Validation of a Nested PCR assay for detection of *Xanthomonas axonopodis* pv. *dieffenbachiae* in anthurium tissues in a European multicenter collaborative trial

**X. a. pv. dieffenbachiae** *(Xd):* a quarantine organism in Europe *(EPPO A2 list)*

**Comparison of methods**

1. **1**st step of the NF EN ISO 16140:2003 european standard(4)
2. **N-PCR** assay and EPPO reference method 2004(5) were more efficient *(relative accuracy > 95%, detection threshold 10⁴ CFU.mL⁻¹)* than DAS-ELISA and IF

   - **N-PCR**(3) included in the EPPO diagnostic protocol revised in 2009(2)

**Collaborative trial**

1. **2**nd step of the ISO 16140:2003 standard
2. project presented at the European Standing Committee on Plant Health
3. objective: determine the variability of the results obtained by several labs and compare results with those obtained during the methods comparative study

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**15 European participating laboratories**

Organizing lab: 1 CIRAD UMR PVBM and 2 LNPV, La Réunion, France
1 Plant Protection Service, Wageningen, The Netherlands
2 ERSAP, Pozzuolo del Friuli, Italy
3 ArBoPavé, University of Naples, Portici, Italy
4 SPA, Oloomouc, Czech Republic
5 FERA, York, United Kingdom
6 Benaki Phytopathological Institute, Kifissia, Greece
7 CLPQ, Voluntari-Ciunty, Romania
8 IFR, Sanremo, Italy
9 FCDON Réunion, La Réunion, France
10 LDA972, Martinique, France
11 ENSE, Battipaglia, Italy
12 Laboratorio da Sanidade Vegetal, Canary Islands, Spain
13 LNPV, Angers, France
14 ILVO Plant, Merelbeke, Belgium

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**Statistical significance tests:** MA no test available, too few discordant results ([4](#)) between the reference method and the expected theoretical results. **N** results obtained when laboratory A was excluded from the statistical analysis. This laboratory obtained half of the false positive results from the sample contaminated with the saprophytic strain. When this laboratory is kept in the analysis, there is a significant difference from the expected theoretical results ([p<0.001](#)).

**Results obtained with ambiguous results analysed as theoretical expected results.**

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**Two methods are particularly remarkable:** N-PCR and reference methods

- **significant difference from the expected theoretical results:**

<table>
<thead>
<tr>
<th>Ref. method</th>
<th>N-PCR</th>
<th>DAS-ELISA</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>absence</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>positive</td>
<td>no** (p&lt;0.05)</td>
<td>yes** (p&lt;0.001)</td>
<td>yes** (p&lt;0.001)</td>
</tr>
</tbody>
</table>

- **significant variation between laboratories:**

  - **NA:** no results obtained
  - **no** (p<0.05): results obtained, no statistically significant differences
  - **yes:** results obtained, statistically significant differences
  - **yes** (p<0.001): results obtained, statistically significant differences

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**Conclusions on the results of the collaborative trial**

- N-PCR and EPPO reference method 2004: excellent results for AC, SP and SE (> 95%) and no significant differences from the expected theoretical results and between labs
- DAS-ELISA results explained by the lack of performance on the sample 10⁴ CFU.mL⁻¹
- IF results: an important variation between laboratories: only labs with IF experience for detection of Xad in anthurium obtained correct results

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**Conclusions on the whole validation study**

- N-PCR equivalent to the reference method. Its high specificity makes the pathogenicity tests to anthurium optional, drastically reducing the amount of time for result delivery (2 days vs. 10 days). ET medium replaceable by NCTM for isolation step
- I-ELISA not recommended for detection purpose
- DAS-ELISA not recommended for detection on asymptomatic samples and for identification within the framework of the EPPO scheme
- IF requires appropriability by laboratories before routine analyses

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**References:**