

USDA-APHIS Procedures for Submitting Domestically Detected Suspect *Ralstonia solanacearum* Positives Based on ImmunoStrip Testing

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Purpose: The purpose of this document is to provide USDA guidance on the subject of procedures for submitting suspect *Ralstonia solanacearum* positive samples.

Symptomatic, i.e., wilting, plants can be tested for the presence of *Ralstonia solanacearum* bacteria using immuostrips available from commercial providers listed below, or by ELISA testing. Positive reactions will only indicate the presence of *R. solanacearum*, but not what race is present. *R. solanacearum* race 3 biovar 2 is considered a federal quarantine pest and is not known to occur in the United States. *R. solanacearum* race 1 is considered endemic to the southern United States and is not a federal quarantine pest.

The following commercially available immunostrips are approved by APHIS for field level screening:

1. [Rs ImmunoStrip® Test from Agdia, Inc.](#), Elkhart, IN
2. [Potato Brown Rot Pocket™ Pocket Diagnostic](#), from Forsite Diagnostics Ltd, York, UK

Because of changes in the [Federal Select Agent Services](#) regulations, all races and biovars are select agents. Once a sample is determined to be positive for *R. solanacearum*, the sample must be handled according to the Select Agent Program regulations. There are Agriculture Select Agent Services (AgSAS) forms necessary depending on your course of action to A) destroy the sample or B) send it for further testing. The appropriate form(s) need to be completed and approved by the AgSAS. See below which provides the specific requirements for how you handle *R. solanacearum* positive samples and forms.

Here are links to the forms:

APHIS/CDC Form 4a info is here: [Reporting the Identification of a Select Agent Guidance for completing APHIS/CDC Form 4](#)

APHIS/CDC Form 2 info is here: [Request to Transfer Select Agents Guidance for completing APHIS/CDC Form 2](#)

These forms should be completed by the appropriate persons and sent to the following e-mail address: agsas@aphis.usda.gov or according to the instructions on the form and the guidance below.

Questions about the Select Agent Program should be directed to the above e-mail address or call: 301-851-3300 option 3.

There are two choices for plants disposition that show a positive *R. solanacearum* using the immunoStrip or ELISA: A) destroy the sample, or B) send for further testing at an approved laboratory (the only US approved laboratory for this purpose is the CPHST Beltsville Laboratory).

Plant samples testing positive for *R. solanacearum*:

- A) May be destroyed according to the select agent regulations if they are not from a foreign source, are not unusual hosts, do not show unusual symptomatic reactions, or are from areas in the US where *R. solanacearum* is known to occur.
 - a. An APHIS/CDC Form 4a (Report of Identification) is sent to the AgSAS and the sample is destroyed by the diagnostic lab. No further action required.

- B) Samples fitting the following criteria will require further testing at the CPHST Beltsville Laboratory, approved for confirmation and select agent handling:
 - 1) For positive testing (*R. solanacearum*) samples with a connection to a foreign source (i.e., imported plants), it is required that the sample(s) be forwarded to the USDA-APHIS-PPQ CPHST Beltsville Laboratory to be tested for *R. solanacearum* race 3 biovar 2. This must be done under the select agent regulations listed below.
 - a. an (APHIS/CDC Form 2 (Request to Transfer) must be sent to AgSAS and AgSAS approval required before sample shipping.
 - b. An APHIS/CDC Form 4a (Report of Identification). This Form is submitted by the confirmatory lab to AgSAS.
 - c. Sample destroyed at both diagnostic and confirmatory labs
 - d. Sample retained
 - i. If registered for agent, notify the AgSAS of retention and put in inventory
 - ii. if not registered, request a written authorization from AgSAS to exclude the sample from select agent regulations. This written authorization applies only to races 1 & 2.

 - 2) For other domestic detections of *R. solanacearum*, samples should be forwarded to the CPHST Beltsville Laboratory from unusual hosts, unique symptomatic reactions, and/or from areas in the country where *R. solanacearum* is not known to occur. The usual cultivated hosts of *R. solanacearum* race 3 biovar 2 include geranium (*Pelargonium* sp.), tomato, potato, pepper, and eggplant.
 - a. an (APHIS/CDC Form 2 (Request to Transfer) must be sent to AgSAS and AgSAS approval required before sample shipping.
 - b. An APHIS/CDC Form 4a (Report of Identification). This Form is submitted by the confirmatory lab to AgSAS.
 - c. Sample destroyed at both diagnostic and confirmatory labs
 - d. Sample retained
 - i. If registered for agent, notify the AgSAS of retention and put in inventory
 - ii. if not registered, request a written authorization from AgSAS to exclude the sample from select agent regulations. This written authorization applies only to races 1 & 2.

