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Portable test kits for diagnosing potato diseases

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INTRODUCTION

Misdiagnosing a potato disease can be very costly if unnecessary fungicide applications or other control measures are implemented. There are many real-life examples in which proper identification of a disease came only after avoidable inputs were applied or management practices changed.

Identification of samples submitted to a laboratory can take several days to complete. It would be beneficial for growers, crop consultants, and extension personnel to have rapid and simple disease identification test kits for verifying their initial disease diagnoses in the field. These test kits could also be helpful in eliminating disease misdiagnoses and determining whether the problem is caused by physiological or chemical factors rather than by a pathogen.

TEST KIT EVALUATION

A University of Idaho and Miller Research program sponsored by the Idaho Potato Commission evaluated the use and reliability of commercially available pathogen test kits. The objective of this program was to evaluate the reliability and practicality of the test kits for the potato industry. Multiple test kits for numerous diseases were used and assessed (see box at right), and test kit results were compared to results from traditional laboratory diagnostic methods. Ease of use and storage requirements were also assessed for each test kit. Test kits typically have a 1-year expiration date. Approximate expense for each test kit at the time of the study ranged from $4 to $12, depending upon company and type of test kit.

Overall, the test kits accurately identified the pathogen causing the problem and in some cases ruled out other potential pathogens. The test kits were relatively easy to use and typically provided a result within 3 to 5 minutes.

These kits will provide an additional tool for greater accuracy, efficiency, and sustainability in potato disease management. Due to the potential for false negatives and/or false positives, however, it is imperative to have samples subsequently verified by university personnel or an independent laboratory. These experts may also provide management suggestions or input on the situation.

TEST KIT AVAILABILITY AND SOURCES

Sources of kits used in the study were Agdia, Inc., Bioreba AG, and Pocket Diagnostic. Other companies also sell test kits.

Kits evaluated in the study

Potato virus Y (PVY)
Potato virus Y^N (PVY^N)
Alfalfa mosaic virus (AMV)
Spongospora subterranea f. sp. *subterranea* (powdery scab)
Botrytis species (gray mold)
Phytophthora species (late blight and pink rot; the available test kits will not distinguish between the two)
Pythium species
Rhizoctonia species (stem canker and black scurf)

Available kits not evaluated in this study

Potato leafroll virus (PLRV)
Erwinina amylovora
PVA
PVM
PVS
PVX
Ralstonia solanacearum

MAKING AN INITIAL DISEASE DIAGNOSIS

Since each test kit is specific to a particular pathogen, you must select an appropriate test based upon the foliar or tuber symptoms. Initial visual diagnosis of a problem begins with a systematic approach to determine the cause. This approach includes inspecting all plant parts and comparing them to healthy/unaffected plant parts, determining if a pattern of symptoms is apparent, and looking for pathogen sporulation, bacterial exudate, or other structures produced by the pathogen. Common initial questions to ask include:

- Does the disease or problem follow a pattern associated with the row or planting?
- Does the disease or problem occur in areas of overwatering?
- Does the disease or problem appear following an unusual or extreme weather event?
- Does the disease or problem affect other plants or weeds around that area?

Asking questions and collecting crop history can help narrow the possibilities and help determine which pathogen test kit to use. For more information on diagnosing crop problems refer to "Diagnosing Field Problems," and for information on diagnosing storage diseases, refer to *Diagnosis and Management of Potato Storage Diseases* (see the further readings section of this publication). Both of these articles can be found at the University of Idaho Potato Storage Research Facility website (http://www.kimberly.uidaho.edu/potatoes).

USING PATHOGEN TEST KITS

Several manufacturers produce serological test kits, and the kits vary in materials and procedures. Most of these test kits can be used in the field, storage, or office; a laboratory is not required. Some test kits require components to be refrigerated, have vials or pouches that need to stand upright during testing, necessitate scissors, or require addition of buffer from a central container. It may be best to become familiar with the process prior to using the kit in the field.

