

European Union
Reference Laboratory
for Pests of Plants on
Viruses, Viroids
and Phytoplasmas



Co-funded by the
European Union

Key insights from nanopore high-throughput sequencing training

Anja PECMAN, Veronika BUKVIČ, Ana VUČUROVIĆ, Denis KUTNJAK, Nataša MEHLE

14.1.2026, Wageningen

Background

- **HTS** from **research** (2012 →) to use for analysis of **official samples** (2015 →)



Situation:

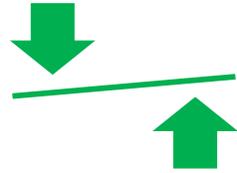
- Only 10 samples per year
- Illumina – outsourcing sequencing
 - to reduce costs: combining samples
 - sequencing at the end of the season
 - waiting for results
 - unclear how to achieve ISO17025 accreditation



How to establish and validate an HTS test for the detection of plant viruses that would be suitable for laboratories with relatively low sample throughput?

Background

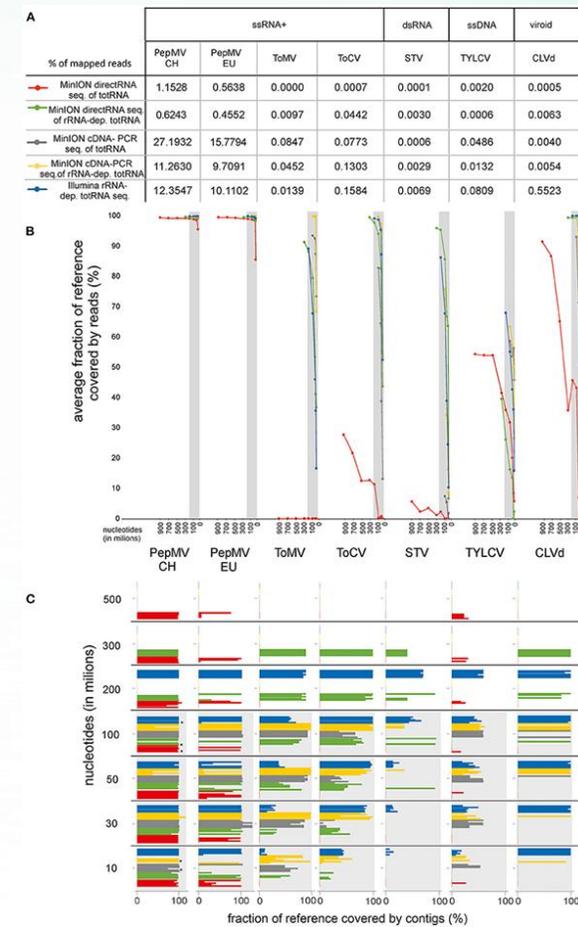
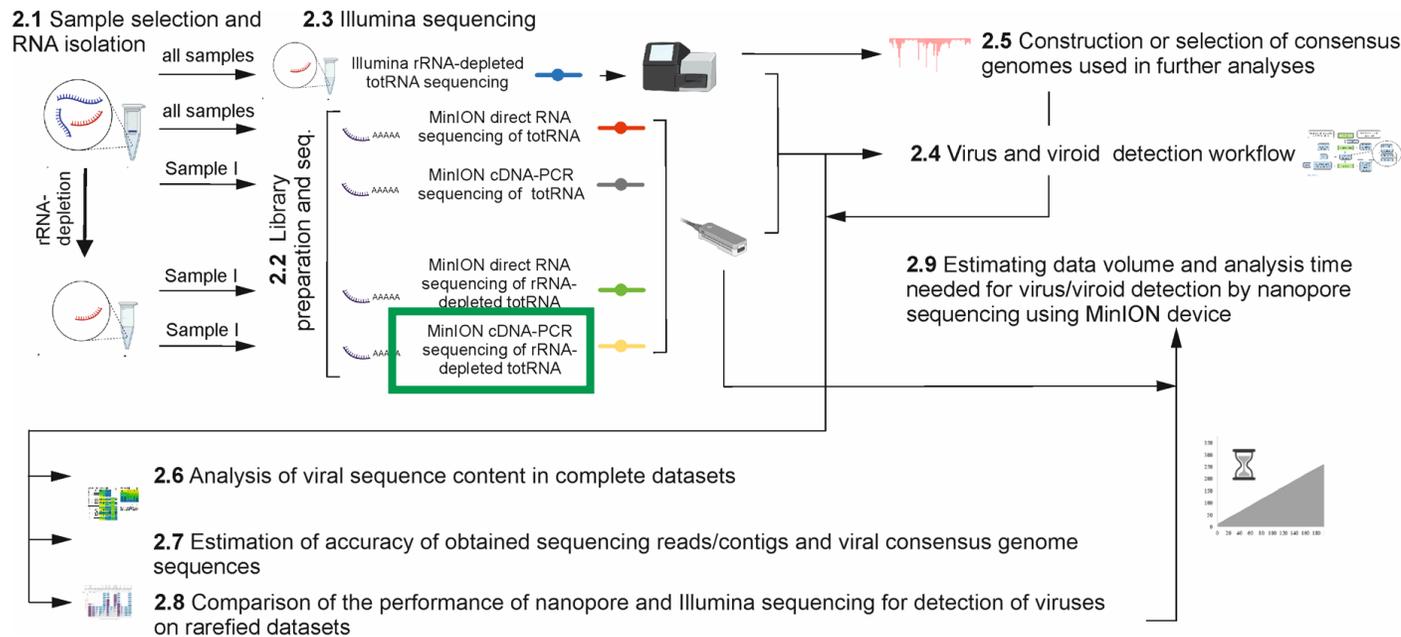
- 😊 Accurate
- 😊 Lots of experience
- 😊 Low cost per base
- 😊 Stable protocols and chemicals
- 😞 Expensive machine
- 😞 Expensive for maintenance



