Chapter 3
Material Platform for the Assembly of Lateral Flow Immunoassay Test Strips

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3.1 Introduction

Lateral flow immunoassays provide a convenient and relatively inexpensive means of performing biological and chemical testing. The technology is used to support existing and emerging markets and applications including:

Infectious Diseases	Clinical Diagnostics
Drugs of Abuse	Forensic Science
Human/Animal Health	Therapeutic Monitoring
Genetic/Cardiac Markers	Agriculture
Environmental Safety	Water/Food/Dairy Safety/Processing
Bio-Defense	Biotech Quality Control

And with many more uses being discovered daily, this exciting technology has almost unlimited potential.

Material selection is a critical criterion for the success of any lateral flow platform. A test strip is typically composed of a plastic backing with an adhesive, a sample pad, a conjugate pad, a nitrocellulose membrane, an absorbent pad, and printed tapes for identification. This chapter will outline the materials that form the backbone or platform of a testing device including backing basics, adhesives, liners, backing format, sub-assembly concepts, cover tapes, and laminating and processing recommendations.

3.2 Selection and Assembly of Materials Used in Test Strips

3.2.1 Backing Basics

Many of the components of a lateral flow immunoassay test strip, such as nitrocellulose, fiberglass pads, and cellulose sample and absorbent pads, are

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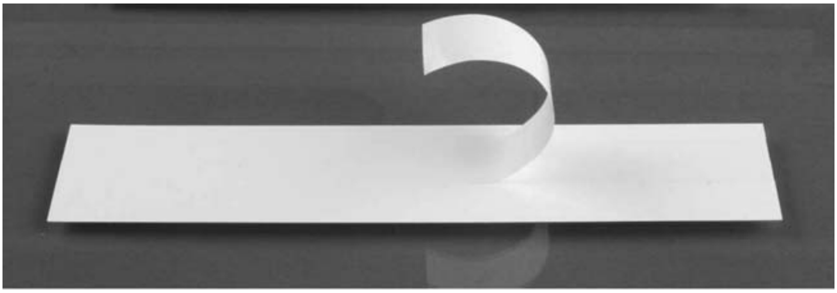

Fig. 3.1 A typical backing with part of release liner partially peeled back. (Photo courtesy of G&L Precision Die Cutting, Inc. San Jose, CA, USA)

delicate in nature. Therefore, test strips need a supporting backbone or platform to run on. This platform is commonly referred to as the backing or backing card as shown in Fig. 3.1.

The backing is designed as a custom platform to fit the specific requirements of each test. A traditional backing consists of three components – a semi-rigid plastic, a pressure-sensitive adhesive, and a release liner. The most common plastics used for lateral flow immunoassay tests are polystyrene, vinyl (poly vinyl chloride or PVC), and polyester. Custom materials are also available from experienced converters and in vitro diagnostic (IVD) component suppliers like G&L Precision Die Cutting (San Jose, California), Millipore, Inc. (Billerica, Massachusetts), Adhesive Research, Inc. (Glen Rock, Pennsylvania), and Whatman Inc. (Florham Park, New Jersey). Commonly available thicknesses are:

a) 0.005 inch for polyester
b) 0.010 inch for polystyrene, polyester, and vinyl
c) 0.015 inch for polystyrene and vinyl

The thickness of the backing material is a major consideration for the design of a test strip. Thinner backings in the 0.005 to 0.010 inch thickness are typically used for test strips that go into a holder, cup, or plastic housing. Films in the 0.010 to 0.015 inch range and thicker materials are usually preferred for stand-alone test strips. Backing materials greater than 0.015 inch in thickness should only be used if absolutely necessary since thicker backing materials can cause difficulty in assembly because of the propensity of the materials to curl. It can also create issues during cutting of the material into individual test strips. Thicker materials will require greater cutting force that can cause ridge marks along the edge of the strip or twist the strip, which can affect sample flow. In general, the backing material should be thick enough to provide support to the strip during manufacturing and assembly, but not too thick that it creates irregularities when cutting assembled material to the finished test strip size, or adds unnecessary cost to the product.

Custom films, absorbent materials, and rigid plastic are also used sometimes as backing materials for very special applications. In initial product design, one may wish to research or consult with one or more experienced converters to best determine the materials that are available for use in the new product.

