THE COMPLETE GUIDE TO

ELISA

INTEGRA

FOREWORD

The enzyme-linked immunosorbent assay (ELISA) is considered the gold standard of immunoassays available today. It is a popular plate-based method for detecting and quantifying peptides, proteins, antibodies or hormones, and is used in a wide range of both research and applied settings, including clinical applications, such as the detection of viral infections.

We have compiled this detailed eBook – consisting of in-depth educational articles, relevant app notes and customer testimonials – to help you understand how ELISAs work, the materials required to perform them, and their limitations in comparison to other immunoassays, such as western blot. We also highlight what you should consider in order to run ELISAs successfully, and demonstrate how our solutions can help you to enhance the throughput of your lab and become an ELISA expert.

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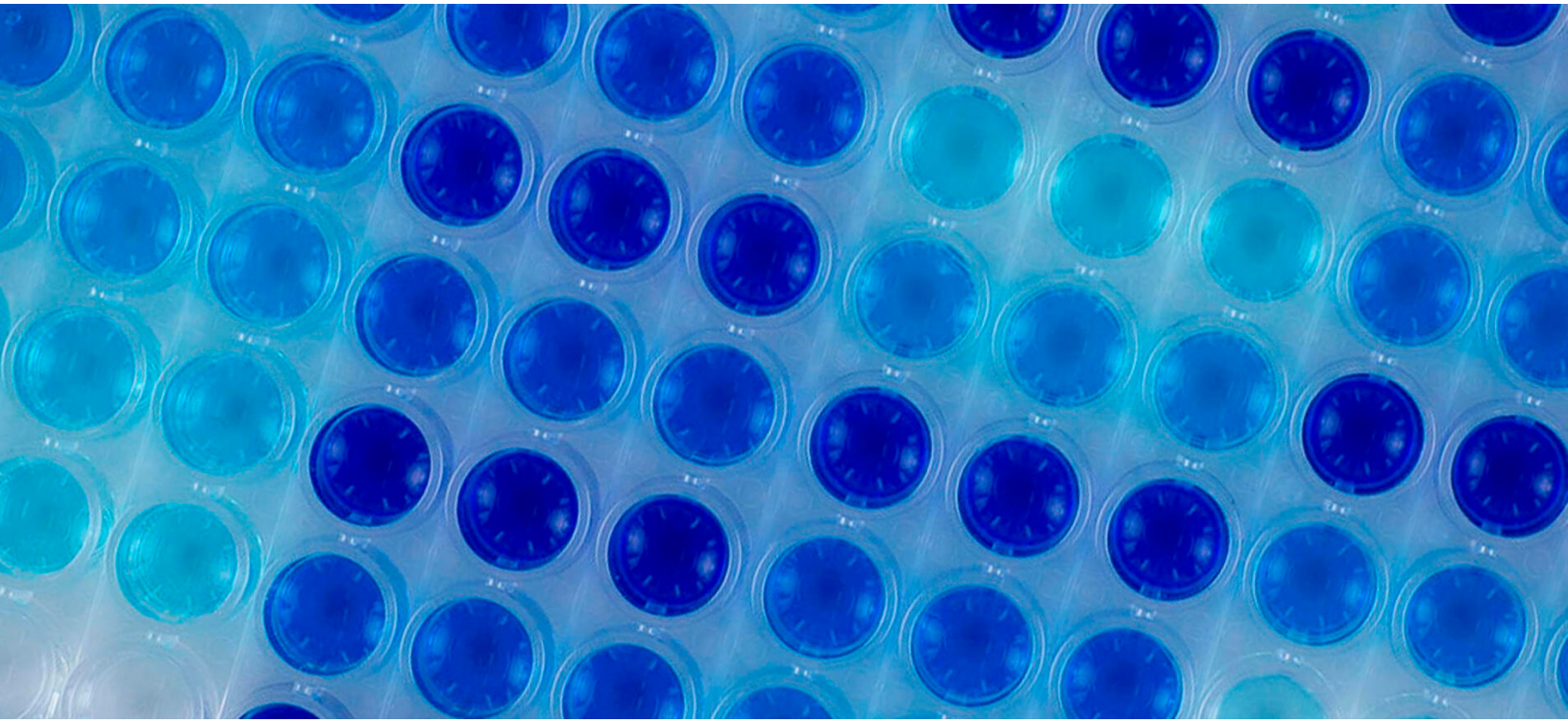


CHAPTER 1:
What you need to know about ELISAs

In this chapter, we will cover the different types of ELISAs, analysis of ELISA data, and the different reagents and equipment required to perform these tests in the lab. We will also explain the differences and similarities between ELISAs and western blotting, and their respective pros and cons, to allow you to make an informed decision on which technique to use for your specific application.

1.1 An introduction to the different types of ELISA tests

ELISA stands for 'enzyme-linked immunosorbent assay', and ELISA tests are mainly used in research and clinical applications, including detecting viral infections such as hepatitis B virus (HBV), hepatitis C virus (HCV) or human immunodeficiency virus (HIV). In this article, we will outline the different types of ELISAs, explain how to analyze the assay data, and give an overview of the reagents and equipment required.

What is an ELISA?

Let's start with a general definition of an ELISA before looking at different assay methods:

ELISA

An ELISA test is a type of enzyme immunoassay (EIA). These highly specific and sensitive assays are used to detect concentrations as low as 0.01 nanograms of antigen or antibody per milliliter. When performing ELISAs, antigen-antibody complexes are immobilized to a solid surface. An enzyme is covalently attached to one of the molecules in the complex, and the subsequent addition of an enzyme-specific substrate results in a colored reaction product.

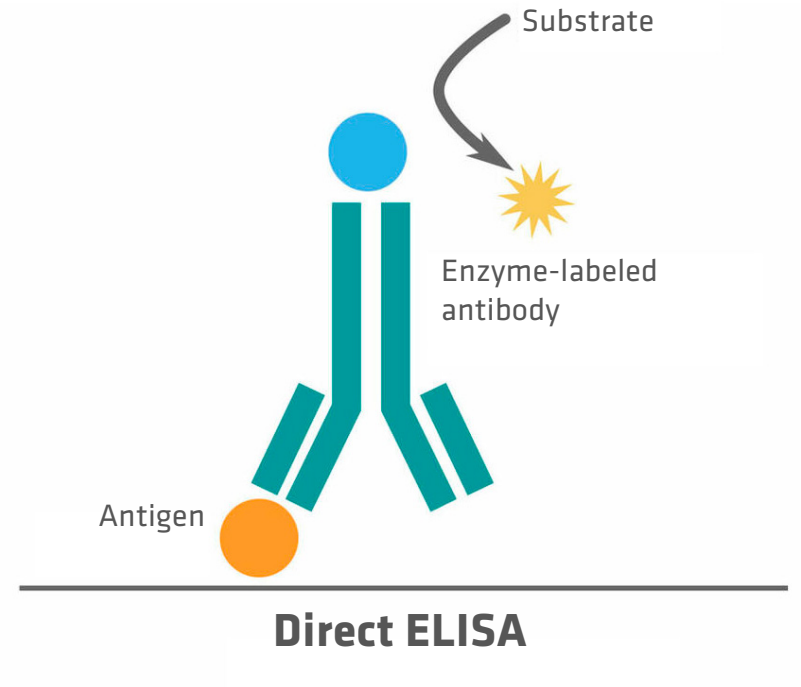
Different types of ELISA

The four main types of ELISAs are direct, indirect, sandwich and competitive. All of these tests are commonly performed in 96 well plates, using the bottom of the wells as the solid surface to immobilize the antigen-antibody complexes. The differences between the four types – and their advantages and disadvantages – are discussed in the following sections.

Direct ELISAs

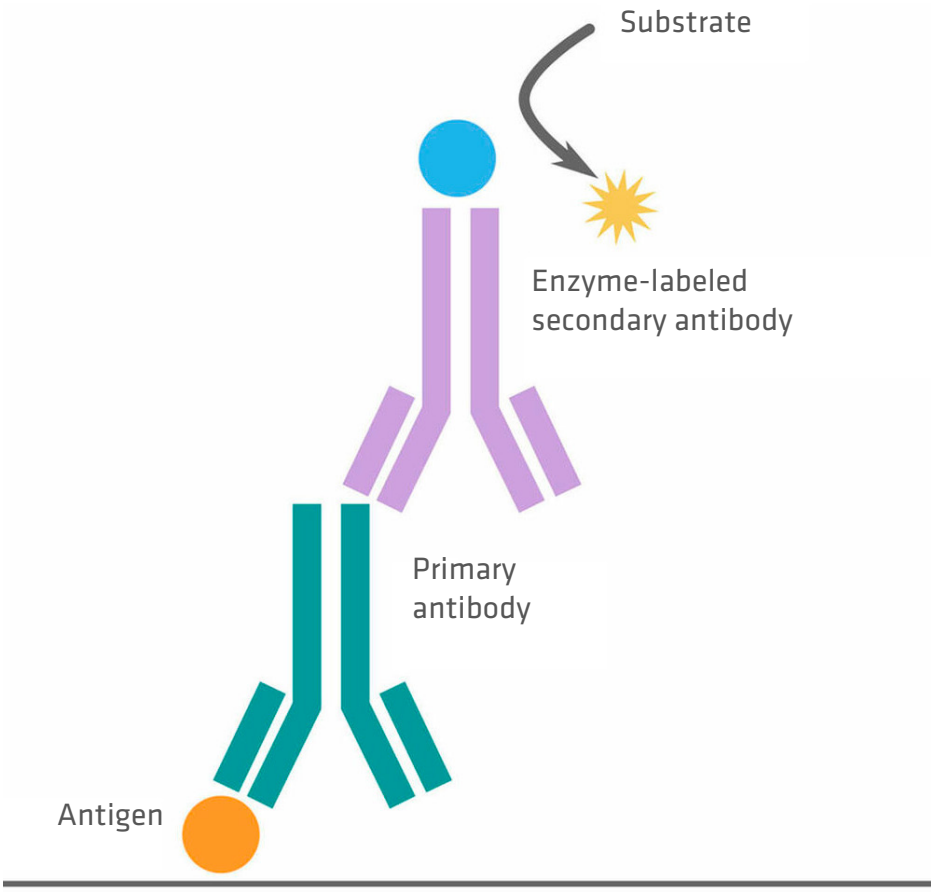
The direct ELISA protocol can be used to detect antigens, and consists of four steps:

1. **Plate coating:** Dilute your samples using a coating buffer, then pipette them into a microwell plate. After incubation, discard the coating solution and wash the plate with a wash buffer. The proteins contained in your sample – including the antigen of interest – are now immobilized to the plate.
2. **Plate blocking:** Dispense blocking buffer into the plate and incubate it. The blocking buffer will bind to any remaining protein-binding sites in the coated wells, reducing subsequent non-specific binding of antibodies to the plate. Wash your plate again.
3. **Antibody incubation:** Transfer enzyme-labeled antibodies to the plate, then incubate. The antibodies will bind to the antigens of interest in wells that contain the analyte. Following incubation, wash away unbound antibodies with a wash buffer.
4. **Detection:** Add an enzyme-specific substrate to the plate. The enzymes covalently attached to the antibodies will start producing a colored reaction product. After adding a stop solution to terminate the color development, read the absorbance of each well with a plate reader. The signal intensity allows you to determine whether a sample contains the antigen of interest, and at what concentration.



Indirect ELISAs

The only difference between a direct and indirect ELISA is that the antibody incubation process is divided into two steps. After coating and blocking the plate, primary antibodies that bind to the antigens of interest are added to the plate, which is then incubated. Following a wash step, enzyme-conjugated secondary antibodies – which bind to the primary antibodies but not to the target antigens – are added. After incubating the plate a second time and washing off unbound secondary antibodies, the detection step can be performed.

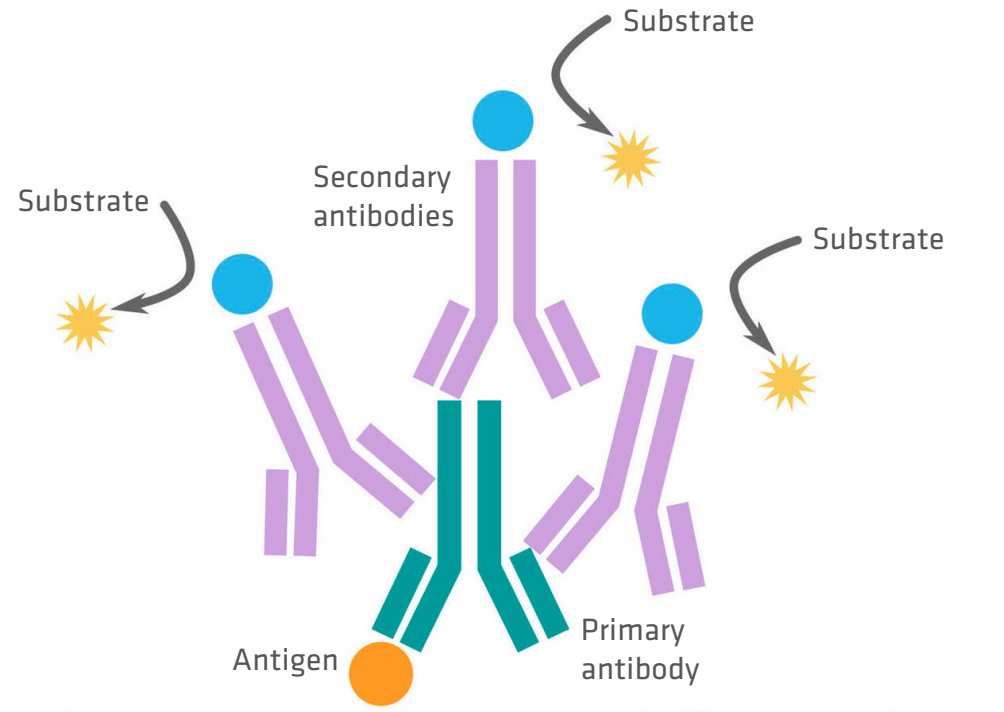


Indirect ELISA

Direct vs. indirect ELISAs

The advantage of direct ELISAs is that they save time and reagents, as the protocol is slightly shorter. In addition, the risk of cross-reactivity is reduced. Cross-reactivity occurs when antibodies bind non-specifically to epitopes on molecules that are not the antigen of interest. Because direct ELISAs use only one antibody instead of two, this risk is lower.