Some test kits include a pouch with an insertable test strip, whereas others call for placing a drop of tissue extract into a depression or well. The three types of kits and step-by-step guides to their use are on pages 4–6. Essentially, the overall process is the same for each kit:

1. Make an educated guess as to which pathogen is causing the disease symptom. Each kit is specific to a particular disease, except for the *Phytophthora* kit, which will identify whether the sample is positive for late blight or pink rot but will not distinguish between the two.
2. Select the test kit (or kits) that best fits the observed symptoms. For instance if you are concerned that foliar symptoms are due to late blight, select the *Phytophthora* kit. If you get a negative result, follow with the *Botrytis* kit.
3. Select a sample of leaf tissue (quarter size) or tuber tissue (nickel size) that shows the symptom in question. If possible, collect a sample at the interface between infected and healthy tissue.
4. Place the tissue sample in the pouch or vial and macerate it with liquid buffer for a specific amount of time as indicated in the directions.
5. Test the liquid either with a test strip or by transferring some of the liquid into a plastic well. After a specific amount of time, the test will show one line if the test was accurately performed (the control line). A second line will appear if the tissue was positive for the pathogen being tested (figure 1). In other words, one line equals negative for the pathogen, and two lines equals positive for the pathogen. Be careful to avoid adding too much tissue extract since this may overwhelm the test and create a void test (no control line).

Figure 1. Two types of test kits (one well and one strip) used for diagnosing PVY and PVY^{N}. Note the quarter-sized sample of macerated foliage in the pouch with buffer. The test strip shows a positive (control line at top and test line at bottom) result for PVY. The well-style kit (white plastic tray) indicates a negative test (control line but no test line) for PVY^{N}. Laboratory results verified the sample to be PVY$^{N:O}$.

For more detailed information on how to use the kits selected for this study see pages 4–6.

ACCURACY OF TEST KITS

PVY and PVY strains

PVY test kits from the three companies were accurate in identifying the presence or absence of PVY. PVY test kits displayed broad reactivity and correctly detected PVY^{O}, $PVY^{N:O}$, and PVY^{NTN}. The kits tested were reliable and accurate, and using a general PVY test kit identified infection by common strains of PVY that are observed in Idaho and the Pacific Northwest. There is no need to use a specific PVY^{N} test kit to identify any of the various PVY strains.

Phytophthora species (late blight and pink rot)

Phytophthora test kits accurately identified the pathogen causing foliar late blight and tuber and stolon pink rot infections. The test kits identified tubers affected by pink rot (caused by *Phytophthora erythroseptica*) even when the tubers were succumbing to secondary invasion by bacterial soft rot.

Several *Phytophthora* test kits were used, and they accurately gave negative results for foliar late blight (caused by *Phytophthora infestans*) in grower samples. This had the immediate benefit of determining no need for foliar fungicide applications. If the kit is negative for late blight, however, an additional test kit for *Botrytis* may be warranted since foliar symptoms of these two diseases can be similar.

Spongospora subterranea f. sp. *subterranea* (powdery scab)

Several tuber samples were tested for powdery scab, caused by *Spongospora subterranea*, using the available test kit. This test kit produced accurate results and is very useful to determine if tuber lesions are due to powdery scab or something else such as common scab (figure 2).

Pythium species

Test kits were accurate in diagnosing tuber infection with *Pythium*. Severely decayed potatoes that developed significant secondary bacterial soft rot still tested positive for *Pythium*, indicating the initial source of the breakdown (figure 3). This test kit will be helpful in identifying the cause of tuber breakdown in storage.

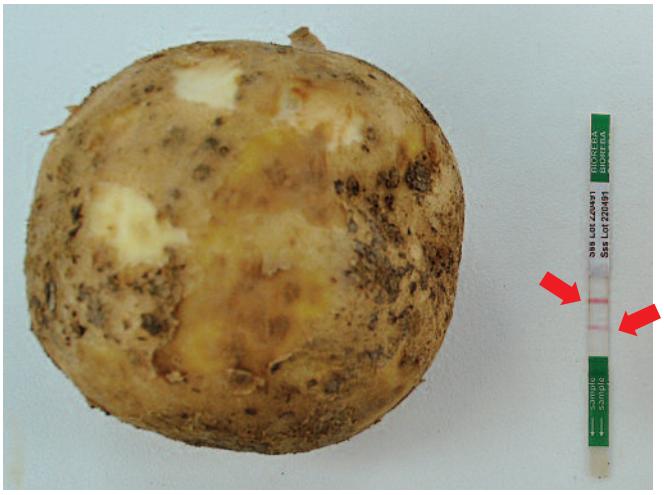


Figure 2. Pouch-type test kit strip showing a positive result (both a control and test line) for powdery scab, *Spongospora subterranea* f. sp. *subterranea*.