- 😊 Fast turnaround
- 😊 Cheap machine
- 😊 Maintenance makes sense even for smaller throughput labs
- 😞 Higher error rate
- 😞 Changing protocols and chemicals

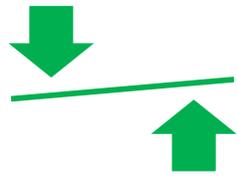
Background

- Comparing different nanopore sequencing protocols with Illumina sequencing (total RNA)
- Which performs the best for virus detection in plants and how comparable it is to Illumina?



Systematic Comparison of Nanopore and Illumina Sequencing for the Detection of Plant Viruses and Viroids Using Total RNA Sequencing Approach

Background



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Nanopore sequencing has been found to provide comparable results to Illumina sequencing, while being faster and better suited for small laboratories



Accreditation
(April 2024)



External audit
(November 2023)

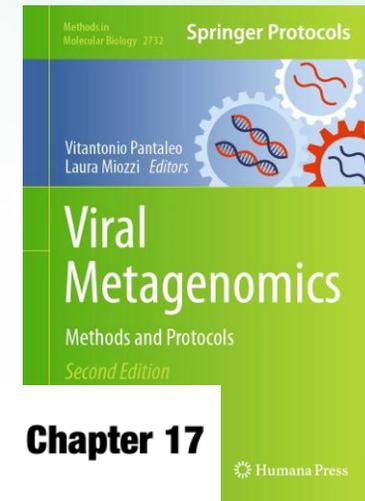
2nd Internal audit
(May 2023)

1st Internal audit
(April 2023)

Validation
(selected virus)



Lab setup
Setting up controls
SOP
Results reporting



Chapter 17

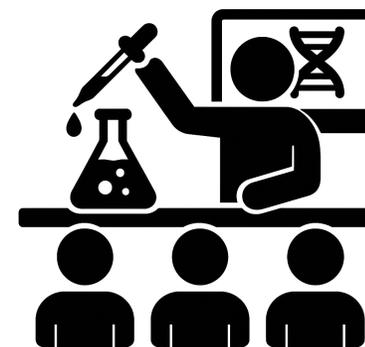
Detection of Plant Viruses Using Nanopore Sequencing Based Metagenomic Approach