Despite the longer protocol and higher risk of non-specific binding due to cross-reactivity, indirect ELISAs have many advantages too. For example, they are more sensitive, because multiple secondary antibodies can bind to one primary antibody, amplifying the detectable signal.



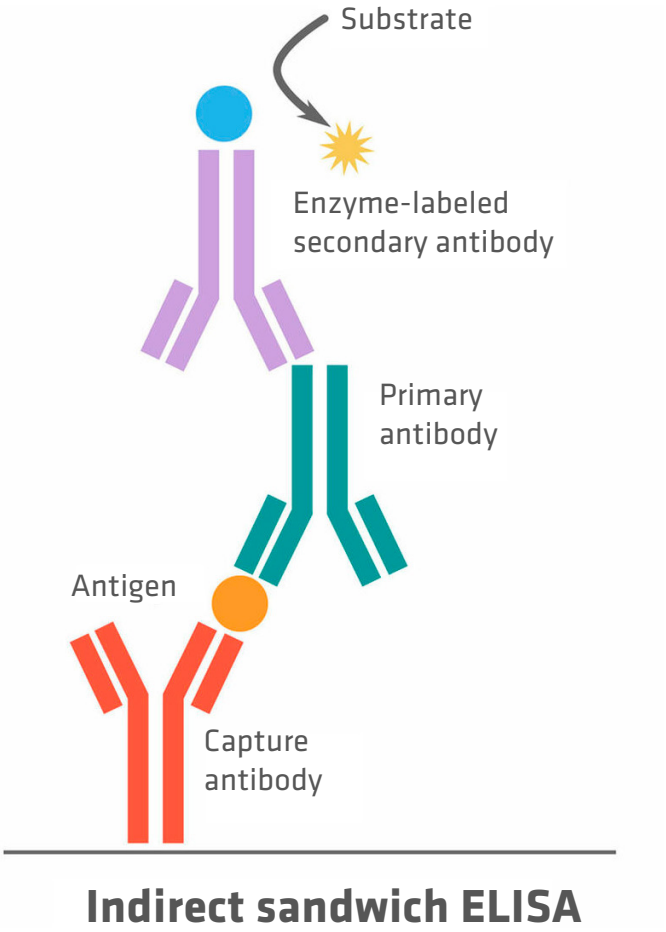
Signal amplification (indirect ELISA)

Moreover, indirect ELISAs offer greater flexibility. A secondary antibody can be specific to several primary antibodies, as long as they are the same type and from the same host species. This means that the same labeled secondary antibody can be used in different indirect ELISA applications, whereas several labeled primary antibodies are needed to perform different direct ELISA protocols.

Another advantage of indirect ELISAs is that they can not only detect antigens, but also antibodies. This requires coating the plate with antigens that bind to the antibodies of interest, then adding the samples in place of the primary antibodies during the first antibody incubation step.

Sandwich ELISAs

For sandwich ELISAs, the plate isn't coated with antigens, but with capture antibodies specific to the antigen of interest. After blocking the remaining protein binding sites with a buffer that is designed to bind to the sites not occupied by the capture antibodies, the samples can be added to the wells. During incubation, the antigens of interest bind to the capture antibodies. Next, the plate is incubated either with enzyme-labeled detection antibodies (direct sandwich ELISA) or with primary antibodies followed by enzyme-labeled secondary antibodies (indirect sandwich ELISA). Please note that the capture and primary antibodies for indirect sandwich ELISAs need to be from different host species to increase specificity.



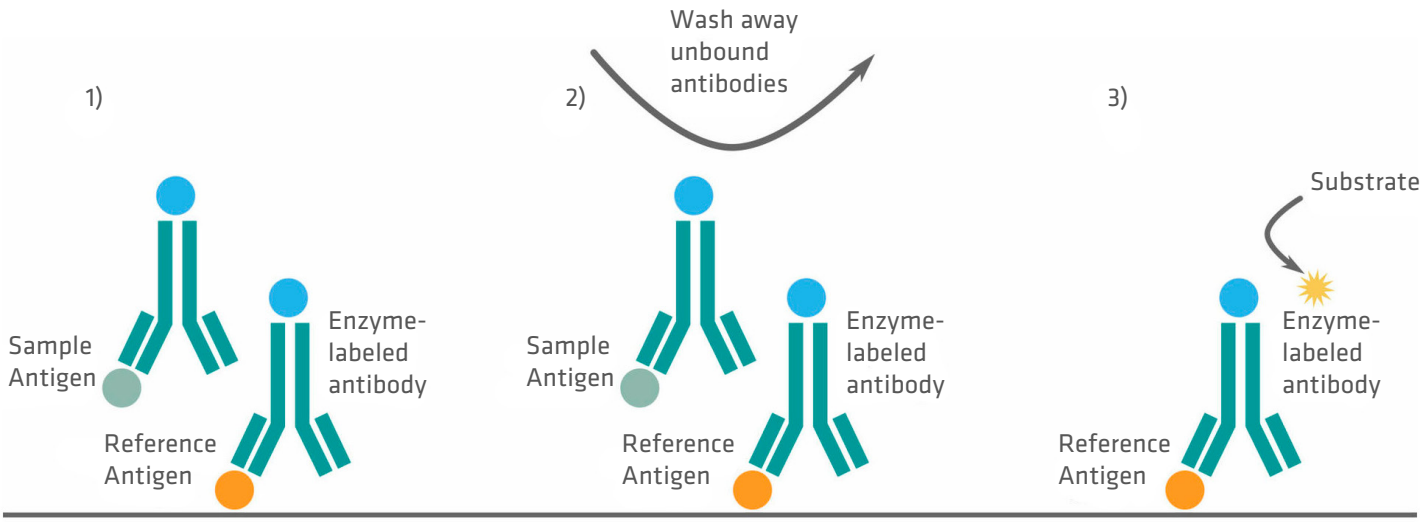
The advantage of sandwich ELISAs is that they are highly specific. The two antibodies capturing an antigen bind to different epitopes of it, making it almost impossible for both antibodies to bind non-specifically. Thus, sandwich ELISAs are the perfect method for complex samples. A disadvantage of sandwich ELISAs is that it can sometimes be difficult to find two antibodies that work well together while binding to different epitopes of the same antigen.

Competitive ELISAs

In competitive ELISAs, also known as inhibition ELISAs, the amount of labeled molecules is limited, and the sample antigens or antibodies compete with reference antigens or antibodies to bind to these labeled molecules. This means that the analyte concentration is measured by the detection of signal interference. Direct, indirect and sandwich ELISAs can all be adapted to this format, but the steps and reagents of a competitive ELISA protocol can vary. To perform a competitive direct ELISA, you could, for example, proceed as follows:

- Coat the plate with a reference antigen and block the remaining binding sites with a buffer.
- Incubate your sample that has an unknown antigen concentration with a limited amount of labeled antibodies. If the antigen concentration in the sample is low, a large portion of the antibodies will not be able to bind to an antigen, and *vice versa*.
- Add the sample-antibody mixture to the coated wells and incubate the plate. The antibodies that couldn't bind to a sample antigen in the previous step will now bind to a reference antigen (**step 1** in the image below).
- Wash your plate. Antibodies bound to sample antigens will be washed away because they aren't immobilized to the plate (**step 2** in the image below).
- Add a substrate (**step 3** in the image below) and measure the colored reaction product. The stronger the color change, the less antigen was present in the sample, and *vice versa*.

The advantages and disadvantages of a competitive ELISA depend on the base ELISA (direct, indirect, competitive) selected.



Competitive direct ELISA

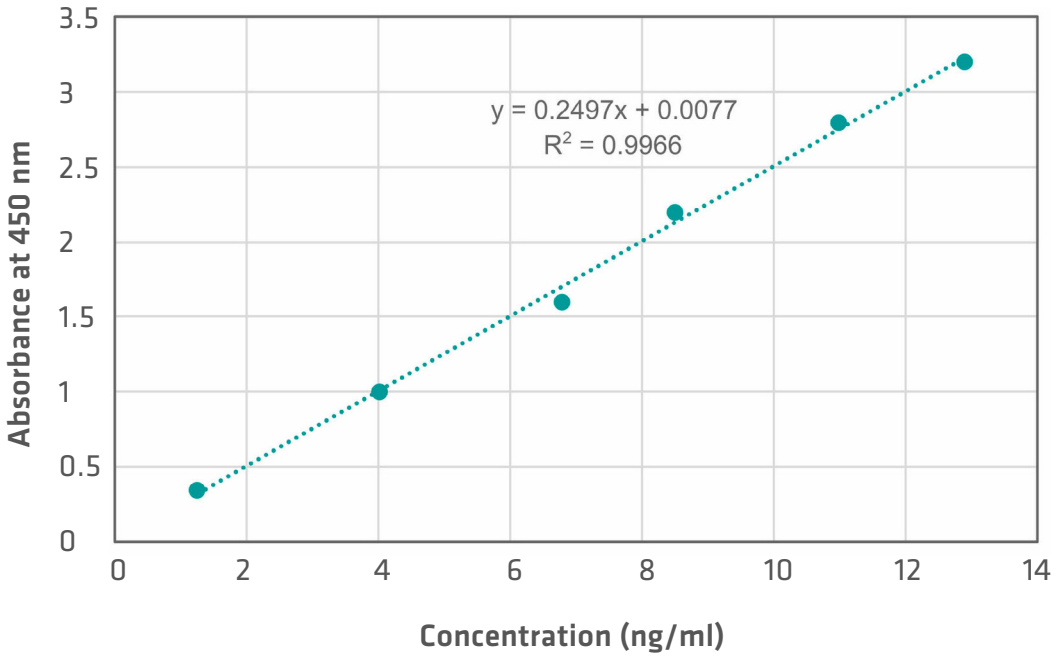
How to analyze ELISA results

After looking at the working principle of the different types of ELISAs, let's now talk about data analysis. ELISAs can either deliver qualitative, semi-quantitative or quantitative results:

- **Qualitative results:** Is the antigen or antibody present in a sample or not?
- **Semi-quantitative results:** Is the antigen or antibody concentration in sample A higher or lower than in sample B?
- **Quantitative results:** What is the antigen or antibody concentration in a sample?

To obtain reliable results, you should include negative and positive controls in every ELISA plate that you set up. Negative controls will allow you to check for false positive results caused by non-specific binding or contamination, whereas positive controls confirm that the test is working as intended, even if all the samples are negative.

If you want to obtain quantitative results, you also need to set up a standard curve. To do so, serially dilute a sample with a known amount of the antigen or antibody of interest with a diluent buffer. After running the ELISA, plot the known concentrations against the obtained absorbance values, using curve fitting and data analysis software to find the curve that best fits your data. This will give you an an equation that you can use to calculate the unknown antigen or antibody concentrations in your samples. For example, if a linear plot is the curve that fits your data best, the standard curve will show the concentration on the x-axis and the absorbance values on the y-axis,[1,2] with R^2 indicating how closely the data points fit the trendline. Values greater than 0.99 allow you to get accurate results.[1,3]



The equation for the linear regression line of the standard curve (y = mx + b) will then allow you to calculate the antigen or antibody concentration of your samples. As y corresponds to the absorbance and x to the concentration, the equation for the linear regression line is equivalent to:

Absorbance = m(concentration) + b

Solving this equation for the concentration will give you the formula:

Concentration = (Absorbance - b) / m

For example, if your equation for the linear regression line is y = 0.2497x + 0.0077 (as in the graph above), a sample with an absorbance value of 2 would have a concentration of 7.9788:

7.9788 = (2 - 0.0077) / 0.2497

When performing quantitative ELISAs with complex samples, you should also include a spike control. A spike control is a serial dilution of a sample with a known amount of antigen or antibody of interest in serum instead of diluent buffer. Comparing the absorbance values of the spike control and the standard curve will allow you to see whether proteins other than the antigens or antibodies of interest in your sample hinder antigen-antibody binding, leading to an underestimation of the target concentration.

Note that the negative and positive controls, standard curves, spike controls and samples should all be run in duplicate or triplicate. This lowers the number of samples that can be analyzed per plate, but increases assay reliability. The chance that factors such as pipetting errors remain unnoticed is much lower when working with average absorbance values of duplicates or triplicates during data analysis, as you would notice high deviations of the mean if only one of the wells in the set was affected. An acceptable deviation of the mean for duplicates is a value of 20 % or lower[2].

