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In-line processing trends for lateral-flow immunoassay manufacturing

In-line methods answer the demands for quantitative tests and higher volume manufacturing. **BY THOMAS C. TISONE, HELENE CITEAU, AND BARBARA McINTOSH**

Historically, IVD companies have produced lateral-flow immunoassays (LFIAs) by using batch methods in research and development and manufacturing processes. However, with the drive toward achieving quantitative LFIAs and the demands for higher-volume manufacturing, LFIA production is transitioning to in-line methods for research and development and manufacturing. Implementing in-line methods into research and development minimizes development costs and risks during the scale-up transition to manufacturing.

This article reviews current trends in manufacturing processes that IVD companies are implementing to achieve higher performance reproducibility from test to test, as measured by coefficient of variability (CV), and better efficiency for high-volume manufacturing. This article also focuses on processes such as application of various chemistries onto supporting materials, drying of such porous materials, and laminating and cutting all processed materials into finished test strips that are adequate for LFIAs.

Introduction to Lateral-Flow Immunoassays

The lateral-flow immunoassay format was initially developed in the



The Auto Laminator by BioDot Inc. (Irvine, CA)

1970s. In 10 years, it became a standard platform for various point-of-care (POC) immunological tests. The benefits of this format were the following factors:

- Ease of use.
- Small amount of sample required.
- Adequate level of sensitivity.
- Manufacturability on a large scale.
- Stable final-product shelf life at room temperature.

- Able to implement with a reader technology.
- Ease of regulatory approval (since the format was well known in the market).
- Inexpensive to manufacture.

For many years, the lateral-flow immunoassay format was very popular and was implemented in many tests. However, recent market needs have created higher demands, requiring LFIA tests to become more than

qualitative tests. The evolution of lateral-flow tests toward true quantitative formats is an area of active research and development, especially for tests for diseases with high societal costs such as cholesterol, cardiac arrest, diabetes, and HIV testing.

A lateral-flow immunoassay device is composed of various materials and reagents (see Figure 1). The standard components include a backing card, membrane, sample pad, absorbent pad, conjugate pad, and sometimes a wick. Modern lateral-flow tests have several improvements over the traditional model. For example, the sample pads have become efficient blood separators, allowing assays to be run with whole-blood samples without any sample centrifugation or clotting.^{1,2}

Due to some constraining patent issues, many lateral-flow assay manufacturers have recently invested in research and development to develop multiplexing assays on complex platforms for specific applications. There is also an acute effort to improve the design of current lateral-flow substrates to increase reproducibility and simplicity of use, such that a venipuncture sample would suffice.

Existing Manufacturing

Processes. There are many ways to process LFIA tests, depending on how they will eventually be used. Most IVD manufacturers use either an in-line process or a batch process, which has been far more common until recently. The batch process starts with a roll or sheet of each material (i.e., membrane, sample pad, conjugate pad, wick material) and individual backing cards. Each material is processed independently, entailing considerable operator interference. Once all of the materials are processed, they are laminated onto the backing cards. The cards are then cut into 4–7-mm strips that are assembled into the final test cassette.

The in-line process starts with a roll of all of the materials, and usually involves three types of modules. The first is the reagent dispensing and

