Document Control Number WI-B-T-4-3	**WORK INSTRUCTION** USDA, APHIS, PPQ, CPHST-Beltsville Laboratory, Bldg. 580, BARC-East, Beltsville, MD 20705	Revision Number **Revision 4**
Effective Date: **Apr 22, 2013**	**DAS ELISA for Detection of *Plum Pox Virus* using Agdia PPV ELISA kit**	**Page 1 of 14**

This work instruction describes detection of *Plum Pox Virus* (PPV) **by screening laboratories** using the Enzyme-Linked Immunosorbent Assay (ELISA) kit from Agdia Inc. (Elkhart, IN). The kit has been successfully tested with PPV isolates representative of six (6) known PPV subgroups (strains): PPV-C, PPV-D, PPV-EA, PPV-M, PPV-Rec and PPV-W in leaves, fruit and flowers.

This instruction includes ELISA testing recommendations taken directly from Agdia's PPV ELISA manufacturer instruction (see section I) with information added for use in USDA regulatory programs. It is expected that you will use this work instruction for PPV ELISA testing of **leaf samples**.

*Note: ELISA testing from flowers and fruit samples is **not** considered to be a routine testing and is **not** recommended for testing by screening laboratories.*

I. References and Work Instructions

https://orders.agdia.com/Documents/m243.pdf

***Retired May 2007**: WI-B-T-4-1 Supplemental Instructions for ELISA for Plum Pox Virus*

II. Introduction to PPV DAS ELISA

Agdia's PPV ELISA is a Double Antibody Sandwich (DAS) ELISA. Test wells of a micro titer plate are coated with antibodies specific to PPV. Samples are added to the micro titer plate. If PPV is present in the sample, it is bound by the antibodies and captured on the micro titer plate. After sample incubation, the plate is washed to remove any unbound sample. A PPV-specific polyclonal antibody conjugated to alkaline phosphatase is added and binds to any captured PPV. After a brief incubation the plate is washed to remove any unbound conjugate. PNP substrate is added to the micro titer plate. If the alkaline phosphatase conjugate is present a color will be produced signifying the presence of PPV. While color reactions can be observed visually, we highly recommend measuring the reaction quantitatively using a spectrophotometer (ELISA reader) in order to improve the detection of low concentration positives.

III. Equipment, Materials and Reagents

A. Equipment

1. Homogenizer: Homex 6 homogenizer (Bioreba #400004 or 400005) or hand grinder tissue homogenizer with circular bearings (Agdia #ACC 00900, Bioreba # 400010) (Hand grinder can also be attached to drill press)
2. ELISA plate reader (Spectrophotometer), capable of reading 405 nm (any vendor)
3. Dedicated calibrated pipettors (P10, P50, P200, P1000, 8 or 12 channel multi-channel)
4. Plate washer bottle (Agdia #ACC 00520)
5. Magnetic stirrer plate and magnetic bars (any vendor)
6. Analytical balance, capable of weighing 0.3 to 33 grams (any vendor)

B. Materials

1. PPV ELISA Kit (Agdia, #SRA 31505, available as 50, 500, 1000 or 5000 test wells)
2. PPV-positive control strip (Agdia #SPC 31505) *(Note: some positive control strips are included with the ELISA kit)*
3. Negative control (Agdia #LNC 31505) *(Note: Healthy* Prunus *leaf tissue could be used if available)*
4. Buffer pack, alkaline phosphatase, DAS/Compound (Agdia # ACC 00113) contains 10X carbonate coating buffer, 5X PNP substrate buffer and tablets, and PBST wash buffer powder (*These items are not included with the ELISA kit.)*
5. Sample extraction (mesh) bags: 12 x 14 cm, (Agdia #ACC 00930, Bioreba #430100, or Neogeneurope.com, #05-002)
6. Airtight container for incubations (i.e. plastic box with airtight lid)
7. Sealing film: SealPlate™ (i.e. LabSource #P98-241)
8. Sterile filter (barrier) pipette tips for the corresponding pipettors (any vendor)
9. Wide bore tips for loading ground plant samples (i.e. LabSource #P23-149)
10. 1.5ml disposable micro centrifuge tubes (any vendor)
11. Disposable multi-chanel solution basins (i.e. LabSource #P53-611)
12. Plastic transfer pipettes (*optional, any vendor*)
13. Ice
14. Gloves, disposable (any vendor)
15. Paper towels (any vendor)
16. Basin to collect sap runoff
17. Bleach to treat sap runoff
18. Distilled or deionized water