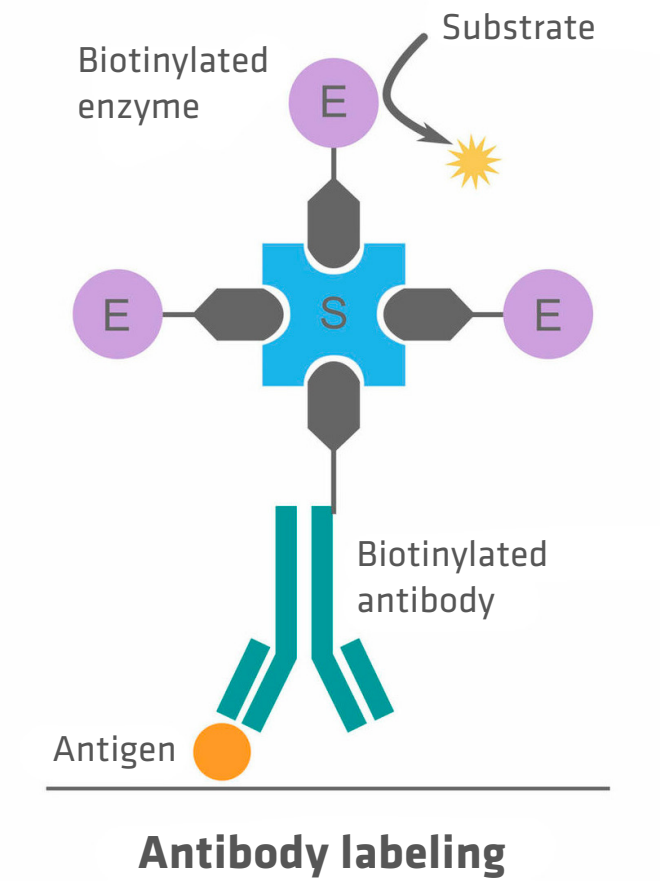
Reagents for ELISAs

The reagents needed for an ELISA test depend on the protocol, but generally include coating, blocking and wash buffers, enzyme-conjugated antibodies or antigens, a substrate and a stop solution. For the detection of common antigens and antibodies, you can usually purchase ready-to-use ELISA kits that contain everything you need.

The most widely used reagents in these kits are:

- **Coating buffer:** The two most common coating buffers are PBS and bicarbonate. They stabilize the antigen or antibody used to coat the plate during incubation.[4]
- **Blocking buffer:** To prevent non-specific binding of detection antibodies to the plate surface, remaining protein binding sites need to be blocked. This is achieved with blocking proteins such as bovine serum albumin (BSA), non-fat dry milk (NFDM), casein or caseinate, normal serum or fish gelatin.[5]

- **Wash buffer:** Wash steps to remove unbound materials are mostly performed with PBS containing a small concentration of a nonionic detergent, such as Tween® 20.[4]
- **Enzyme-conjugated antibodies or antigens:** The antibodies or antigens required will depend on the analyte. To label the antibodies or antigens with an enzyme, a streptavidin-biotin bridge is generally used to link the antibodies or antigens to the detection enzyme.[6] The two most common enzymes used are horse radish peroxidase (HRP) and alkaline phosphatase (ALP).[7]



Antibody labeling

- **Substrate and stop solution:** The substrates and stop solutions are enzyme specific. The substrate for HRP contains TMB (3,3′,5,5′-tetramethylbenzidine), substrate buffer and hydrogen peroxide, and the reaction can be stopped with sulfuric acid. The substrate for ALP is a p-nitrophenyl phosphate (pNPP) solution,[4] and the reaction can be stopped with NaOH.[8]

Equipment for ELISA

To perform an ELISA, you need pipettes to add the reagents and samples to your microwell plate, an incubator to hold it at a constant temperature, a plate washer to remove unbound molecules, and a plate reader for assay analysis.

If you're working in a lab with a limited budget or low throughput, there might be no plate washer at hand. In this case, you can also use pipettes in combination with an aspiration system for the wash steps.

On the other hand, high throughput labs might be looking for solutions to increase their productivity. In this case, various pipetting solutions may come in handy, including adjustable tip spacing pipettes to transfer samples between different labware formats, 96 channel pipettes to simultaneously add reagents to every well, or small benchtop pipetting robots to automate workflows.

Conclusion

ELISAs have been used since the 1970s, and are considered the gold standard of immunoassays.[9,10,11] We hope that this article has helped you to get an overview of the most relevant aspects related to this important application.

1.2 ELISA vs. western blot: a comparison of two common immunoassays

If you work in a laboratory and perform immunoassays, you have probably had to choose between the enzyme-linked immunosorbent assay (ELISA) and western blot techniques at some point. Both approaches have their pros and cons, so deciding which one to use can be challenging. This article takes a closer look at these techniques, to help you select the most appropriate method for your lab's workflow.

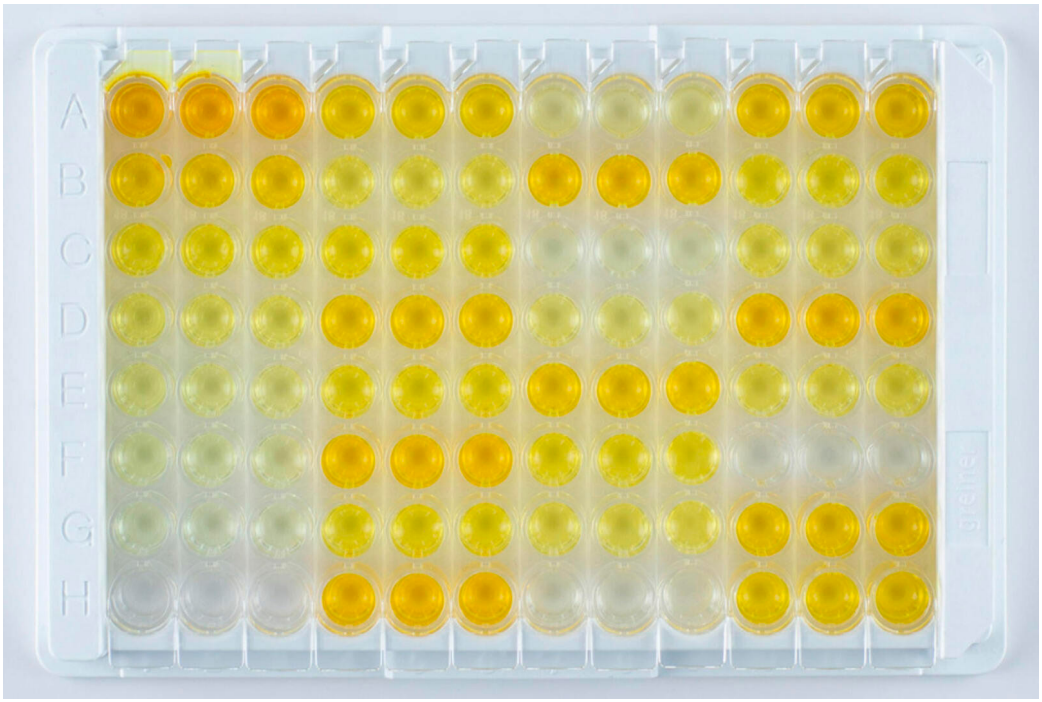
What are immunoassays?

Immunoassays rely on the antibody-antigen binding mechanism that occurs naturally in the immune system. An antibody produced by the adaptive immune response is specific to a certain antigen only, and immunoassays take advantage of this specificity to identify a molecule of interest – typically a protein – in a sample.

Many different types of immunoassays have been developed over the last few decades, including the ELISA and western blot, two common methods that we will compare and contrast in this article.

ELISA

ELISAs are highly specific and sensitive assays used to detect concentrations of as little as 0.01 nanograms of antigen or antibody per milliliter of sample.[1] The four main types of ELISA – direct, indirect, sandwich, and competitive – are all usually performed in 96 well plates, using the bottom of the wells as a solid surface to immobilize antigen-antibody complexes. Since an enzyme is covalently attached to one of the molecules in the complex, the subsequent addition of an enzyme-specific substrate results in a detectable colored reaction product if the antigen or antibody of interest is present in the sample. ELISAs can either deliver qualitative, semi-quantitative or quantitative results.



Western blot

Before delving deeper into the details and methodologies involved in western blotting, we'd like to share the story behind its name with you.

The western blot was developed in 1981 by Walter Neal Burnette, a postdoc in the Nowinski lab at the Fred Hutchinson Cancer Center in Seattle. The name 'western blot' alluded to the Southern blot invented in 1975 by Ed Southern, and the northern blot invented in 1977 by James Alwine. He chose the ‘western’ and not ‘eastern’ direction descriptor, to make a geographical reference to the location of his lab in Seattle, on the West Coast of the United States.[2,3]

Now that you know the origin of its name, let's have a look at how this technique works.

What is a western blot?

In a nutshell, the western blot (also called an immunoblot) is a technique used to detect a specific protein, such as an antigen, in a sample consisting of a mixture of proteins. To this end, the proteins in the sample first need to be denatured and separated by size.

After transferring the proteins to a membrane, antibodies are used to detect the protein of interest, and the result is visualized using a colorimetric, chemiluminescence or fluorescence analysis method. Radioactive detection is also possible, but is now only rarely used due to health and safety risks.

For those who aren't familiar with the technique, we've explained the various parts of western blotting in more detail below.

Steps of a western blot

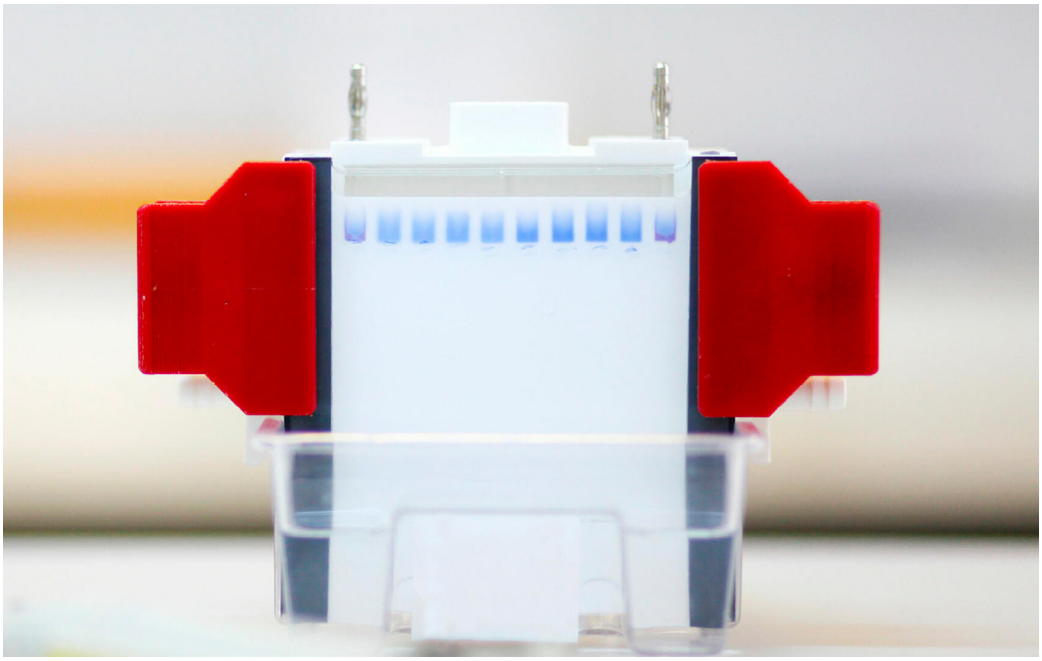
We can break western blotting down into three main steps:

- Separation of proteins by size
- Transfer of proteins to a membrane
- Labeling of proteins using antibodies

The first step is achieved using gel electrophoresis. Samples are loaded into wells on top of the gel. An electrical current is then applied to the gel, causing the proteins to travel through it. Two factors influence how fast the proteins in the sample travel: size and charge. To separate the proteins according to their size, they need to have a proportionally uniform charge, therefore, they are treated with sodium dodecyl sulfate (SDS) which causes proteins to unfold into linear chains and imparts a negative charge.[4]

The porous polyacrylamide composition of the gel allows smaller proteins to migrate faster than larger ones, resulting in the formation of bands containing proteins of the same size. The polyacrylamide concentration determines the size of pores in the web of the gel, and can be adjusted to ensure even distribution of proteins and a satisfactory electrophoresis result. Generally, larger proteins separate easily in a gel with a low percentage of polyacrylamide, and smaller proteins require a gel with a high percentage of polyacrylamide. Please note that unpolymerized acrylamide is carcinogenic and mutagenic, and should always be handled in a biosafety cabinet using appropriate personal protective equipment.

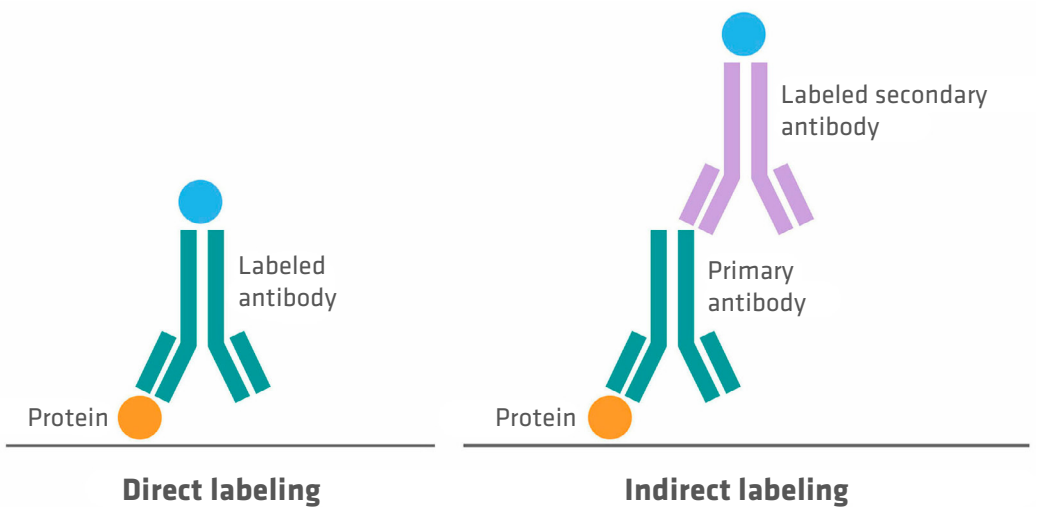
In addition to your samples, you also need to add a molecular marker containing several proteins with known molecular weights to the gel. This will allow you to see if electrophoresis has been successful, and whether the subsequent transfer of the proteins to the blotting membrane has been effective. Moreover, it will enable you to estimate the length of your proteins of interest, as you can compare their migration path through the gel with the distance travelled by the proteins of the molecular marker. The method is often abbreviated as SDS-PAGE (**s**odium **d**odecyl **s**ulfate **p**olyacrylamide **g**el **e**lectrophoresis) because gel electrophoresis for western blotting uses SDS and polyacrylamide.



