A flexible scope on phytosanitary diagnostics

Mariëtte Edema, Arjen Werkman, Marianne van der Blom, Annelien Roenhorst.

National Reference Centre (NRC)
National Plant Protection Organization (NPPO-NL)
The Netherlands
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Fixed accreditation scopes

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Type of material</th>
<th>Method(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Lycopersicon esculentum/</em> <em>Solanum lycopersicum</em> (tomato)</td>
<td>Isolation of <em>Clavibacter michiganensis</em> subsp. <em>michiganensis</em> in symptomatic material (stem) from tomato by plating on semi-selective media. Identification of <em>Clavibacter michiganensis</em> subsp. <em>michiganensis</em> by IF, real-time PCR and pathogenicity test</td>
</tr>
</tbody>
</table>

2. *Andean potato latent virus* (APLV)  
3. *Phytophthora ramorum*  
4. *Thrips palmi*  
5. *Ditylenchus dipsaci*
Diagnostic results 2014

- **# organisms**: 1200
- **# organism-host combinations**: 2000
- **# accredited**: 200
<table>
<thead>
<tr>
<th>Fixed scope</th>
<th>Flexible scope</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Process is fixed</td>
<td>- Process is flexible</td>
</tr>
<tr>
<td>- Detailed description of type of test, organism(s) and matrix</td>
<td>- Generic description</td>
</tr>
<tr>
<td>- Based on validation</td>
<td>- Flexibility concerning object, or matrix or sample.</td>
</tr>
<tr>
<td></td>
<td>- On-going validation</td>
</tr>
</tbody>
</table>
Targeted samples

Non-targeted samples
Tomato seed sample for PSTVd


Grind & extract RNA extraction

Real-time RT-PCR

Read test results

(+)/-

Targeted samples

RT-PCR
Seq analysis

Report
Pepper fruit: necrotic spots

CMV, PMMoV, TSWV (bioassay)

Prepare Inoculum

Bioassay

Interprete test results

ELISA CMV

Select further tests

Report

Non-targeted samples
Key principles

Validation

Expertise

Quality Assurance
Results

Organisms under accreditation

Quality management system

Audits

Validation

Expertise

Assurance
Microbiological methods

Morphological methods

Diagnostic process

1st

+/- controls

slide/voucher key

2nd test
2nd specialist

blind sample
2nd test
2nd specialist

blind sample
throughout process

Proficiency test

Contra expertise

Proficiency test

Method level

Individual / test level

3rd

Proficiency test

Proficiency test

Proficiency test
<table>
<thead>
<tr>
<th>YEAR</th>
<th>Method</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>Bio-assay</td>
<td>Non-targeted</td>
</tr>
<tr>
<td>2017</td>
<td>ELISA</td>
<td>?</td>
</tr>
<tr>
<td>2018</td>
<td>PCR</td>
<td>?</td>
</tr>
<tr>
<td>2019</td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>2020</td>
<td></td>
<td>?</td>
</tr>
</tbody>
</table>
### Flexible accreditation scopes

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Type of material</th>
<th>Method(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Plant material and cultures</td>
<td>Identification of Plant pathogenic bacteria using Isolation, IF, Real-time PCR, PCR, Pathogenicity testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Arthropods</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Nematoda</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Oomycota</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Plants</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Plant viruses and viroids</td>
<td></td>
</tr>
</tbody>
</table>
## R-VIR-000-001 Overview of viruses and viroids under accreditation

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Matrix</th>
<th>Bioassay</th>
<th>DAS-ELISA</th>
<th>PCR</th>
<th>Real-time PCR</th>
<th>PCR-Sequencing</th>
<th>Electron microscopy</th>
<th>r-PAGE</th>
<th>Date of addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tymovirus</td>
<td>Andean potato latent virus</td>
<td>leaves</td>
<td>TYMO_20160315_TPO</td>
<td>TYMO_20160315_ELISA</td>
<td>+</td>
<td></td>
<td>TYMO_20160315_SEQ</td>
<td></td>
<td>+</td>
<td>20160315</td>
</tr>
<tr>
<td>Tymovirus</td>
<td>Andean potato mild mosaic virus</td>
<td>leaves</td>
<td>TYMO_20160315_TPO</td>
<td>TYMO_20160315_ELISA</td>
<td>+</td>
<td></td>
<td>TYMO_20160315_SEQ</td>
<td></td>
<td>+</td>
<td>20160315</td>
</tr>
<tr>
<td>Pospiviroid</td>
<td>Potato spindle tuber viroid</td>
<td>leaves</td>
<td></td>
<td>POSPI_20160315_PCR</td>
<td></td>
<td>POSPI_20160315_RPCR</td>
<td>POSPI_20160315_SEQ</td>
<td></td>
<td>+</td>
<td>20160315</td>
</tr>
<tr>
<td>Pospiviroid</td>
<td>Tomato chlorotic dwarf viroid</td>
<td>leaves</td>
<td></td>
<td>POSPI_20160315_PCR</td>
<td></td>
<td>POSPI_20160315_RPCR</td>
<td>POSPI_20160315_SEQ</td>
<td></td>
<td>+</td>
<td>20160315</td>
</tr>
</tbody>
</table>

**validated test**

**support test, not validated**
Preparation

Review

Adding to scope

Check

Approval

Changing scope

Approval

Addition to scope