IV. ELISA Kit Limitations and Notes

- Expiration: The test should be used **within 1 year of purchase**. Please record the lot numbers and expiration dates of the capture antibody (Ab) and the alkaline phosphatase (AP) enzyme conjugate.
- Storage: Test results may be weak or the test may fail if storage instructions are not followed properly.
- Buffers: Do not store 1X buffers from day to day. Buffers should be warmed to room temperature prior to use, with the exception of GEB4 buffer which must be chilled before and during use.
- Inspect wash buffer (concentrate or 1X) for bacterial growth. If any, prepare new batch of PBST in a clean container.
- Sample Dilution: ELISA performance is very dependent on the recommended proper sample dilution of 1/10 tissue to grinding buffer ratio.
- Sampling: **PPV virus in infected trees can be unevenly distributed in the tree** and will be at lower concentrations as environmental temperatures increase. These factors can limit the ability of detecting the virus. Sampling from throughout the tree canopy,

selecting symptomatic tissue if possible, and testing in spring and early summer will increase the chances of detecting the virus.

- Please read all reagent and antibodies labels before preparing reagents or antibody dilutions. **The antibodies may have different dilution ratios in different reagent sets.**
- All antibodies and enzyme conjugates should be prepared in a container made of a material such as **polyethylene or glass** that does not readily bind antibodies. Do not use polystyrene, polypropylene or polycarbonate.
- Designated containers for capture Ab, AP enzyme conjugate and substrate are recommended if reusable. Never use the same container for the AP enzyme conjugate and substrate even if washed!
- All incubations should be done using a humid box (line an air-tight container with wet paper towels).
- Always use aseptic techniques when opening and removing reagents from vials and bottles. Keep the plate covered except when adding reagents, washing or reading. Always use new pipette tips for each reagent.
- Concentrated antibodies (capture Ab and AP enzyme conjugate) should be mixed by carefully pipetting up and down before dispensing. Concentration gradients could form due to prolong storage in glycerol.
- ELISA plates can be hand washed or washed using an automatic plate washer. We recommend that plates are first hand washed 3 times after sap and AP enzyme conjugate steps with 5-6 additional washes on the automatic plate washer.
- Air bubbles in the reaction wells could cause uneven coating or reagent interaction and are known to cause elevated O.D. or inconsistent results. Inspect each plate after loading each reagent or sample for bubbles. If bubbles are found, dislodge them by firmly holding the plate with one hand, and gently tapping the side of the plate with the flattened palm of the other hand in a horizontal motion. Do not tap the plate on the bench: this can cause splashes and well to well contamination.
- Processing large number of plates requires working in batches or assigning processing numbers to the plates for timely processing.

V. Leaf Samples

- Collected leaf samples must be processed as soon as possible. Field samples can be stored at 4°C **up to seven days** prior to processing. Prolonged storage will result in degradation of the virus and unreliable test results.
- Samples that are partially decayed (have a brown appearance or are moldy) should **not** be tested due to potentially decreased absorbance values. These materials must be recollected.

- If collected leaf samples are wet, they should be blotted dry with clean paper towels prior to storage at 4°C.
- If samples arrive warm to the touch, place them at 4°C for several hours prior to processing. Our experience has shown that the titer will be diminished if you do not chill the samples first to stop degradation.
- We strongly recommend wearing gloves when handling samples and changing them as often as possible. Changing gloves between samples in high throughput situations may not be practical so in those situations, we recommend changing gloves between sets of samples, based on the samples loaded on each plate or from the same property. We strongly suggest wearing gloves when handling a suspect positive sample.
- Samples for PPV need to be kept cold (4°C) for virus integrity; however, leaf samples that are to be ELISA tested should **not** be stored in a freezer.