3.2.2 Backing Format

Plastic backings are available in pre-cut card form (or called sheets) and roll form. Cards are the most common and flexible format for many manufacturers, but there is an increasing demand for backing in roll form for use on automated or semi-automated equipment. Backing materials have a propensity to curl, causing liners and adhesives (see below) to pinch or wrinkle if not handled properly. Precautions should be taken in determining the format that will actually work best for a specific application. There are some general guidelines to consider for the two formats:

a) Backing cards allow for a wide range of manual assembly options. They are easy to handle and promote flatness of the material. They can also be partially assembled and easily stored for future use or finishing. Cards can be a very effective format and are still the most popular format today. However, cards cannot be easily automated and will generally be labor-intensive to assemble.
b) Backing in roll form can be used on an in-line automated assembler for high-volume production. However, because lateral flow laminations (see below) generally result in a fairly thick laminate with varying heights, it is not recommended that the materials be rewound after being laminated. Therefore, the laminated materials need to be cut either into cards or into test strips in-line. If the latter case is chosen, a system should be designed so that the laminating portion can still be done at high speeds.

3.2.3 Diagnostic-Grade Adhesives

One of the most important issues to consider in choosing a backing for a lateral flow application is the adhesive used on the backing material. Not all adhesives are created equal and given the expense of most other lateral flow components; this is not an area to cut costs on. Special attention should be made to choose an adhesive with the following characteristics:

- Does not build up excessively on blades during cutting
- Does not migrate into conjugate pads, sample pads, or backed or unbacked membranes (i.e., an inert adhesive)
- Compatible with biological components of the test and does not affect the stability of reagents or membranes (especially for acrylic adhesives, see below)
- Compatible with a stable protective release liner
- Specially formulated diagnostic-grade pressure-sensitive adhesive

It has been shown that rubber and industrial acrylic adhesives can leach chemicals, solvents, and other additives. This property may affect the bonding of materials, or the chemistry of the test, causing false positive or false negative results. Solvents and plasticizers, common in acrylic adhesive systems, are especially prone to migrate out of the adhesive. Users should always seek out a diagnostic-grade adhesive from an experienced supplier or converter to avoid such complications.

Pressure-sensitive adhesives, commonly referred to as “psa”, are used in most lateral flow applications. These adhesives form a bond when pressure is applied. No heat, water, or other outside chemicals are required. Typically, a psa will “wet out” or cure and achieve its ultimate bond over the course of 24 hours. This is important when using substrates that are difficult to bond to, such as a fiberglass pad. If components are not bonded well or are falling off during the process of cutting the assembled materials into test strips, the cause could be that not enough time has been allowed for the materials to form a sufficient ultimate bond.

3.2.4 Liners

In order to protect the adhesive before and during assembly, a protective liner must be placed over the adhesive. The liner can be a paper or a film treated for release characteristics. A paper liner must be further treated for moisture resistance. The release agent is typically a silicone coating to allow for easy removal of the liner from the adhesive.

For in vitro diagnostic (IVD) applications, the liner is generally scored with “backsplits” to aid in device assembly. A backsplit is a custom, pre-cut score in the liner, sometimes referred to as a kiss cut, backsplit, or back score. A proper backsplit will be cut completely through the liner, just barely touching the adhesive. A backsplit should never be cut through the plastic backing as this jeopardizes the integrity of the material. Figure 3.2 depicts an example of the

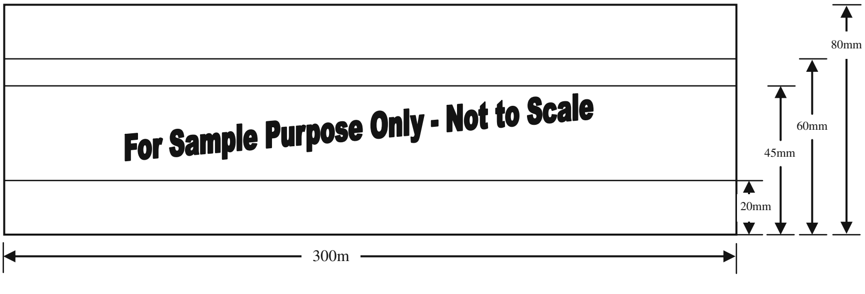

Fig. 3.2 Dimensions for a typical lateral flow immunoassay test strip (for sample purpose only, not drawn to scale)

dimensions for a typical lateral flow immunoassay test. Note that all dimensions are measured from the bottom.

The various components from bottom to top are: the absorbent pad (20 mm), the nitrocellulose membrane (25 mm), the conjugate pad (15 mm), and the sample pad (20 mm). A custom cutting die for scoring the backsplit, often referred to as "tooling", is made for each unique product configuration. This is a one-time-only investment, as the tool can be used over and over again.

