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INTERNATIONAL POTATO CENTER

Standard Operational Procedure

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Detecting Sweetpotato Viruses with the Doctor Vida Device.

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INTRODUCTION

Loop-mediated isothermal amplification (LAMP) is a single-tube technique used to amplify specific nucleic acid sequences. It offers a low-cost alternative for disease detection and can overcome several limitations of PCR-based methods, particularly the high cost and complexity of thermal cycling equipment.

LAMP employs four primers—two internal (forward and reverse) and two external—and a DNA polymerase with strand-displacement activity to generate products with single-stranded loop structures. These loops enable subsequent rounds of amplification without the need for thermal denaturation. The addition of a third primer pair (loop primers) can further accelerate the reaction (Fig. 1, top). LAMP is highly efficient, capable of producing up to 10^9 copies of the target sequence in under one hour, all at a constant temperature.

Like PCR, LAMP can detect RNA targets by incorporating a reverse transcription step to synthesize cDNA. This can be performed in a single tube using a thermostable reverse transcriptase, allowing for a combined reverse transcription–LAMP (RT-LAMP) reaction.

LAMP is more tolerant than PCR to inhibitors present in crude plant extracts, reducing the need for extensive nucleic acid purification (e.g., CTAB-based extraction). This makes it suitable for field applications. For instance, the Doctor Vida device (STAB Vida, Portugal) is a portable, battery-powered platform capable of real-time fluorescence monitoring of isothermal amplification reactions, enabling on-site diagnostics (Fig. 1, bottom).

