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EPPO STANDARD ON DIAGNOSTICS

PM 7/161 (1) *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* pv. *fuscans*

Specific scope: This Standard describes a diagnostic protocol for *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* pv. *fuscans*.[1] This Standard should be used in conjunction with PM 7/76 Use of EPPO Diagnostic Standards.
Specific approval and amendment: Approved in 2025–05. Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

Common bacterial blight of bean was first described in the United States in 1893, and one of the bacterial pathogens was isolated by Smith in 1897 (Zaumeyer, 1930). The disease has since been observed worldwide in areas where beans are cultivated. For an updated geographical distribution consult EPPO Global Database (EPPO, 2024).

The bacteria causing common bacterial blight are genetically diverse but share a common host (*Phaseolus vulgaris* L.) (common bean), on which they cause similar symptoms on leaves, pods, stems and seeds. Among these strains, some produce a brown pigment on tyrosine-containing medium and were described as fuscous strains (Burkholder, 1930). Upon description of the genus *Xanthomonas* (Dowson, 1939), fuscous and non-fuscous strains were classified as *Xanthomonas phaseoli*, then as *X. campestris* pv. *phaseoli* (Dye et al., 1980). Based on DNA homology, the pathovar *phaseoli* was transferred to *X. axonopodis* (Vauterin et al., 1995), with fuscous strains forming a variant within this pathovar. Molecular divergence between fuscous and non-fuscous strains led to the fuscous strains being renamed as *X. fuscans* subsp. *fuscans* (Schaad et al., 2005). Later on, amplified fragment length polymorphism (AFLP) analysis revealed two non-fuscous genetic lineages (GL2 and GL3) only isolated from bean in La Réunion, (FR) and Tanzania that grouped with the fuscous strains (Alavi et al., 2008), and that belong to *X. axonopodis* rep-PCR group 9.6 as defined by Rademaker et al. (2005). Additional non-fuscous strains isolated from lablab bean (*Lablab purpureus*) in Sudan and Zimbabwe form yet another genetic lineage ('lablab') within the *X. citri* subcluster also defined as *X. axonopodis* Rademaker group 9.5 (Aritua et al., 2015). Following a taxonomical revision of the species *X. axonopodis*, the bacterial pathogens responsible for common bacterial blight of bean were classified as *X. phaseoli* pv. *phaseoli*, while the fuscous strains and the three distinct lineages (GL2, GL3, and 'lablab') were classified as *X. citri* pv. *fuscans* (Constantin et al., 2016). However Chen et al. (2021), based on differences in pathogenicity, indicated that the 'lablab' genetic lineage may be a distinct pathovar inside the *X. citri* species.

Phaseolus vulgaris is the main host of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*. Natural infections have been also reported on diverse other legume species such as Lima bean (*Phaseolus lunatus*), members of the *Vigna* genus, and on few other legumes.

Contaminated seeds are the primary inoculum source for the pathogens causing common bacterial blight of bean (Darrasse et al., 2007). Hence, the use of pathogen-free seeds is essential in an effective management strategy and requires appropriate sampling, detection, and identification methods.

