

10 top tips

for lateral flow assay (LFA) development

When designing your own lateral flow assay, there are several variables to consider: with the choice of detection reagents, assay materials, and manufacturing methods all influencing the accuracy and reliability of your test.

Use this checklist to review ten essential considerations when developing your LFA, which will give you a deeper insight into lateral flow assays to streamline your development process.

1

Know your target molecule

The biology of your target molecule will be the crucial factor in developing the biology of your test. Is it a large or small molecule? What antigen or other ligand will bind specifically to your target but not other molecules? The kinetics of the binding reactions between your analyte and the antibodies used in the test will determine your assay's optimal flow time, which will influence your choice of nitrocellulose (NC) membrane. A keen understanding of the biology of your target molecule will shape every choice in the design of your LFA.

2

Know your sample type

The biological source of your sample will influence the design of your test and help you determine which materials you should use in your assay.

The type and volume of liquid samples (e.g., blood, urine, or saliva) are two parameters that will help you choose which sample and absorption pads are most appropriate for your test.

[Start developing your lateral flow immunoassay with our diagnostic services](#)

Pick your test type

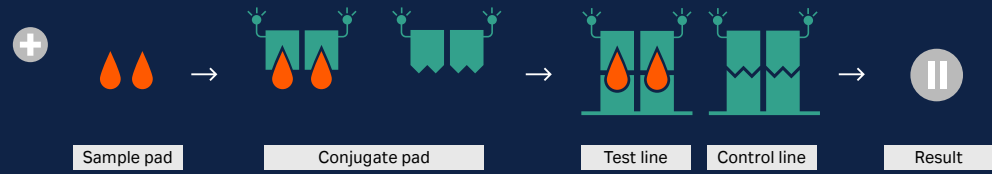
The size of your target molecule will be a key factor in determining the type of LFA you choose.

There are two approaches to lateral flow assay tests: sandwich and competitive.

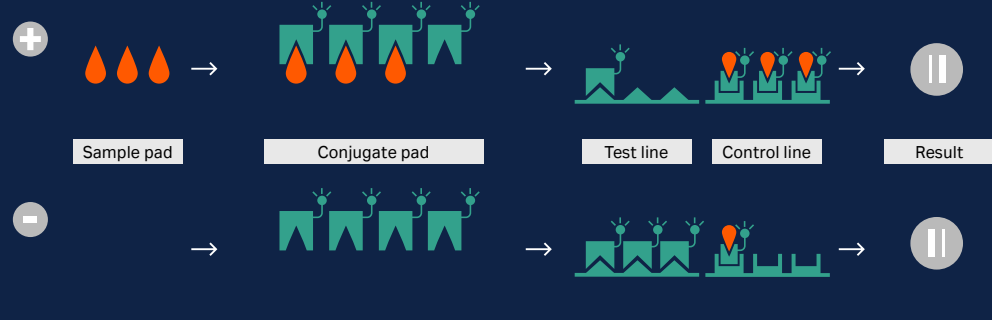
Sandwich assays rely on the binding of two separate antibodies to different regions of the same analyte molecule, making them well-suited for detecting high molecular weight analytes.

Competitive assays can detect smaller molecules, such as mycotoxins or cortisol, and larger analytes, such as insulin.

Sandwich



Competitive



Target Label-conjugate antibody Imm. captured antibody

Fig 1. Comparison of sandwich and competitive lateral flow assay design.

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Materials matter!

Choosing the right sample pad, conjugate release pad, and nitrocellulose membrane are critical to developing sensitive and reliable lateral flow assays.

Sample pads are commonly made from either cotton or bound glass fiber. Cotton has a lower wicking rate and is suitable for low sample volumes. Bound glass is appropriate for separating red blood cells (RBCs) from plasma. Using glass fibers with a higher diameter can also prevent hemolysis, the bursting of RBCs that turns plasma red and prevents accurate assay readouts.