The second stage of the western blotting process involves transferring the migrated proteins to a blotting membrane. The most commonly used technique is electrophoretic transfer, where the gel and membrane are sandwiched between filter paper and placed between electrodes, after which an electric field moves the proteins from the gel to the membrane.[5]

The third step of a western blot protocol – protein labeling – is very similar to an ELISA. It requires you to incubate the membrane with a blocking buffer, which binds to any remaining protein binding sites to reduce subsequent non-specific binding of antibodies to the membrane. After washing the membrane you can opt for either a direct or indirect labeling method.

In the direct method, the membrane is incubated with labeled antibodies that bind to the proteins of interest, before washing unbound antibodies away. The indirect method uses two antibodies, a primary and a secondary antibody. In the first step, the membrane is incubated with unlabeled primary antibodies that bind to the proteins of interest. After washing, a second incubation is performed, using labeled secondary antibodies that bind to the primary antibodies. This is followed by a final washing step to remove any unbound molecules.



There are risks and benefits to both labeling protocols. Direct binding is a shorter process, which can save time and reagents. It also reduces the risk of non-specific antibody binding since it only uses one antibody.

On the other hand, indirect western blotting is more sensitive because several secondary antibodies can bind to one primary antibody, amplifying the detectable signal. The indirect method also offers greater flexibility, as secondary antibodies can be specific to several primary antibodies of the same type and from the same host species. This means that the same labeled secondary antibody can be used in different indirect western blot applications, whereas several labeled primary antibodies are needed to perform different direct protocols.

Western blot analysis

To analyze a western blot, you need to measure the signal produced by the labeled antibodies. The detection steps and equipment differ depending on whether the antibodies are tagged with an enzyme or a fluorophore.

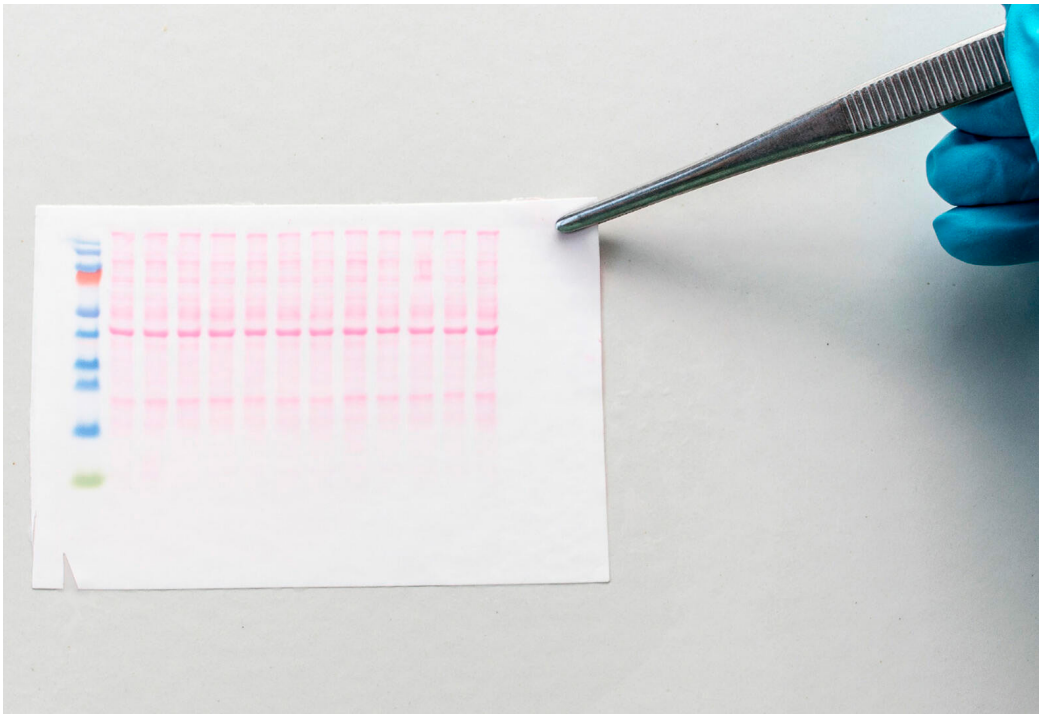
Enzymatic detection

You can use either colorimetric or chemiluminescence detection methods to analyze your western blot if your antibody is labeled with an enzyme.

Colorimetric methods

Colorimetric methods require the application of an enzyme-specific chromogenic substrate to the membrane; the reaction catalyzed by the enzymes attached to the antibodies will produce a colored reaction product. A stop solution is added to terminate the color development, and the chromogenic products of the enzymatic reaction are visible as bands to the naked eye.[6]

A colorimetric western blot analysis can be performed quickly and cost-effectively because you don't need any special detection equipment. It is, however, not very sensitive. Proteins in the nanogram range are required to produce a visible band.[6]



Chemiluminescence methods

Chemiluminescence detection methods also require an enzyme-specific substrate, but one that is luminescent instead of colorimetric. This means that the enzymatic reaction on adding substrate to the membrane will produce light as a by-product. The light can then be detected using an X-ray film or a charge-coupled device camera (CCD).[6]

The main advantage of chemiluminescence detection is its sensitivity; it can be used to detect and quantify as little as femtograms of the protein of interest. However, a downside of this technique is that light is only emitted briefly, when the enzyme converts the substrate into a product, and therefore detection needs to be performed immediately after substrate addition.[7]

Fluorescence detection

If your antibodies are labeled with fluorophores, you don't need to add any substrate to the membrane. Instead, you can image the membrane directly using a fluorescence imaging system, where a light source excites the fluorophores and the fluorescent signals emitted are detected by wavelength-specific filters and digital camera systems.[8]

Tagging antibodies with fluorophores instead of enzymes has several advantages. Firstly, tagging individual antibodies with different fluorophores allows for the simultaneous detection of various proteins with similar molecular weights (multiplexing). Secondly, as fluorescent signals are still detectable on membranes after months of storage at room temperature, this method offers a lot of flexibility when it comes to analyzing assay data. Thirdly, fluorescence detection is simple to perform because you don't need to add any substrate. The only disadvantages of this detection method are the need for a special imaging system, and reduced sensitivity compared to chemiluminescence detection.[7]

ELISA vs. western blot

Now that you understand how to conduct and analyze the two immunoassays, how should you decide which technique to use?

Generally speaking, an ELISA is easier and faster to perform than a western blot because the protocol is shorter. Moreover, ELISAs are better suited for high throughput labs because they can be performed with lower sample volumes and are usually carried out in 96 well plates, allowing for workflow automation.

When it comes to obtaining reliable quantitative data in an ELISA, a simple standard curve – serial dilutions of a known target protein – can be set up, and its equation can be used to calculate the antigen or antibody concentration of samples based on their absorbance values.

Performing a quantitative western blot can be more demanding. It requires the consideration of several factors, such as overloading of samples, membrane saturation and signal saturation. Additionally, internal loading controls like a housekeeping protein are required, data must be normalized, and the background signal must be subtracted for quantitative analysis.

However, even though performing a western blot can be complex and time-intensive, they are preferred over ELISAs for some applications. The first advantage of a western blot is that they are highly specific, and are sometimes used to rule out false positives and confirm positive ELISA results in clinical settings. This is due to the initial protein size separation which ensures that non-specific signals from analytes – which would likely be a different size – can be easily detected as a second band on the membrane.

Secondly, a western blot generates more information about the target protein and the sample in general. Rather than simply identifying the presence or absence of an analyte in a sample, western blot can also determine the length of a protein and analyze sample purity. Therefore, the slightly longer and more tedious protocol may be worthwhile if your assay requires more extensive information.

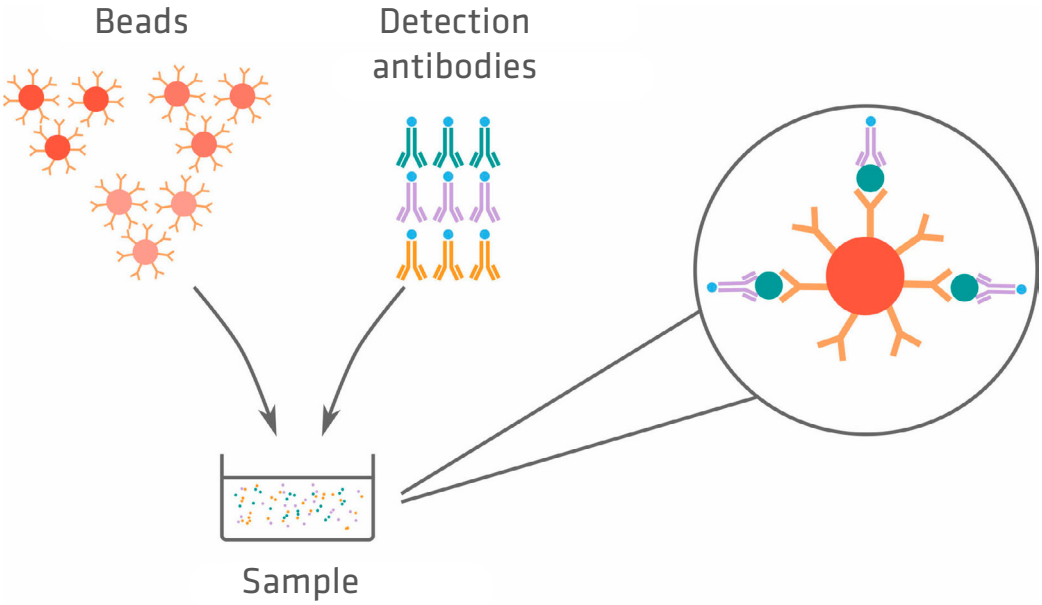
A third advantage of western blotting – although only true if the detection antibody is labeled with a fluorophore – is its ability to detect several proteins per sample in parallel.

Alternatives to ELISAs and western blotting

We have determined that the ELISA and western blot methodologies have their individual benefits and disadvantages for various laboratory applications. However, these techniques fall short when looking to perform more complex high throughput multiplexing immunoassays: the enzyme-labeled antibodies used in ELISAs don't allow you to detect several analytes in parallel, and fluorescent western blots are only suitable for low throughput settings. Luckily, alternative techniques like bead-based immunoassays and protein microarrays have been developed for just this purpose.

Bead-based immunoassays

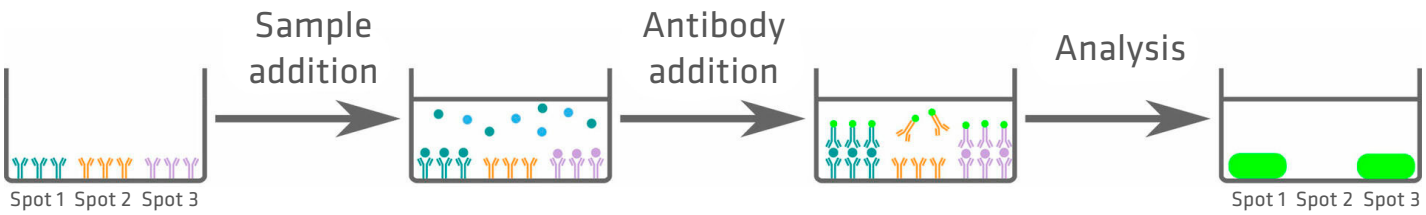
To perform a bead-based immunoassay, you need as many bead sets as you have analytes of interest. Each bead set is coated with capture antibodies specific for a certain analyte, and labeled with a red fluorescent dye with a unique intensity. Different detection antibodies are also needed for this assay. These are labeled with a green fluorophore, and bind to the analytes of interest. When the bead sets and detection antibodies are added to a sample and incubated, they form complexes with the analytes of interest.



These bead complexes can then be analyzed using flow cytometry, during which individual complexes pass through a red and a green laser beam. The red laser identifies the signal intensity of the fluorescent dye, and can consequently tell which type of analyte the bead is supposed to bind. The green laser is then used to determine whether analytes and detection antibodies are bound to the beads, by detecting fluorophores on the detection antibodies.[9,10,11]

Protein microarrays

Protein microarrays (also called protein chips) are comparable to performing a lot of sandwich ELISAs simultaneously on a single solid surface. The solid surface – which can be a membrane, a glass slide or the well of a microplate – is pre-spotted with different capture antibodies specific for the analytes of interest. When incubating the solid surface with a sample, the analytes, if present, will bind to the capture antibodies and can then be detected with labeled antibodies. As you know that spot 1 is supposed to bind analyte A, spot 2 analyte B, etc., the pattern generated after analysis will allow you to determine which analytes are present in your sample.