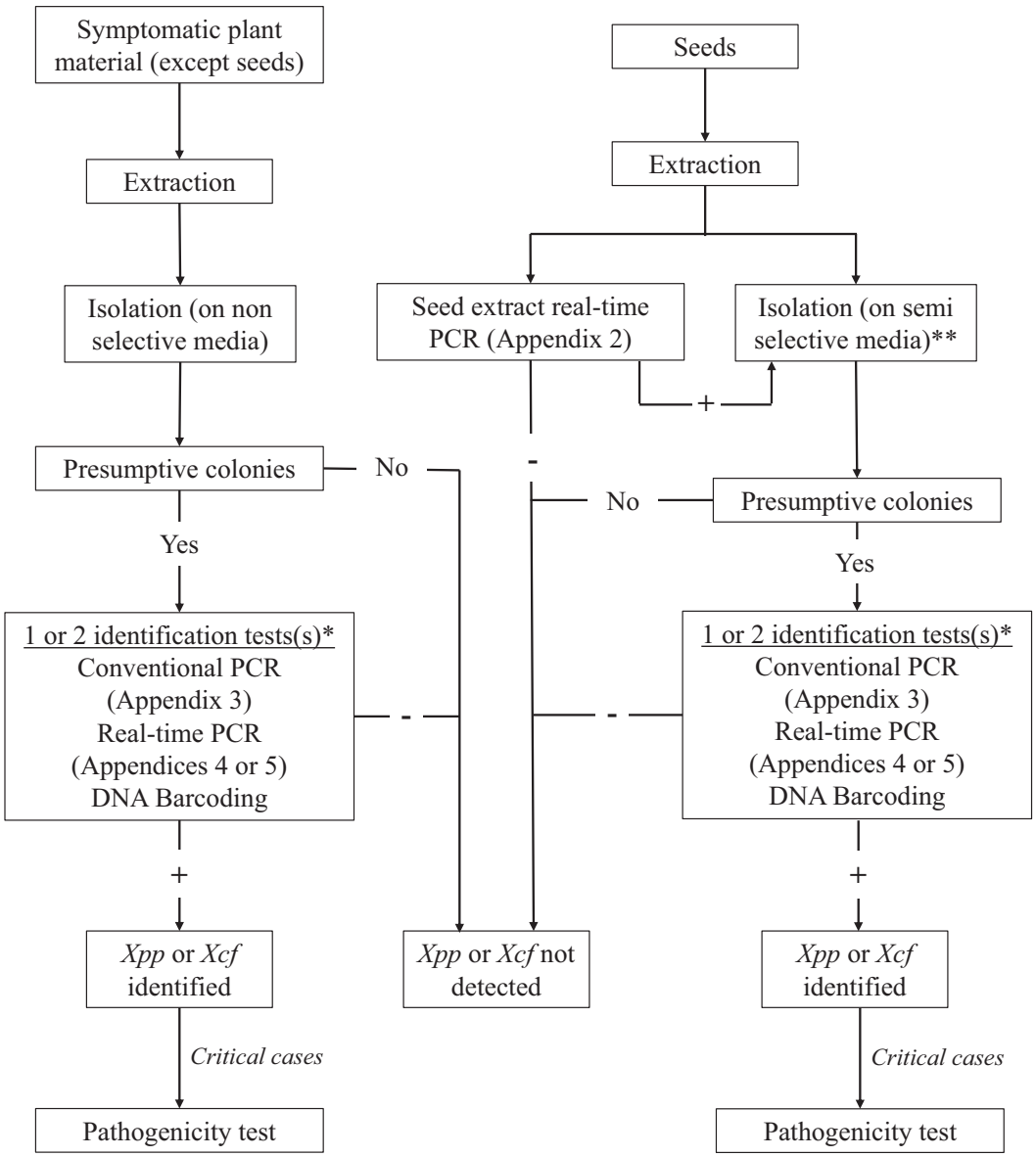
A flow diagram describing the diagnostic procedure is available in Figure 1.

2 | IDENTITY

Name: *Xanthomonas phaseoli* pv. *phaseoli* (Smith, 1897) Constantin et al. (2016).
Other scientific names: *Xanthomonas phaseoli* (Dowson, 1939); *Xanthomonas campestris* pv. *phaseoli* (Dye et al., 1980); *Xanthomonas axonopodis* pv. *phaseoli* (Vauterin et al., 1995).
Taxonomic position: Bacteria; *Pseudomonadota* (previously known as *Proteobacteria*); *Gammaproteobacteria*; *Lysobacterales* (previously known as *Xanthomonadales*); *Lysobacteraceae* (previously known as *Xanthomonadaceae*); *Xanthomonas*.
EPPO Code: XANTPH.
Phytosanitary categorization: EPPO A2 no. 60, EU RNQP (Annex IV[2]).

[1]*Use of brand names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.*

[2]Commission Implementing Regulation (EU) 2019/2072.



*For isolates obtained from lablab beans only barcoding is recommended due to the risk of false negatives with other molecular tests, For other matrices: 1 molecular test is sufficient when using barcoding or the real-time PCR (Appendix 4 or 5).

