White Paper

MERCK

Overcoming the challenges that delay development of your lateral flow assay

How fast-access support services can get your assay launched sooner

Novice and expert *in vitro* diagnostics (IVD) manufacturers agree that developing, optimizing, and launching a lateral flow assay (LFA) test is never routine nor easy. Although the plethora of test strips that popped up during the COVID-19 pandemic might give the illusion these assays are simple, developers know—or soon learn—that development of those streamlined plastic cassettes poses key technical challenges that can delay assay launches.

Lateral flow test strips are complex devices (**Figure 1**), and designing and making them requires significant knowledge about diverse fields including biochemistry, immunochemistry, membrane engineering, and nanomaterials. Experience suggests an assay team is likely to encounter one or more development challenges for which external expertise might be beneficial.

This white paper offers strategies to address these challenges for companies or researchers wanting to convert an existing IVD to a lateral flow design or to develop a new LFA. This includes those familiar with lateral flow diagnostics who don't have the complete in-house expertise to develop a test, as well as those looking for on-demand technical support to ensure a rapid path to commercialization.

The Basic Strip Design

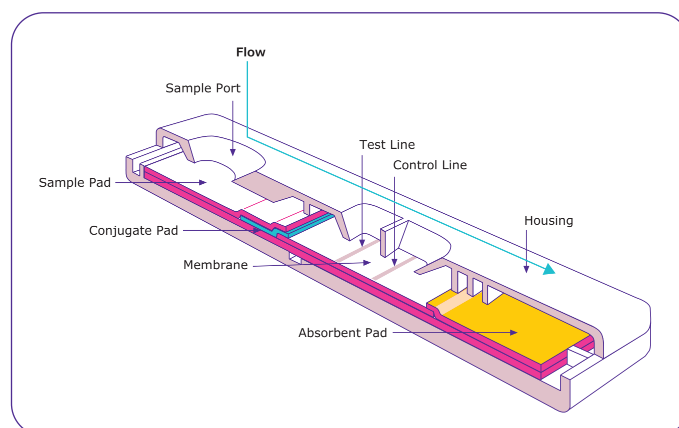

Figure 1. A basic lateral flow assay strip design.

Among the numerous areas in the process where support might be needed, these are the top three consistent challenges:

1. Chemical challenges
2. Physical challenges
3. Membrane materials and assembly

Variation can exist in the chemicals, the materials, the way the device is assembled, the stability of the test, and how the materials interact with the packaging in which they’re housed. It is the sum of all these sources of variation that will determine the strength or weakness of an LFA.

The Life Science business of Merck operates as MilliporeSigma in the U.S. and Canada.

1. Chemical challenges

Manufacturing starts with aqueous reagent preparations, which are applied to the porous materials and dried. The key reagents are spatially separated in a finished test strip and must remain this way, and dry, throughout the shelf life of the device. Once they are wetted with a sample, they must solubilize and be biochemically active as soon as the sample reaches them. The two main chemical challenges involve ensuring both mobile and immobile reagents are properly distributed throughout the LFA before and during a test run.

Mobile reagents may be unevenly distributed during a test run

While the capture reagents of the test and control lines need to be immobile, many other reagents move along the test strip with the sample. Mobile reagents on the membrane include unbound surfactants, excess blocking agents, and any soluble components that are applied with the capture reagents, such as buffers, surfactants, or salts. Within the conjugate pad, the detector particles, including any buffer salts or solubilization agents, are mobile. Within the sample pad, soluble additives are mobile.

> The concentration profile of any mobile reagent is dynamic and constantly changing at any given location as the test strip runs.

For example, the distribution of surfactant is uniform across the surface of an unused membrane (**Figure 2**). As a sample migrates through the test strip, it concentrates the surfactant directly behind the leading edge of the liquid, as much as tenfold. Understanding the distribution of each reagent as it moves along the test strip is particularly important for quantitative tests. The ability to form an immunocomplex at the test and control lines is determined, in part, by the chemical background in which the reagents are coming into contact with each other.

