MODULE 9: Identification of Gram-Negative Bacilli

LEARNING OUTCOMES

1. Discuss the purpose of identifying medically important Gram-negative bacteria.
2. Name several tests used to identify enteric and non-enteric Gram-negative bacilli.

INTRODUCTION

Identification of Gram-negative bacteria is initially based on distinguishing enteric (intestinal) bacteria from those that are non-enteric. Enteric bacteria, particularly those members of the family *Enterobacteriaceae*, are important causes of urinary, wound, blood, and hospital-acquired infections. These organisms are Gram-negative bacilli and include many genera: *Escherichia, Proteus, Citrobacter, Serratia,* and *Klebsiella* to name but a few.

Following a Gram stain, biochemical identification schemes usually begin with tests to detect production of key metabolic enzymes involved in respiratory pathways. Initially, the nitrate reduction and oxidase tests separate Gram-negative bacilli into two major groups based on the fermentation or oxidation of sugar (Figure 9.3). Secondary tests to identify genus and species include the IMViC series (indole, methyl red, Voges-Proskauer, and citrate tests), production of urease, and/or growth characteristics on specialized media.

Commercial systems with multi-test capabilities, such as the Enterotube™ and API strip, provide results of many tests with one inoculation procedure (Figure 9.1). These tests have multiple wells of media and require only one inoculation of the culture. Following incubation, reactions are interpreted as positive or negative, and compared with known results of a particular organism.

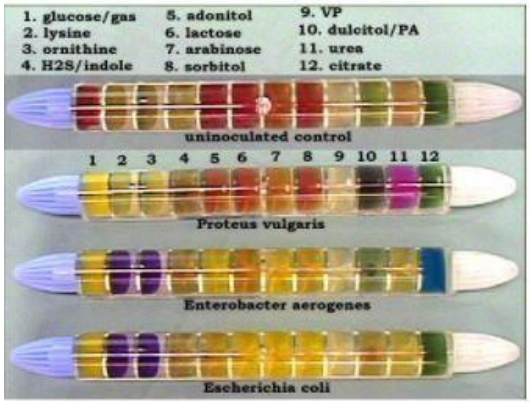

Figure 9.1: Rapid systems such as the Enterotube™ provide results of multiple biochemical reactions with a single inoculation.

Other rapid identification systems are based on principles of immunology, where antigens, or proteins found on the bacterial cell surface, are bound by specific test antibodies. Test antibodies are also proteins and usually attached to an indicator, such as a colored latex bead or fluorescent marker, in which a positive result creates a visible reaction (Figure 9.2). Direct or indirect fluorescent antibody kits utilize fluorescent microscopes to visualize bacterial antigens that are bound together by antibodies.

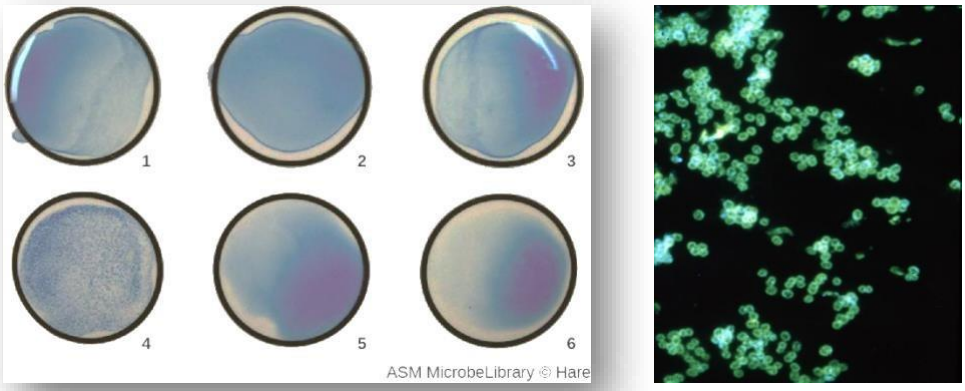

Figure 9.2: Immunological reactions include visible agglutination (left, well 4) and immunofluorescence (right).