Anja Pecman, Nataša Mehle, and Denis Kutnjak

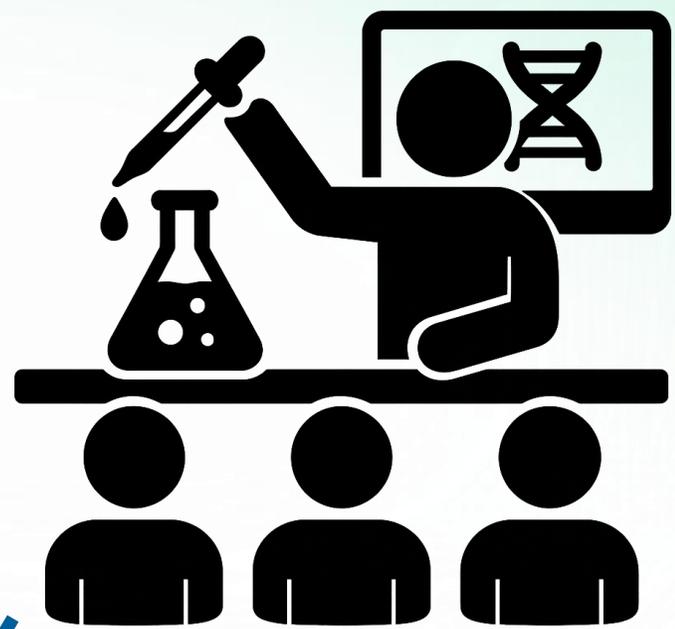
DOI: 10.1111/epp.13026

ADDENDUM

Addendum – New Supporting information for PM 7/151
Considerations for the use of high throughput sequencing in plant health diagnostics



Hands-on Training on Nanopore HTS



Announcement
17.2.2025



Participant Requirements:

- Basic knowledge of PCR
- Familiarity with sequence analysis

Application
7.3.2025



8 Applications
from 6 Countries

Selection
10.3.2025

Selection Criteria:

- Priority to those with more experience
- To those planning to seek accreditation in nanopore sequencing in the near future.

Hands-on Training
17.-28.11.2025

3 Participants
from 3 Countries



Anja Pecman & Veronika Bukvič

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Ednar Wulff, DK

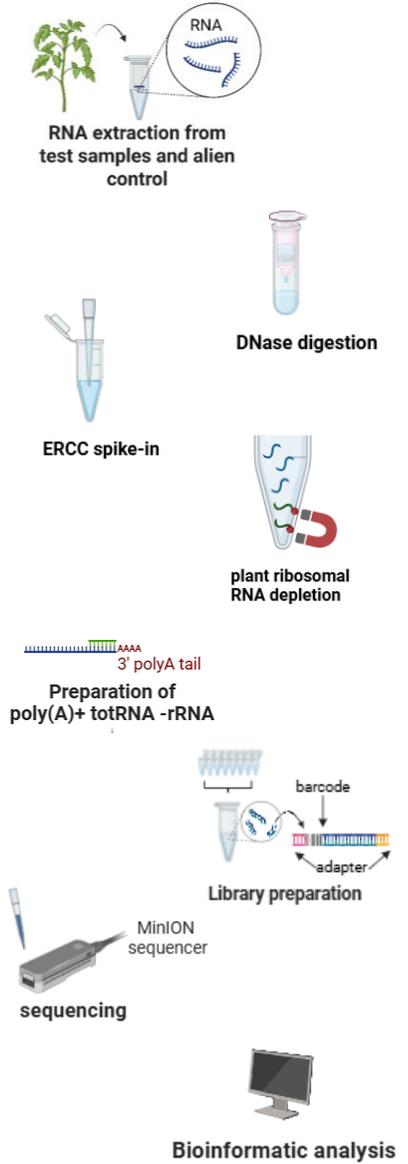
Chiara Pohn, AT

Šárka Linhartova, CZ



WORKFLOW

CONTROLS



NAD5	PvEV	ERCC	RCS	Bioinfo control
✓	✓			
		✓		
			✓	
				✓

nad5 – an internal positive control for RNA extraction, tested by RT-qPCR (Botermans et al., 2013).

PvEV – alien control; RNA extracted from *Phaseolus vulgaris* infected with endornaviruses (Kesanakurti et al., 2016), processed in parallel with samples. Used to monitor cross-talk and detect low-titer viruses.

ERCC RNA Spike-In Mix (Invitrogen) – an internal control added to every sample to evaluate all steps following DNase treatment.

RCS – RNA Calibration Strand (by ONT); a high-titer artificial spike-in used to monitor the success of library preparation and sequencing, and for cross-talk estimation.

Bioinformatic pipeline control (PvEV) – an alien control from a previous run, used to evaluate the bioinformatics pipeline.



