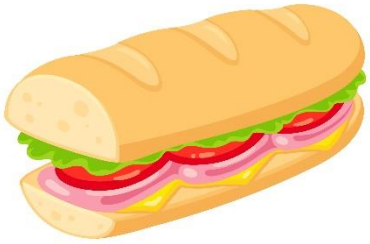
READING PASSAGE

A Look inside the Lab: ELISA Plate Reader
The Common Features of ELISAs and Sandwiches



What is this called where you live? A hoagie? A sub? A hero? Regardless of what you call it, you expect this sandwich to have a series of layers that make your mouth water and your tastebuds dance. You likely also personalize it, so it has just the right combination of ingredients for maximum enjoyment.

Enzyme-linked immunosorbent assays, or ELISAs, share some things in common with these sandwiches. Like hoagies (what they are called in Philly!), ELISAs rely on a series of layers and they, too, are easily "personalized," so that scientists can use them in an array of situations. Let's take a closer look.

What is an ELISA?

An ELISA is a lab technique that can detect and measure a protein of interest in biological samples. The protein of interest can be part of a pathogen, like a virus, or part of an immune response, like an antibody. Detection relies on two important relationships:

- Interactions between antigens and antibodies that provide information about the sample. Antigens are parts of a pathogen that cause an immune response. Antibodies are products of the immune response that can recognize and bind to the antigen.
- Interactions between enzymes and substrates that cause color changes. Enzymes are proteins that cause a chemical reaction, and substrates are the molecules that are affected by enzymes during the reaction. In the case of ELISAs, an enzyme-substrate combination that causes a color change is used. For example, horseradish peroxidase (HRP) is a common enzyme used in ELISAs. It causes the substrate tetramethylbenzidine (TMB) to turn blue. After a short period of time, an acid is added to stop the reaction, causing the samples to turn yellow.

By linking enzymes to antibodies that recognize the antigen, these two interactions are connected, allowing scientists to visually learn about their samples through color change. ELISA plate readers that detect different wavelengths of light, measure the amount of color in samples, allowing scientists to calculate numerical differences between samples.

What are the types of ELISAs?

Whatever your favorite sandwich, it likely has more than one layer. ELISAs also use layers. The number of layers depends in part on the kind of ELISA being completed. Four types of ELISAs can be described:

1. Direct ELISA – The first ELISA developed was the direct ELISA. It was created in 1971 by two different groups of scientists with the same idea. The sample being tested is added to a plate to make the bottom layer. Then an enzyme-linked antibody that is specific for the antigen in the

sample is added. Following an incubation period, the sample plates are washed to remove any antibody that is not attached to the antigen, and a substrate is added to cause the color change. In sum, this type of ELISA has three layers: the antigen (in the sample), the enzyme-labelled antibody, and the substrate.

2. Indirect ELISA – The indirect ELISA, developed in 1978, uses two antibodies. Like the direct ELISA, the plate is coated with antigen. But, in this type of ELISA, the sample is the antibody that is specific for that antigen. For example, the scientist might want to measure the level of antibodies to a virus that are present in a person's blood. After incubating the antigen with the patient sample (containing antibodies), another antibody is added. This second antibody is linked to an enzyme, and it is able to attach to the antibodies that were in the patient's sample. Finally, a substrate is added to cause color change, so the results can be detected. In sum, this type of ELISA has four layers: the antigen, the antibody in the patient's blood (sample), the second antibody (with an enzyme attached), and the substrate.

3. Sandwich ELISA – The sandwich ELISA was developed in 1977 and in this type, the bottom layer on the plate is an antibody that will attach to the antigen being measured in a sample. For example, if a person has a respiratory virus, like COVID-19, the scientist might want to measure how much virus is in an infected person's nose. So, the bottom layer would be an antibody that binds to COVID-19. Once the antigen has attached to the antibody layer on the plate, another type of antibody that will bind to the virus is added. This antibody is linked to an enzyme that will cause color change when the substrate is added. So, this type of ELISA also has four layers: antibody, antigen (in the sample), enzyme-linked antibody, and substrate.

4. Competitive ELISA – Developed in 1976, the competitive ELISA starts with adding antigen to the bottom layer of the plate. But in the next step, the sample with antibodies and the enzyme-linked antibodies are added at the same time and compete with each to attach to the antigen on the bottom layer of the plate. If the sample has large quantities of antibody, less enzyme-linked antibody will be able to bind to the antigen on the plate. As such, when the substrate is added and the color changes, the less color that develops, the greater the quantity of antibody in the sample. In sum, this ELISA has three layers: antigen, either antibody of interest (sample) or enzyme-linked antibody, and substrate.

What are ELISAS used for?

ELISAs are used in a variety of ways. Research scientists often use them to learn about experimental samples to inform their research questions. ELISAs are also frequently employed in medical settings, where they can be used to measure or detect a variety of things, including:

- Antibodies in the blood, such as during an infection
- Levels of tumor markers
- Hormone levels, such as to detect pregnancy
- Disease outbreaks or exposures
- Contaminants in donated blood or other samples
- Drug use or abuse

As you can see, ELISAs are a versatile tool for scientists and clinicians.