Conclusion

In conclusion, ELISAs and the western blot are two immunoassays that can be used to detect the presence of a protein in a sample. Each method has its pros and cons; ELISAs are easier, faster to perform, and better suited for high throughput labs, the western blotting technique is more specific, can provide additional information about the target protein and the sample under analysis, and allows for multiplexing. The decision as to which method to use depends on the application. Moreover, there are alternative techniques such as bead-based immunoassays and protein microarrays that may be more suitable when performing high throughput multiplexing assays.

CHAPTER 2: INTEGRA Biosciences' ELISA solutions

ELISAs are the mainstay for a wide range of studies but, unfortunately, generally involve multiple time-consuming and tedious processing steps that require consistent pipetting – from well-to-well and plate-to-plate – to ensure success. On top of this, the repetitive nature of this work can also frequently result in user fatigue and handling mistakes.

Fortunately, with the right tools, you can quickly and easily ramp up your throughput, reduce errors, increase consistency and improve the reproducibility of your results. Here, we will demonstrate how INTEGRA's range of liquid handling solutions can help you to save time and elevate your ELISA workflows for more successful experiments.

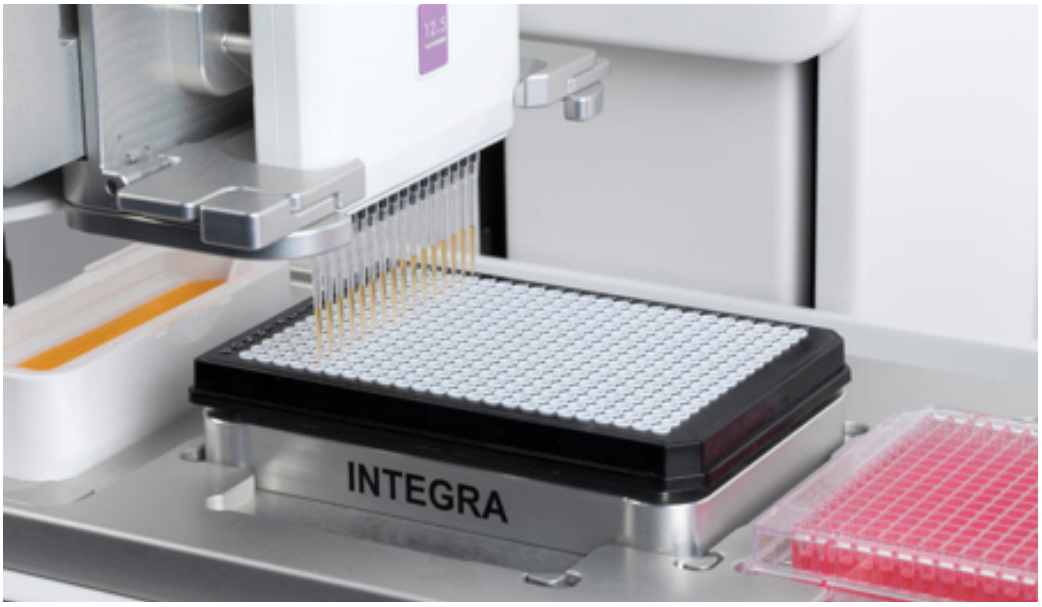
Handheld electronic pipettes

The VIAFLO 96 and VIAFLO 384 handheld electronic pipettes can vastly improve ELISA workflows by enabling the transfer of samples and reagents into 96 or 384 wells simultaneously. All the steps needed to run the protocol can be saved as a custom program on the instrument, which then guides the user with easy to follow prompts. This streamlines the workflow and eliminates user variability. These instruments can also quickly fill multiple plates using the Repeat Dispense mode, or can be used for partial plate processing, providing even higher throughput. Crucially, the VIAFLO 96 and VIAFLO 384 offer all of these benefits while still being as easy to use as traditional handheld pipettes.

Learn more about **VIAFLO 96** and **VIAFLO 384**

Pipetting robots

The ASSIST PLUS pipetting robot automates the ELISA process, and can be easily adapted to the requirements of your specific application. Pipetting settings and assay set-up options – including volumes, sample predilutions and plate layouts – can easily be modified using our VIALAB software, making sure the system is always perfectly suited to your assay, even for partial plate processing. Various samples and tube types – as well as multiple reagent vessels – can be accommodated on the deck, and you can mount any of our 25 electronic pipettes or the D-ONE single channel pipetting module onto the ASSIST PLUS. This combination of flexibility and automated, optimized processing improves productivity, reproducibility and consistency.

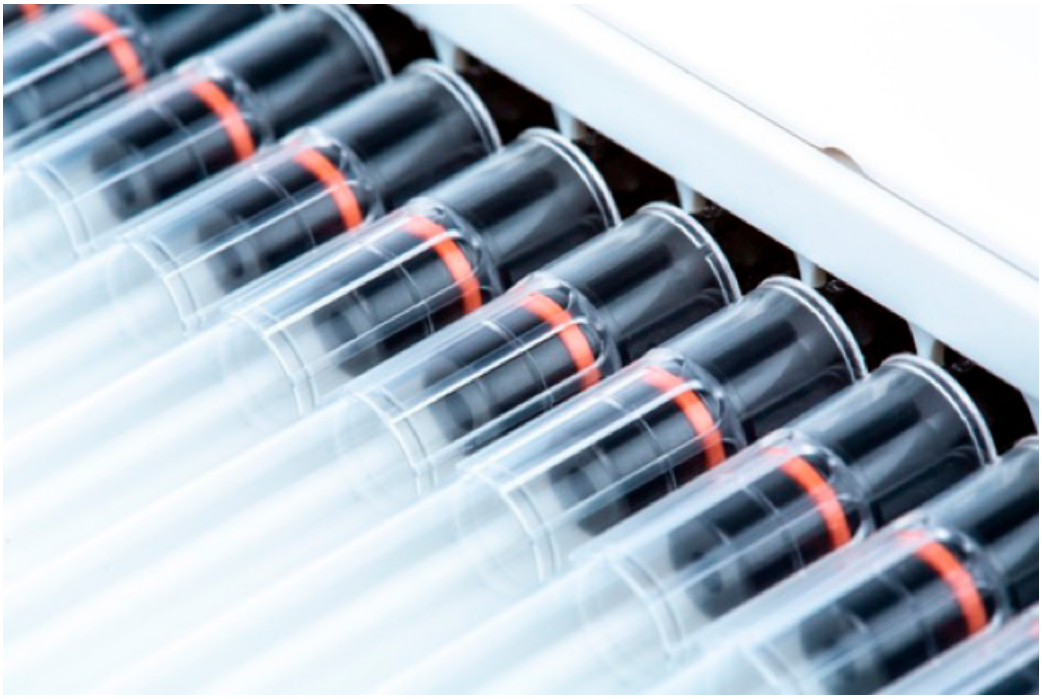


Learn more about **ASSIST PLUS**

Learn more about **D-ONE**

Pipette tips

ELISA buffers often contain surfactants – like Tween® 20 – which tend to interact with standard pipette tips, forming a thin liquid film on the inner wall. This leads to inaccurate and inconsistent pipetting results, as well as loss of precious reagents. INTEGRA's low retention GRIPTIPS are made from a unique polypropylene blend that offers heightened hydrophobic properties, reducing the residual tip volumes for maximum sample recovery.



Learn more about **GRIPTIPS**

Aspiration systems

There are many advantages to using an aspiration system to remove buffers from samples, especially for 96 well plate formats, where there are multiple wells to process. The VACUSIP portable aspiration system provides safer, faster and more convenient waste removal than a multichannel pipette, is simple to operate, and comes ready to use straight out of the box. It's an affordable and versatile tool that helps to increase productivity for ELISA workflows, while also protecting the wellbeing of users.

Learn more about **VACUSIP**

CHAPTER 3: Application notes

Our pipetting instruments are used across a broad spectrum of life sciences applications, and we endeavor to share knowledge and experience of using our products with the wider scientific community. To this end, we have compiled an extensive database over the years, which contains a wide range of thorough and useful application notes. Here are some of the most relevant app notes for ELISA protocols and workflows.

3.1 Performing an ELISA with the ASSIST PLUS pipetting robot

Enzyme-linked immunosorbent assays with ASSIST PLUS pipetting robot

The enzyme-linked immunosorbent assay (ELISA) is a standard method used to detect and quantify peptides, proteins, antibodies or hormones in a sample. It consists of multiple repetitive steps that are time consuming and tedious to perform manually. The ASSIST PLUS pipetting robot allows this process to be automated, which not only increases the reproducibility of your results, but also gives you more time to focus on your science. Any VIAFLO or VOYAGER electronic pipette can be automated using the ASSIST PLUS; the VOYAGER adjustable tip spacing pipette enables reformatting of samples from one labware type to another in the blink of an eye. All the steps needed to run an ELISA are saved on the pipette as a VIALAB program – the smart and easy-to-use pipetting automation software of the ASSIST PLUS. Simply place the labware on the deck, choose the program corresponding to the ELISA step and let the ASSIST PLUS do the work.

Key benefits

- Optimal pipette settings – including tip immersion depth, pipetting speeds and angles – maximize the consistency and reproducibility of the ELISA.
- The full automation capability of the ASSIST PLUS frees highly valuable time that you can use for more important tasks.
- Repeat Dispense and Multi Aspirate steps can be used for fast dispense and removal of reagents to speed up the process. The automatic Tip Change ensures assay contamination is avoided.
- VOYAGER and VIAFLO electronic pipettes, in combination with the ASSIST PLUS, provide unmatched pipetting ergonomics.
- The ASSIST PLUS pipetting robot is perfectly adapted to handle different plate layouts, increasing the flexibility of your work depending on your needs.
- Various sample input tubes can be used. The samples are easily transferred to the assay plate using multichannel and adjustable tip spacing pipettes, increasing the assay productivity while avoiding reformatting errors.

Overview of the sandwich ELISA steps and corresponding programs:

The ASSIST PLUS is used to perform a sandwich ELISA. The pipetting robot operates a VOYAGER 8 channel 1250 µl electronic pipette with 1250 µl sterile, filter, low retention GRIPTIPS. The use of low retention GRIPTIPS guarantees optimal liquid recovery when pipetting ELISA buffers that contain surfactants, such as Tween® 20.

Below is an example set-up for a sandwich ELISA with a standard curve and 24 samples in triplicate. The pipetting programs are prepared with the VIALAB software. The protocol is divided into eight programs that guide the user through the eight steps of the ELISA.

Step-by-step procedure

1. Coat the ELISA plate

Adding the capture antibody to coat the ELISA plate.

Place the capture antibody, prediluted in the coating buffer, in a 10 ml polypropylene multichannel reagent reservoir. Select and run the first VIALAB program, 1_E_Coating. The pipette automatically transfers 100 µl of the capture antibody into the ELISA plate using the Repeat Dispense mode. The plate is ready to be incubated.

Figure 1: Adding the capture antibody to coat the ELISA plate.

2. Block the plate

Blocking the ELISA plate's non-specific binding sites.

Select the VIALAB program 2_E_Blocking and set up the deck with the required labware.

The program incorporates all the necessary pipetting steps, including removing of the coating buffer from the plate followed by washing three times with 200 µl of the washing buffer. In our example, we included a 15 second incubation time, which can be easily adjusted to your protocol using the VIALAB software. The use of the Repeat Dispense and Multi Aspirate modes speeds up this fully automated process. At the end of the washing steps, the user is prompted to blot the plate against clean paper towels – the only manual step of this ELISA protocol. After confirming that this step has been completed, the ASSIST PLUS pipetting robot continues by adding the blocking buffer into the ELISA plate using the Repeat Dispense mode. Finally, the pipette informs the user that the plate is ready for incubation.

Figure 2: Blocking the ELISA plate's non-specific binding sites.

3. Prepare your samples

Diluting your samples 1:10.

In this example, centrifuged blood samples are stored in EDTA collection tubes placed in an INTEGRA rack. The plasma is diluted 1:10 with the dilution buffer.

Select and run the 3_E_Sample_Preparation program. The pipette automatically fills the microcentrifuge tubes with 900 µl of the dilution buffer. This is followed by transfer of the plasma samples from the EDTA tubes to the microcentrifuge tubes, and careful and thorough mixing. The samples are then ready to be used.

Figure 3: Diluting your samples 1:10.

Tips:

- Using a VOYAGER adjustable tip spacing pipette together with the ASSIST PLUS allows automatic and error-free sample reformatting.
- The ASSIST PLUS pipetting robot ejects and loads the tips automatically, eliminating any risk of sample cross-contamination.

4. Add your controls and samples

Adding the controls and diluted samples to the ELISA plate.

Prepare the deck of the ASSIST PLUS. Select and run the 4_E_Sample_Addition program to remove the blocking buffer and subsequently wash the plate. In our example, each sample is added to the ELISA plate in triplicate. The ASSIST PLUS pipetting robot uses the Repeat Dispense mode to transfer the triplicate samples into the plate, replacing the pipette tips before aspirating the next series of samples.

Figure 4: Adding the controls and diluted samples to the ELISA plate.

Tip:

Each pipetting step is done in exactly the same way, ensuring the reproducibility of the assay from row to row, and plate to plate.

5. Add the detection antibody

Adding the diluted detection antibody to the ELISA plate.

After incubation, prepare the deck and select program 5_E_Detection_Antibody. The addition of the detection antibody is performed automatically by the ASSIST PLUS. Incubate the plate again.

Figure 5: Adding the diluted detection antibody to the ELISA plate.

6. Add the enzyme conjugate

Adding the enzyme conjugate to the ELISA plate.

Set up the ASSIST PLUS deck. Select program 6_E_Enzyme_Conjugate, which includes the removal of the previous solution, the three washing steps, and the addition of the enzyme conjugate to the ELISA plate. The plate is ready for incubation.