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EURL-Virology

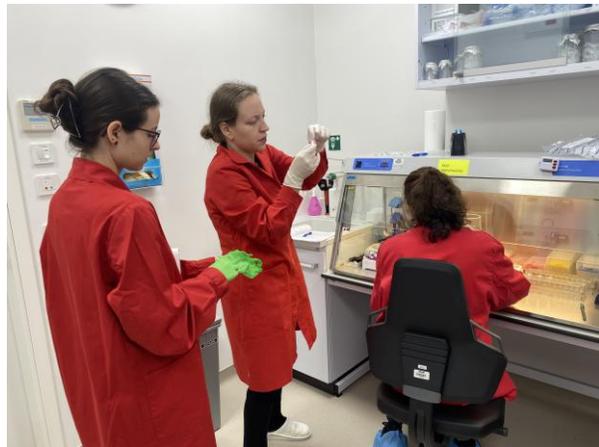
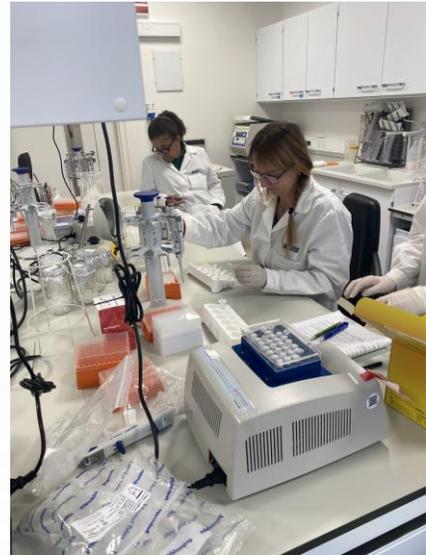
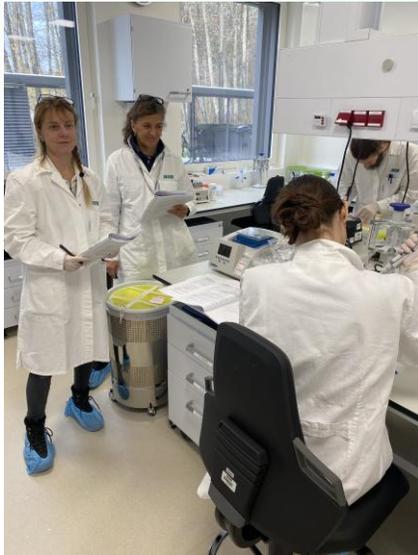
(European Union Reference Laboratory for Pests of Plants on Viruses,
Viroids and Phytoplasmas)

NANOPORE HIGH-THROUGHPUT SEQUENCING: HANDS-ON TRAINING

Course materials 17.-28. NOVEMBER 2025

- Work with **real samples** (each participant were asked to send an RNA sample before training)