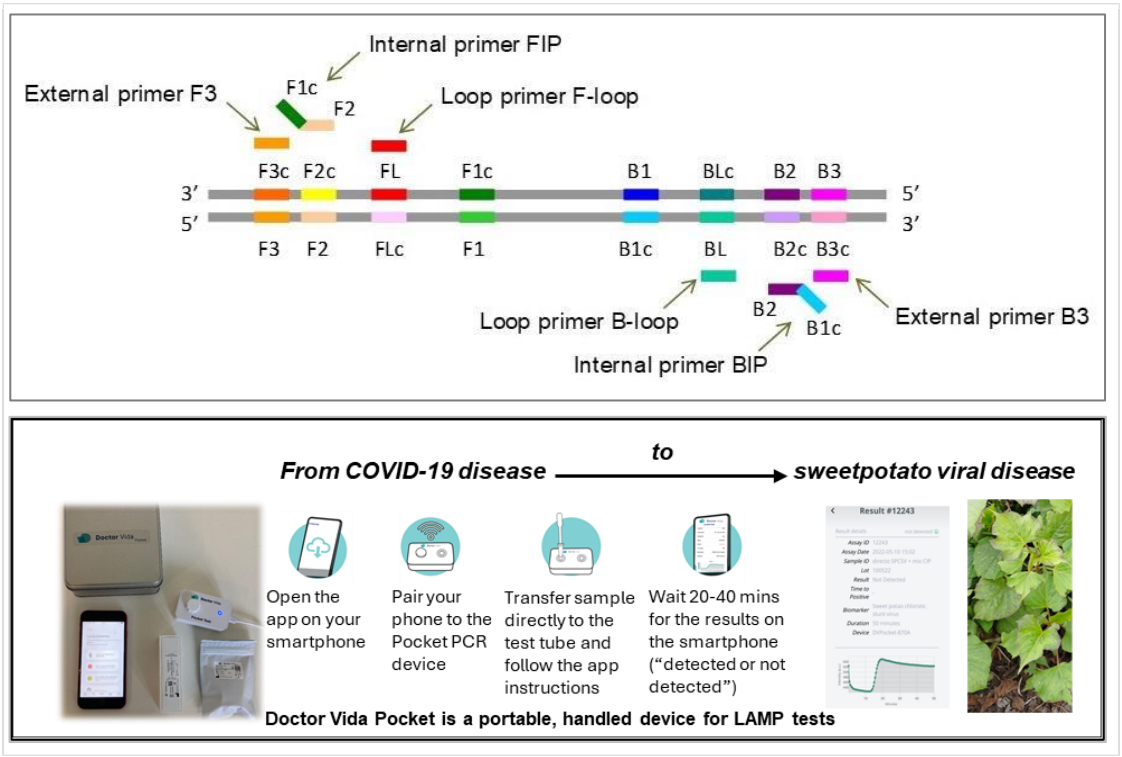


Figure 1. (Top) Primer set used in LAMP, consisting of two outer (external) primers—Forward Outer Primer (F3) and Backward Outer Primer (B3); two inner (internal) primers—Forward Inner Primer (FIP) and Backward Inner Primer (BIP); and two loop primers—Loop Forward (LF) and Loop Backward (LB). **(Bottom)** Workflow of LAMP testing using the Doctor Vida device, which enables real-time fluorescence monitoring of the amplification reaction. (*Source: Adapted from STAB VIDA*).

SCOPE

This procedure outlines the steps for conducting a field-deployable LAMP assay using the Doctor Vida device to diagnose the major viruses infecting sweetpotato worldwide—SPFMV, SPCSV, and SPLCV (including related sweepoviruses). It covers the full workflow, from sample collection and crude sap extraction to LAMP reaction setup, operation of the Doctor Vida device, and result recording and interpretation.

SAFETY

Always wear gloves when performing sap or nucleic acid extraction and when preparing Master Mix reactions to minimize the risk of RNase contamination. Use only clean, RNase-free disposable pipette tips with all supplied reagents. Thoroughly wash containers, glassware, and other equipment after use. Keep all reagent containers tightly closed when not in use.

MATERIALS

Plant material

- Field-grown plants: fresh and dried leaves.

Plant material is considered suitable for RNA extraction if it meets the following criteria:

- Fresh leaves collected on the same day or the previous day and stored at 4 °C.
- Dried leaves that retain a green color; browning indicates degradation or oxidation of the sample.

Reagents

Chemical & reagents	Supplier	Catalog	Storage conditions
Polyethylene glycol average mol wt. 200 (PEG 200)	SIGMA-ALDRICH	P3015-500G	Room temperature
KOH	Merck	105033	Room temperature
Alkaline PEG buffer Alkaline PEG200 reagent to use as a lysing agent for sample maceration. PEG captures inhibitors present in the extraction. The alkaline pH denatures nucleases in the extraction which could degrade target nucleic acid and inhibit the detection.			Room temperature (dark bottle or cover with foil)
Nuclease-free water Nuclease-free water (NFW) to dissolve/ mix primers and prepare Master Mix for LAMP reaction			Room temperature (Fridge at 4°C when it is already dispensed in Eppendorf tubes)
Isothermal Master Mix The Isothermal Master Mix allows for florescence detection of the product on the genome. It is tolerant to the inhibitors present in the plant tissue	OptiGene, UK	ISO-DR004-RT	Fridge at 4°C (Freeze at -20°C once opened)
Hexadecane	Sigma-Aldrich	H6703-100ml	Room temperature
SuperScript III reverse transcriptase-200U/µl	Invitrogen	18080-044	Freeze at -20°C

Primers for SPFMV, SPCSV, SPLCV (sweepovirus), and COX (Invitrogen):

SPFMV Primers	Sequence (5' to 3')	Final conc
SPFMV F3	TACAACGTAAMCTTGACTGATATGAGT	0.2 µM
SPFMV B3	GTTATGTATATTTCTAGTAACRTCAGT	0.2 µM
H SPFMV FIPv2*	TGCRGCTGCYTTCATCTGYAWWTGTGGATATGCATTTGATTTYTAYGAGCT	2 µM
H SPFMV BIP*	AAGAATGCGMRWAATCGGTTGTTTGGGCCTCTCCGTATCYTCTTCTT	2 µM
SPFMV LF	TTCTTTAGCACGTGYAGGKG	1 µM
SPFMV LB	TGGAYGGAAACGTCTCCAC	1 µM