Surfactant in the Membrance

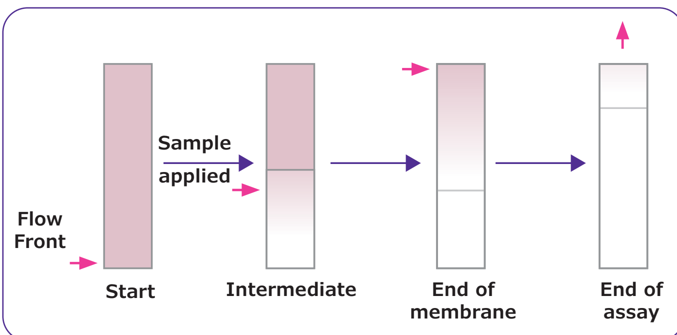

Figure 2. Surfactant movement in the membrane.

Reagent concentrations may also vary through the depth of the membrane

It can also be challenging to ensure that the distribution of soluble chemicals at the test and control lines is uniform through the depth of the nitrocellulose membrane. Variations in the microenvironment surrounding the protein immobilized along the test line can have an impact on the ability to generate a signal in specific regions of the test line.

Where the various reagents are loaded onto lateral flow test strips is complex and must be optimized—they can't simply be copied from another assay format (**Figure 3**).

- **Test and control lines** - Capture molecules are added in a weak buffer. This might include a free protein, like BSA, which improves adsorption of proteins to the nitrocellulose membrane when antibody concentration is low.
- **Conjugate pad** - A weak buffer and carbohydrates act as a release agent. An aqueous sample dissolves these reagents, releasing the detector particles into the actively moving fluid.
- **Sample pad** - Contains buffer, salts, surfactants to ensure uniform particle movement, carbohydrates, and free protein. Other additives are sometimes used, including those that break down the mucopolysaccharides in saliva. This mixture modulates the chemical composition of the sample, drives flow of the sample, and supports formation of immunocomplexes.
- **Absorbent pad** - This is untreated and typically made of paper.

Location of Added Chemistries

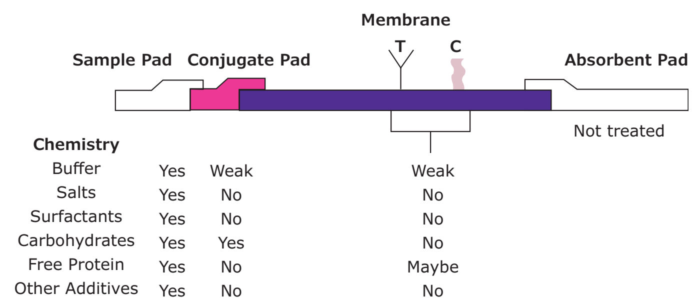

Figure 3. Location of added chemicals.

MilliporeSigma helps you choose appropriate reagents

We apply a set of rules for sourcing the best available agents for your assay based on decades of experience. These rules include:

- Recipes that take into account water evaporation. We recommend using solutes at concentrations no greater than what's necessary for consistent performance and to prevent denaturing antibodies.
- Choosing the highest quality reagents available, even for common ingredients like sodium chloride. Beware of potential contaminants and lot-to-lot variation, especially for antibodies, other biological components, and custom-synthesized molecules.
- Using the highest quality water available (e.g., ≥18.2 MΩ resistivity) and avoiding reverse osmosis or distilled water.

2. Physical challenges

Many physical attributes and manufacturing steps affect sample flow. Here are a few of the challenges you may encounter when developing the physical structure of your LFA.

- **Choosing the correct material properties**

The sample pad, conjugate pad, membrane, and absorbent pad are porous, each with a specific pore size. The bed volume—the volume of liquid that can be absorbed by each one—is dependent on the material's thickness and the amount of solids it contains.

- **Improper housing design causes lack of sensitivity or specificity**

The design of the plastic cassettes has an important role in determining the flow path of the liquid and the ability of the sample to migrate from one end of the strip to the other. Improper design can lead to a lack of sensitivity or specificity at the test and control lines.