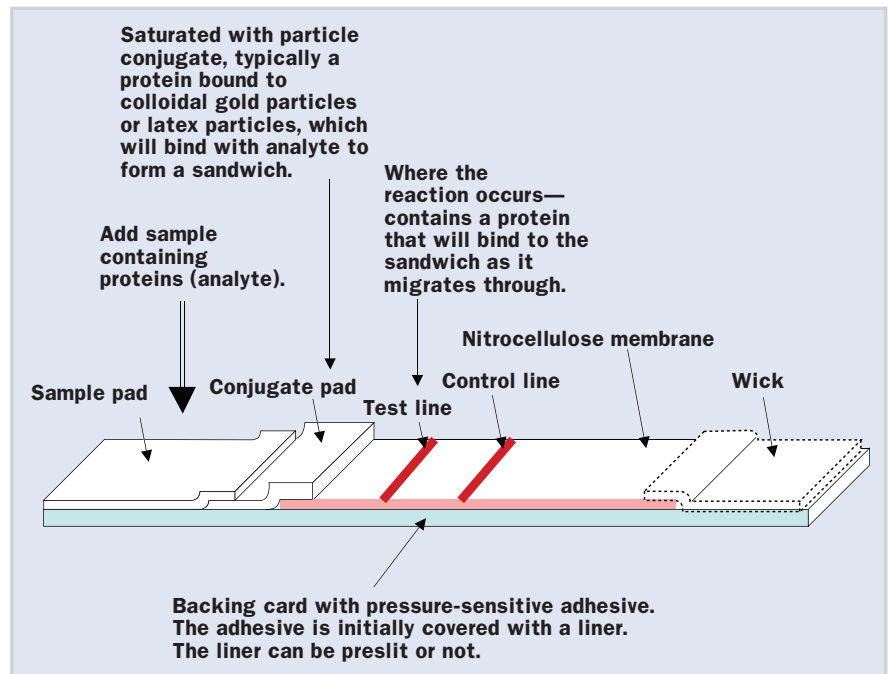


Figure 1. A typical lateral-flow immunoassay test.

drying module, the second is the slitting module, in which processed webs are cut down to narrower widths, and the third is the lamination module. A slitting module is needed only when the materials are provided as wide webs instead of narrow rolls.

Batch Process

In a batch process, a roll of membrane is cut into 300-mm strips. LFIAs use nitrocellulose membranes, which represent the analytical part of the final product. To be sufficiently sensitive and stable, the nitrocellulose membrane must exhibit high protein-binding selectivity and protein-stabilization potential. Lines of proteins are deposited onto the membrane by either a contact or noncontact method, and serve as test and control lines.

After dispensing, the membrane is dried and then blocked to prevent nonspecific binding. The blocking step may be done by impregnation in a dip tank or by spraying, and the membrane may be dried in an oven at 37°C for one hour. While the blocking step is commonly done, it is optional and may be omitted depending on a test's design.

Available in a roll format, the conjugate pad is made of glass fiber or

polyester material and should be cut into individual sheets. It is very hydrophobic, and should be pretreated to render it hydrophilic and capable of absorbing a colloidal gold or latex solution. This solution contains the conjugate antibody that forms the second part of the assay. As the sample being tested moves through the conjugate pad, the conjugate antibody is released and binds to the sample.

Pretreatment can be done by impregnation in a dip tank or by spraying, in which the conjugate pad is dipped and agitated into a solution designed to make it hydrophilic without affecting the antibodies. In some cases, manufacturers of conjugate pads may treat them in advance to render them hydrophilic (e.g., by adding PVA), eliminating the need for additional pretreatment. The pad is then blotted to remove excess fluid and dried in an oven at 37°C for one hour.

Following these steps, the gold or latex particle solution containing the conjugate antibody is dispensed onto the pad by using an aerosol-type spray.³⁻⁶ An alternative method involves adding the antibody solution to the pretreatment solution, and dipping the pad into the resulting bath. While this method saves one

processing step, it does not result in the same penetration of conjugate antibody into the pad.

Also available in a roll format, the sample pad is made of cellulose material and should be cut into individual sheets. The sample pad absorbs any fluid applied to it and makes it compatible with the rest of the assay (e.g., by changing its pH, filtering it, separating out some of its constituents, etc.). For some applications, the sample pad should be pretreated by dipping it into a specific buffer, blotting it, and drying it for one hour at 37°C. The wick material is also available in a roll or sheet format, and should be cut into individual sheets.

Limiting Factors. The batch method is the same for both research and development and manufacturing processes, and can be scaled to higher-volume manufacturing by using duplicate systems. However, the batch process requires multiple operator interventions and includes several steps that cannot be well controlled and cause variability in the final product, which affects the quantification of the LFIA. Such variability emerges during the dispensing, blocking, drying, and assembling steps for all LFIA test components.

In particular, the blocking and pretreatment steps for the membranes, conjugate pads, and sample pads are areas of concern. The dipping process involved in these steps is poorly controlled, which leads to variability in the results. Another significant area of concern is the drying process. Batch ovens do not produce uniform temperatures, resulting in a poorly controlled drying process especially when wet components are placed in them. Reproducible drying is important since it eliminates variability in the resolubilization of the constituents. Moreover, drying membranes in an oven is cumbersome and slow, and affects the overall manufacturing process and cost.

Converting a batch process into an in-line process involves multiple transformations of several steps in the batch

process. By doing so, the impregnation process and drying times are considerably shortened. But despite such limitations, the batch process is still readily used and is adequate for producing qualitative LFIA formats.

Throughput Consideration. With the batch process, manufacturing volumes can be as high as 2 million to 3 million parts per year. However, manufacturing volumes can be more than 10 million parts per year by using a set of in-line equipment. In addition, the in-line approach results in lower CVs compared with the batch process.