** It is recommended to use the same seed extract for the screening test and isolation. After the seed extract real-time PCR, isolation should be performed on PTSCA.

FIGURE 1 Flow diagram describing the diagnostic procedure for *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) and *Xanthomonas citri* pv. *fuscans* (*Xcf*). This flow diagram is intended to provide an overview of the diagnostic process and may not cover all possible scenarios.

Name: *Xanthomonas citri* pv. *fuscans* (Schaad et al., 2005) Constantin et al. (2016).
Other scientific names: *Xanthomonas phaseoli* (Dowson, 1939); *Xanthomonas campestris pv. phaseoli* var. fuscans (ex Burkholder, 1930; Dye et al., 1980); *Xanthomonas axonopodis pv. phaseoli* var. fuscans (Vauterin et al., 1995); *Xanthomonas fuscans* subsp. *fuscans* (Schaad et al., 2005).

Taxonomic position: Bacteria; *Pseudomonadota* (previously known as *Proteobacteria*); *Gammaproteobacteria*; *Lysobacterales* (previously known as *Xanthomonadales*); *Lysobacteraceae* (previously known as *Xanthomonadaceae*); *Xanthomonas*.
EPPO Code: XANTFF.
Phytosanitary categorization: EPPO A2 no. 61, EU RNQP (Annex IV[2]).