Figure 6: Adding the enzyme conjugate to the ELISA plate.

7. Add the substrate

Adding the TMB substrate and incubating the plate until the color develops sufficiently.

Select and run program 7_E_Substrate. The ASSIST PLUS removes the previous buffer then washes the plate six times before adding the TMB substrate. Incubate the plate at room temperature until the color is sufficiently developed. The color of the solution changes from transparent to blue in wells where the samples have reacted with the antibodies. The color intensity is dependent on the sample concentration.

Figure 7: Adding the TMB substrate and incubating the plate until the color develops sufficiently.

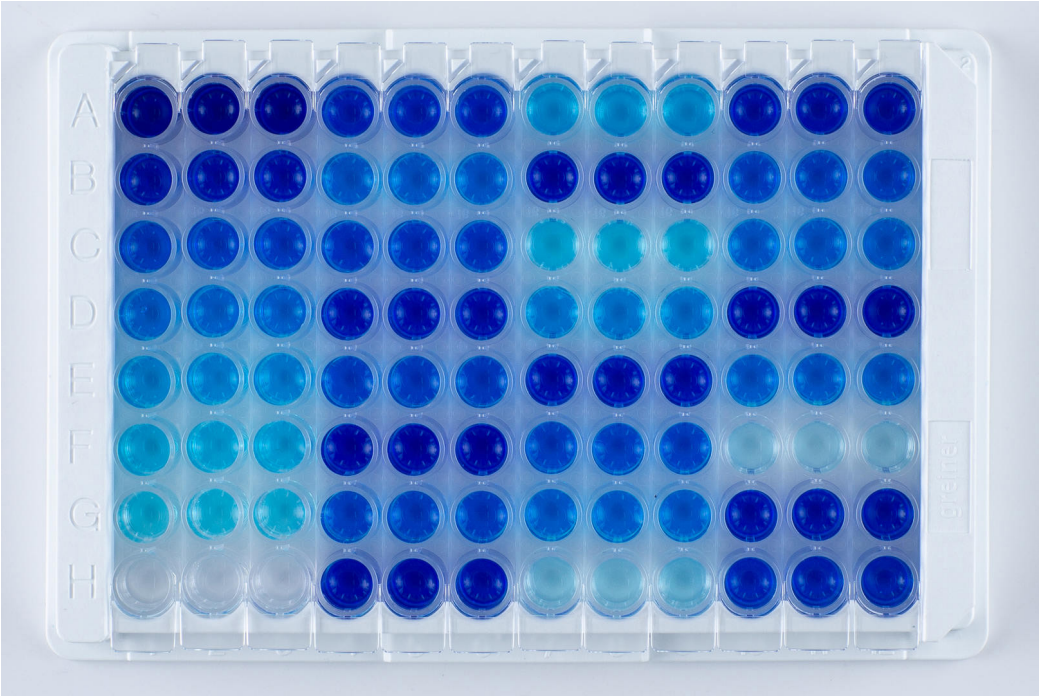


Figure 8: Example of a sandwich ELISA plate after incubation with TMB substrate, showing positive (blue) and negative (clear) reactions of the triplicate samples with the antibodies. The color intensity directly depends on the sample concentration.

Tips:

- The pipetting robot automatically processes the plate, regardless of the multiple and repetitive pipetting steps, freeing up time for you to concentrate on other tasks.
- The ASSIST PLUS tells you when to add the TMB substrate into the corresponding row of the reservoir, preventing the photosensitive substrate from being exposed to light for too long a period of time.

8. Stop the reaction

Adding the stop solution to the plate before detection.

Select and run the final program, 8_E_Stop_ Solution.

The ASSIST PLUS adds the stop solution to the sample triplicates in the plate; the color changes from blue to yellow in the wells where the samples reacted with the antibodies. The plate is now ready for detection.

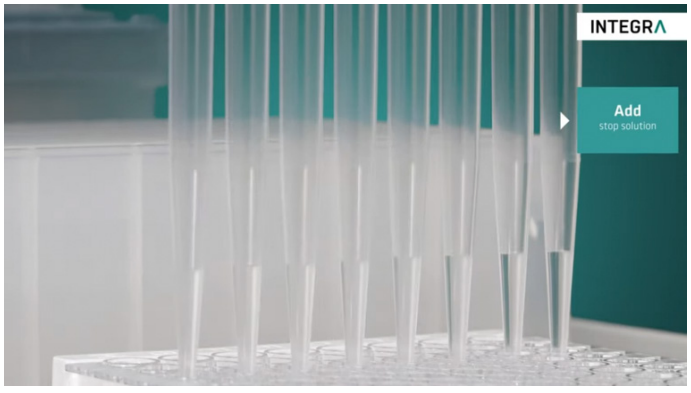


Figure 9: Adding the stop solution to the plate before detection.

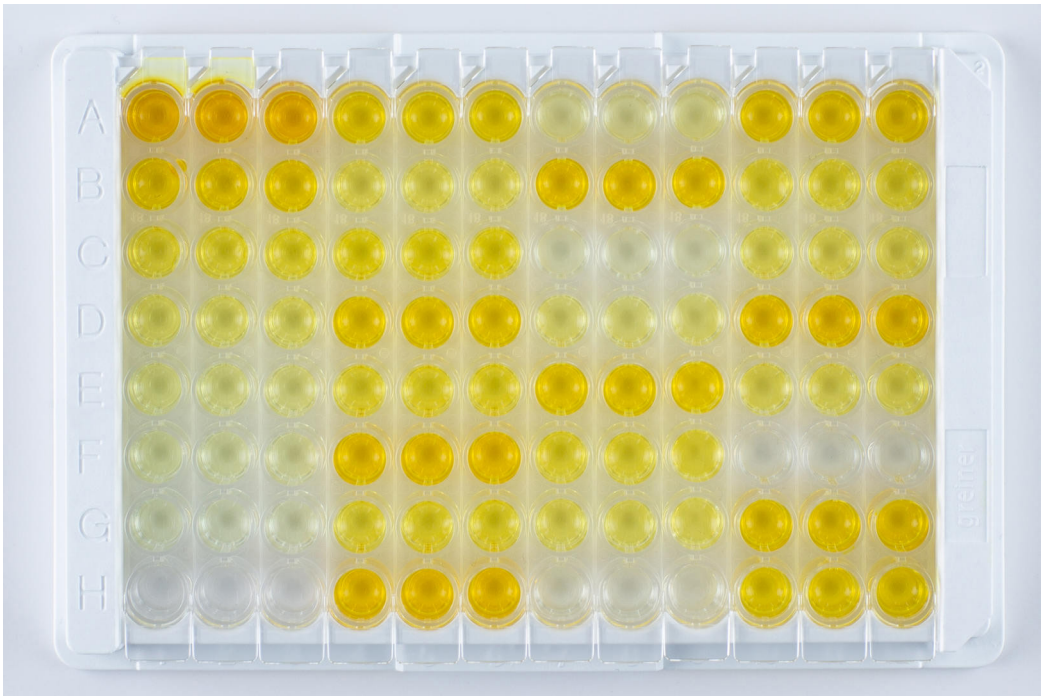


Figure 10: Example of a sandwich ELISA plate using a TMB substrate after addition of the stop solution, showing positive (yellow) and negative (clear) reactions of the triplicate samples with the antibodies.

Remarks

Partial plate

If your particular ELISA doesn't require processing of 96 samples, the ASSIST PLUS is able to work with any number of columns. Simply adapt the VIALAB program to fit your need.

VIALAB software

The VIALAB programs can be easily adapted to your specific labware and protocols.

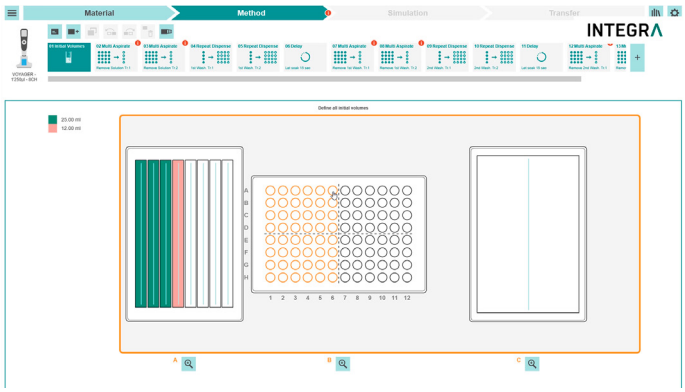


Figure 11: Partial plate and VIALAB software.

Conclusion

- ELISAs can be fully automated using the ASSIST PLUS pipetting robot, offering users increased walk-away time.
- Optimized pipetting settings and tip immersion, together with the use of low retention GRIPTIPS, guarantee the consistency and reproducibility of the ELISAs.
- Using the ASSIST PLUS pipetting robot allows various sample tube types and multiple reagents to be accommodated on the deck, for improved productivity and unrivaled flexibility.
- Automatic Tip Change avoids any assay contamination while using the Repeat Dispense and Multi Aspirate modes whenever possible speeds up the process.
- Thanks to the VIALAB software, the pipetting programs can be easily adapted to specific protocols and labware.

For more information and a list of materials used, please refer to our website.

3.2 Performing an ELISA with the VIAFLO 96 or 384 handheld electronic pipette

Enzyme-linked immunosorbent assays with the VIAFLO 96 and VIAFLO 384 handheld electronic pipettes

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay that is commonly used to quantify and detect peptides, proteins, antibodies or hormones. Well to well and plate to plate consistency and reproducibility are key success factors for ELISAs, which consist of multiple pipetting steps that are time-consuming and tedious to perform manually. Using the VIAFLO 96 or 384 handheld electronic pipette can vastly improve ELISAs. The 96 channel pipetting head makes the whole process faster, less error prone and much more reproducible compared to traditional single- or multichannel pipettes by transferring the samples and reagents into all 96 wells of the plate at the same time. The VIAFLO 96 and VIAFLO 384 electronic pipettes offer clear gains in productivity, consistency and reproducibility while remaining as easy to use as traditional handheld pipettes.

Key benefits

- The VIAFLO 96 and 384 allow easy and rapid simultaneous transfer of all samples for optimal productivity, reproducibility, and massive time reduction.
- All of the steps and settings needed to run the ELISA protocol can be saved on the pipette as a custom program, with easy to follow prompts to guide the user through the process.
- The pipetting heights are saved to avoid any crashes and scratching of the coated surface.
- For higher throughput assays, multiple plates can be quickly filled using the Repeat Dispense mode of the VIAFLO 96 or 384.

Overview of the sandwich ELISA steps and corresponding programs:

The following protocol shows an example set-up for performing a sandwich ELISA using the VIAFLO 96 or 384 handheld electronic pipette together with a three position stage. In this protocol, position A is dedicated to the GRIPTIPS box and the liquid waste reservoir, position AB to reagent reservoirs containing the various reagents and washing solutions required, and position B to the ELISA plate. A 96 channel pipetting head (10-300 µl) is used together with 300 µl sterile, filter, low retention GRIPTIPS. Customized VIALINK programs are provided for performing a sandwich ELISA with the VIAFLO 96 or 384.

Note: The VIALINK programs provided can be easily adapted to any other ELISA type, e.g. direct, indirect or competitive ELISAs, by just adding or removing steps from the protocols supplied.

Step-by-step procedure

1. Coat the ELISA plate

Adding the capture antibody to coat the ELISA plate.

Place the capture antibody, prediluted in the coating buffer, into a 150 ml automation-friendly reagent reservoir. Select and run the VIALINK E_REAGENTS program, then simply follow the instructions on the pipette. Pipette 100 µl of the capture antibody into the ELISA plate and then incubate it.

Figure 1: Adding the capture antibody to coat the ELISA plate.

Tips:

- A Z-height limit is defined to ensure an optimal tip immersion depth, preventing both air entering into the tips during the aspiration step and the pipette tips from touching the bottom of the plate. We also recommend setting the Tip Align support strength to 3 for this application.
- To increase your throughput, the Repeat Dispense mode of the VIAFLO 96 or 384 electronic pipette can be used to rapidly prepare several plates at once. The VIALINK program E_REAGENTS_3 PL is an example of a multiple plate filling protocol.

2. Block the plate

Blocking the ELISA plate's non-specific binding sites.

Figure 2: Blocking the ELISA plate's non-specific binding sites.

Select the VIALINK program E_REAGENTS to remove the coating buffer from the plate, then use the E_WASH program to wash the plate three times with 200 µl of washing buffer from a 300 ml automation-friendly reagent reservoir. Firmly blot the plate against clean paper towels. To block the ELISA plate, select the corresponding E_BLOCK program and transfer 300 µl of the blocking buffer from a 150 ml automation-friendly reservoir into the plate before incubation.

Tips:

- All wells are treated simultaneously and in the same way. This ensures not only the well to well consistency of the assay, but also the reproducibility from plate to plate.
- The Z-height is set 1 mm above the bottom of the plate, preventing the tips from scratching the coated surface, which can lead to inaccurate or inconsistent assay results.

3. Add the controls and samples

Adding the diluted samples and controls to the ELISA plate.

Figure 3: Adding the diluted samples and controls to the ELISA plate.

Select and run the E_BLOCK program to remove the blocking buffer, then wash the plate using the E_WASH program. In the current example, the controls and samples are dispensed into a 96 well plate. The E_REAGENTS program allows rapid, simultaneous transfer of all samples and controls into the ELISA plate before incubation.