SPCSV Primers	Sequence (5' to 3')	Final conc
SPCSV F3_A	CCGATTATGATGGTTCCGATT	0.2 µM
SPCSV B3_A	CGGCGAAAGTCTTCCTAC	0.2 µM
H SPCSV FIP_A*	TGACATACGATGCGACAGCCGGAAGTCGTCATAGATTGGATT	2 µM
H SPCSV BIP_A*	CGCGTATGCTGACAGATCTCTTATTATGAGCGCGAAGCAA	2 µM
SPCSV LF_A	CACCTGAAGTACAAATGCTGTG	1 µM
SPCSV LB_A	ATGCTGATGCTGAATCTCTGT	1 µM

Sweepo Primers	Sequence (5' to 3')	Final conc
Sweepo F3	TTGCCAGTCCTTCTGGGC	0.2 µM
Sweepo B3	GTAATTTAGATAGGATWTTTTCWCC	0.2 µM
H Sweepo FIP*	GAAGGCCCAAGYAGAATAGGCAATTTAGGTATTGGGGGTTGACGT	2 µM
H Sweepo BIP*	ATCCATSACATTYTCAGRGCCCTCCTTCTGTITATTCTTCICCTT	2 µM
Sweepo LF	TACAGCAACAGTGCTTGGTAT	1 µM
Sweepo LB	ARTCRCTGATAATGTCAGGWAC	1 µM

COX Primers	Sequence (5' to 3')	Final conc
COX F3	TATGGGAGCCGTTTTTGC	0.2 µM
COX B3	AACTGCTAAGRGCATTCC	0.2 µM
COX FIP*	ATGGATTTGRCCTAAAGTTTCAGGGCAGGATTTCACTATTGGGT	2 µM
COX BIP*	TGCATTTCTTAGGGCTTTCGGATCCRGCGTAAGCATCTG	2 µM
COX LF	ATGTCCGACCAAAGATTTTACC	1 µM
COX LB	GTATGCCACGTCGCATTCC	1 µM

*FIP/BIP preferably HPLC grade purification. Other primers work well as desalted.

NOTE: *Different LAMP primers are used depending on the virus target.*
A LAMP assay targeting the plant cytochrome oxidase gene (COX) is also available and serves as a positive extraction control, helping confirm that a negative result is not due to extraction failure.

Supplies
Plastic (crystal clear polyethylene) bags: Sample bags 4"x6"x6 (wide, long and thickness, respectively. Thickness in thousandth of an inch). For collecting and macerating leaves samples
Hand roller
Small glass tube (1 cm diameter) For cutting leaf discs
Fine-tip permanent marker To annotate/identify samples, tubes, etc
0.5- or 0.2-ml tubes (for dispensing nucleic acid free water or for preparing Master mix reaction)
Tips whit filter (1 ml, 200 µl, 10 µl)
0.2 ml PCR tubes, clear flat cap (Axygen PCR-02-C x 1,000)
Pipettes set (2, 20, 100, 200, 1000 µl)
Inoculation loop (1 µl) For diluting sample sap and placing it in the LAMP reaction (in the field)
Criobox For storing primers, Isothermal Master Mix, etc.
Equipment
Doctor Vida device – It is the primary tool as LAMP instrument for the isothermal reaction. ***NOTE:*** *cycling conditions can be set in a real-time PCR (qPCR) machine to run large number of samples in the lab. For this, you will need standard 0.1 or 0.2-ml PCR tubes (depending on the qPCR machine requirements)*
Smartphone – to connect to and operate the Doctor Vida device One smartphone can operate up to four Doctor Vida devices simultaneously. ***NOTE****: A smartphone with internet access, Wi-Fi connectivity, and Bluetooth capability is required.*
Portable power bank (10,000mAh) – act as power supplier to connect devices.
USB 2.0 (4-Port) hubs – accessory to connect four devices at once.
Multiplugs – accessory for plugging four devices at once.
Power adapters – accessory to facilitate plugging four devices at once
Freezer (-20°C) – for storing primers, enzymes, iso-thermal master mix.
Refrigerator /fridge – to keep nuclease-free water dispensed in Eppendorf tubes, collected samples until their processing.
Laminar Flow chamber – for dissolving and preparing primers stock.

