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TITLE AND ID.	SOP FOR DETECTION OF SWEET POTATO VIRUSES USING NCM–ELISA TE/02	
DEPARTMENT	Virology	
EFFECTIVE DATE	2 nd Feb 2023	Version Number: 02

Approval and authorizations.

Completion of the below signature blocks signifies the review and approval of this document.

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Required by: 1st March 2024

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1.0 INTRODUCTION

NCM-ELISA is an immuno-enzymatic test that uses nitrocellulose membranes as a support for the reagents used in the serological reaction. The test is as sensitive as the direct double antibody sandwich ELISA (DAS-ELISA), easier to perform, and can be completed in a shorter period of time. The samples can be dot blotted onto the nitrocellulose membrane and stored for several weeks before continuing with the test; or they can be sent to another laboratory for development. All the steps are done at room temperature (25°C ±4) for a period of two days.

The test consists of:

- I. Blotting a small amount of the test sample onto the membrane.
- II. Blocking areas not utilized by the samples.
- III. Reacting the virus particles with specific antibodies (IgG).
- IV. Detecting the virus specific antibodies with enzyme-labeled antibodies by means of an appropriate substrate.
- V. Virus detection is based on colour development. The intensity of the coloration is proportional to the virus concentration and is stable for a long period of time. The membrane can be easily stored.

1.1 SCOPE:

This procedure describes the process testing sweet potato viruses from sampling, sample preparation, analysis and interpretation of the results. The procedure covers, detection of the following sweet potato viruses: Sweetpotato feathery mottle virus (SPFMV), Sweetpotato mild mottle virus (SPMMV), Sweetpotato latent virus (SPLV), Sweetpotato mild speckling virus (SPMSV), Sweetpotato virus G (SPVG), Sweetpotato chlorotic fleck virus (SPCFV), C-6

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virus (C-6), Sweetpotato chlorotic stunt virus (SPCSV), Sweetpotato collusive virus (SPCV; former name Sweetpotato caulimo like virus, SPCaLV) and Cucumber Mosaic Virus (CMV).

1.2 SAFETY

The assay does not pose any serious health and safety risks. However,

- I. A laboratory coat should be worn at all times when working in the laboratory.
- II. Do not touch the nitrocellulose membrane with bare hands. Wear dry and clean gloves and/or use forceps because finger prints give false positive reactions.
- III. Be careful when manipulating hydrochloric acid 37% since it is severely corrosive and can cause burns. Avoid contact with the product; manipulate the product only with adequate ventilation or inside fume hood. In case of contact with eyes immediately flush them with plenty of water and seek medical attention.

3.0PROCEDURE

3.1SAMPLING

- I. Samples will be collected from grafted setosa plants (Refer to SOP on grafting TE/01),
- II. From each grafted plant pick 3 leaves (top, middle, lower) and place in a polythene bag to constitute one sample, Select, if possible, those showing symptoms. (This must be done on the same day the test is performed)
- III. Samples to be carried in a pre-cooled box
- IV. Samples will be received at the sample reception and issued with laboratory order numbers.

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3.2 SAMPLE PREPARATION

- I. From each leaf sample cut a disk approximately 1 cm in diameter. To perform this, position each leaf within the upper part of the plastic bag and using a small test tube, cut a leaf disk by exerting pressure on the outside of the plastic bag.
- II. Eliminate the remaining part of the leaves and grind the leaf disks with 3 ml of extraction buffer (1 ml of extraction buffer per leaf disk). Grind the tissue completely using a large test tube or a roler
- III. Let the bag stand in an upright position for 30-45 minutes at room temperature until the plant sap phases out (this can be achieved by placing the bags in a large beaker).

3.2 SAMPLE APPLICATION ON NITROCELLULOSE MEMBRANE (DOT BLOTTING)

- I. Cut the nitrocellulose membranes to the required size depending on the number of samples. Label the membranes by writing the name of the virus (or number coding each virus), date and name of analyst at the bottom and pre-wet the membranes in TBS for at least 5 minutes prior to use.
- II. Pipette 20 µl sample (plant sap) into each square on the nitrocellulose membrane using a micropipette. Take care not to pipette plant tissue. Using a clean micropipette tip for each sample, repeat the process until all the samples have been spotted. Record the order/number of the samples as they were spotted onto the membrane on the [NCM-ELISA record sheet](#)

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- III. Transfer the membrane onto a dry Whatman filter paper No.4 and let it dry for 30minutes, in between two paper towels.

3.3 SEROLOGICAL TEST

(Reagents and their preparation for this test are described in annex 2 and 3)

- I. Dip the dry nitrocellulose membrane and the positive control strips in blocking solution for 1 hour at room temperature with gentle shaking (50 rpm).
- II. Add the 1st antibody in TBS + 2 % milk. Incubate overnight at room temperature with gentle shaking (50 rpm).
- III. Wash nitrocellulose membrane in TTBS three times for 3 minutes each with rapid shaking
- IV. Add the 2nd antibody (GAR) in TBS + 2 % milk. Incubate for 1 hour at room temperature with gentle shaking (50 rpm).
- V. Wash nitrocellulose membrane in TTBS four times for 3 minutes each with rapid shaking
- VI. When ready to use, prepare the NBT/BCIP substrate solution (prepare immediately before use).
- VII. Incubate the nitrocellulose membrane for 30 minutes (1 to 1.5h for SPCSV) in NBT/BCIP substrate solution at room temperature with gentle shaking .
- VIII. Stop the color development by discarding the NBT/BCIP substrate solution and immersing the nitrocellulose membrane in distilled water. Wash nitrocellulose membrane in tap water three times for 3 minutes each.
- IX. Dry the membranes before recording.