VI. ELISA procedure

A. Coating an ELISA plate with PPV-antibody solution

1. Prepare a humid box

   Prepare a humid box by lining an airtight container with a wet paper towel. Keeping test wells in a humid box during incubation and between washes will help prevent evaporation.

2. Determine the number of 96-well plates you need to coat, based on the number of samples being tested. Maximum of 45 samples and 3 controls could be loaded on each plate (duplicate wells loaded).

3. Prepare capture antibody

   The capture antibody is provided as a concentrated solution and must be diluted with carbonate coating buffer before use. The recommended antibody to buffer ratio is given on the label. Please read label for appropriate dilution rate and note that the 96 test well reagents have a different dilution ratio than larger reagent sets.

   a) First prepare the volume of 1X carbonate coating buffer needed for the test. One hundred (100) µl of carbonate coating buffer is needed for each test well. A full plate will require 10 ml. Dilute the 10X carbonate coating buffer concentrate with distilled water (1 ml 10X concentrated to 9 ml distilled water).

   b) Mix the antibody by pipetting up and down or gently flicking the tube and then add the appropriate volume of concentrated capture antibody to the carbonate coating buffer at the dilution on the label. Pipette up and down to transfer the entire capture antibody from the tip to the solution. Mix the solution well before loading.

***Example 1**: To prepare 10 ml of capture antibody with specified 1/200 dilution, dispense 10 ml of 1X carbonate buffer into appropriate container and then add 50 µl of the concentrated Ab to the buffer.*

***Example 2**: To prepare 10 ml of capture antibody with specified 1/100 dilution, dispense 10 ml of 1X carbonate buffer into appropriate container and then add 100 µl of the concentrated Ab to the buffer.*

4. Coat plate(s)

Pipette 100 µl of the diluted capture antibody into each well using multichannel pipette for large number of samples. Tap the plate as described in Section IV to remove bubbles. Carefully apply SealPlate™ film tightly to the top of the plate. Inspect the seal to ensure complete adherence to prevent evaporation.

5. Incubate plate(s)

Incubate the plate in a humid box for 4 hours at room temperature (RT) or overnight in the refrigerator (4°C).

Note: Do not store coated plates longer than 24 hours. Use freshly coated plates immediately. If long term storage is desired, contact Agdia about postcoat buffers.

Prepare GEB4 buffer and samples during this period of time (see sections B and C).

6. Wash plate(s)

Once incubation is completed, empty the plate by firmly shaking the liquid from the plate into a sink or waste container. Turn plate right side up again and forcefully fill the wells completely with 1X PBST. Then quickly dump the plate again. Repeat 2 times. Hold the plate upside down and tap firmly on a folded paper towel on a benchtop to remove excess liquid.

B. General extract buffer 4 (GEB4)

GEB4 buffer is used to extract samples. Prepare fresh GEB4 buffer (see recipe below) each time after coating the ELISA plate, before handling the samples. Prepare only what will be required for that day.

GEB4 Buffer powder	33 g
Distilled water	1000 ml (1 liter)
Tween 20	20 ml or 20 g

1. Shake the bottle containing dry GEB4 powder to mix well. Weigh specified amount of powder, then transfer to a beaker containing a magnetic bar and place on a stir plate.

2. To make 1 liter of GEB4 sample extract buffer, add about 100 ml of distilled water to the powder and mix into smooth slurry. While mixing, slowly add the remaining volume of distilled water. Add Tween 20 to the solution. It is easier to add Tween 20 by weight than by volume due to the high viscosity of the solution. **Stir the buffer for at least 30 minutes**.

3. Chill the grinding buffer on ice for **a minimum of 30 minutes** so it is ice cold prior to use. Larger buffer volumes will require longer chilling time.