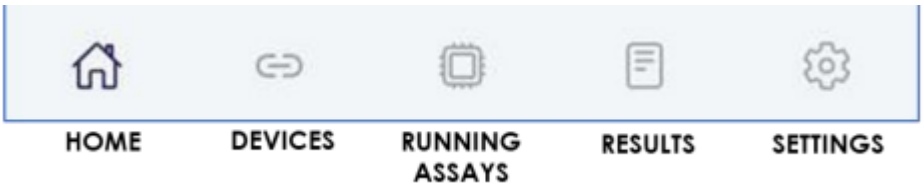
PREPARING MATERIALS AND PAIRING SMARTPHONE WITH THE DEVICE BEFORE CONDUCTING LAMP ASSAY IN THE FIELD

It is important to ensure that all devices and materials are ready before going to the field for on-site detection.

Devices:

1. Fully charge the portable power bank(s) (100%).

2. Install and configure the Dr. Vida Pocket PCR app on your smartphone.
   - Carefully follow the manufacturer's instructions. For a visual guide, refer to the instructional videos provided by STAB VIDA (video 1, video 2) to assist with the installation and setup process.
   - The app is available for download on both Google Play Store and the iOS App Store.
3. Create an account if you don't already have one.
   - Tap on "New user? Create account", complete the form, and select "Create account".
4. Log in with your registered email and password.
   - ***NOTE:*** *Ensure your email address is valid—otherwise, you will not receive the test results*.
5. Open and familiarize yourself with the menu options displayed at the bottom of the screen:



6. In the "DEVICES" menu, check and select the available devices to perform tests. Only devices recognized via Bluetooth and their status will be displayed. You can connect to four (4) devices and operate them simultaneously
   - Select the desired device(s), then click "Connect" and "Continue". The devices will now be ready to run tests.
   - Ensure that:

     (i) the selected device is turned on and not connected to another phone, and

     (ii) Bluetooth is activated on your mobile phone (if not, refer to the troubleshooting section).
7. Set up the Doctor Vida device according to the manufacturer's instructions.

Materials:

8. Double-check that you have all the necessary primers, reagents, and consumables required for conducting the LAMP assay.
9. If needed, prepare Alkaline PEG200 buffer, a lysis reagent for sample extraction composed of Polyethylene Glycol 200, potassium hydroxide (KOH), and autoclaved distilled water.
10. **Prepare the Alkaline PEG200:**
    - Use the following formula: 60 g Polyethylene glycol (PEG 200) + 930 µl 2 M KOH + 39 ml autoclaved distilled water.
    - Adjust the pH (if needed) by adding drops of KOH or NaOH until the pH reaches approximately 13.3.
    - Store at room temperature in a dark bottle or wrap the container in foil to protect from light.
11. **Prepare the 10x primer mix:**

    Primers (oligos) are typically supplied lyophilized. Rehydrate them using TE buffer or nuclease-free water (NFW) to prepare a 100 µM stock solution (label the tube accordingly).
    - Before opening: Briefly spin the tubes in a microcentrifuge to collect the oligos at the bottom.
    - Rehydration: Add the specified volume of TE buffer or NFW to each tube. Use TE

buffer for long-term storage

- Mix and store: Vortex or pipette to mix thoroughly. Store at –20 °C.
- Prepare working stocks: Make 100 µl of a 10× primer mix (for each virus and COX gene). Use the dilution formula for the calculations: $C_1V_1 = C_2V_2$, where
  C1 = initial concentration (100 µM)
  C2 = final concentration (e.g., 10 µM)
  V2 = final volume (e.g., 100 µl)
  Solve for V1 = volume of stock needed

LAMP Primers	**Final concentration in LAMP reaction**	**For 10x concentration**	**Volume from 100 µM** (stock solution) required **for preparing 10x stock**	**Volume of TE buffer/** NFW **to add for 10x primers mix***
F3	0.2 µM	2.0 µM	2 µl	36 µl (The total volume of the primers mix is 64 µl)
B3	0.2 µM	2.0 µM	2 µl	
FIP	2.0 µM	20.0 µM	20 µl	
BIP	2.0 µM	20.0 µM	20 µl	
LF	1.0 µM	10.0 µM	10 µl	
LB	1.0µM	10.0 µM	10 µl	

* To prepare the 10× primer mix, add 36 µl of TE buffer (or nuclease-free water, NFW) to the 64 µl of combined primer components (as previously indicated), bringing the final volume to 100 µl.

