**Sandwich ELISA**

This assay is used to quantitate the amount of antigen (o) present in a sample. The antigen being measured is “sandwiched” between two antibodies. The color developed is proportional to the amount of antigen in the sample.

1. Add antibody ( ) to plate. Incubate. Wash.
2. Add solution to be tested for antigen(o) to plate. Incubate.

Wash. The antigen will stick to the antibody on the plate.

1. Add enzyme-linked antibody ( ) to plate, which will bind to the antigen. Incubate. Wash.
2. Add substrate to plate.
3. Interpret color development.

**Indirect ELISA**

This assay is used to quantitate the amount of antibody present in a sample. The antigen is bound by the antibody being measured, which is then bound by the secondary antibody. The antigen- binding portion of the antibody being measured is attached to the antigen while the secondary antibody attaches to the effector portion of the antibody being measured. The color developed is proportional to the amount of antibody in the sample.

1. Add antigen(o) to the plate. Incubate. Wash.
2. Add serum to be tested for antibodies. Patient antibodies **( )**will bind to antigen(o). Incubate. Wash.
3. Add enzyme-linked antibody ( ) to plate. Incubate.

Wash. This second antibody is against the first antibody.

1. Add substrate to plate.
2. Interpret color development.

**Competitive ELISA**

This assay is used to quantitate the amount of antigen or antibody present in a sample. The assay below depicts the detection of the antigen amount in a sample. The color developed is inversely proportional to the amount of antigen in the sample. For example, less color develops with a higher amount of sample antigen.

1. Add antigen(o) to plate. Incubate. Wash. Plate is now coated with antigen.
2. Add enzyme-linked ) PLUS sample antigen(o) to plate.

The sample antigen competes with the antigen bound to the plate for the enzyme-linked antibodies. Incubate. Wash.

1. Add substrate to plate.
2. Interpret color development.