C. Sample and Control Preparation

1. Sample and tissue control preparation

Note: If healthy and/or PPV-infected tissue controls are available, please prepare them after the samples, following the same preparation procedure described below.

a. Label a set of grinding bags

b. A laboratory sample should consist of no more than 8 leaves. Stack the leaves one on top of another. **Unlike Agdia's instruction**, tear a portion of the leaves nearest the petiole end, along the mid rib on **one side** of the leaf, leaving the other side available for confirmation testing if necessary (see diagram in the Technical Note on page **13** of this WI). Leaves can also be cut with a disposable, single-edged razor blade. If cork borers or scissors are used, they must be decontaminated to prevent sample-to-sample contamination that could affect subsequent testing. (Cutting implements can be decontaminated by soaking in a 10% bleach solution for at least 3 min, followed by a wash in tap water and a second wash in distilled water to remove the bleach solution.)

c. Weigh **0.3g to 0.5g** (+/-10%) of each sample and place in the corresponding mesh bag. For Agdia's grinding bags, place plant sample between the mesh layers. Keep bags with samples **on ice** until all samples are prepared.

d. Add **pre-chilled** GEB4 grinding buffer to each bag in 1:10 ratio (tissue weight in g: buffer volume in ml). *Example: A sample weighing 0.5 grams requires 5 ml of GEB4 buffer.* (Chilled buffer helps to prevent virus degradation.)

Note: *A minimum of 300 µl of ground sample or control is needed: 100 µl of the ground sample is loaded per test well x* ***two test wells per sample,*** *plus an additional amount to assure easy dispensing.*

Note: We do not recommend volumes higher than 5 ml due to the bag capacity. Larger buffer volumes may cause spill-over and potential cross contamination between the samples.

e. Using a tissue homogenizer device, grind tissue samples **one at a time.** Grinding 2 or more samples together (stacking samples' bags) could cause insufficient tissue grinding or spill-over and potential sample-to-sample contamination. Tissue must be ground completely to release the virus. Insufficient grinding could cause false negative especially for low titer positive samples.

f. After grinding, keep bags of ground sap buried in ice until loading. (Once ground, we recommend placing the bags in the ice at an angle of 45° to facilitate sap collection in one corner. Please note that the Agdia grinding bags have no mesh in one of the corners: a convenient place to gather the sap.)

g. All processed samples should be loaded into a prepared plate (from step A.6) within 1-2 hours of grinding. Ground samples should NOT BE stored overnight for loading the next day. Storage of ground samples can lower the absorbance readings due to sample degradation.

2. PPV Positive Control (strip)

Before opening the container of control strips, let the container warm at room temperature for 15 minutes. This maintains the shelf life of the strips. Do not allow the container to remain open. Keep tightly sealed between uses. We recommend taking the container with the positive control strips out of the cold storage just before washing the plate, prior to loading the samples.

Each control strip requires 500 µl of GEB4 buffer. Dispense the required amount of GEB4 sample extract buffer into a micro centrifuge tube or other container. Dip the pad end of the strip(s) into the buffer and let it sit for 5 minutes. Use the strips to stir the buffer and discard after that.

The control strip can only be used once, after which it should be discarded. Do not store the positive control solution. It should be discarded after one day.

3. Negative Control (healthy peach) preparation

Please use the manufacturer's instruction provided with the purchased negative control to prepare and store the negative lyophilized ELISA control. Once re-hydrated, the vial of negative (healthy peach) control should be **kept on ice** until needed unless negative control is dispensed in individual tubes and aliquots stored at -80°C. Do not leave the

vial/tube of negative control open and/or at room temperature while loading multiple plates; keep the vial on ice with lid closed.

4. Grinding buffer control
   Each plate must include at least two test wells of the GEB4 buffer that was used for sample preparation. Please keep extraction (grinding) buffer in closed container while loading multiple plates to prevent contamination.

D. Loading the plate(s)

1. Prepare a loading diagram for each plate. **Each plate must include one positive and at least two negative controls** (purchased negative control and/or fresh healthy tissue, and GEB4 buffer control).

2. Before loading, mix each sample well by pipetting up and down or by gently massaging the sap in the sample bag. We highly recommend using **wide-bore** tips as it makes sap loading easier and ensures more consistent results between the duplicate wells.

3. Plates should be loaded as quickly as possible; prolonged loading time can cause poor reproducibility in duplicate wells and between samples.