NOTE: *Do NOT store oligonucleotides in NFW at 4°C as they may degrade over time. For long-term storage, use TE buffer and store at –20 °C. TE buffer helps protect DNA by chelating divalent cations (e.g., Mg^{2+}), which are required for nucleases to degrade DNA.*

CONDUCTING LAMP ASSAY IN THE FIELD

NOTE: *A visual guide is available showing how to perform LAMP in the field, using Genie III device as a reference.*

Sweetpotato leaf samples

12. Collecting leaves and labelling bags:

    Collect and clearly label leaf samples in plastic bags. This should be done on the same day the test is performed.

    For each plant to be tested, make a composite sample by taking one leaf from the top, middle, and bottom sections of the plant.

13. Cutting leaf discs:

    From each leaf sample, cut a 1cm diameter leaf disc. To perform this, Place the leaf inside the upper part of the plastic bag. Then, using a small test tube, press firmly on the outside of the bag against a hard surface (e.g., table) to cut the disc. Discard the remaining leaf tissue.

    NOTE: *Viruses such as SPCSV and SPLCV are typically concentrated in the leaf veins. To improve detection, cut discs near the leaf base, close to the petiole, where veins converge.*

14. Adding extraction buffer into bag:

    Add 1 mL of alkaline PEG buffer to each labeled maceration bag containing 3 leaf discs. This represents a sample dilution of approximately 1:10 (w/v).

NOTE (bulk testing): *When many samples are tested, a bulk of 5 samples can be combined for high-throughput testing. In this case, collect one leaf from the middle level of each plant and cut one disk per leaf. Add then 2 ml of the alkaline PEG buffer to the bulked samples (5 discs).*
In this case, a positive result indicates that at least one sample is infected but does not identify which one.

15. Crushing the sample:

    Manually crush the leaf discs inside the bag. Use a test tube or a wooden dowel to grind the tissue thoroughly until a uniform sap is produced.

16. Diluting de extract 1:10 (v/v) with nuclease-free water (NFW):

    Mix 1 µl of crude extract with 9 µl of NFW, mix thoroughly, and use 2 µl of this diluted sap as the template for the LAMP reaction.

LAMP Assay Setup

Set up in a location protected from the wind, as wind can cause aerosol movement between samples, leading to cross-contamination.

NOTE: *Clearly label all 0.2 ml reaction tubes to keep your samples organized during fieldwork.*

Preparation of Master Mix reaction

Set up a clean reaction area. Place the labeled 0.2 mL PCR tubes on a rack with the lids open and pointing away from the pipetting hand.

17. Preparing the Master Mix (MMix) reaction:
    - Pipette the required volumes of each component according to the LAMP master mix formulation (see table below).
    - Calculate the total volume based on the number of samples plus one extra to compensate for pipetting loss.

LAMP Master Mix reaction for sweetpotato viruses:

Component	Initial conc	Vol per rxn (µl)	Final conc.	Vol for "n +1" rxn (e.g. 9 +1)
Isothermal Master Mix (ISO-DR004-RT)	10 x	31.30	1x	313.00
Primer Mix		5.22	1x	52.20
Nuclease-free Water (NFW)		9.81		98.10
SuperScript III RT diluted 1/50 (4U/µl)		1.67		16.70
Volume of Master mix reaction		**(48.00)**		**(480.00)**
Target (diluted leaf extract - see step 13)		2.00		
Hexadecane (Sigma-Aldrich)		50.00		
Total volume		**100.00 µl**		

NOTE 1: *The RT Isothermal Master Mix dried reagents are stable for a minimum of twelve months when stored cold (4°C). After resuspension, it is recommended to aliquot the mix for storage at -20°C if the mix is not used immediately.*

NOTE 2: *SuperScript III reverse transcriptase-200U/µl (Invitrogen, USA). Addition of this extra (fresh) enzyme improves LAMP assay.*

NOTE 3: *Hexadecane is added carefully on the top of the Master Mix reaction to prevent evaporation.*

18. Add 2 µl of the 1:10 diluted leaf extract (target, **from Step 16**) to the corresponding tube containing 48 µl of the LAMP MMix reaction.
19. Close the tube caps starting with the negative control and ending with the positive control.
    Always add and close the positive (target) control last to minimize the risk of cross-contamination in case of spills during closure.
20. Set the LAMP amplification program to 65 °C for 40 minutes using the smartphone connected to the Doctor Vida device.