Tips:

- Adding all the samples at once ensures optimal productivity and reproducibility of the ELISA. If your samples are stored in microcentrifuge tubes, using a VOYAGER 8 channel 300 µl adjustable tip spacing pipette allows you to quickly reformat them from the tubes to the plate at the simple touch of a button, while reducing transcription errors when compared to a single channel pipette.
- ELISA buffers often contain surfactants, such as Tween® 20. Using low retention GRIPTIPS reduces the residual volume in the tips for maximum sample recovery.

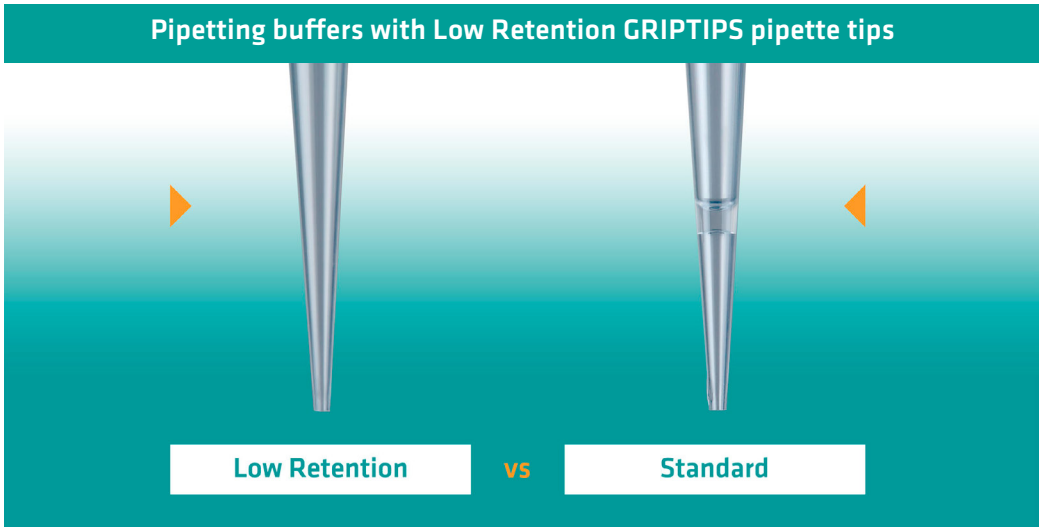


Figure 4: The image highlights the advantages of using low retention GRIPTIPS (left) vs. standard GRIPTIPS (right) when pipetting buffers containing surfactants.

4. Add the detection antibody

Adding the diluted detection antibody to the ELISA plate.

After incubation, select the E_REAGENTS program to remove the sample buffer, then use E_WASH to wash the plate three times. Place the detection antibody into a 150 ml automation-friendly reagent reservoir on position AB. Run the E_REAGENTS program to rapidly transfer the detection antibody into the ELISA plate before incubation.

Figure 5: Adding the diluted detection antibody to the ELISA plate.

5. Add the enzyme conjugate

Adding the enzyme conjugate to the ELISA plate.

Repeat step 4 to add the enzyme conjugate. Incubate the plate.

Figure 6: Adding the enzyme conjugate to the ELISA plate.

6. Add the substrate

Adding the TMB substrate and incubating the plate until the color develops sufficiently.

Remove the buffer then thoroughly wash the plate six times before adding 100 µl of TMB substrate to the ELISA plate. Incubate the plate at room temperature; the color of the solution changes from transparent to blue in wells where the samples have reacted with the antibodies. The color intensity depends on the sample concentration.

Figure 7: Add the TMB substrate and incubate the plate until the color develops sufficiently.

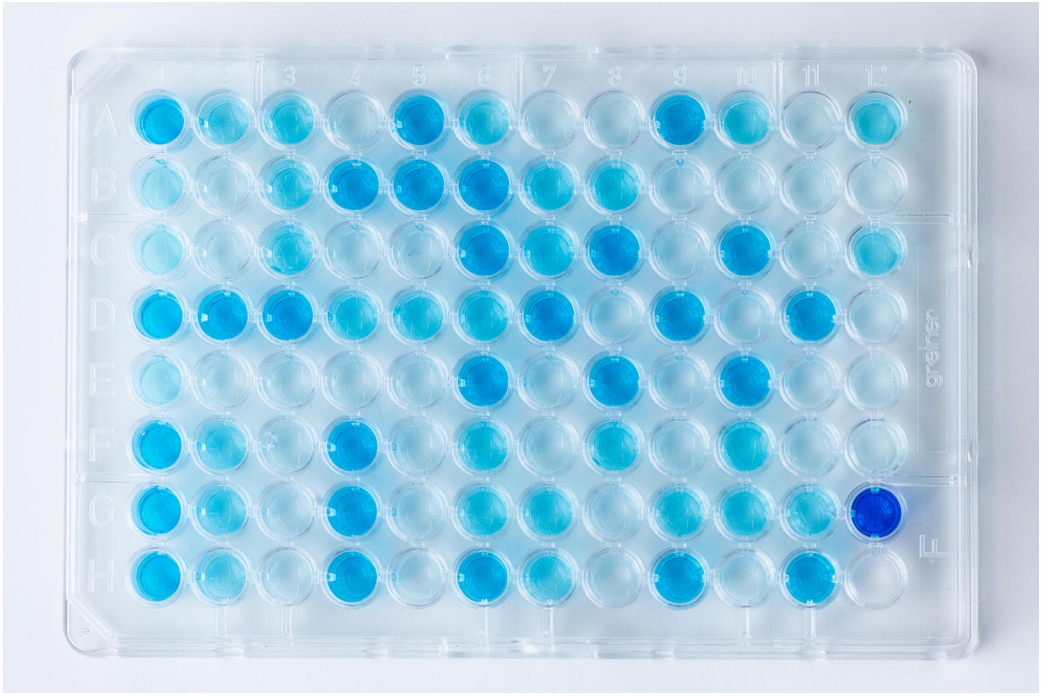


Figure 8: Example of a sandwich ELISA plate after incubation with TMB substrate; positive (blue) and negative (clear) reaction of the samples with the antibodies. The color intensity directly correlates with the sample concentration.

7. Stop the reaction

Adding the stop solution to the plate before detection.

Use the VIALINK E_REAGENTS program to add the stop solution to the plate; the color changes from blue to yellow in wells where the samples have reacted with the antibodies. The plate is now ready for detection.

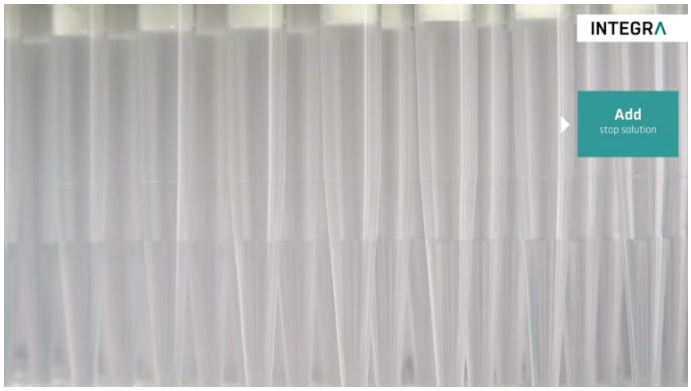


Figure 9: Adding the stop solution to the plate before detection.

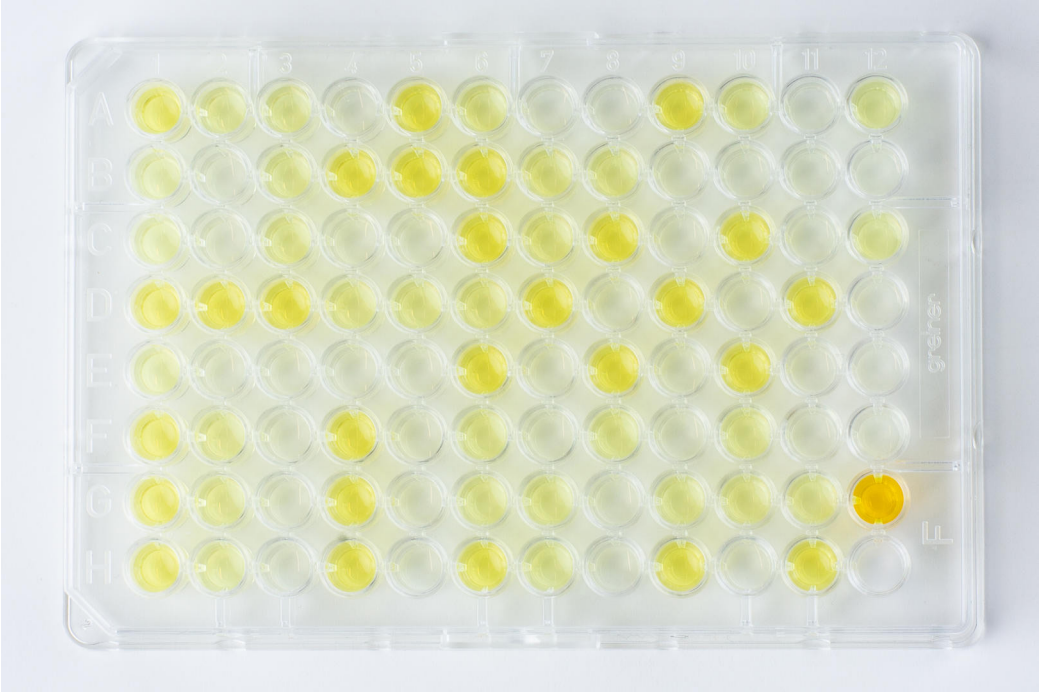


Figure 10: Example of a sandwich ELISA plate after incubation with TMB substrate and addition of the stop solution; positive (yellow) and negative (clear) reaction of the samples with the antibodies. The color intensity directly correlates with the sample concentration.

Remarks

Partial plate

If your particular ELISA doesn't require processing of 96 samples, the VIAFLO 96 or 384 is able to work with any number of tips loaded, giving you the benefit of simultaneous and precise dispensing of a smaller number of samples.

Automation

The instrument can also operate on its own, reducing user interaction, which in turn improves ergonomics and reproducibility. This also makes the VIAFLO 96 or 384 ideal for use in tight spaces, such as under a laminar flow cabinet.

Conclusion

- The VIAFLO 96 and VIAFLO 384 electronic pipettes allow all wells of a 96 well plate to be treated at the same time, offering optimal consistency and reproducibility of results from well to well and plate to plate.
- Thanks to their unique operating concept, the VIAFLO 96 and VIAFLO 384 electronic pipettes are as easy to use as any traditional handheld pipette.
- Optimized pipetting settings ensure easy and fast sample and reagent transfers, while avoiding the risk of damaging the coated surface of the plate.
- The VIAFLO 96 and VIAFLO 384 electronic pipettes are adaptable to your needs. Work with a reduced number of tips for partial plate processing, or automate processing for improved ergonomics in tight spaces, such as a laminar flow cabinet.

For more information and a list of materials used, please refer to our website.

CHAPTER 4:
Customer testimonials and product stories

Our range of innovative liquid handling products has helped many laboratories to achieve success with their ELISA projects, improving lab throughput and meeting their exciting research goals. But don't just take our word for it! Here is a small selection of real-life experiences from a few of our valued customers, along with a key product story, which illustrate why INTEGRA Biosciences' pipetting solutions and labware are the best choice for your ELISA workflow.

4.1 VOYAGER adjustable tip spacing pipette accelerates immunology research at the University of Basel

The Department of Biomedicine at the University of Basel specializes in a number of key research areas, including neurobiology, infection and immunity, cancer, stem cell biology and regenerative medicine. Korcan Ayata is a member of the gastroenterology group – led by Professor Doctor Jan Nies – which focuses on researching mucosal immunology and gastroenterology. The group is using INTEGRA's VOYAGER adjustable tip spacing electronic pipette to increase the efficiency of their workflows and reduce pipetting errors.

The role of immune cells

The immune system plays a critical role in everyday life and disease, and Korcan's group is specifically interested in the role of immune cells in inflammatory conditions, such as inflammatory bowel diseases (IBD) and colitis-associated colorectal cancer. He explained: "We mainly work with human patient samples and transgenic mouse models, looking at how monocytes enter the tissue and differentiate into macrophages to have pro- or anti-inflammatory effects during the disease course. We are primarily looking for the cytokines and cytokine receptors expressed by macrophages and epithelial cells, and studying their interactions."

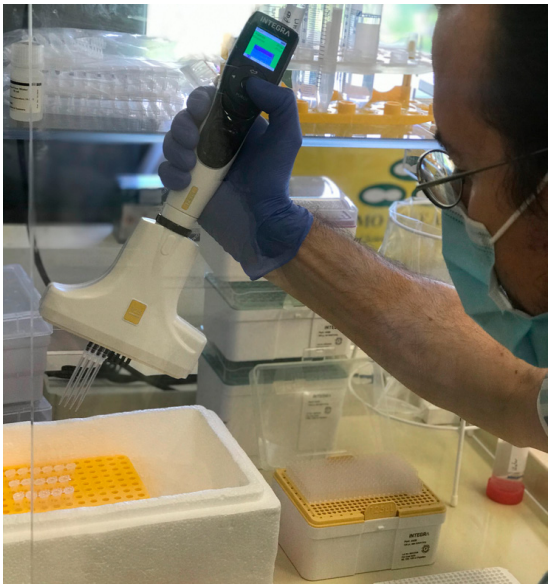


Photo courtesy of the University of Basel

Increasing workflow efficiency

The group's normal workflow involves extracting RNA from samples and converting it to cDNA, before conducting qPCR, or running ELISAs for serum samples. Korcan continued: "Most of our samples come from the Swiss IBD cohort, and we're usually analyzing biopsies and blood, looking at criteria such as electrolytes, minerals, trace elements or antibodies. Our work involves transferring many samples between different well formats. Previously, we were conducting this work with single channel pipettes, which was a laborious and error-prone process."