4. Pipette 100µl of each prepared sample or control per well using wide-bore tips according to your plate layout onto a coated and washed plate (step VI-A-6). Each sample and control is loaded into 2 wells adjacent to each other, to improve the accuracy of the assay. Change tips between each sample/control.

5. ELISA controls should be loaded after the samples **with positive controls being loaded last.** We recommend loading positive controls in the rightmost column.

6. When using non-certified plates, we recommend loading buffer in the outside (edge) wells to avoid 'edge effect'. This is less critical when using certified plates.

7. Cover the plate with SealPlate™, place in the humid box and incubate at 4°C for at least 16 hours **or** 2 hours at RT.

E. Washing the plate to remove plant sap (1X PBST)

When it is time to wash the sap out of the plate:

1. After an overnight incubation, first remove the plate from 4°C and allow it to warm up for a few minutes so the sap will be easier to wash out.

2. To wash out sap, first empty the plate into a basin to catch the sap/buffer runoff by firmly shaking the plate over the basin. Wash the wells with 1X PBST several times (usually 3) over the basin until green is no longer visible. When the green sap is **no longer visible,** then wash 8 more times (counting out loud is helpful).

3. To hand wash, hold the plate at a 45° angle in your left hand (if you are right-handed), with column twelve at the lowest end to prevent contamination from the positive control well (if loaded on columns 11 and/or 12). Quickly and forcefully wash out the wells. After all washes are complete, tap the plate firmly on clean, dry paper towels several times to remove as much moisture as possible.

4. If using automatic plate/strip washer: Three **hand** washes are recommended to first remove the sap before doing 8 additional washes on the washer to reduce plate background.

   Note: Add appropriate volume of household bleach to the basin with runoff solution in order to make 10% bleach concentration. Treat for 20-30 minutes to deactivate potentially positive sap before pouring down the drain.

F. Prepare AP enzyme conjugate

Note: Always prepare enzyme conjugate within 10-15 minutes of use.

1. The AP enzyme conjugate is supplied as a concentrate and must be diluted with RUB3 enzyme conjugate diluent before use. The recommended conjugate to buffer ratio is given on the label to achieve the appropriate dilution.

   Please use disposable or **dedicated** container for preparation of conjugate working solution.

2. Dispense the appropriate volume of RUB3 enzyme conjugate diluent into a dedicated container. Remember 100 µl of diluted enzyme conjugate is needed for each test well. Add appropriate amount of AP conjugate to diluent.

   Example 1: To prepare 10 ml of AP conjugate with specified 1/200 dilution, dispense 10 ml of RUB3 buffer into appropriate container and then add 50 µl of the concentrated enzyme conjugate to the RUB3 buffer.

Example 2: To prepare 10 ml of AP conjugate with specified 1/100 dilution dispense 10 ml RUB3 buffer. Into appropriate container and then add 100 µl of the concentrated enzyme conjugate to the RUB3 buffer.

3. After adding the enzyme conjugate to the diluent, mix thoroughly by pipetting up and down to transfer all of the conjugate from the tip into the solution. Then mix the entire solution well by swirling the container or using clean glass rod. It is important to mix the enzyme conjugate solution well.

4. Add 100 µl of the diluted AP enzyme conjugate into each test well. Tap the side of the plate to remove bubbles. Cover the plate with SealPlate™, place in the humid box and incubate for 2 hours at RT.

G. PNP substrate incubation and plate reading

Prepare PNP substrate 10 -15 min before the end of the incubation in step F-4.

Note: Do not touch the PNP tablets or solution, or place fingers inside the reagent reservoirs. Phosphatase contaminants from human skin will utilize the PNP substrate and develop color before addition to the test plate. Do not use metal forceps for counting PNP tablets. Use plastic forceps or shake tablets into the bottle cap. Do not expose PNP substrate to strong light. Light or contamination could cause background color in negative wells.

1. Prepare working solution of the PNP substrate buffer by diluting it 5 times in a container large enough to ensure good mixing. Each well needs 100 µl of PNP substrate. Tablets will dissolve faster if the tube is placed on a shaker at slow motion for several minutes. Dark glass containers are recommended for larger volumes.
    a. Each PNP tablet makes 5 ml of substrate.
    b. Full plate requires 10 ml of substrate.
    c. For a full 96-well plate, add 2 ml of 5X substrate buffer to 8 ml of dH2O in a plastic tube (i.e.: 15 ml Falcon tube with screw-on cap) wrapped with aluminum foil. Add two (2) PNP tablets.