- **Sample make-up can adversely affect reaction kinetics**

Viscous liquids (e.g., serum) will flow more slowly than more fluid liquids (e.g., urine), affecting the kinetics of the reaction at the test line and, thus, the specificity of test results. Samples that contain more solid particles or, in the case of whole blood, a large cell load are said to have a high particle load and can clog porous materials.

- **Misalignment of materials can disrupt flow path**

If the design or misalignment of materials prevents contact between two adjacent materials, the continuous flow path will be disrupted.

- **Misassembly can lead to flow variability**

Contact and consistent overlap between the materials, as well as compression within the housing, must be maintained to minimize flow variability from strip to strip.

- **Cutting can damage fragile materials**

Proper cutting equipment protects fragile porous materials while cleanly cutting the hard plastic adhesive card on which they're mounted.

- **Improper overlap of components**

The configuration of components is important in terms of the overlap of each component and where to apply compression. Long overlaps can create dead space that slows down sample migration, leading to slower conjugate release and lower sensitivity (**Figure 4**). Conversely, minimal overlap between the sample pad and conjugate pad, and the conjugate pad and the membrane, leads to more uniform flow through the entire conjugate pad and quicker release of the conjugate.

Configuration Effects on Flow Path

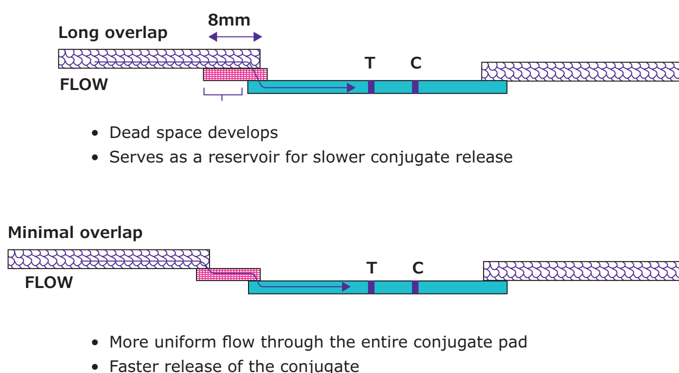

Figure 4. Configuration effects on flow path.

Excessive compression can crush membranes

Compression is required at all overlaps to ensure contact is maintained and to prevent curling of the sample pad and conjugate pad, which can interfere with contact with other components (**Figure 5**). Minimal overlap requires three compression bars. Since the membranes are porous, they can be crushed if too much pressure is applied and even create gaps between materials that come before a compression point.

Configuration Affects Where Compression is Required

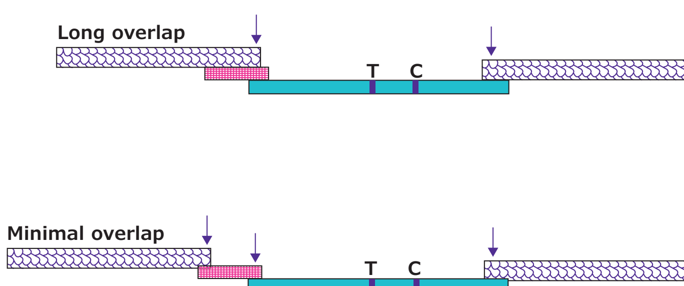

Figure 5. Ideal placement of overlaps and compression bars.

MilliporeSigma addresses the physical challenges of creating and assembling an LFA

From choosing the ideal physical components, to cutting membranes and assembling a test strip, there are multiple challenges MilliporeSigma can help you overcome. Most of this is science, while some is the application of strategies hard-won through experience. Working with a partner who has done this for decades can be invaluable to save you time.

3. Membrane materials and assembly

Choosing membrane materials, which can be made of nitrocellulose, cellulose and synthetic fibers, or glass fibers, is critical to the function of an LFA. Membrane materials affect a sample's capillary flow time (CFT) and, as such, can alter an assay's sensitivity and specificity.

