Immunology

Field Trip Background

Background Information

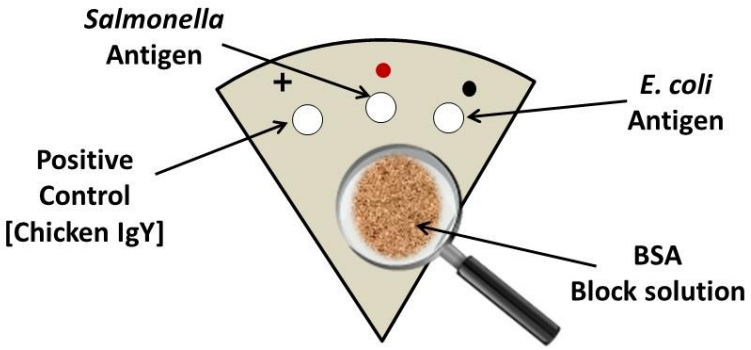
Immunology is the study of the immune system. The immune system is a network of cells and organs that produce substances, such as **antibodies** (Ab), which defend the body against attack by "foreign" substances called **antigens** (Ag). Antigens can be viruses, bacteria, proteins or other foreign compounds. When the immune system works properly, it protects your body against infections. If the immune system malfunctions, it can produce undesirable effects that range from minor allergies to serious autoimmune diseases. In autoimmune diseases such as rheumatoid arthritis and multiple sclerosis (MS), an individual's antibodies attack the body's own organs.

Antibodies, also called **immunoglobulins (**abbreviated as **Ig**), are protein molecules produced by the B-lymphocytes (white blood cells) that bind very tightly and specifically to the antigens they recognize. Medical tests have been developed that use this specific binding of antibody and antigen (Ab:Ag) to diagnose infectious diseases by detecting the presence of the relevant antibody or antigen in the patient's blood. Commercial antibody production involves injecting the specific antigen into animals such as mice, rabbits, goats, etc. After several weeks, the animal should be producing antibodies against the antigen, and blood is drawn from the animal. The serum containing the antibodies is separated from the blood cells, and the specific antibodies are further purified. This process produces only the antibodies for a specific antigen, which can then be used in a diagnostic test to detect the presence of that antigen.

Using Immunoglobulins for Diagnostic Molecular Detection

The BTC Institute Immunology Field Trip instructs students how to set up a diagnostic test called a **dot blot**. In this experiment, a bird-and-reptile-specific class of immunoglobulin, **IgY**, is isolated by students from egg yolk. (IgYs are deposited in the egg yolk by a laying hen to provide the developing chick with passive immunity to pathogens that the hen has been exposed to.) For the Immunology field trip, antigens from two common microbes (*E.coli* and *Salmonella)* have been spotted onto a **nitrocellulose membrane** to create the dot blot.

Dot Blot Membrane



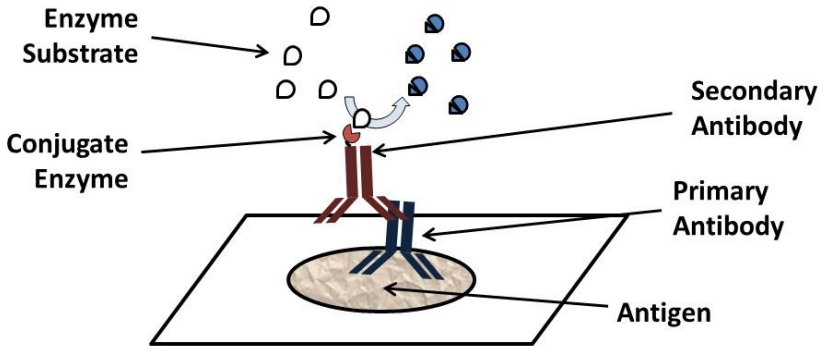
The student-isolated IgY antibodies are incubated with the antigen spots on the dot blot. If the hen that laid the egg was exposed to *E.coli* or *Salmonella,* then IgY antibodies will be present, and should bind to the spotted antigens.

Using Immunoglobulins for Diagnostic Molecular Detection (continued)

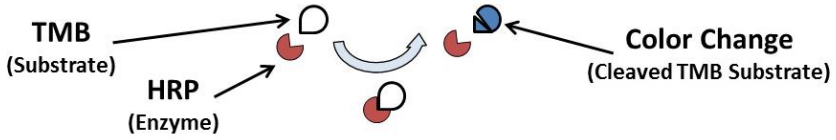
Because the chicken IgY antibodies are not labeled, a **secondary antibody** is used to trace them. The secondary antibody is created by injecting chicken IgY antibodies into a rabbit. The rabbit's immune system mounts a response to the foreign chicken IgY molecules by creating IgG antibodies that are specifically-shaped to recognize the chicken IgY molecule.

The secondary antibody is labeled with an **enzyme.** This enzyme causes a chemical conversion which results in a color change when the complex is exposed to the appropriate substrate. The secondary antibody will bind to any IgY antibodies bound to *E.coli* or *Salmonella* antigen on the surface of a student's dot blot.

Secondary Antibody Binding



Antigen: Proteins from *E. coli* or *Salmonella* bacteria
Primary (1°) Antibody: Chicken IgY antibody purified from egg yolk
Secondary (2°) Antibody: Anti-Chicken IgY Rabbit IgG antibody conjugated to HRP enzyme
Conjugated Enzyme: Horseradish Peroxidase **(HRP)** enzyme attached to 2° antibody
Enzyme Substrate: TMB* is a molecule that is cleaved by the HRP enzyme



When HRP acts on TMB, the result is a **color change** from clear to a greyish-blue
*-TMB is: 3,3',5,5'-Tetramethylbenzidine

If a color change is seen on a student's dot blot, it indicates that the:

- student's IgY antibody purification was successful
- purified IgY antibodies bound to the *E.coli* and *Salmonella* antigen spots on their membrane
- rabbit IgG secondary antibody recognized and bound to the chicken IgY
- HRP enzyme connected to the secondary antibody combined with the TMB substrate to cause the color change

During the laboratory, instructors will discuss how clinical laboratories use antibodies to detect various infectious diseases. If you have any questions about this field trip, please give us a call, or bring your questions along and we can discuss them during the lab. Thank you for your interest in BTC Institute's Biotechnology Field Trips program!

Brief Outline of Laboratory Activity:

Part I: Block Membrane

- ✓ Immerse membrane in a Bovine Serum Albumin (BSA) solution to block nitrocellulose membrane from binding non-specifically to proteins.

Part II: Purify IgY Antibodies from the Chicken Egg Yolk

- ✓ Remove large proteins and lipids from the yolk. Do this using low-concentration Polyethylene Glycol (PEG).
- ✓ Use a higher concentration of PEG to precipitate the smaller IgY antibodies from the rest of the molecules in solution.

Part 2: Detect the Antigens.

- ✓ *E*. *coli*, *Salmonella*, and a chicken IgY positive control have been blotted onto and permanently bound to a membrane.
- ✓ Using two antibodies, one that students purify and one that was made specifically to allow us to "see" the proteins, students will detect the antigens on the membrane. This immunodetection technique is referred to as a “dot blot”.

References for Antibodies:

Everything and then some (http://antibodyresource.com/educational.html)
Computer graphics of Antibody structure
(http://www.path.cam.ac.uk/~mrc7/mikeimages.html)

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