2. While PNP tablets are dissolving, wash the plate to remove the enzyme conjugate 8 times with 1X PBST. Inspect the wells for air bubbles. Tap firmly on the paper towel to remove remaining wash buffer and any air bubbles. If air bubbles are still present, they may be broken with a clean pipette tip. Carefully wipe the bottom of the plate to remove any liquid, bubbles, lint, or smudges.

3. Dispense 100 µl of the prepared PNP substrate into each test well. Tap the side of the plate carefully with the palm of your hand to dislodge air bubbles. Incubate plates in a

drawer or dark room for 60 minutes at room temperature. Plates should be protected from direct or intense light. (There is no need to cover the plate with SealPlate™ or place in humid box at this step).

4. Before the end of the incubation:
   a. Turn on the spectrophotometer/ELISA reader and allow a warm-up period according to manufacturer specifications.
   b. Set the wavelength to 405nm.
   c. If necessary, wipe the bottom of the plate again with a paper towel to remove moisture.
   Make sure there are no bubbles in the test wells because they can interfere with the readings. If bubbles are observed, gently break the bubble using a clean pipette tip (one tip per well). Any remaining bubbles should be noted on the loading diagram.
5. Read each plate after 60 minutes and record the O.D. Immediately review the readings for potential problems (significant well-to-well variation). If any are observed, visually *inspect the plate, remove the bubbles or condensation, if any, and read the plate again.*

VII. Results interpretation

Results interpretation is conducted based on the 60 min reading.

1. Negative (healthy peach) Control and Buffer Control Readings

The negative control and buffer control wells must be **colorless** when inspected visually. If color develops, the test is invalid and all of the samples of the plate plus the controls must be re-tested.

Cut-off determination
Calculate the O.D. average of the two wells of negative control from Agdia (or healthy Prunus, if available). This value = '**A**'.

Then determine the threshold (**cut-off**), '**B**', by multiplying '**A' x 2.0= 'B'**.

2. Positive Control Readings

The averaged positive control O.D. on each plate must be positive **(O.D. >B) (yellow)** for the ELISA run to be valid. If a plate's positive control fails to be positive then none of the ELISA results on that plate are valid and all the samples loaded on that plate must be retested using newly ground sap.

Note: O.D. range for the healthy peach control, PPV positive or/and buffer control is not provided by the manufacturer. Table 1 below provides controls range (average +/- 3x STDEV) established in the 2012 confirmatory tests (around 1300 samples tested) and 2012 NPPLAP PPV Proficiency Testing Panel results of 13 individual diagnosticians from 8 laboratories. Provided for reference only!

Table1. ELISA controls range (Ave O.D. +/-3x STDEV) at 405 nm after 60 min incubation without background subtraction.

Controls (Agdia)	2012 Confirmatory tests	2012 PPV PT test
Negative cntr (healthy peach)	0.095 +/- 0.071	0.096 +/- 0.053
Positive cntr	3.385 +/- 1.806	3.555 +/- 2.040
Buffer (GEB4) cntr	0.089 +/- 0.021	0.089 +/- 0.042

3. Sample Readings

Determine each sample's O.D. value by averaging the readings of the samples duplicated wells.

- O.D. of the duplicate wells for the sample could vary by no more than 50% or the sample should be re-tested.
- If O.D. of one of the wells for the same sample is above the cut-off (**2.0 x A**) and the other is below the cut-off, the sample should be re-tested.

Interpret each sample result by determining if the average sample O.D. is less than, equal to or greater than "**B**".

- Any sample that is ≥ B is considered suspect positive.
- Any sample that is < B is considered negative.