Determining the locations of all of the components in a lateral flow immunoassay test including overlaps is critical to the success and reproducibility of a test strip. An important item that should not be overlooked in determining the overlaps and backsplit locations is the tolerance on the widths of the materials. Typical tolerances on the width of the sample pads, conjugate pads, membrane, and absorbent pads are in the ±0.25 mm to ±0.50 mm range and will vary by manufacturers. Typical tolerances for the width of the backing are ±0.35 mm and those of the backsplit locations are ±0.005 mm. It is important for the success of a test that the overlaps are correctly defined in a way compatible with the dimensions of the test strip and taken into consideration the limits of the tolerance.

3.2.5 Sub-assembly and Contract Manufacturing

Experienced converters and suppliers often provide a number of value-added features and sub-assembly options to help customers get their products to the market faster. Sub-assembly services may include custom lamination of customer-supplied materials such as nitrocellulose membrane, conjugate pads, absorbent pads, and cover tapes. They may also offer the assembly of active materials in dry rooms. Close tolerance laminating of components often translates into improved consistency and accuracy of a lateral flow immunoassay test. It can also reduce the high-waste factors typically associated with hand lamination. Overall, it decreases costs by allowing a manufacturer to eliminate or reallocate extra labor and to handle production spikes in volume without adding staff. In addition, it may free up time to let researchers focus their development efforts on chemistry and design of the test strip and ultimately get their products to the market faster.

In addition to the sub-assembly type services, converter partners may offer custom options like notching the backing cards for registration, improving placement of components, zone coating of adhesives, and producing adhesive free areas on the card.

As the market moves toward quantitative tests or improved sensitivity of tests, many manufacturers search for better tolerances on the width of raw materials. An experienced converter partner can provide narrow web slitting of lateral flow components often to a closer tolerance than available from the raw material supplier.

3.2.6 Cover Tapes

Printed films are used as a top laminate or as a cover tape in many lateral flow applications. Typical uses for cover tapes include:

a) Test identification
b) Arrows to indicate orientation of the test strip
c) Stop lines to indicate the upper limit in which the strip should be immersed into the liquid sample
d) Trade names
e) Identification during manufacturing
f) Holding down fragile components (i.e., fiberglass pads)

Cover tapes materials are generally thin, flexible film tapes with adhesive on one side and printing on the other. The film can be either clear or opaque white and a Flexographic printing process is used to print virtually any color, text, or symbol on it. A unique print plate is needed for each design. Printing can be positive print where the ink is the color of the text, or reverse print where the text is in white surrounded by color.

Gaining popularity is the practice of printing cover tapes in different colors for each product a company manufactures. This allows easy identification of the actual test during manufacturing when several test strips on the same platform are similar in size and base materials, but with different chemistry. Often, the finished test strips are assembled into housing, which does not show the identification tape in the final packaging.

Cover tapes need to be evaluated for performance whenever a change is made to a test including materials, formulation, and test strip cutting. For example, a change from a cellulose-based pad to a glass fiber–based pad may significantly improve the performance of the test strip, but the cover tape may not be aggressive enough to bond to the new fiber glass pad that has a very open cell structure. Another common challenge occurs when the method of cutting test strips is changed. Going from a guillotine test strip cutter to a rotary cutter may improve manufacturing efficiencies. However, since many male/female rotary test strip cutters twist the strip during the cutting process, this may cause the cover tape to pull away from the taping components or even delaminate entirely from the strip.

3.2.7 Assembly and Laminating

The assembly of a lateral flow immunoassay test is as varied as the tests themselves. There are hand assembly, hand-made jigs, light box, vacuum shell systems, large, automatic in-line lamination systems, and everything in between. All methods are effective and can be adapted to meet a variety of manufacturing requirements.

Hand assembly is sometimes designed so that only the backsplits in the liner guide the assembler to where to put the components. A jig or light box will show lines indicating where each component goes and aid in manual hand assembly.

Several companies offer clam-shell style laminators that use pin locators, formed trays, and vacuum pressure. The components are placed on one side with the proper overlaps and then the clam shell is closed, pressing the two sides together and laminating the materials onto the backing card. In addition to these kinds of batch laminators, equipment manufacturers also make custom in-line lamination systems. A properly designed in-line system provides individual control to exert proper tension to the various components that make up the lateral flow immunoassay test.

The wide variety of equipment and design support allows researchers to scale-up quickly from bench, through pilot production to full manufacturing with a minimum of process development issues.

Regardless of the assembly method, whenever possible, time should be allowed for all of the materials to form an adequate bond once laminated. A period of a few hours will suffice for many tests, but some tests with high surfactant loads or fiberglass pads or others difficult to bond to substrates may require a longer period of time.

3.3 Conclusion

In the development of lateral flow immunoassay, most attention has been paid to finding a good detection method or seeking the best antibody or antigen. However, as we have shown above, one should ensure the compatibility of basic components like the backing, adhesive, and cover tape in order to produce a consistent and high-quality product.