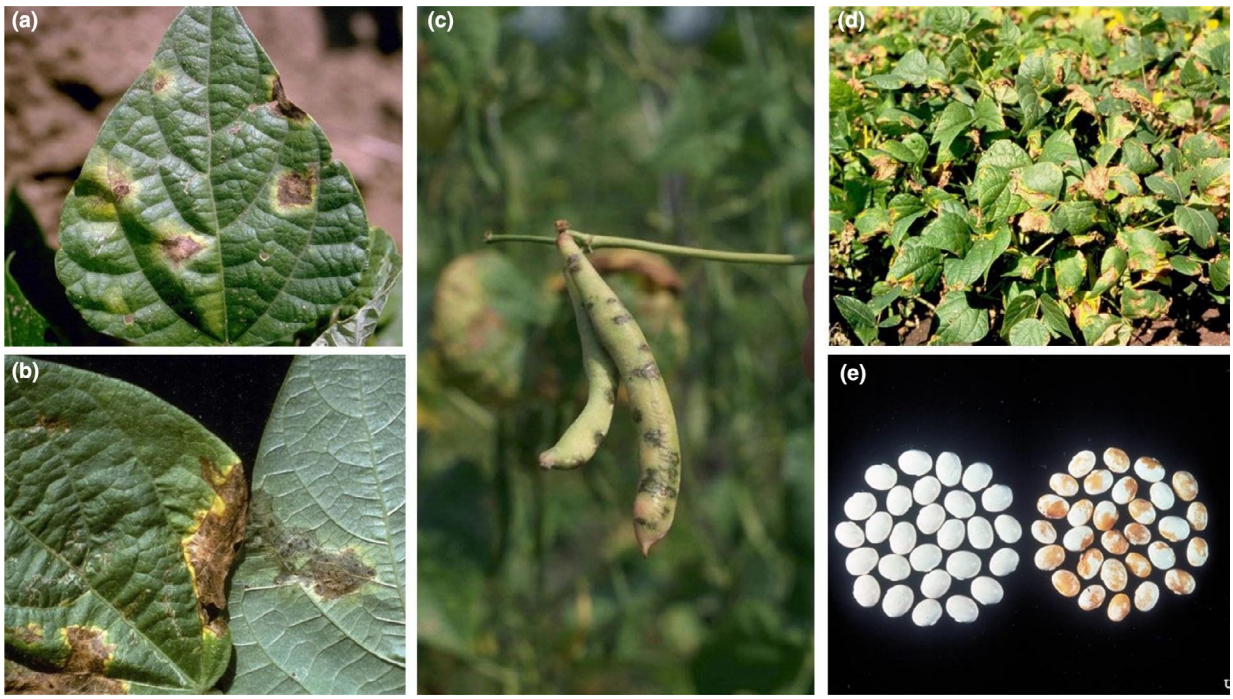


FIGURE 2 Symptoms caused by *Xanthomonas phaseoli* pv. *phaseoli* in common bean (*Phaseolus vulgaris*). (a) Leaf spots with necrotic dry brown lesions surrounded by a narrow yellow halo on a common bean leaf infected by *X. phaseoli* pv. *phaseoli*. (b) Bean leaves showing advanced symptoms caused by *X. phaseoli* pv. *phaseoli*. (c) Water soaked lesions on dry bean pods caused by *X. phaseoli* pv. *phaseoli*. (d) Bean plants showing common bacterial blight symptoms caused by *X. phaseoli* pv. *phaseoli* in the field. (e) Discoloration of common bean seeds caused by *X. phaseoli* pv. *phaseoli* infection (on the right) compared to healthy seeds (on the left). Courtesy: Photos A to D: Howard F. Schwartz, Colorado State University (USA, Bugwood.org); photo E: V.R. Wallen, Agriculture and Agri-Food Canada (Canada, Bugwood.org).

3 | DETECTION

3.1 | Symptoms

Symptoms caused by *X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans* (Gilbertson & Maxwell, 1992; Zaumeyer, 1930) occur on all aerial parts of bean plants - leaves, stems, pods and seeds. Examples of symptoms are shown in Figure 2.

Leaf symptoms first appear as small water-soaked spots, which gradually enlarge and coalesce into large necrotic dry brown lesions usually surrounded by a narrow yellow halo (Goodwin & Sopher, 1994; Figure 2a,b). These leaf lesions can progress to give a burnt appearance and may result in defoliation and death of the plant (Figure 2d).

Stem infection is less common and begins as water-soaked spots, which turn into reddish-brown longitudinal lesions. The bacteria can invade the xylem, and wilting may occur if sufficient bacterial numbers develop in the xylem.

Pod symptoms begin as water-soaked spots that develop into sunken reddish-brown spots, with possible bacterial ooze under humid conditions (Figure 2c).

Seed symptoms appear as yellow or brown spots on the seed coat, particularly around the hilum area in case of vascular infection (Figure 2e). The seed discoloration may not be visible on dark-seeded varieties. Severely infected seeds may be shrivelled, affecting their germination rate and seedling vigour (Darrasse et al., 2018). Infected seeds may also be symptomless.

When grown from infected seed, seedlings are usually asymptomatic if infected with relatively low population numbers (Darrasse et al., 2007). In more severe infections, seedlings can present water-soaked symptoms on the stem, cotyledons and/or primary leaves (Gilbertson & Maxwell, 1992). In some cases, seedlings will have injured or entirely destroyed growing tips.

Symptoms of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* can be confused with those caused by other pathogens, such as *Curtobacterium flaccumfaciens* pv. *flaccumfaciens or Pseudomonas savastanoi* pv. *phaseolicola*. The only means to ascertain that the observed symptoms are caused by *X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans* are bacterial isolation and molecular identification.

3.2 | Detection in symptomatic plant material

3.2.1 | Test sample requirements and extraction

For symptomatic plants, the leaf surface can be sterilized by wiping it with 70% ethanol. Pieces of symptomatic leaf tissues, including spots or necrosis, are

removed with a disinfected scalpel blade. The symptomatic tissue parts are cut aseptically into small pieces and immersed in approximately 4–5 mL of 10 mM sterile phosphate buffered saline (Appendix 1). The mixture is then incubated for approximately 15 min on the bench to allow bacteria to diffuse into the buffer, generating the leaf extract. After incubation, the leaf extract is transferred into new tubes for further analysis.