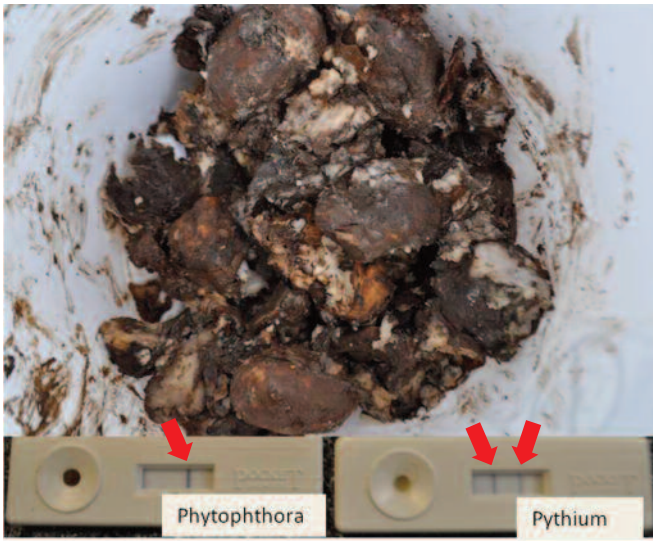
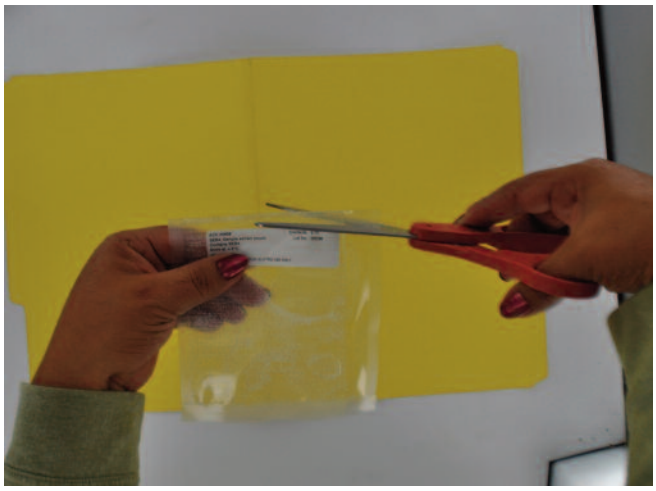


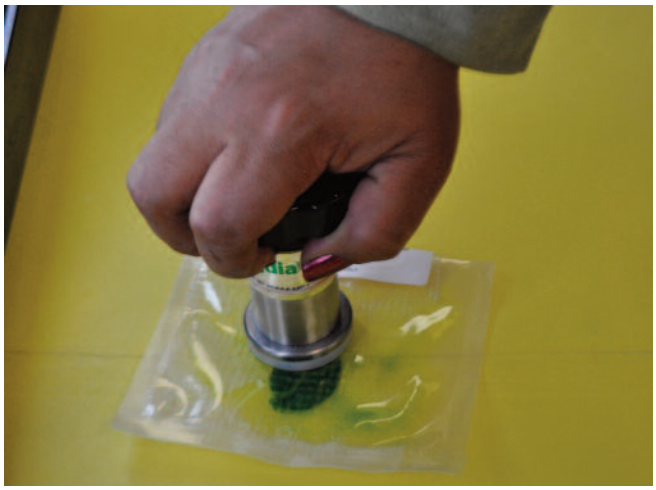
Figure 3. Severely rotted sample from a commercial storage tested negative for *Phytophthora* spp. and positive for *Pythium* spp.

USING POUCH-TYPE TEST KITS (TYPE 1)



Step 1. Cut off the top of the mesh bag with a clean utensil.

