NAPPO
North American Plant Protection Organization
Organización Norteamericana de Protección a las Plantas

NAPPO Conference Call Report

Expert Group:	Seeds-ToBRFV	
Location:	Video conference – Zoom meeting	
Date:	February 24, 2022	
Chairperson	Beatriz Xoconostle (CINVESTAV)	
Participants:		
Huimin Xu (CFIA)	Geoffrey Dennis (APHIS – PPQ)	Samantha Thomas (US Industry)
Kevin Ong (TAMU)	Daniela Alejandra Bocanegra Flores (SENASICA)	José Manuel Cambrón Crisantos (SENASICA)
Stephanie Dubon (APHIS – PPQ)	Jennifer Nickerson (CFIA)	Ángel Ramírez Suárez (SENASICA)
Vessela Mavrodieva (APHIS – PPQ)	Rick Dunkle (US Industry)	Pamela Ross (CFIA)
Ed Podleckis (APHIS – PPQ)	Jessica Berenice Valencia Luna (SENASICA)	Sofia Baez (NAPPO)
Stephanie Bloem (NAPPO)	Nedelka Marín-Martínez (NAPPO)	Maribel Hurtado (NAPPO)
Alonso Suazo (NAPPO)		

Summary

Project:	A pilot for the harmonization of diagnostic protocols for seed pests focuses on *ToBRFV*
General comments:	The NAPPO Secretariat thanked EG members for joining the call. The NAPPO TD agreed to take notes and write the meeting report. The EG agreed to record this session for report purposes.
Item 1:	Updates
Consensus:	*Terms of Reference document* (Ed Podleckis, APHIS – PPQ) The EG Chairperson informed the EG the document is almost completed and will be ready by March 3, 2022. The ED informed that the ToR will be presented to the EC for approval in the next NAPPO Management Team meeting scheduled for March 29, 2022. EG members will be allowed to review the document before it is presented to the EC. *Manual for participating labs* (Alonso Suazo, NAPPO TD) The first draft of the manual has been completed. The document is being updated with the feedback received from some EG members. An updated version of the manual should be available

	in March. Copies of the manual will be shared with the EG before producing the final version. *Panel design* (Vessela Mavrodieva, APHIS – PPQ) Development of ToBRFV – positive sample B and PPC: • Tests using real time PCR were conducted to evaluate different methods to prepare samples B and Positive Processing Control (PPC). • Methods with the lowest sample variability were selected. • Selected methods were used to produce 100 bags each one containing 3 grams of seeds for sample B and PPC. • 15% of randomly selected samples were validated for Samples B and PPC. The samples were divided in half and evaluated by two diagnosticians. RNA was extracted from each sample and tested with all five protocols (samples tested in triplicate for real time PCR methods and in duplicates for conventional PCR methods). The purpose of this test was to make sure that low sample variability is observed with all methods tested. For quality control purposes, the lab records the lot number of reagents and serial numbers of the equipment. • Data is compiled for samples B and PPC. • Sample B is tested for five 10-fold dilutions. Each dilution was tested with all methods. • Homogeneity was evaluated using real time PCR. Standard deviation is kept under 2. Validation of samples D, E and Negative Processing Control (NPC). • Samples D, E and NPC are packed and ready to be shipped. Transcripts • Transcripts were received from the CINVESTAV lab in Mexico and additional transcripts were developed, quantified, and tested in the APHIS – PPQ lab in Maryland. • Produced, quantified, and tested for three target regions: RdRp, MP and CP. • Tested in 10-fold serial dilutions, 5 dilutions for samples A and the calibrator. Proper level of dilution was selected. • Stability testing was done using five different temperatures: -80°C, -20°C, 4°C, and room temperature. • Stability in transportation is being evaluated. • Transcripts for the ToMMV are being developed and will be validated. • Validation tests will be done for sample A and the calibrator using all five methods. Verification by another lab and determination of sample stability

	during transportation:
	• Aliquots of samples B and PPC were sent to Dr. Kevin Ong's laboratory in TAMU where RNA was extracted to confirm the validation results from the USDA lab in Maryland.
	• Verification of the validation work was also done for the Transcripts in the TAMU lab.
	• Validation results in both labs are comparable.
	• To ensure transcript stability, transcripts were shipped in dry ice.
	
	Reagents for RT-PCR assays for participating laboratories were purchased with NAPPO funds. Reagents have been received and packing will start on the week of March 7.
	
	Boxes and data loggers were also purchased for the TAMU laboratory.
	
	Recommendations to maximize sample stability:
	• Store samples at -80°C or -20°C.
	• Start tests as soon as samples are received.
	• Avoid constant freezing and thawing of samples.
	
	Document on data collection analysis, and results (Geoffrey Dennis – APHIS-PPQ).
	
	• Three different documents will be prepared. Two for data analysis and results and one for data collection.
	• Result analysis document:
	  ○ Will cover the definitions, equations, and validation categories.
	  ○ Includes details of the sample design and data analysis.
	• Data analysis using the APHIS – PPQ portal includes two documents:
	  ○ General instructions and description of the portal.
	  ○ The second document will include details based on the panel each participating lab will receive. Each step on the data entry is described.
	Examples of data entry were provided.
	
	Additional notes:
	• Geoff encouraged EG members to fill and send the form to grant access to the portal and to organize a training session.
	• The ED suggested standardizing the nomenclature including the lab id, sample id, etc…

Item 2:	Preliminary tests for participating labs.
Consensus:	The EG agreed that participating labs should run a preliminary test before the ring test to ensure the extraction protocols each lab use is working with the selected protocols. An extra set will

	be included in the panels for labs to run preliminary tests. Additional reagents for the preliminary tests will be covered with NAPPO funds. Panels and the additional material for the preliminary tests will be shipped preferably together to avoid the need for additional import permits. Data generated during the preliminary tests will not be uploaded into the data collection portal.
Item 3:	Panels shipment logistics
Consensus:	The EG expressed concerns about shipping the panels outside the U.S. and getting those panels on time with the material stable to the destination. Of particular concern was the shipping with dry ice and if airlines will allow to carry packages with dry ice. Notes from the EG: • Shipping with carriers like UPS and FedEx sometimes results with problems in customs. • Better if someone transport the sample and provide authorization letters to introduce the material.

Next Steps		
Responsible Person	**Action**	**Date**

Next Meeting	
Location:	Zoom meeting – Video conference.
Date:	March 23, 2022 from 2:00 – 4:00 pm EST.

Proposed Agenda Items
1.
2.