Operating the Doctor Vida device

Carefully follow the manufacturer's instructions.
One smartphone can simultaneously operate up to four Doctor Vida devices.

21. Prepare the Doctor Vida device before starting the tests (see Fig. 1, bottom)
    - Place the device on a flat and stable surface. Uneven placement may affect test performance.
    - Connect the device to a 5VDC, 2A power source, either through a wall outlet or a fully charged portable power bank (Fig. 2). Allow a few minutes for the device to reach a stable temperature.
    - Ensure the device is paired via Bluetooth with a smartphone that has a stable Wi-Fi connection.
    - Remove the lid and leave the device powered on for approximately 30 minutes before the first use of the day to allow temperature stabilization.

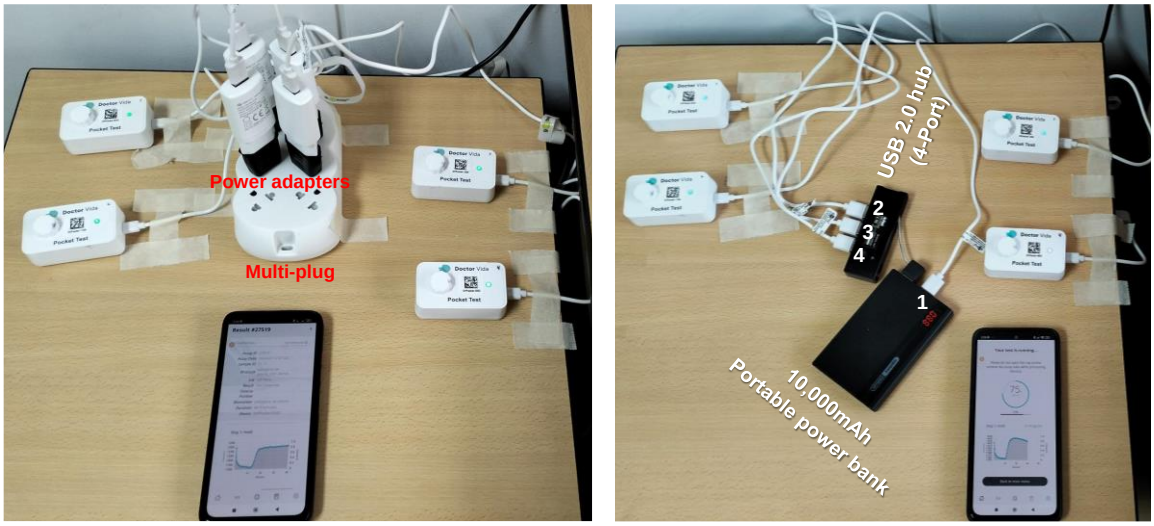


Figure 2. Simultaneous LAMP testing using four Doctor Vida devices. **(Left)** Devices connected to a wall outlet for electrical power. **(Right)** Devices powered by a portable power bank—one connected directly and three connected through a USB 2.0 (4-port) hub.

22. Run the test using the smartphone interface:
    - From the "HOME" screen, select the test type: "Other (R&D)".
    - Tap "Select device" or use the QR code icon to scan the QR code located on the label of the Doctor Vida device (top or bottom).
    - Then, select the option to scan the QR code of the reagents (see Fig. 3).
    - Tap "Read Sample's ID" to manually enter sample information by filling out the form fields: "Sample ID", "Type of sample", "Report Issuance – Assay without report at the end".
    - Tap "Next step" and follow the on-screen instructions.

- Carefully review the entered information before starting the test.
- When ready, press “Start Test”. The system will perform an automatic check to ensure all conditions are met.