It's important to determine that the conjugate release pad you choose will store the detection reagents without damage or aggregation over the shelf life of the test strip. Choosing hydrophilic materials with an open structure can support efficient liquid flow through your immunoassay.

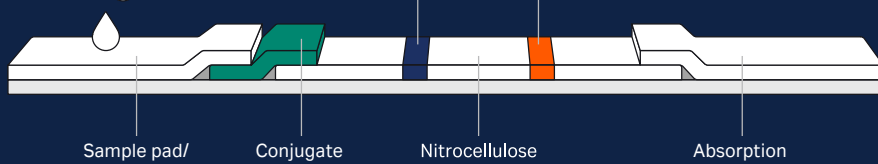


Fig 2. Lateral flow assay components

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Test membrane flow rates

Nitrocellulose (NC) membranes are one of the most critical components of a lateral flow assay; their interactions with your sample will directly affect test performance.

To help ensure a good balance between assay speed and sensitivity, try testing the flow rate of your sample with a range of different NC membranes. The membrane you choose should allow adequate time for the interaction of analyte and reagents at the test and control lines but also provide results within your chosen timeframe.

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Test, and test again!

NC membranes contain surfactants that could denature analyte proteins and affect antibody-antigen binding reactions. So, testing your detection and capture reagents on a range of membranes with different surfactants before you decide which is the best membrane for your test is a good practice.

[Find out more about membrane selection for lateral flow immunoassays](#)

Monoclonal or polyclonal antibodies?

The detection and capture of antibodies in your lateral flow assay can be polyclonal or monoclonal.

Monoclonal antibodies are generally preferred as capture antibodies, as they have minimal batch-to-batch variability and are less prone to problems in supply than polyclonal antibodies.

Monoclonal antibodies are also the preferred detection reagents, as their recognition of a single epitope provides a consistent binding reaction with minimal cross-reactivity. Polyclonal antibodies can be unsuitable for detection as their recognition of multiple epitopes might lead to antigen cross-linking that can prevent the analyte from entering the NC membrane.

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Maximize your assay sensitivity

After choosing antibody binding regions on an analyte, the next step involves screening the set of potential antibody clones and measuring the amount of antibody-antigen complex formed after incubation. But what's the best way to do this screening step?

- Enzyme-linked immunosorbent assays (ELISAs) are a common way of screening antibodies. However, this assay's long duration means that it cannot identify antibodies that bind quickly to the target antigen, which is an essential requirement for high-sensitivity assays.
- Using optical biosensors, such as surface plasmon resonance (SPR) or bio-layer interferometry (BLI), can give you a more accurate measure of antibody binding kinetics and help you maximize the sensitivity of your lateral flow assay.

[Read our knowledge article, The path to high-quality immunoassay reagents.](#)

Understand your detection method

While fluorescent molecules and enzymes can work as labels, some of the most widely used are those that produce a direct, visible signal, such as gold nanoparticles and latex beads. As well as having good optical characteristics, gold nanoparticles have good antibody compatibility and long-term stability, important benefits when your tests might be stored for extended periods.

Your choice of label will determine your choice of reader — and vice versa. For visible labels, electronic optical readers can measure analyte concentration, with detectors available in benchtop and handheld formats. Recent developments have also led to systems that provide analysis software in convenient smartphone apps for visible detectors such as gold nanoparticles.

Whichever method you decide on, by testing your assay—reader and analyte concentrations of analyte, you can measure the lower and upper limits of quantification and obtain the reader's measuring interval. Take these limits into account to minimize the risk of under- or overestimating the sensitivity of your lateral flow assay.

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A final word on testing

As you've probably figured out by now, iterative testing is part of the development process. This can be time-consuming, but an experienced LFA services provider can help shorten the development cycle and support you with efficient methodologies, infrastructure, and knowledge gained from experience.

For more guidance on generating reliable lateral flow assays, read our blog on [custom lateral flow assay development](#).

To discuss any challenges you might be facing with lateral flow assay development, our diagnostics specialists are ready to help you.

Ready for the next step?