Demystifying Diagnostics: The Agdia ImmunoStrip®

Part Three: Component Parts

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The ImmunoStrip® is composed of multiple functional components that must work together systematically to be effective. Altering a single component can alter test performance and final result. Therefore, it is imperative to understand the functional roles of these components to understand how an ImmunoStrip® works. The majority of Agdia's ImmunoStrip® products are utilized for the detection of plant pathogens present in plant material, such as leaves, roots and stems. And the antigen is typically a protein produced by bacteria, fungi or a virus. This will be the focus of our discussion moving forward. It is important to note that many refer to the target antigen as an **analyte** in the context of lateral flow device function.

As mentioned in part one, lateral flow devices are available in multiple configurations. Moreover, they are available in multiple packages. Many commercial LFD products are packaged within plastic cassettes, including COVID-19 tests and pregnancy tests. As you might know, the ImmunoStrip® is not packaged this way, as the application is dissimilar. Variable applications notwithstanding, the key components and underlying principles of all lateral flow devices remain uniform.

Extraction buffer is used in processing the plant material in question and is the liquid component of the test sample; indeed, this is a discussion on lateral **flow** devices. The extraction buffer is typically composed of blocking agents, surfactants, multiple salts and water. Collectively, these ingredients bind superfluous molecules while facilitating sample flow and cell lysis, releasing antigenic targets (analytes) into solution. Moreover, the buffer stabilizes the sample pH at a level ideal for immunogenic chemical reactions to occur. Extraction buffer for ImmunoStrip® products is available in pre-filled, mesh processing bags and labeled SEB (standard extraction buffer) 1, 4, 9, etc. (**Figure 1.**).

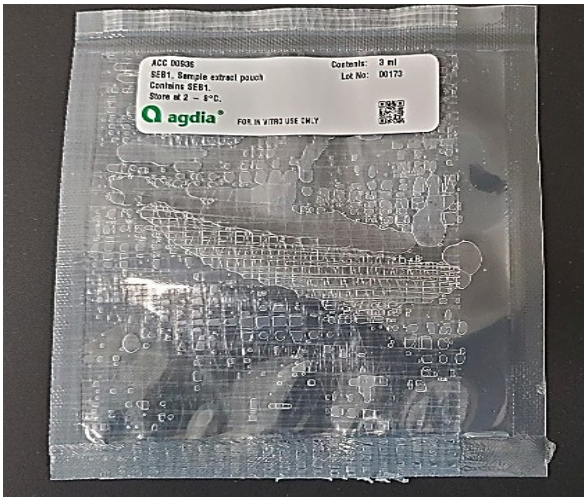

Figure 1. Mesh bag containing extraction buffer.

The **backing card material** is cut into the ImmunoStrip® shape and functions as the foundation to which all other components are adhered, providing a rigid, stable platform. It is made of laminated polyester material and functions in a physical role only. The **sample pad** is proximal to and the first component to contact the liquid sample. It is typically composed of cellulose fibers and contains additional dehydrated surfactants and buffering agents. The primary function of the sample pad is to promote uniform, measured distribution of the sample into the next component, the conjugate pad.

The **conjugate pad** is the first component where an antibody comes into contact with the liquid sample and the antigen if present therein. The conjugate pad is composed of compressed, porous glass fibers and contains the dehydrated **detection or primary antibody**, which is chemically bound (conjugated) to the detector reagent **colloidal gold**. I have established that the detection antibody will bind specifically to the antigen's epitope(s) if it is present. The detector reagent is a nanoparticle and responsible for visualization of signal, i.e., the magenta color present on test and control lines downstream. The

conjugate pad functions as the platform wherein detection antibodies contact the liquid sample uniformly. Moreover, the conjugate pad allows for the uniform transfer of liquid sample combined with the detection antibody conjugate to the membrane.

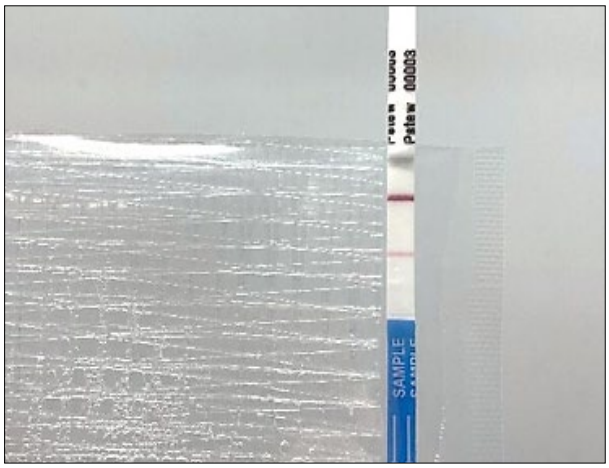

Figure 2. Development of control line (upper) and test line (lower) on ImmunoStrip® in a positive sample.

