



Demystifying Diagnostics: The Agdia ImmunoStrip®

Part Four: Bringing It All Together

Robert Emmitt

Over my last three installments, I introduced everyone to the lateral flow device, discussed antibodies and provided a crash course in the functional components of the ImmunoStrip®. This final installment will bring it all together by providing a stepwise instructional for performing an ImmunoStrip® with an explanation of what is taking place at each step. I recommend referencing installments one through three for more detailed background information.

1. Once you have decided on the tissue sample you will test, you process it in the sample **extraction buffer** in the **buffer-filled bag**. When the tissue is pulverized within the buffer-filled bag, the antigen of interest is released into the solution, if present. The buffer provides a liquid matrix that lyses plant cells, adjusts pH and facilitates constant and uniform flow of the sample (**Figures 1. and 2.**).
2. The ImmunoStrip® is placed vertically in the bag containing the sample as designated by markings on the **cover stock**. This allows the sample to contact the **sample pad**, beginning its journey across the ImmunoStrip® (**Figure 3.**). The sample pad wicks the sample and organizes it for uniform distribution to the **conjugate pad**.
3. The sample is wicked to the conjugate pad where it comes in contact with dehydrated **detection antibody conjugate**. The liquid sample rehydrates and incorporates the antibody conjugate. The antibody component binds chemically to the antigen if it is present, forming a complex of **colloidal gold** conjugated to antibody, which is bound to the target antigen. The sample then flows uniformly into the membrane.
4. The sample flows through the porous **nitrocellulose membrane** via capillary action and encounters the first line of **capture antibody** immobilized in the shape of a thin line. The capture antibody binds to a



Figure 1. Opening the buffer-filled bag



Figure 2. Processing your plant sample



Figure 3. Placing test strip in liquid



second site on the antigen if present, forming a large **immunocomplex** of two discrete antibodies with the antigen sandwiched in between. At this point, these complexes are no longer in solution, as they are bound irreversibly to the membrane. The reaction that takes place between the colloidal gold and membrane is visualized via a magenta color change, forming the **test line**.

5. The remaining sample moves across the membrane until it comes in contact with the second capture antibody. This antibody does not bind to the antigen but to the remaining detection antibody conjugate still in solution. This binding forms the **control line** in the same way the test line was formed. Since the reaction at this site does not require the antigen, it occurs whether or not the sample is positive or negative. The control line should always appear; if it does not appear, the result is invalid (**Figures 4. and 5.**).
6. The test concludes as the remaining sample is pulled into the **absorbent pad** where it is physically bound and cannot flow farther or back.

There you have it, probably more than you ever wanted to know about the inner workings of an ImmunoStrip®. Nevertheless, I hope you find all four installments interesting and informative.

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.



Figure 4. Positive result signified by presence of control and test lines.

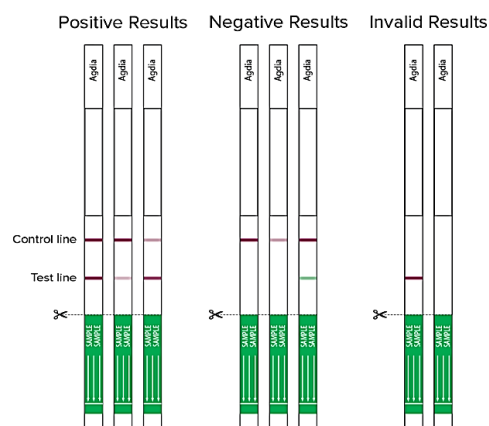


Figure 5. Interpretation of ImmunoStrip® results