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- X. Record the results on the [NCM-ELISA record sheet](#).
- XI. Take a photo of the developed membrane and keep them for reference.
- XII. After the test has been completed and concluded, store the NCM membranes between two dry filter paper sheets for a duration of six months and dispose thereafter.

RESULTS INTERPRETATION

Positive reactions are those showing different grades of purple color, faint color indicates mild reactions and no color change indicates negative reactions. Record the reactions on the [NCM-ELISA record sheet](#) along with the responses of the samples. Record the positive reaction with a "+ ve", negative reactions with a "– ve" and mild reaction with a "± ve".

3.4 INTERNAL QUALITY CONTROL

- I. Use the positive control strips provided in the NCM-ELISA kit for each of the viruses
- II. Negative controls are extracted sap from virus-free sweet potato plants or *Ipomea setosa* grown in an isolated greenhouse. Record the negative reaction with a "-ve".
- III. Both negative and positive controls are used every time a NCM-ELISA test is performed

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4.0 REVISION LIST

Current Revision number	Supersedes revision number	Reason for review	Reviewers name
01	00	New	Edith Avedi Maureen Mwangangi
02	01	Inclusion of KENAS review comments	Maureen Mwangangi

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ANNEXES

1.0 MATERIALS AND REAGENTS

1.1 BUFFERS AND STOCKS

NAME	COMPOSITION	STORAGE CONDITIONS
TBS pH 7.5 (2,000 ml)	Tris Base 4.84 g (0.02 M) NaCl 58.44 g (0.5 M)	Stable at 4 ±3°C for one month.
TTBS (2,000 ml)	TBS 2,000 ml Tween-20 1.0 ml (0.05 %)	Store at 4 ±3°C
Extraction buffer	TBS buffer containing 0.2 % sodium sulfite	Store at 4 ±3°C
Blocking solution	TBS + 2 % milk + 2 % TRITON X-100	Prepare fresh for each test
Substrate buffer pH 9.5 (500 ml)	Tris Base 6.05 g (0.1 M) NaCl 2.92 g (0.1 M) MgCl ₂ ·6H ₂ O 0.51 g (0.005 M)	Dissolve Tris Base, NaCl and MgCl ₂ ·6H ₂ O in 450 ml distilled water. Adjust pH to 9.5 with concentrated HCl (37%). Add up to 500 ml with distilled water.
Antibody and conjugate solution	TBS buffer containing 2% milk	Prepare fresh for each step.
NBT Stock solution	NBT 40 mg N,N-dimethylformamide (70%) 1.2 ml	Mix well and store at 4 ±3°C, protected from light (dark bottle or cover it with foil).
BCIP Stock	BCIP 20 mg	Mix well and store at 4 ±3°C,

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solution	N,N-dimethylformamide 1.2 ml	protected from light (dark bottle or cover it with foil).
Substrate solution	Substrate buffer 30ml NBT stock solution 90 <i>ul</i> BCIP stock solution 90 <i>ul</i>	Add the NBT stock solution in 30 ml of substrate buffer. Afterwards add the BCIP stock solution drop by drop while stirring (it is NBT/BCIP substrate solution).

1.2 OTHER REAGENTS

SATCHET CODE	NAME	STORAGE
	Antibodies for each virus to be tested	Store at 4 ±3°C
# 9	GAR: Goat anti-rabbit IgG alkaline phosphate conjugate (BIO-RAD).	Store in a dark and cold place at 4 ±3°C. Do not freeze.
# 11	NBT: Nitro blue tetrazolium (BIO-RAD).	Store in a dark and cold place at -20°C
# 11	BCIP: 5 Bromo-4-chloro-3-indolyl phosphates (BIO-RAD).	Store in a dark and cold place at -20°C.
# 12	DMF : N,N-dimethylformamide	Store at room temperature
# 4	MILK : Powdered cow milk	Store at room temperature
# 2	HCl : Hydrochloride acid	Store at room temperature

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1.3 MATERIALS AND EQUIPMENT

Name	Storage Conditions / Use
Nitrocellulose membranes (BIO-RAD)	Store in a dry place at room temperature.
Filter papers (Whatman # 4).	Store at room temperature
Sample bags (polythene 4 X6X6) (wide, long and thickness respectively).	Use one for each sample
Small tubes of 1 cm diameter (glass).	Use to cut disks from leaf samples.
Large test tubes or piece of round wood	Use in sample maceration.
Micropipettes (20, 200 and 1000 µl)	Store in a dry place at room temperature.
200 and 1000ul tips	Store in a dry place at room temperature.
Surgical forceps	Use them when manipulating the membranes, Store in a dry place at room temperature.
2000, 1000, 500, 250ml containers or glass bottles	Store in a dry place at room temperature.
graduated cylinders (1000ml, 500ml, 250ml, 100ml and 50ml)	Store in a dry place at room temperature.
Distilled water	Store at room temperature
Refrigerator 1 – 7°C	1 – 7°C

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Deep freezer -20°C	-20°C ±3
pH meter	Store in a dry place at room temperature.
Analytical balance	Store in a dry place at room temperature.
Orbital shaker	Store in a dry place at room temperature.
Magnetic stirrer	Store in a dry place at room temperature.
Plastic containers	Use them when incubating and washing the membranes.

1.4 REAGENTS PREPARATION PROCEDURES

See NCM-ELISA kit instructions manual

2.0 RECORDS

1. NCM-ELISA record sheet

7.0 REFERENCES

1. NCM-Elisa kit Instruction manual for sweetpotato viruses detection (CIP)