Although identification of bacteria through biochemical testing has been traditionally used in microbiology laboratories for decades, genetic tests are now widely available for detecting many pathogens. Genetic tests are particularly useful for identifying fastidious bacteria or those that are slow growing, such as *Mycobacterium tuberculosis*, which may take weeks to months to cultivate on media.

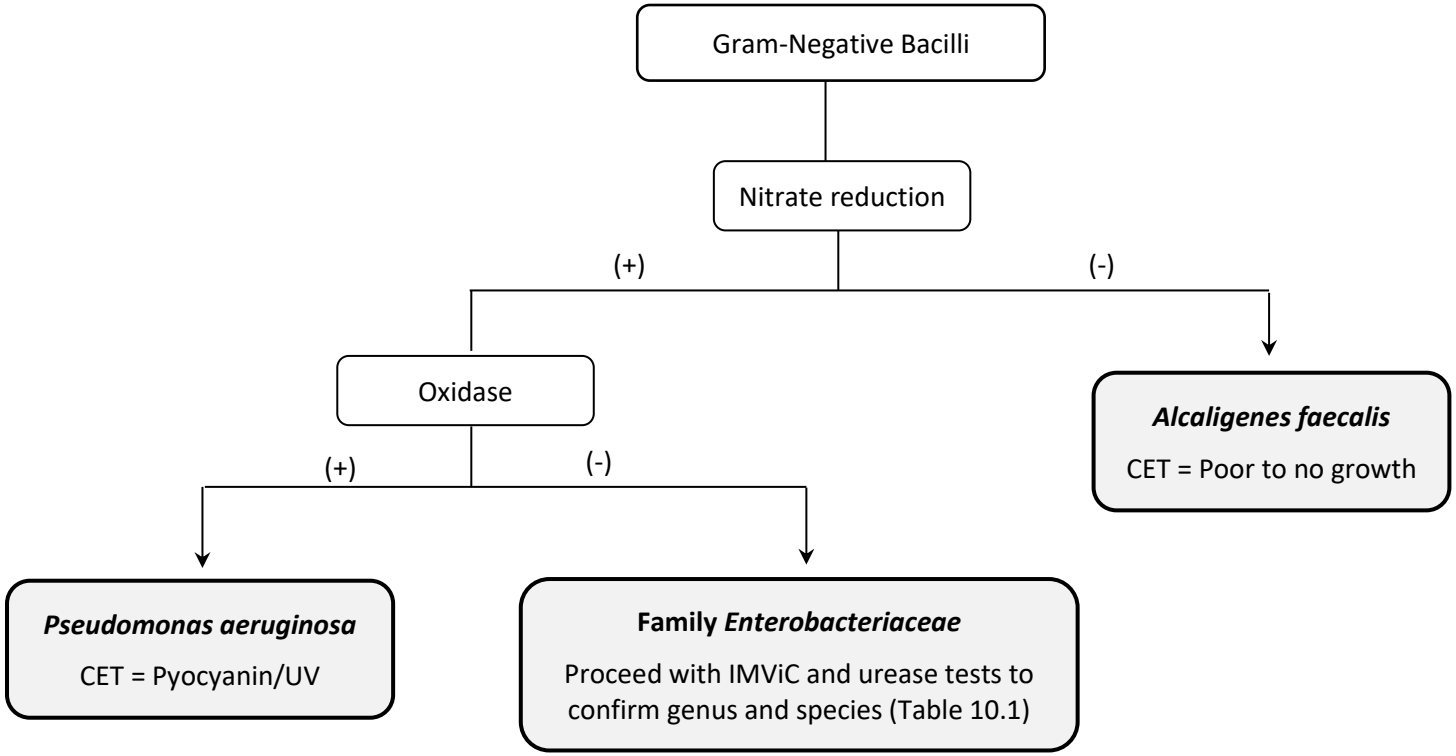

Figure 9.3. Differential testing of Gram-negative bacilli.