NOTE: *If the Bluetooth or Internet connection is lost during the test, your data will not be lost. Do not unplug the device. To resume: close and reopen the app, go to the "Devices" screen to reconnect the devices in progress. Then Tap on the device and click “Resume rehearsals".*

Figure 3. Labell showing the QR code of the reagents **(**LOT – CIP Peru, corresponding to OptiGene master mix) and the LAMP test conditions configured at 65°C for 40 minutes (for plant pathogens). Fluorescence is detected using a blue filter.

23. Insert the reaction tube into the Doctor Vida device:
    - Ensure the tube is tightly closed before insertion.
    - Unscrew the cap (counterclockwise), insert the tube, and screw the cap back on (clockwise).
    - The test will start automatically.

    NOTE: *Do not move the device during the run, as this may affect result accuracy.*

24. Viewing the Results:
    - Tap “Results” on the app interface.
    - After the run is complete, the device may take up to 2 minutes to transfer the data.
    - Tap on each listed test to view its result summary (see Fig. 4)

Results interpretation: Positive amplification

- A positive result is indicated by a typical sigmoid ("S"-shaped) fluorescence curve. The signal begins with a slow increase, followed by a rapid (exponential) rise, and finally plateaus—forming a characteristic “S” curve (see Fig. 4).
- If this curve shape does not appear, the test should be repeated. It is also recommended to run a complementary assay, as irregular amplification curves may suggest non-specific amplification or other issues.
- Time to Positivity (TTP) can be calculated manually. It corresponds to the point where fluorescence increases most rapidly.
- Any detectable amplification—regardless of the TTP value—should be considered a positive result.

  NOTE: *TTP can serve as an indirect indicator of viral concentration. A shorter TTP suggests a higher viral load, since amplification proceeds more quickly when more target nucleic acid is present.*

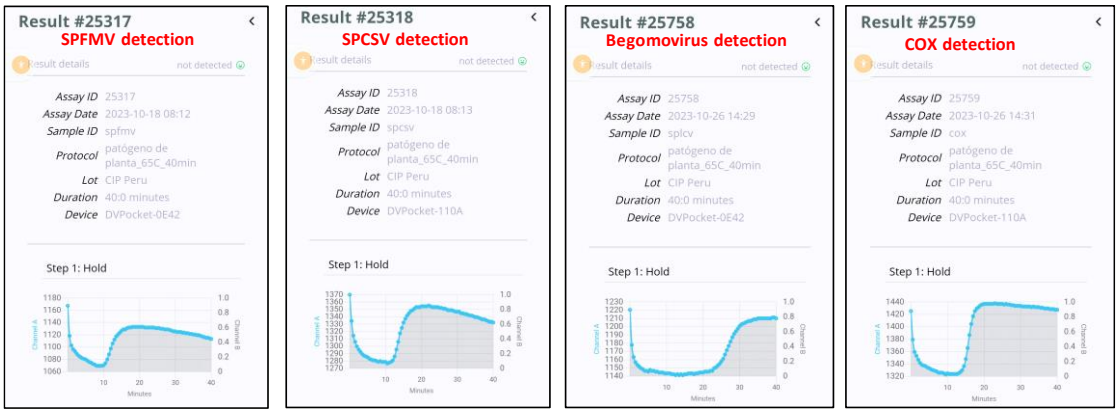


Figure 4. Results are shown as amplification plots (fluorescence vs time during amplification), which are displayed on the smartphone screen.

INTERNAL QUALITY CONTROL

Control sample

Positive (target) and negative (no target) control from virus-infected plant and "healthy" plant, respectively, are included in the LAMP reaction.

- **Positive (target) control:**
  Derived from a virus-infected plant. Confirms that the LAMP assay is functioning properly and can detect the target virus.
- **Negative (no-target) control:**
  Derived from a "healthy" (virus-free) plant. Ensures there is no contamination or non-specific amplification in the reaction.

Host Plant Control – Cytochrome Oxidase (COX)

A LAMP assay targeting the cytochrome oxidase (COX) gene of the host plant has been developed. This control is used to validate the success of nucleic acid extraction, particularly in samples that yield a negative virus result. Detection of COX confirms that the sample contains amplifiable plant nucleic acid.

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