the sample into other quadrants and the loop was not cooled sufficiently between streaks, the bacterial sample may have been killed by the heated loop. Finally, the preparer of the streak plate may have simply overlooked the completion of the procedure and did not streak the later quadrants.

1. a. The use of a stain such as Lactophenol cotton blue enhances the contrast of a specimen to be viewed under a microscope. Unstained cells can be indistinct when viewed under a microscope, no matter how strong the magnification or powerful the resolution. Staining a sample enables the development of a contrast between the sample and the background, making for easier visualization.

b. Viruses are too small to be observed with light microscopy. Thus, other forms of microscopy, such as fluorescence microscopy, must be utilized. In fluorescent microscopy, the viral particles are made observable through the use of fluorescent stains.

The visualization of multiple organisms within a specimen can be accomplished using light microscopy. Bacteria and fungi are large enough to be observed using this technique. If viral particles are to be observed in the sample, other forms of microscopy would need to be used. Depending on the specimen, dark field microscopy may be used, as it permits rapid recognition of the cell’s structure.

To view cellular structures such as organelles, phase contrast microscopy is one technique that can be utilized. As light passes through cellular structures of differing densities, the light is altered and this pattern can be used to produce an image. An electron microscope can also be used to view organelles, given the size of these structures.

1. The Gram stain is a differential stain. This staining procedure uses two different dye colors to differentiate between gram-positive and gram-negative bacteria. When a Gram stain results in both pink and purple cells, the results indicate a mixed sample of both gram-positive and gram-negative cells. In this case, because the cells are bacilli, the cells are rod-shaped.

In order to identify the pathogen further, selective and differential media may be utilized. Selective media, such as MacConkey agar, encourage the growth of gram-negative bacteria, while mannitol salt agar selects for certain gram-positive organisms. The resulting growth on selective media can help the clinician begin to determine the possible pathogen. Differential media also helps clinicians identify organisms in mixed samples. For example, blood agar helps to identify certain bacteria based on the hemolytic activity. Additional tools for identification, such as biochemical tests and genetic analysis, can also be employed.

Therefore, although the original Gram stain results showed a mixed culture of gram-positive and gram-negative bacteria, further tools are available to help identify the pathogen.