In-Line Process

When IVD manufacturers encounter limitations in producing their LFIA tests due to test-to-test variability, they can turn to an in-line process, an alternative to the batch process. The in-line method is designed to meet the specific needs of demanding applications and provides lower CVs because of tighter-tolerance processes.^{3,4} It allows multitasking between dispensing (contact or noncontact) and impregnation (in a dip tank) applications. It also has the capacity for in-line drying and quality control monitoring.

The current drive toward developing novel test formats and quantitative tests requires higher demands on the dispensing, blocking, drying, cutting, and laminating processes, the manufacturing of the tests, and the equipment. In order for lateral-flow-based assays to meet such demands, IVD manufacturers should select reagents, substrates, and materials that are compatible with those processes. For developing LFIA with a high level of complexity and reproducibility, adopting an in-line approach provides a robust and controlled manufacturing process.

Compared with batch processes that require multiple interventions from qualified operators, the in-line approach can be fully automated, modular, and configured based on individual application needs. It minimizes the risk of operator failure, and

provides more control and symmetry over the process compared with batch processing. The following are various modules that can be configured into an in-line reel-to-reel system (starting with a roll of raw material and ending with a roll of processed material), or an autolamination system (starting with several rolls of processed material and ending with laminated cards).

Web Control and Dispensing. This module consists of a payout reel with tension and web speed control. It also includes web tracking systems that locate the web edge relative to the dispenser positions and align the web edge to a common position on the take-up reel. The purpose of the tracking systems is based on the camber inherent in the rollstock of materials that are knife slit from the master rollstock. The camber is the edge deviation from a straight line that is inside a roll-formed piece and becomes apparent as the piece is unrolled.

The camber results in a drift of the web edge relative to a fixed reference point of 2–5 mm over the length of a 50-m roll. Taking the example of test and control lines dispensing on nitrocellulose, the result of this drift is that the line position can be offset from the membrane edge by as much as 2–5 mm. This displacement is a source of not only increased product variability but also offsets in the positions of the test and control lines once the final product has been inserted into a plastic cassette.

Different types of dispensers driven by tandem pumps can be mounted to the control system.^{4,5} The tandem pump is a configuration of two syringe pumps that are connected to one dispenser and are working in an offset mode to alternately fill and dispense each pump. This provides a constant dispense output over long web lengths. As mentioned above, the test and control lines could be striped on the nitrocellulose membranes using either contact or noncontact method dispensing. While the contact dispenser represents a cheaper

Parameters	BioJet	FrontLine
CV	9.5%	13.6%
Minimum line width	0.61 mm	0.33 mm
Maximum line width	2 mm	1.35 mm
Minimum dispensing rate	0.75–0.5 $\mu\text{l}/\text{cm}$	<0.5 $\mu\text{l}/\text{cm}$

Table I. Coefficient of variability (CV) of the FrontLine contact dispensing method versus the BioJet noncontact dispensing method.

alternative that necessitates little maintenance, it is not designed for quantitative tests.

For example, FrontLine dispensers by BioDot Inc. (Irvine, CA) drag a meniscus of fluid on top of a nitrocellulose membrane at a speed slow enough to allow the meniscus to be absorbed by the porous and hydrophilic membrane, and draw a line of sample into the membrane.

By contrast, the BioJet dispenser by BioDot ejects drops of defined volume with high reproducibility and improved coefficient of variability on the drop volume compared with FrontLine's contact approach (see Table I).⁶⁻⁸ The antibody or proteinaceous reagent inside each drop binds instantly to the nitrocellulose membrane due to its high protein-binding property. With the appropriate center-to-center drop pitch and drop volume, the drops overlap to form a continuous line.

The dispensing module also accommodates a camera system for inspecting dispensed lines (see Figure 2). The test and control lines can be assessed for continuity, position relative to an edge, and position relative to each other. Those parts of the lines that fail to meet inspection criteria are marked with a visible ink and rejected when the corresponding laminated card is cut for final assembly.

Dip Tank. This module consists of a reagent reservoir with a roller system to impregnate a moving web, and includes a refill system controlled by a reagent-level sensor. The refill system maintains a constant, slow flow of fluid into the tank, ensuring a consistent level of fluid and a more consistent solute concentration than a batch tank, in which solute drag is uncontrolled. This module can also

be provided with an enclosure and, when appropriate, a dedicated fume-exhaust system.

Dry Tower. The dry towers have a vertical web path to minimize the footprint or length of the composite machine. The dry tower can also be configured horizontally for drying formats that cannot be accommodated with the vertical design. Drying module features include the following details:

- Variable path length.
- Small footprint with a vertical six-foot dry path.
- Independent temperature-control zones along the web path with a temperature range of ambient up to 80°C.
- Forced-air convection from normal to web surface.
- Noncontact surface temperature sensors along the web path.
- Independent convection flow paths from blower to web surface with exterior venting.