Consult the attached flowchart on page 4 of this document to see a summary of required steps for handling suspect samples and making the proper notifications/documentation.

A) For samples being destroyed, meeting the stated criteria:

1. If identified as *Ralstonia solanacearum*,
2. and no further race/biovar determination is required at an approved lab because the plant is not associated with a foreign source, not an unusual host, and from an area of the country where the organism is known to occur,
3. then an [APHIS/CDC Form 4a-Report of Identification](#) will be completed,
4. this Form 4 will be sent to Ag Select Agent Services (AgSAS) by fax or mail as indicated on the Form,
5. the sample will be destroyed.
6. No further action by the lab is required (destruction records for the pathogen must be kept and maintained for a minimum of three years).

B) For samples requiring further testing at an approved laboratory:

If a positive *R. solanacearum* sample requires further testing to confirm if it is race 3, biovar 2 at an Ag Select Agent Program registered laboratory (CPHST Beltsville Lab) because the sample is connected to a foreign source or is an unusual host or from an area in the country where the organism is not known to occur; then once you have submitted the **APHIS/CDC Form 4a Reporting the Identification of a Select Agent** and the **APHIS/CDC Form 2-Request to Transfer Select Agents**, and only after you have received notification that the select agent transfer has been approved by the Ag Select Agent Services, at that point you send the sample to the CPHST Beltsville Laboratory at the address below. The step-by-step procedures are summarized below.

1. If identified as *Ralstonia solanacearum*,
2. and further race/biovar determination is necessary at an Ag Select Agent Services registered lab because the plant is associated with a foreign source, or an unusual host and or from an area in the country where the organism is not known to occur,
3. the diagnostic lab completes sections C & D of the [APHIS/CDC Form 4a Reporting the Identification of a Select Agent, and completes an APHIS/CDC Form 2 Request to Transfer](#),
4. and sends the APHIS/CDC Form 2 to the Ag Select Agent Services for approval of the transfer,
5. once approved the diagnostic lab completes the APHIS/CDC Form 2 and notifies the CPHST Beltsville Lab of the intended shipment and shipping information,
6. then sends the sample and copy of the Form 2 in the shipment to the CPHST Beltsville Lab,
7. then the CPHST Beltsville Lab will receive the sample, complete the APHIS/CDC Form 2 paperwork and send the completed paperwork to the Ag Select Agent Services and the diagnostic lab,
8. then the CPHST Beltsville lab will determine if it is *R. solanacearum* race 3 biovar 2 or not,
9. and then the CPHST Beltsville lab will complete the APHIS/CDC Form 4a-Reporting of Identification of a Select Agent,
10. and send the APHIS/CDC Form 4a to Ag Select Agent Services,
11. the CPHST Beltsville Lab will notify the Domestic Diagnostics Coordinator of the test outcome, who will then report the results according to the Pest Identification Notification Protocol.

Sample Preparation and Sending Procedures

When sending the sample you can send the whole plant including roots, but they must be washed free of soil. At a minimum, enclose 1 gram of symptomatic stem/crown tissue. Include completed hard copies of [PPQ form 391 in the shipping container](#) and APHIS/CDC Form 2. Be sure to include cool packs in a sturdy insulated container and send by overnight carrier (do not send on a Friday) to the following address:

Sample Diagnostics
USDA-APHIS-PPQ-CPHST
BARC-East, Bldg. 580
Powder Mill Road
Beltsville, MD 20705-2350

Phone: (301) 504-7100, VOIP: (301) 313-9200

Group E-mail Address: APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@aphis.usda.gov

Once packaged and sent, please send an e-mail message to the group e-mail address above with a pdf of the completed PPQ form 391 and the overnight carrier tracking number.

Results Reporting

Results will be reported through the PPQ National Identification Services Domestic Diagnostics Coordinator and the National Survey Coordinator, according to the [Pest Identification Notification to the States protocol](#). Sample submitters and originating laboratories will receive results through their State Plant Regulatory Officials (SPROs) or State Plant Health Directors (SPHDs).

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***Ralstonia solanacearum* select agent notification process flow diagram**

This flow diagram is only for how to proceed and meet the select agent regulations. The determination on what to be destroyed or further tested can be found in B) 1 and 2 of this document.