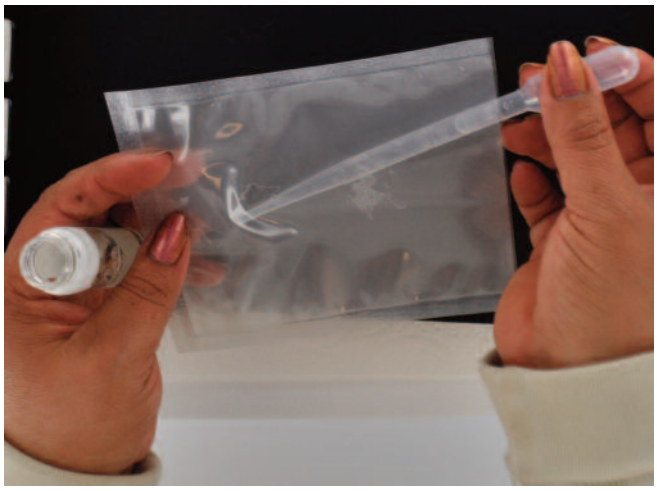
Step 2. Place the quarter-sized symptomatic leaf sample or nickel-sized tuber tissue sample between the mesh linings.



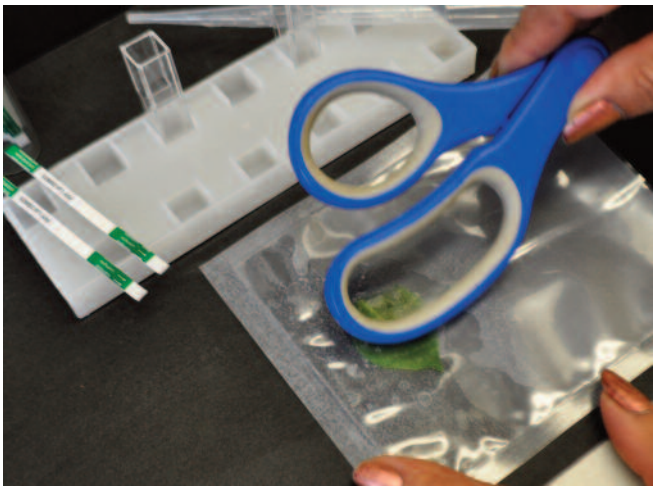
Step 3. Rub the bag with a handheld homogenizer or blunt instrument (pencil, scissors handle, etc.) to crush the tissue and produce an extract.

Step 4. Place the strip into the pouch sample-end first. Submerge no more than ¼ inch of the strip in the extract. Wait up to 30 minutes for test results. One red line (as above) indicates the test worked but results are negative. Two red lines indicate a positive result. No line indicates a faulty test.

USING POUCH-TYPE TEST KITS (TYPE 2)

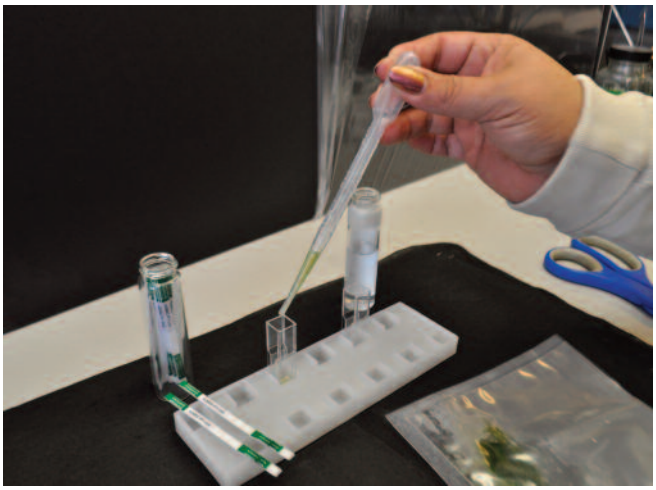


Step 1. Select a quarter-sized symptomatic leaf sample or nickel-sized tuber sample showing signs of infection and place it in the extraction bag either before or after adding buffer with the provided disposable pipette.