The dry path for each tower is six foot long and is composed of six heater zones, three in each tower (see Figure 2). The air input for the convection drying comes from three fans with variable speed control. The air flow from each fan has an independent flow path that directs the air to both sides of the web and exits through a vent on top of the dry tower. In this manner, dry air is continuously fed to the moving web surfaces along the web path. The dry path and drying time are

increased by adding extra dry towers.

The temperature can be controlled and can range from room temperature to 80°C. Noncontact IR temperature sensors are positioned along the dry path to measure the web surface temperatures. Due to the endothermic nature of evaporation, the web temperature does not reach the convection air temperature inside the drying tower until the moisture has been completely removed. This allows for high temperatures during the initial drying process and lower temperatures (around 40°C) when the web temperature approaches the convection air temperature. The kinetics of the drying process in an in-line method are enhanced compared with a batch oven.

Lamination and Cutting. The in-line system's modular design can accommodate adding other types of modules for value-added processes. For example, when materials are provided as wide webs, a module can cut the webs into strips rather than re-roll them. A laminating module can laminate the reagent-treated webs to other materials such as adhesives with release liners, plastic backing, etc. Such laminating modules will laminate membranes to a plastic backing after the test and control lines have been dispensed.

In-Line Slitting. This module is used to slit wide webs into narrower web formats. The machine consists of a reel feed system, a rotary blade system, and a number of take-up reels.



Figure 2. An auto-laminator system by BioDot Inc. with backing card feeding module, dispensing module, and laminating module.

The take-up reels are designed to be transferred directly to the in-line lamination systems. The rotary blade system is composed of a series of cutting blades with spacers such that the cut width can be customized by using spacers of different widths. For example, a 100-mm-wide web of conjugate material could be sprayed with 10 conjugate lines, then dried, rerolled, and taken to the slitter where it is slit into 10 narrower webs, each 10 mm wide. Impregnated webs may also be processed as 100-mm-wide webs and then slit to the appropriate lengths for automatic lamination.

In-Line Lamination. These modules assemble the various component layers that have been pretreated, slit to the appropriate widths, and rerolled into a laminated card or roll. Such rolls are laminated onto a plastic backing once the pre-cut liners have been released. Similar to the reel-to-reel systems, the in-line lamination systems have a modular design to accommodate a wide range of LFIA (see Figure 3). The different types of lamination modules include the following characteristics:

- *Membrane lamination.* This module consists of a feed reel for the membrane with tension and speed control, a take-up reel for the membrane protective cover when required, and a lamination roller. An important feature of this module is its web tracking system that senses the membrane edge and aligns it to a defined position on the plastic backing. This station can laminate both backed and unbacked membranes.
- *Multiple material feeds.* This module includes three-roll feeds for the conjugate, sample, and absorbent pads with lamination rollers. Take-up reels for various release liners may also be included. Mechanical guide systems can reference the different materials to the plastic backing.
- *Vision inspection.* A vision system inspects the different laminate



Figure 3. A reel-to-reel system by BioDot Inc. (Irvine, CA) with camera control and dry towers.

placements. Those out of position are ink marked for removal during the final cutting process.^{8,9}

- *Reroll/cut.* This module cuts the laminate into strips using a guillotine cutter or rerolls it for feeding into the strip cutting operation. If the reroll option is selected, the system is configured with a large-diameter core to prevent any stress damage from the rolling to the laminated card.
- *Additional options.* Other modules include adding plastic overlays to the top of the laminate and performing dispensing operations at any point during the lamination process.

Conclusion

While there are currently several methods for processing LFIA tests, IVD manufacturers are primarily using batch and in-line processing. However, even though batch processing remains a valid method for lateral-flow assay manufacturing, its inherent deficiencies that are due to multiple operator interventions prevent it from obtaining CVs as low as in-line processing. With the current market's demanding specifications for LFIA tests, IVD companies are investing into new processes to produce rapid diagnostic tests with better CVs.

Controlling the complete process is crucial, from quantitative dispensing of reagents, blocking, and drying to aligning materials onto backing cards. In order to meet such manufacturing requirements, research and development laboratories are also

transitioning to in-line systems to produce adequate lateral-flow assays. Moreover, the volume of rapid diagnostic tests is exploding, pushing IVD manufacturers to take into account higher throughput considerations. In-line processing can offer advantages for the development and manufacturing processes of rapid diagnostic tests.

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