# **Exercise 13**

# Serology: Agglutination Testing of Specific Bacteria

The Use of in vitro Antigen-Antibody Interactions

## **INTRODUCTION:**

Student Learning Objectives: After completing this exercise students will:

- 1. Define serology, agglutination, antibody, and antigen.
- 2. Describe the principles of antibody-antigen interaction and its use in serology.
- 3. Describe the serological classification of selected Enterobacteriaceae members.
- 4. Demonstrate the methods used to identify exposure to bacterial strains or subtypes.

### Activities for today:

- Do the slide agglutination test on *S. typhimurium* using the H flagellar antigen.
- Do the slide agglutination test on *S. typhimurium* and *E. coli* using the O somatic antigen.

## <u>Materials</u>

Exercise to be done in pairs

Slant cultures of the following bacteria: Salmonella typhimurium and E. coli Salmonella typhimurium H antigen reagent Salmonella typhimurium H antibodies (antiserum) reagent Salmonella typhimurium O antibodies reagent 0.85% sterile saline Toothpicks Glass slides Beaker with disinfectant Loop

#### Introduction

Antigens are usually made of a specific protein produced by a cell. Antibodies are also proteins, produced by cells of the immune system (B lymphocytes) which bind specific antigens. In principle, one antibody binds only one specific antigen. Antibodies are produced in response to a specific organism with that antigen which has invaded the body, in an attempt to neutralize the invading organism by binding to its antigens and thus stimulating the immune system's defenses. This antigenantibody interaction can be used in different ways; one would be to identify if the person has been exposed to the antigen, which could aid in the diagnosis of an illness. Here, serum from the person is mixed with Salmonella antigens, and if the antibodies to the antigens are present, clumping, or agglutination occurs. Another way is to identify specific organisms by adding a solution containing the organismspecific antibodies to a saline suspension of organisms. If the target antigen is present, the antibodies will bind and cause an agglutination reaction, seen as clumping of the cells to antibodies and to each other. If the antigens are absent, no agglutination occurs, thus the sample is negative for that particular organism. Bacteria belonging to the family Enterobacteriaceae are often classified to the subspecies level using serology. The flagellar antigen (H) and somatic antigen (O) are used to classify the species into serotype strains. For example, *Salmonella* has several serotypes, which is important in the identification of outbreaks of disease. *E. coli* is another example where serotyping is important, because certain serotypes of this organism, which is part of the enteric microbiota in warm blooded animals can be pathogenic to humans (example; E. coli O 157: H 7).

In this exercise, you will demonstrate serological tests for *Salmonella typhimurium* using the H and the O antigens and antibodies.

### **Procedure:**

- Demonstration of a positive and negative agglutination test
- 1. Using a wax pencil, draw 2 circles on a glass slide.
- 2. In the first circle, add one drop of saline and one drop of *Salmonella typhimurium* H antigen (Flagellar antigen).
- 3. Mix well using a toothpick, discard toothpick in the beaker containing the disinfectant.
- 4. In the second circle, add a drop of the H antigen and a drop of the H antiserum and mix well using a toothpick.
- 5. Rock the slide gently to cause a slight swirling motion in the mixtures, and observe for clumping or agglutination.
- 6. Draw your observations below.



- Which of the circles is the positive control? \_\_\_\_\_ Negative control? \_\_\_\_\_

In the circle below, draw the antigen-antibody interaction which led to the clumping.



## - Demonstration of the specificity of an agglutination test

- 1. Using a wax pencil, draw 3 circles on a glass slide.
- 2. In the first circle, add one drop of saline and one drop of *Salmonella typhimurium* O antiserum.
- 3. Transfer a small amount of E. coli from the slant using the loop.
- 4. Flame the loop
- 5. Mix well using a toothpick, discard toothpick in the beaker containing the disinfectant.
- 6. In the second circle, add one drop of saline and one drop of *Salmonella typhimurium* O antiserum only.
- 7. Mix well using a toothpick, discard toothpick in the beaker containing the disinfectant.
- 8. In the third circle, add a drop of saline and a drop of the O antiserum.
- 9. Using the loop, transfer some *Salmonella typhimurium* from the slant.
- 10. Mix well using a toothpick, discard toothpick in the beaker containing the disinfectant.
- 11. Rock the slide gently to cause a slight swirling motion in the mixtures, and observe for clumping or agglutination.
- 12. Draw your observations below.



- Where did the agglutination reaction occur?

Draw the reactions in the circles below showing the antigen-antibody interactions.