3.2.2 | Isolation

An aliquot of 50–80 µL of the resulting suspension, along with its 1:10, 1:100, and optionally 1:1000 dilutions, are plated on a suitable non-selective agar medium (e.g. Wilbrink's, King's B, YPGA, ND or YDC; Appendix 1). Each dilution, including the undiluted extract, is plated on a separate plate, resulting in a total of 3 to 4 plates per medium. The colony morphology is observed after 2–3 days of incubation at 28°C. *X. phaseoli* pv. *phaseoli* colonies on agar plates are circular, convex, mucoid and yellow (Figure 3). *X. citri* pv. *fuscans* colonies are similar but a bit slower growing. Comparison with reference strains (positive control) on the same medium is recommended. The fuscous strains of *X. citri* pv. *fuscans* produce a brown, diffusible pigment when grown on tyrosine-containing media (Figure 4).

3.2.3 | Screening tests

A Loop-Mediated Isothermal Amplification (LAMP) test was developed for the detection of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* in plant material (De Paiva et al., 2020) but further experience in the EPPO region and further validation data are needed (e.g. inclusion of strains from various geographical origins) before it can be recommended as a screening test.

3.3 | Detection in seeds

For the detection of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* in seeds, bacteria are extracted from seeds and isolated on different selective media. Two procedures can be followed: soaking of the seeds in sterile saline plus Tween™ 20 followed by isolation of the bacteria on MT and XCP1 media, according to the ISTA and ISF method (ISF-ISHI, 2019; ISTA, 2024a) or soaking of the seeds in sterile purified water followed by isolation on PTSCA medium according to the ANSES and GEVES method (ANSES, 2013; GEVES, 2024). A seed extract (SE) real-time PCR with enrichment in liquid media (GEVES, Vilmorin-Mikado & HM Clause, 2023 Appendix 2) can be used as screening tests in seeds before isolation. When a screening test is performed before isolation, it is recommended to use the same seed extract for the screening test and for isolation.

3.3.1 | Test sample requirements

The sample should be placed in a sealed bag to prevent loss of the seeds. The minimum recommended sample size is 5000 seeds. ISPM 31 (IPPC, 2008) reports that for lots larger than 200 000 seeds, a standard sample of 5000 seeds allows a 99% confidence level of detecting an infection at 0.1% with a 95% of efficacy of detection.

The sample should be stored immediately upon receipt at approximately 5°C.

The sample is divided into sub-samples with a maximum subsample size of 1000 seeds. Sample and subsample are prepared according to the thousand seed weight (TSW). The TSW can be calculated based on counting and weighing 3 × 100 seeds or by another validated system.

The tests described below for the extraction and isolation of *X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans* from seeds have not been validated for treated seeds.

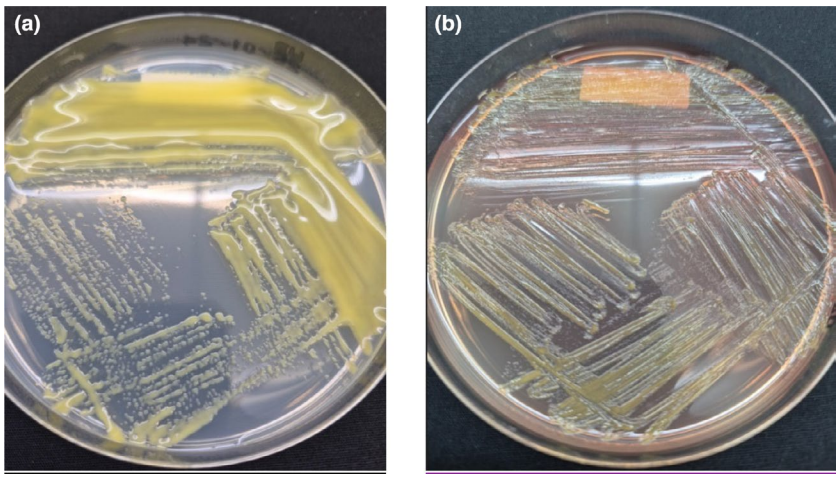


FIGURE 3 (a) *Xanthomonas phaseoli* pv. *phaseoli* on Wilbrink's medium and (b) *Xanthomonas citri* pv. *fuscans* on King's B medium, both after 2 days of incubation at 28°C. Courtesy: NIB (SI).