Improper bed volume can limit the extent of sample analysis

Bed volume is the volume of liquid each material can absorb. It needs to be optimized since only the volume of liquid that moves beyond the test line will be analyzed. While all the liquid remaining behind the test line is necessary to ensure liquid moves to the absorbent pad, any antigen remaining behind the test line does not contribute to the signal. For example, while the total bed volume of an assay may be 105 µL, the volume that passes the test line would be less than 60 µL (**Figure 6**).

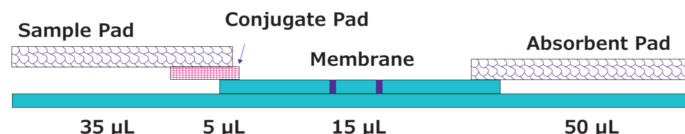

Figure 6. An example of bed volumes of different LFA test strip materials.

Insufficient test strip width can adversely affect assembly

Even the best cutters will damage a portion of the edges of a test strip. For quantitative assays, where you're scanning the test line for signal uniformity, these damaged edges need to be kept away from the scanned zone. As the strip narrows, the number of fibers holding the sample and absorbent pads to the membrane may be insufficient to prevent materials from shifting during cutting.

The biological context of the test, and its necessary sensitivity, need to be kept in mind. Sourcing biological critical raw materials will dictate the sensitivity, specificity, and performance of your final assay.

MilliporeSigma optimizes membranes for LFAs

MilliporeSigma manufactures five Hi-Flow™ Plus membranes, providing a range of choice to optimize bed volume and test strip width. This allows you to choose a membrane that fits the performance requirements of your test (**Figure 7**). For example, a test needing high specificity and low sensitivity would be made with a 'fast' membrane, like Hi-Flow™ Plus 75. The Hi-Flow™ Plus 135 membrane (or its equivalent) is used for the vast majority of tests currently manufactured.

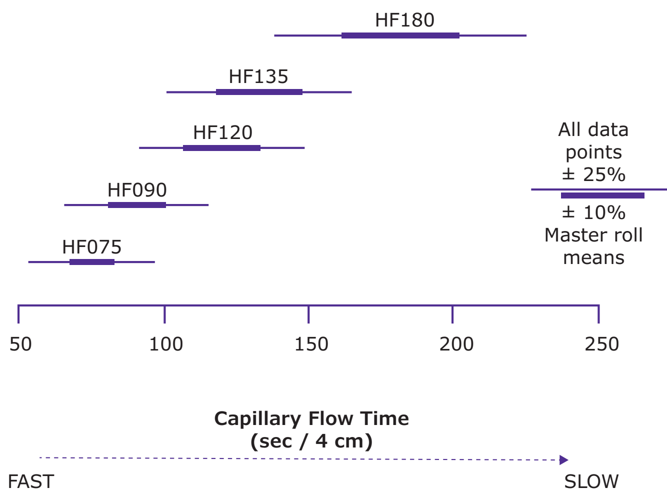

Figure 7. The Hi-Flow Plus membranes manufactured by MilliporeSigma.

In terms of limiting problems with LFA assembly, we recommend visual inspection of the test strips as they are being assembled. It is important to catch mistakes as early as possible to limit wasting valuable material.

MilliporeSigma Assay Development Services

The path to launching a lateral flow assay is fraught technical challenges, miscalculations and misdirections that can take years to navigate. Optimization requires careful consideration of the chemical, physical, and biological attributes of LFA device design and development. MilliporeSigma has the expertise in LFA components and design to rapidly and confidently guide you through the steps. We offer knowledgeable personnel, proven raw materials, and state-of-the-art labs and equipment to save you time and money, getting your LFA to market faster, with the right specificity and sensitivity.

Reduce cost and time to market with on-demand partner support

We partner with experienced IVD developers, providing guidance and support in all potential trouble areas, including selection of materials, design of test strips, and solutions to manufacturing and quality challenges. We also work with customers who need help with the design of an LFA but already have the resources to manufacture it themselves. At one of our regional labs, we can walk you step-by-step through the protocol we've designed for you. During development you can also quickly schedule an appointment with us to work through a problematic step as it arises.