VOYAGER capabilities are second to none

The VOYAGER adjustable tip spacing electronic pipette has proven perfect for the group's highly manual workflow, as the automatic adjustable tip spacing allows users to transfer multiple samples simultaneously, reducing transfer steps and pipetting errors. "We looked at a range of different pipetting systems, and the VOYAGER really stood out, as nothing compared to its adjustable tip spacing abilities. We already had a number of single- and multichannel VIAFLO electronic pipettes, which are well liked in the department, and this made it easier to integrate the VOYAGER into our workflow."

Photo courtesy of the University of Basel

"We mainly use the VOYAGER in qPCR setup, using 9 to 13 mm spacing to transfer samples from 1.5 ml tubes to eight tube strips. After the reverse transcription reaction, we transfer the cDNA to 384 well qPCR plates using 4 mm spacing. The repeat dispensing function is really beneficial too, allowing us to distribute different qPCR master mix solutions from 1.5 ml tubes to 384 well plates. We're also using the VOYAGER in cell culture and ELISA applications, for the addition of reagents from 1.5 and 2 ml tubes to 24, 48 and 96 well plates, plus the transfer of samples between different plate formats. The GRIPTIP pipette tips are the perfect partner to our VOYAGER; the tight connection between pipette and tip ensures nothing is loose or leaks, and this means less errors are made. Overall, the VOYAGER saves us a huge amount of time compared to using manual pipettes, and our throughput has significantly increased since using it – it really helps to accelerate our research."

4.2 “How did we manage without a 96 channel pipette?”

Researchers at the University of North Texas (UNT) discovered INTEGRA’s VIAFLO 96 electronic pipette by accident – and it has transformed their lab! The team is now using the system to increase the throughput of their ELISA, Luminex and flow cytometry workflows, as well as to improve the reproducibility of their results.

The Applied Physiology Laboratory at UNT focuses on research in two key areas – physiologic and immunologic consequences of weight change, and using nutritional countermeasures to maximize immune health after exercise – and performs analysis and testing for other research groups in related fields. Prof Brian McFarlin explained: “We conduct a lot of multiplexed analysis of protein and RNA biomarkers, and this requires precise pipetting. For one study, we were performing 50 flow cytometry preps per blood sample, and we had over 2000 samples. The work was only between one graduate student and myself, and we quickly decided that we needed a solution to reduce the amount of manual pipetting we were carrying out!”

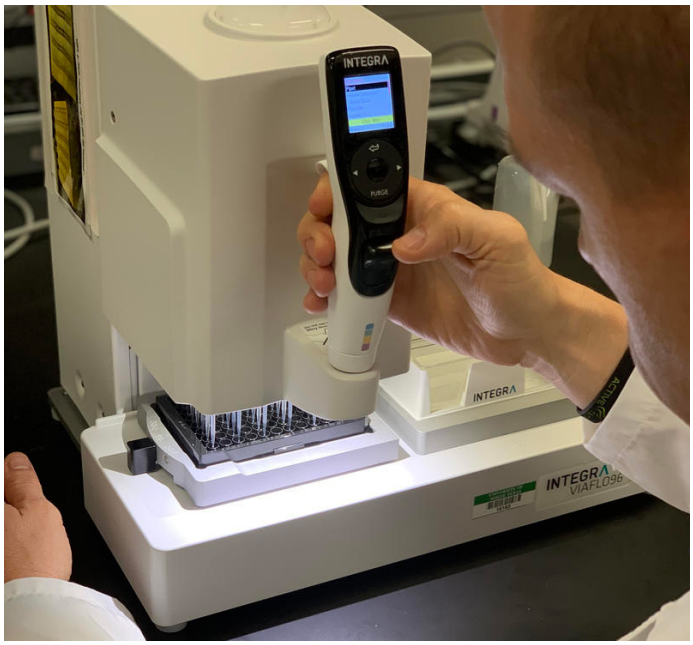


Photo courtesy of University of North Texas

Brian continued: “We looked around the market, but the solutions on offer were fixed volume systems, meaning we would need up to five separate platforms. I first learned about the VIAFLO 96 multichannel pipette at a biomarker conference before it was even available and, after seeing its ability to change modules to have different volumes, I knew it was just what I needed. The conference’s demo model was packed up and sent to me for a trial, and it’s been in constant use ever since!”

The VIAFLO 96 offers an electronic solution that is as easy to use as a traditional handheld pipette, with improved reproducibility and throughput. “It’s been an absolute lifesaver for our work; we use it for sample transfer from storage tubes to reagent plates and reagent additions, especially for running Luminex® assays and ELISAs. Transferring the assays from handheld pipettes on to the VIAFLO 96 was seamless; INTEGRA was able to support us by writing protocols for specific applications and assisting in the set-up of these programs. The team showed us how to use the VIALINK pipette management software which is very intuitive, and now it’s easy for us to tweak a volume here or there on the protocols. We have since added

VIAFLO electronic pipettes and VOYAGER variable tip spacing pipettes – which are great for our plate transfer work – to our collection. We also have an ASSIST that we use to automate some of our standard curve development, and we're now looking to purchase an ASSIST PLUS pipetting robot for our workflow as well."

"We now exclusively use INTEGRA pipettes for all of our liquid transfer work because of the benefits they offer; not only has our throughput increased tremendously, saving a lot of time, but the performance of our assays has improved. Our results are much more reproducible and there is a reduced risk of error – it's revolutionized our workflow," Brian concluded.

4.3 Making COVID-19 ELISA testing safer and easier with convenient buffer removal

The COVID-19 pandemic led to the development of a number of novel RT-PCR-based tests for the detection of the SARS-CoV-2 virus in patients. As the situation progressed, a need emerged for serology tests to evaluate the immune response of patients, as well as to assess immunity in the general population. These immunoassays became critical in allowing movement restrictions to be eased and for preventing subsequent peaks in infections.

SARS-CoV-2 ELISA tests

Most serological tests are based on sandwich ELISA techniques to detect antibodies against the virus in patients that have recovered from COVID-19. The protocols for these ELISAs require several aspiration steps to remove buffers after incubation and washing, and the safest way to perform this is using an instrument such as our VACUSIP portable aspiration system.

VACUSIP for easy aspiration of small volumes of biological liquid waste

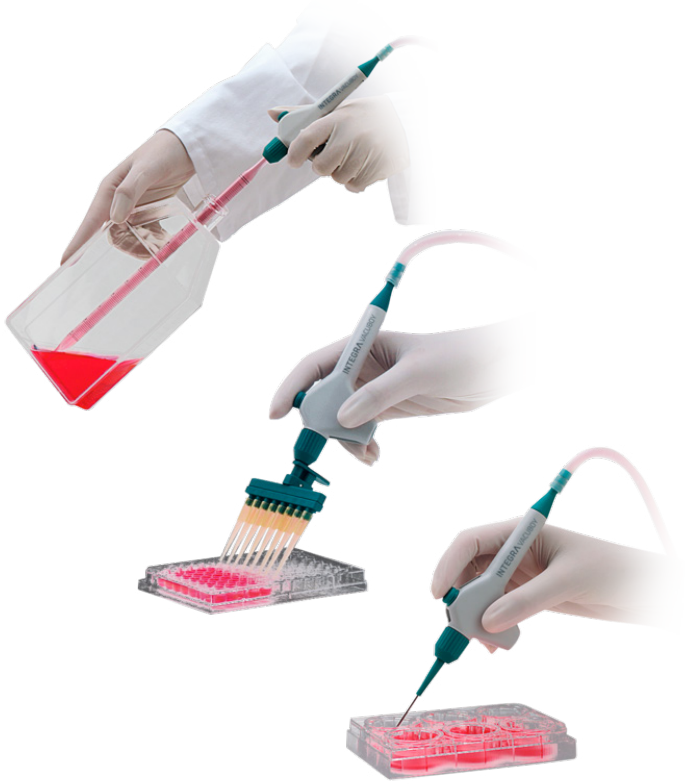
Why use an aspiration system?

There are many advantages to using an aspiration system to remove buffers from samples, especially for 96 well plate formats, where there are multiple wells to process. These include:

- Safer, faster and more convenient waste removal than with a multichannel pipette
- Aspirated liquid waste goes directly into the collection bottle, avoiding the risk of spillages or exposure to biohazardous waste during unnecessary liquid transfer
- Pooled waste can be safely and easily autoclaved, chemically deactivated, or transferred to a larger container

Highlights include:

- **Simple to operate** – consistent and reliable processing of even small liquid volumes via pressure-controlled hand operation
- **Ready to use** – an out-of-the-box solution with integrated silent vacuum pump and optional rechargeable battery
- **Compact design** – with a small footprint and minimal cables/tubing, ensuring it fits onto any bench or into any safety cabinet
- **Safe to use** – with a hydrophobic filter to protect against contamination, and a fully autoclavable liquid path for easy decontamination
- **A choice of waste containers** – including single-use PP and reusable glass bottles
- **Adaptable to your needs** – with a large choice of adapters, including 8 channel adapters for 96 well plates



Why choose the VACUSIP?

The VACUSIP is a convenient and affordable tool for liquid waste removal, helping to increase productivity for ELISA workflows. Its flexibility, ease of use and portability mean that it can be used in a variety of other biochemical and cell-based methods, from high throughput screening and immunofluorescence studies to cell viability and virus entry inhibition assays. Ultimately, our VACUSIP portable aspiration system can be used by anyone, anywhere, speeding up workflows and supporting a range of applications.

CHAPTER 5: Conclusion

By now, you should have all the information you need to become an ELISA expert and run successful experiments in your lab, whatever the application. We hope you found this eBook helpful but, if you'd still like to learn more about this interesting topic, we have a wealth of articles on our website. Whatever your ELISA requirements, INTEGRA Biosciences is always available to answer your questions and provide you with the best workflow solutions.

CHAPTER 6: References

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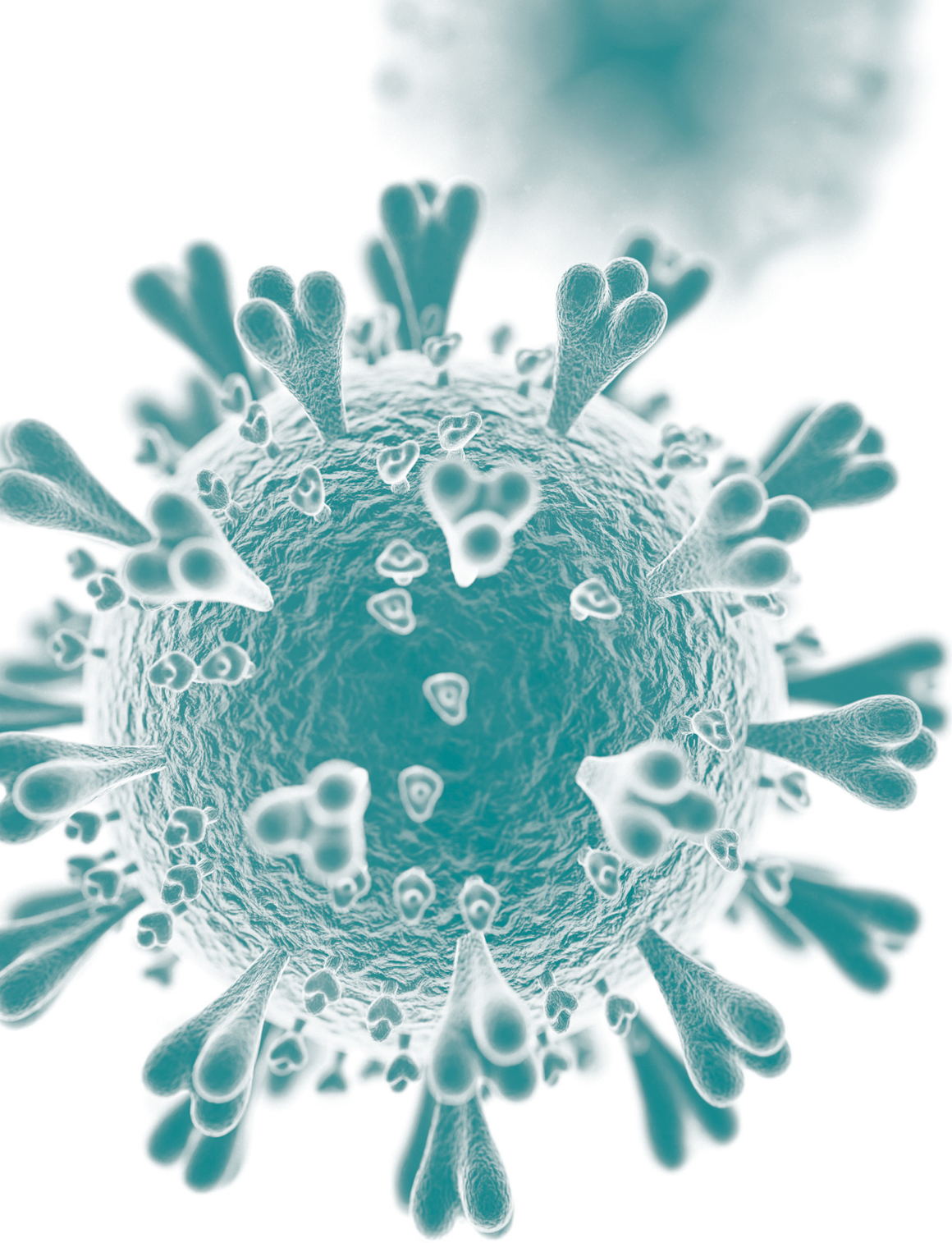

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