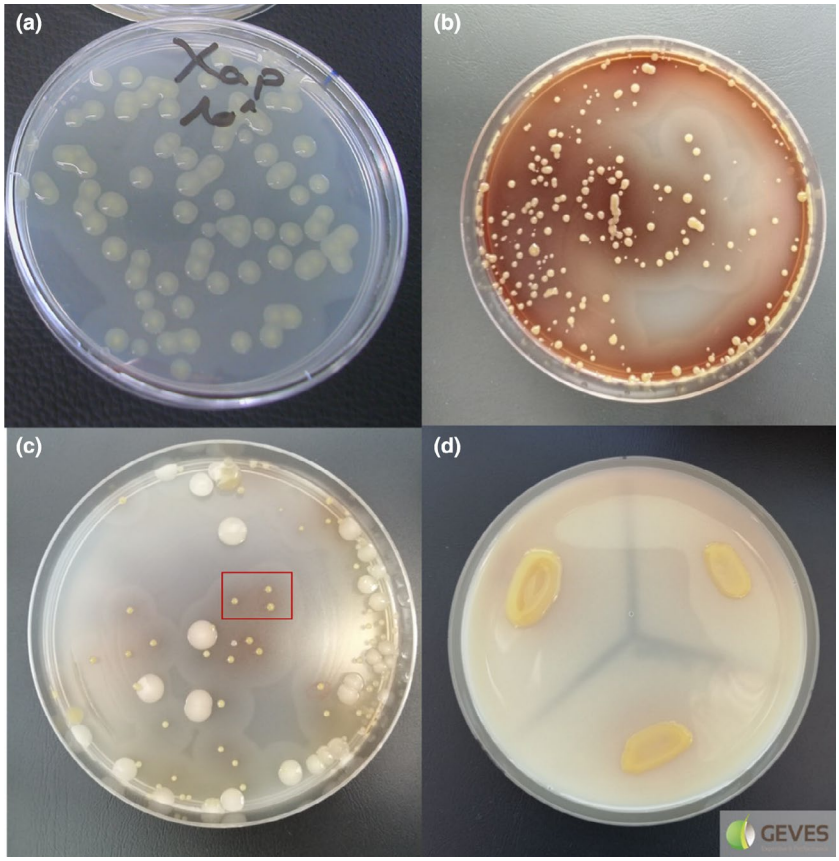


FIGURE 4 *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* pv. *fuscans* on plates after incubation at 28°C (a) Non-fuscous and (b) fuscous *X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans* colonies on PTSCA medium. (c) Example of suspect colonies (in red rectangle) on PTSCA medium and (d) Suspect colonies after transfer on YDC (D). Courtesy: © GEVES (FR).

Treatment may strongly affect the performance of these tests, therefore the use of treated seeds is not recommended. The use of additional spiking controls, that provide information about the reliability of the test result, is described in the Standard in case treated seeds have to be tested (see Section 3.3.3).

3.3.2 | Extraction

The bacteria are extracted from the seeds by soaking seeds. Each sub-sample should be transferred to a plastic bag or container. To perform isolation on MT and XCP1 media (Appendix 1), it is recommended to soak seeds in sterile saline plus Tween™ 20 for 16–18 h at 5°C (±4°C) (ISTA, 2024a; ISF-ISHI, 2019; Appendix 1). To perform seed extract real-time PCR with enrichment in liquid media (Appendix 2) and/or isolation on PTSCA medium (Appendix 1), it is recommended to soak and shake seeds in sterile purified water for approximately 18 h, at 4°C (±4°C) (ANSES, 2013; GEVES, 2024). The volume of liquid (in mL) to be added corresponds to 2.5 times the weight of the seeds (in g).

After extraction, 1 to 5 mL of the seed extract should be collected for screening tests (e.g., isolation on media or molecular screening tests). If there is not enough time to carry out plating alongside molecular testing, the remaining extract can be supplemented with glycerol to a final concentration of 20–30% glycerol and stored in a freezer for subsequent isolation or as a backup. Note that immediate plating is preferable to plating after freezing, as freezing can negatively affect bacterial viability.