Expertly trained personnel in dedicated labs

We are R&D specialists with deep knowledge of assay components and performance optimization for diagnostics. Our dedicated scientific teams and fully equipped, globally distributed labs can support LFA development, provide on-demand technical support, and foster commercialization—anywhere in the world. Our unique resources combine multiple disciplines and we work closely with customers to offer effective, efficient, and cost-effective support for part or all of your assay development with minimal or extensive touchpoints.

Source of high-quality raw materials

You benefit from the cost efficiencies inherent in combining LFA development services with what has long been our core business—the production of the raw materials needed to make reagents and diagnostic devices. With our multisite, global manufacturing and IVD development expertise, we offer supply chain stability for key reagents, guidance on sourcing and regulatory compliance, and efficient optimization processes.

A note on sourcing

An assay is only as reliable as the raw materials used to make it. This is why expert sourcing that ensures consistent quality and supply is critical. The market turbulence of the past few years has made finding the right materials for LFA development and optimization that meet regulatory specifications particularly challenging. Raw materials like antibodies and detector particles have their own challenges given the wide number of potential sources and differing quality standards.

Sourcing antibodies

It can be difficult to source ultra-pure antibodies and other biological materials due to their inherent variability. The challenges of acquiring a high-quality supply of this critical raw material include:

- **Inconsistency from lot to lot** - Antibodies and other biological material can vary between stock solutions, as well as in the additives and contaminating proteins that may be present.
- **Inadequate supply** - There may not be enough antibody for the long-term development of your test.
- **Inability to scale** - Can the supply be scaled to produce amounts needed for commercialization (grams or kilograms)?
- **Lack of legal access** - Is the antibody licensable for commercial applications or is it solely intended for research purposes?

Your antibody search ends here

MilliporeSigma has a comprehensive range of primary antibodies for research and development. We are constantly adding new antibodies to our portfolio.

Sourcing detector particles

As with antibodies, finding a high-quality source of detector particles is critical for the success of your LFA. The most commonly used detector particles are colloidal gold, latex microspheres, and fluorescent tags. The challenges of obtaining appropriate detector particles include:

- **Lack of size uniformity** - There should be a narrow distribution around the mean.
- **Lack of shape uniformity** - All particles should be spherical.
- **No certificate of analysis** - Your commercial supplier should provide a CoA listing the required attributes for your lateral flow test.
- **Instability in storage and/or after conjugation** - Are they stable, in storage, and after conjugation with an antibody?
- **Excessive aggregation** - Clumps of particles may precipitate or, in the case of dimers and trimers, remain in solution. Either way they adversely affect the sensitivity of your assay.

MilliporeSigma helps source, and apply, the ideal detector particles

Choosing the right detector particles can be daunting. Consider colloidal gold and latex microspheres. Given their thousand-fold greater volume, latex microspheres have limitations on how quickly they flow through a membrane and offer no easy visible evidence of aggregation. On the other hand, colloidal gold allows visible detection of aggregation via a color change. Despite this, if we determine latex microspheres are more appropriate for your test, we would recommend bath sonication, followed by microscopic examination to detect aggregates.

Sustainability considerations

The time to consider sustainable practices and component sourcing when developing your diagnostic test is closer than you think. REACH regulations of the European Union are guiding IVD manufacturers toward less toxic reagents for their existing and future assays. Aiming for green solutions isn't just about finding alternatives to banned or restricted chemicals used in IVDs. It also requires sustainability in terms of reduced packaging, waste minimization, and efficient inventory management.

Working with an expert partner can help shorten optimization steps, saving time and materials. Sourcing reagents made regionally or from partners with robust, global supply chains can contribute significantly to sustainability and protect your manufacturing from the type of supply chain disruptions that occur during turbulent markets, as we saw during the COVID-19 pandemic.

A final word

Savvy diagnostic test developers anticipate potential technical roadblocks and secure resources to push through or maneuver around them. Having fast-access diagnostic support services can keep your assay development and launch on track.

Learn more at
SigmaAldrich.com/assaydev

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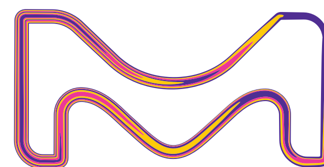

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