In positive samples, complexes of detection antibody conjugate bound to the antigen have formed. These complexes are in solution and ushered into the **membrane** along with the remaining liquid. The membrane is made of nitrocellulose and constitutes the longest and most complex leg of the ImmunoStrip® reaction. The sample moves through the porous structure of the membrane via capillary action, physically contacting two antibodies electrostatically bound to the membrane. These two antibodies are bound to the membrane in discreet lines or stripes running perpendicular to sample flow. The reactions that occur within these areas are visualized as a **test line** and a **control line**.

The antibody immobilized on the test line can be referred to as the **capture or secondary antibody**, and it binds to a second epitope on the antigen. This binding forms an **immunocomplex** of capture antibody and detection antibody conjugate with the antigen sandwiched in between, all of which are now bound irreversibly to the membrane. It is the deposition of colloidal gold into the membrane at this location that causes a magenta line to appear. If the sample is negative, no test line will appear. The sample will continue to flow and contact the second line of different capture antibody, which binds to the detection antibody conjugate, not the antigen. This occurs regardless of antigen presence, forming the control line on all valid tests (**Figure 2.**). The remaining sample material flows through the final length of membrane until it concludes its journey in the absorbent pad.

The **absorbent pad** is placed at the end of the ImmunoStrip®, distal to the sample. It is typically made of cellulose fibers and functions in multiple physical roles. First, the absorbent pad assists in the movement of fluid across the membrane via wicking action. Second, it captures the remaining sample, keeping it from flowing off the strip. And finally, it blocks the captured sample from backflowing on to the membrane. Collectively, the absorbent pad allows for relatively large sample volumes to be used, resulting in increased assay sensitivity (**Figure 3.**).

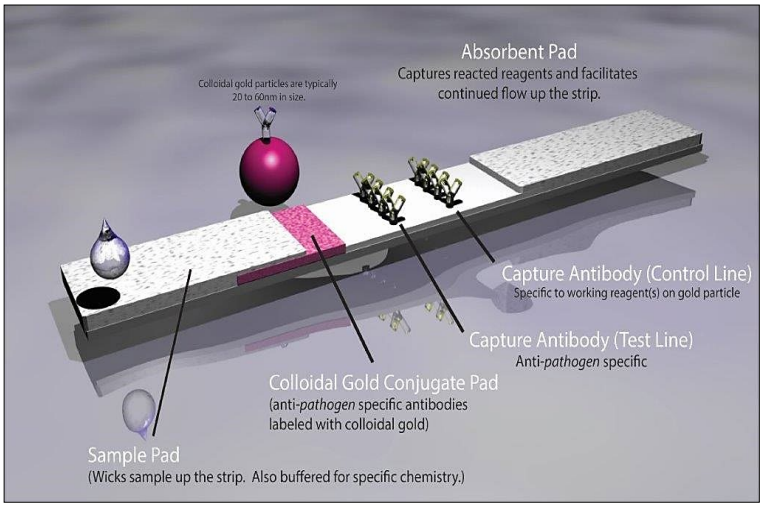

Figure 3. Components of the ImmunoStrip®

Stay tuned for the fourth and final installment wherein I will provide a stepwise explanation of the physical and chemical interactions that take place when performing the ImmunoStrip® assay.

About the author

Robert Emmitt serves as the Domestic Account Manager of Plant Pathogen Diagnostics at Agdia, Inc., where he has been for six years. Robert earned his B.S. in Crop and Soil Science from the University of Kentucky and his M.S. in Plant Pathology from the University of Georgia. Before joining Agdia, Robert spent 18 years in the landscape management industry, culminating as a Plant Health Manager on private estates in Cincinnati and Northern Kentucky, which is where he grew up and became interested in plant health. Moreover, he holds associate degrees in Horticulture and Turfgrass Management and has several years of professional experience in landscape design. Robert can be reached at robert.emmitt@agdia or 574-327-6065.