Laboratory Exercise # 18: Immunological Testing

Purpose:

The purpose of this laboratory exercise is to demonstrate the techniques of immunological testing.

Introduction:

Immunological testing is based on the fact that antigen-antibody reactions are very specific. Antibodies usually react only with the antigen that stimulated their production in the first place, and they are just as specific as an enzyme-substrate reaction. Because of this, one can use known antisera (prepared by animal inoculation or monoclonal antibody technique) to identify unknown antigens such as microorganisms.

The general procedure is to mix a suspension of the unknown antigen with a known antiserum (antibody) for a specific antigen. If that antigen is present then an antigen-antibody reaction occurs. Antigen-antibody reactions may be detected in the laboratory by a variety of techniques. Some of the commonly used techniques for observing antigen-antibody reactions can be described as follows.

**Agglutination** - the antiserum causes bacteria or other particulate antigens to clump together or agglutinate

**Precipitation** - antiserum is mixed with soluble antigen and cloudy precipitate forms at the zone of optimum antigen-antibody proportion

**Enzyme immunoassay** - test antigens from specimens are passed through a tube of membrane coated with the corresponding specific known antibodies and become trapped on the walls of the tube or membrane. Known antibodies to which an enzyme has been chemically attached are then passed through the tube or the membrane, where they combine with the trapped antigens. Substrate for the attached enzyme is then added and the amount of antigen-antibody complex formed is proportional to the amount of enzyme-substrate reaction as indicated by a color change.

The rapid identification of a bacterium is essential in a clinical setting particularly if the pathogen can spread rapidly through the human body causing massive damage. Along with doing two rapid identifications (using test kits) the student is asked to research some diseases that are normally identified by these rapid methods.
Materials:

- Rapid Strep Identification Kit
- Rapid Urine HcG Test Kit
- Plate culture of *Streptococcus pyogenes*
- Plate culture of *Staphylococcus aureus*
- Urine Samples – one + for pregnancy & 1 negative for pregnancy
- Small glass tubes (for Rapid Strep swabs and HcG testing)
- Gloves

Procedure: The students will be working individually at each of the test stations.

1. **Station # 1: Rapid Strep testing**
   a. The student will receive an unknown culturette to be used with the test kit.
   b. Follow the instructions at the station for running the test on your unknown.
   c. Decide whether your unknown is positive or negative for *Streptococcus pyogenes* and record that information on the Immunological Unknown form.
   d. Discard the used swab in the disinfectant beaker and place the tube is placed in a rack on the autoclave table.

2. **Station # 2: Rapid Pregnancy test**
   a. The student will receive an unknown urine sample to be used with the test kit. **Please wear gloves when running this test as the samples are real body fluids!**
   b. Follow the instructions at the station for running the test on your unknown.
   c. Decide whether your unknown is positive or negative for the presence of HcG and record that information on the “Immunological Unknown” form.
   d. Discard the urine sample tube in the sharps container (as the autoclave bags leak) and the test strip and gloves in the autoclave bag labeled “**Human Biological Wastes**”.

3. Students should answer the following questions, the answers for the most part can be found in your textbook, but you may also want to research on the internet. **Please document all sources of your information. This assignment should be typed and should include the answers to your unknown testing.**
Immunological Unknown
(25 points)

Station # 1: Rapid Strep Testing

Unknown #: _____ Result: ________________

Questions:

1. Explain how Streptococcus pyogenes is diagnosed by throat culture on a Sheep Blood Agar Plate. Please be specific.

2. Some Streptococcus pyogenes strains can cause Scarlet fever due to their being infected with a temperate phage.
   a. What is a temperate phage?
   b. What is the name of the toxin that the phage codes for?
   c. What are the clinical symptoms of Scarlet fever?

3. Necrotizing fasciitis (destruction of the flesh) can be caused by some Streptococcus pyogenes infections leading to destruction and death of tissue.
   a. What is superantigen A? What does this antigen cause?
   b. What is exotoxin B? What does this toxin cause?
   c. Why don’t antibiotics or even our immune system have the ability to fight this disease? (Hint: think about circulation!)

4. If Streptococcus pyogenes is left untreated the patient may recover, but several days later may develop Acute Glomerular nephritis.
   a. What causes the inflammation within the glomerulus?

5. A second disease can be related to untreated Streptococcus pyogenes infections, Rheumatic fever.
   a. What are the symptoms of this disease?
   b. A popular theory of this disease is that antibodies are produced against Streptococcus pyogenes that are very similar in specificity to that of the valves of the heart. Why would this affinity be a problem (Hint: What might be activated?)?
Station # 2: Rapid Pregnancy Testing

Unknown #: ________  Result: ________

Questions:

1. What does HcG stand for?

2. Why is the presence of this hormone used to confirm pregnancy?

3. This test kit uses a monoclonal antibody to HcG. How is a monoclonal antibody produced in the laboratory? Please list the steps that are involved.

Another Disease Identified by Rapid Testing:

*Staphylococcus aureus* can cause anything from minor sore throats to boils, these of course, are not life threatening, but other diseases are.

Questions:

1. What are the symptoms of **Scalded Skin Syndrome**?

2. What toxin causes the symptoms observed with this syndrome?

3. Why is there a danger of a secondary infection developing with this syndrome?

4. Why might it be very important to identify this bacterial infection if the patient is a very small infant? (Hint: Think legal issues!)