BALLTECH ON DEMAND™

Ball Seed®

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TECH TRAINING:
ON-SITE PATHOGEN TESTING

Time is money when debating what to spray on diseased crops. However, knowing the target pathogen prior to making a corrective chemical application is not only a legal requirement but also helps provide the best outcome for your crop and wallet. ImmunoStrips, offered by Agdia, provide a quick and easy way to conduct on-site tests for a variety of plant pathogens. This video from Agdia provides a quick tutorial on how to use an ImmunoStrip, but there are some specific considerations to keep in mind to make the most of your testing.

Tip 1: Use the right amount of tissue.

- Agdia recommends using an amount of tissue about the size of a U.S. quarter.
    - Too much tissue can generate a false positive, meaning the pathogen is not present even though the result was positive (Fig. 1).
    - Too little tissue may generate a false negative, meaning the pathogen may be present even though the result was negative.

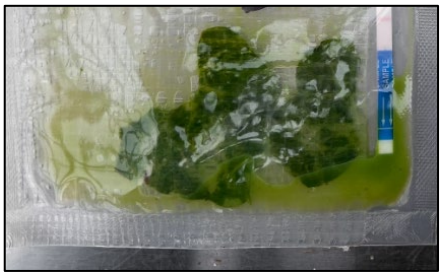

Fig. 1. Excess amount of tissue generated a false positive in this test.

Tip 2: Sample the correct tissue.

- Most pathogens are present and active where healthy and symptomatic tissue meet (Fig. 2).
- The test requires living tissue.
    - Necrotic (brown) tissue is not likely to provide a useful test result.

Fig. 2. Collect samples that contain both healthy and unhealthy tissue.

Tip 3: Confirm results.

- ImmunoStrips for a particular pathogen can yield positive results for other related pathogens.
    - Send a sample to a diagnostic lab that can perform more specific tests and confirm the ImmunoStrip results.
    - Consult with your supplier or a professional diagnostician for assistance.

Consider keeping tests for the following pathogens on hand:

- Tobacco Mosaic Virus (TMV)
- ImmunoComb® for CMV, INSV, TMV & TSWV (Fig. 3)
- *Xanthomonas hortorum* pv. *pelargonii* (*Xhp*)

Fig. 3. Sample showing positive results for multiple viruses.

DEEPER DIVE: THE WHY

Accurate detection and identification of plant pathogens is essential for determining the appropriate course of action. Many diseases result in similar symptoms, but corrective measures are often pathogen specific. Thus, reliable, inexpensive and grower-friendly diagnostic tools are an important resource to ensure crop success. ImmunoStrips are a type of commercially available Lateral Flow Assay (LFA) that utilizes a pouch filled with extraction buffer and strips containing pathogen-sensitive antibodies.

Leaf tissue from symptomatic plants being tested should be harvested, and ***a sample the size of a U.S. quarter*** should be placed in the sample bag. As the sample is ground between the mesh, antigenic substances, such as proteins, antibodies and nucleic acids, are released and can be used to indicate the presence of a pathogen. As an example, some pathogens produce unique proteins or can result in higher concentrations of already-occurring proteins within infected plants.

The presence of a protein that is not normally present in a plant, or a measurably higher concentration of a certain protein that is normally present can both be used as indicators that the plant is infected with the pathogen in question. This emphasizes the importance of using the correct amount of tissue when using ImmunoStrips, as using too much or too little tissue can unintentionally increase or decrease the antigen concentration the ImmunoStrip is testing for. This can generate false negative or false positive results, leading to an inaccurate diagnosis.

When target substances are present in the plant tissue, labeled antibodies in the ImmunoStrip test kit will bind to these substances to form an antigen-antibody complex. Once the test strip is placed in contact with the buffer solution, the solution will migrate up the test strip through capillary action. The buffer solution alone will activate the "control" line regardless of whether the antigen is present. The "test" line contains a secondary antibody that will bind to the first antigen-antibody complex, resulting in the appearance of the magenta line and indicating a positive result.

If ImmunoStrip results are inconclusive, or you want to confirm the results, consider sending additional samples to a diagnostic lab. Several universities and private labs offer disease testing services that utilize more sophisticated methods such as an enzyme-linked immunosorbent assay (ELISA) or quantitative polymerase chain reaction (qPCR), which can provide a more accurate detection.

Final notes for success

- Consider keeping test kits for key diseases on hand and make sure they are not past their expiration date.
- Proper storage is another important consideration. Ideally, tests should be kept refrigerated between 36 and 46°F (2 and 8°C) until used.
- ImmunoStrips may react with other pathogens that are often related to the target pathogen, causing positive test results.
    - For instance, the TMV ImmunoStrip has cross-reactivity with 18 additional viruses. In these cases, sending tissue to a diagnostic lab can provide a specific diagnosis.

For more information, check out these additional resources:

- *Demystifying Diagnostics: The Agdia ImmunoStrip Part 1, Part 2, Part 3 & Part 4*
- *Get Familiar with In-House Disease Testing*