Wet lab procedure in detail



Weekend



Dry lab procedure in detail



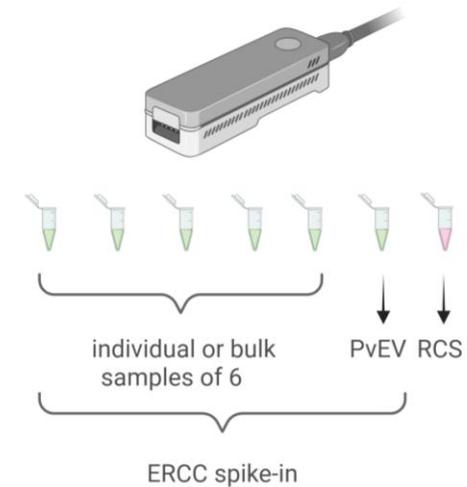
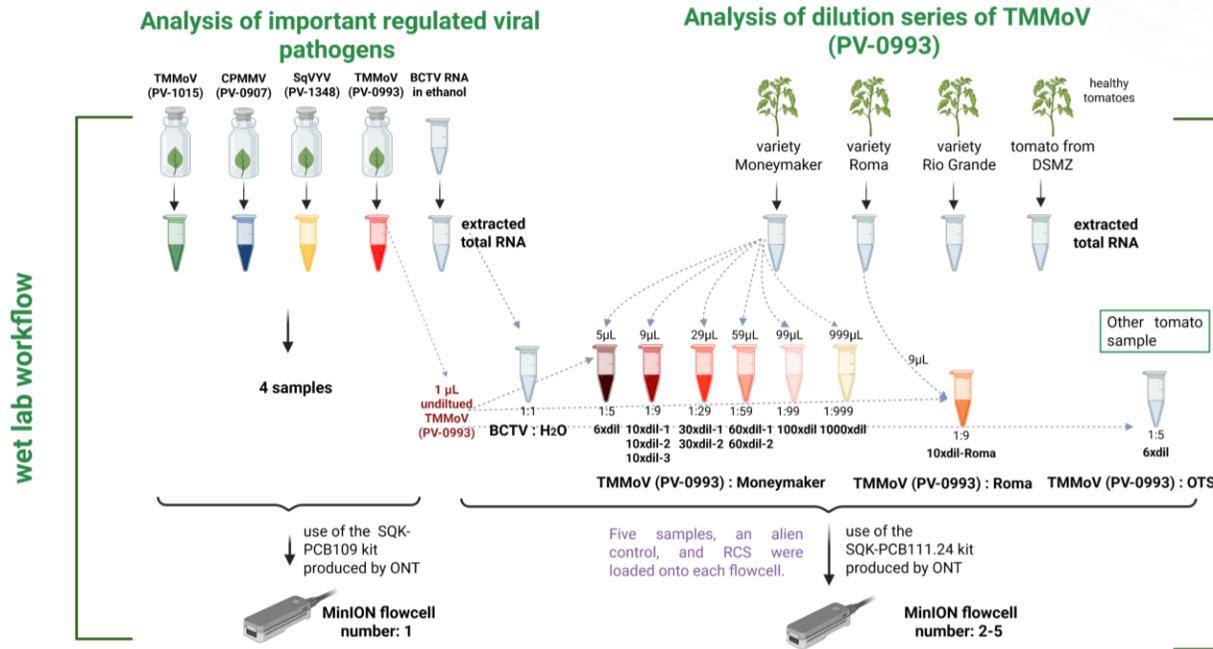
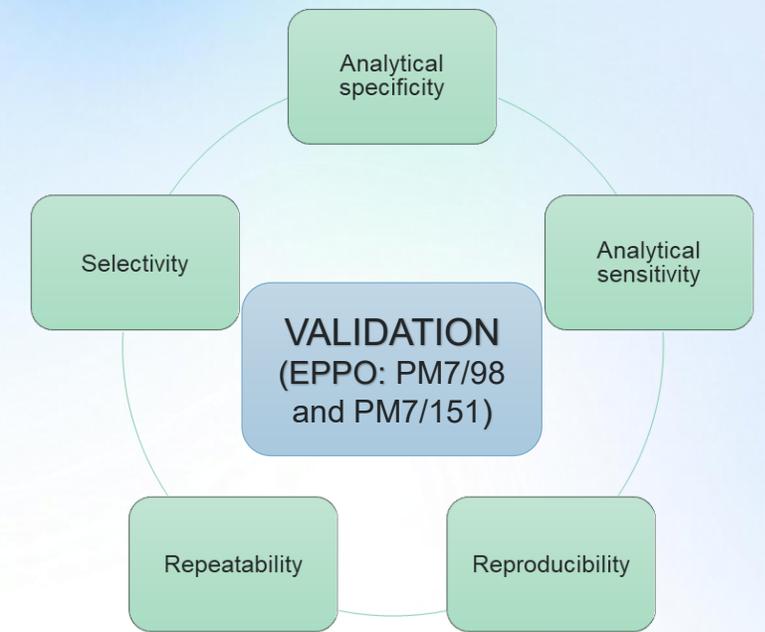
Discussion of critical points for test validation

Example:

matrix → tomato leaves

target → tomato mild mottle virus (TMMoV)

assay → Ribosomal RNA-depleted total RNA using a PCR-cDNA barcoding approach



Virus name	Virus name (Family, Genus)	DSMZ isolate name
TMMoV	tomato mild mottle virus (<i>Potyviridae, Ipomovirus</i>)	PV-1015, PV-0993
CPMMV	cowpea mild mottle virus (<i>Betaflexiviridae, Carlavirus</i>)	PV-0907
SqVYV	squash vein yellowing virus (<i>Potyviridae, Ipomovirus</i>)	PV-1348
BCTV	beet curly top virus (<i>Geminiviridae, Curtovirus</i>)	/

Special thanks to

Anja Pecman
Veronika Bukvič

Chiara Pohn, AT
Ednar Wulff, DK
Šárka Linhartova, CZ

European Commission, Directorate General for Health and Food Safety

Continuing the journey: Future Nanopore HTS hands-on training

16.-27.11.2026

- ❑ Christina Varveri, GR
- ❑ Heiko Ziebell, DE
- ❑ Eva Kovačec, SI

November 2027

The call for applications is expected to be announced by the end of 2026