VIII. Reporting an ELISA positive result

Suspect positive sample test results should be reported immediately to Mafalda Weldon, mafalda.weldon@aphis.usda.gov (USDA-APHIS-PPQ *Plum Pox Virus* Eradication Program Director)

Samples determined by the operations and program managers to need federal confirmation should be sent to the PPQ Laboratory in Beltsville, MD according the PPV action plan. **Prior to shipment, please e-mail APHIS-PPQ CPHST Beltsville Sample Diagnostics** at APHISPPQCPHSTBeltsvilleSampleDiagnostics@we.aphis.gov that samples are being forwarded, providing ELISA testing results for the samples and all three controls.

Remaining leaf tissue from a sample suspected as positive for PPV should not be discarded until it is retested and/or it is determined the sample should be forwarded. (Samples that are determined to test negative should be autoclaved after the test results are reviewed and approved as negative.)

Suspect positive samples should be shipped in an insulated (preferably) cardboard box with enough ice packs to maintain a cool environment for two days. Please do not place samples directly on the ice packs. Use paper towels or newspaper to insulate the samples.

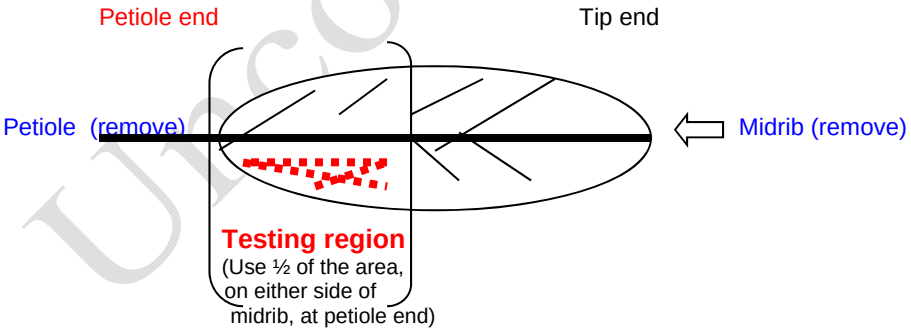
Ship by FedEx or UPS next day delivery to the following address:

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

IX. ELISA Trouble shooting

Problems with ELISA kits should be forwarded to your Agdia representative. Provide them with the kit name, lot number, expiration date and your OD readings. Please, record antibodies, controls and other reagents lot numbers and expiration dates in case they are needed for troubleshooting. Contact the manufacturer for more information on the controls expected O.D. There are many factors that can cause an ELISA to produce inadequate results. See Agdia's website: http://www.agdia.com/campusuite/modules/faq.cfm?grp_id=506&main=0

X. TECNHICAL NOTE: Leaf diagram for area to be sampled for ELISA:



Document Revision History

Status (Original/Revision/Cancelled)	Document Revision Number	Effective Date	Description
Original	Original	2-29-2008	To baseline the work instruction.
Revision	1	01-07-2010	Updated the two Agdia website links. Added note regarding fruit samples being non-routine. Added new PPV-**Rec** strain to detection list. Added info that leaf samples should not be wet prior to storage. Edited some blue font sentences to match the 'm243.3' version of the *Agdia Inc. PPV ELISA User Guide*. Added info on what container material to avoid for preparing antibodies and enzyme conjugate. Added ELISA reader warm-up information. **Revised threshold cut off for positive results to be A' x 2.0 = 'B'.** Removed expected OD for positive controls and changed to follow the greater than threshold rule. PPV Operations Director is in transition. Added leaf diagram technical note.
Revision	2	2-17-2012	Changed results interpretation to use readings taken at 2 hours. Added a note to the confirmatory labs. Updated names and contact information for the PPV Program leadership and CPHST-Beltsville Lab.
Revision	3	5-1-2012	Edited for clarity and content, including adding back in the missing number of washes after green sap was no longer visible (page 8). Added recommendation to 'bleach treat' the first 3 rinses and the basin-collected sap runoff. (page 7). Highlighted 'on ice' (page 6) with red font. PPQ National Program Manager for PPV name updated.
Revision	4	4-22-2013	Provided detailed information on samples and controls handling. Changed results interpretation to use readings taken at 60 min. Removed a borderline determination. Provided reference for the Agdia's kit controls.

Approved by Dr. Mark Nakhla, CPHST-Beltsville Laboratory Director