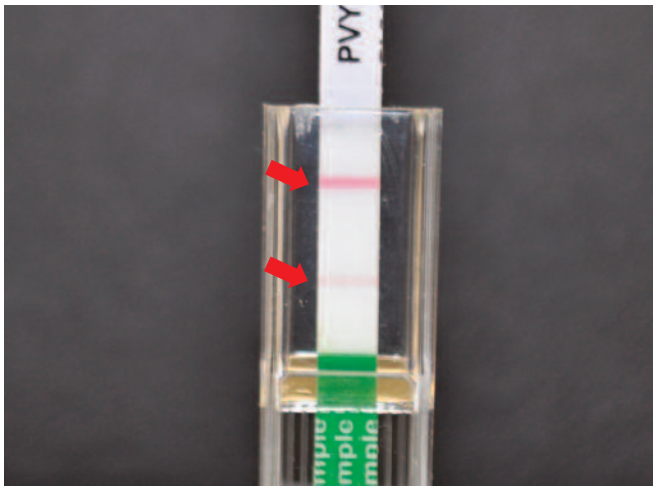


Step 2. Crush the sample with a handheld homogenizer or blunt instrument (pencil, scissors handle, etc.).

Step 3. Remove the extract with the pipette.



Step 4. Put four drops of extract into the provided disposable cuvette.

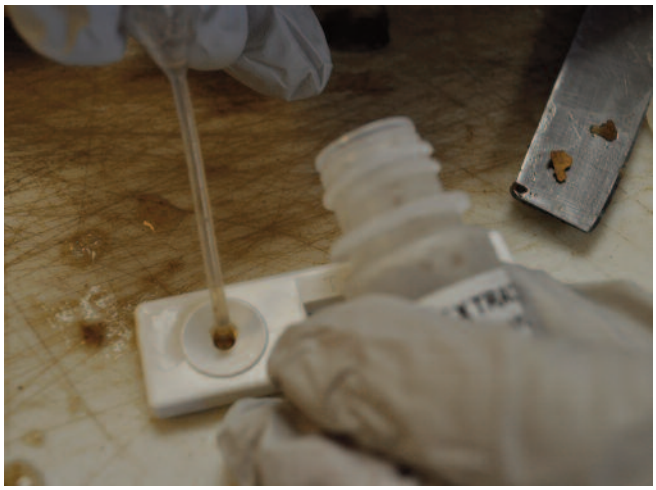


Step 5. Place test strip into the cuvette sample-end first, and wait 2–15 minutes for results. One red line indicates the test worked but results are negative. Two red lines indicate a positive result. No line indicates a faulty test. A faint line or a green line should be interpreted as negative.

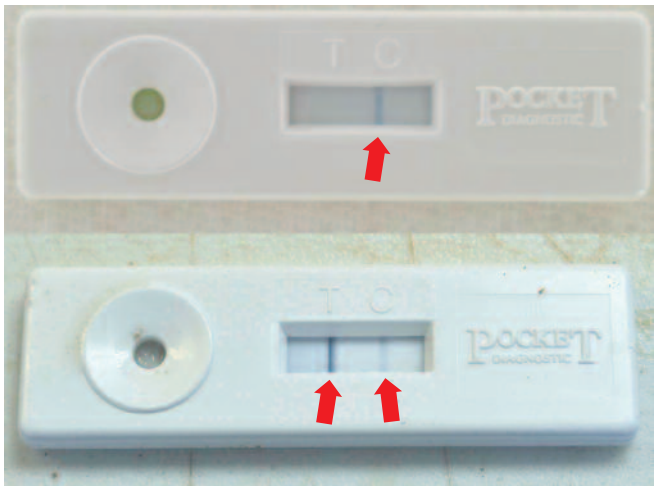
USING WELL-TYPE TEST KITS

Step 1. Select a sample (quarter-sized leaf tissue, nickel-sized tuber tissue), divide it into small pieces, and put it into the provided sample bottle.

Step 2. Shake the bottle for 20 seconds to 3 minutes depending on the fibrousness of the sample.



Step 3. Draw liquid from the bottle with the provided pipette, and place 2–3 drops into the sample well.



Step 4. Read results within 3 to 5 minutes. One line indicates a negative result (top), and two lines show a positive result (bottom).

FURTHER READINGS

Bohl, W., M. Thornton, and J. Miller. Undated. *Diagnosing field problems.* Paper presented at the Idaho Potato Conference, January 18, 2012. Available online at http://www.kimberly.uidaho.edu/potatoes/DiagnosingFieldProblems.pdf

Olsen, N., J. Miller, and P. Nolte. 2006. *Diagnosis and management of potato storage diseases.* CIS 1131. University of Idaho Extension, Moscow. Available online at http://www.cals.uidaho.edu/edcomm/pdf/CIS/CIS1131.pdf

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Photos by Lynn Woodell.

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