3.3.3 | Isolation

3.3.3.1 | *Dilution plating*

For each sub-sample, 100 µL of extract and of dilutions of the extract (1:10 and 1:100 dilutions in e.g. sterile saline solution (Appendix 1)) is plated on semi-selective media (PTSCA, MT, XCP1 depending on the extraction procedure) (See Appendix 1). Plates should be incubated at 28°C (± 3°C) for 3 to 6 days.

3.3.3.2 | *Controls*

The following controls should be included in the dilution plating test:

- Positive Control: a suspension of a reference strain of *X. phaseoli* pv. *phaseoli* and/or *X. citri* pv. *fuscans* is plated on media. The suspension should produce

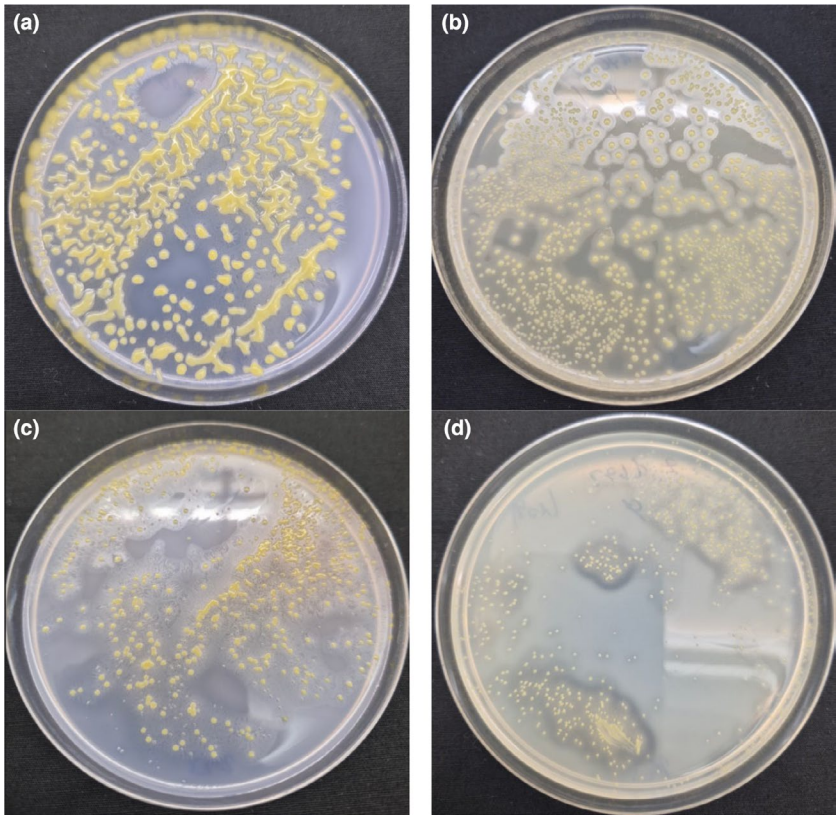


FIGURE 5 *Xanthomonas phaseoli* pv. *phaseoli* (a, b) and *Xanthomonas citri* pv. *fuscans* (c, d) on XCP1 (a, c) and MT (b, d) medium after 6 days of incubation at 28°C. Courtesy: NIB (SI).

isolated colonies (100 µL/plate, recommended 10^4–10^3 CFU/mL, several dilutions can be plated).
- Negative Control: extract from a bag or container without seeds (containing only sterile saline plus Tween™ 20 or sterile purified water) is plated on media. If the soaking step is done in sterile purified water while the dilution of extract is done in sterile saline solution, a second negative control should be done by plating sterile saline solution on media.
- Spiked control: In case of treated seeds (ISTA, 2024b), a spiked control is prepared by diluting a suspension of a reference strain of *X. phaseoli* pv. *phaseoli* and/or *X. citri* pv. *fuscans* in an aliquot of the soaking of a sub-sample. This mix (suspension of *X. phaseoli* pv. *phaseoli* and/or *X. citri* pv. *fuscans*+extract of a sub-sample) should be at the same concentration as the positive control. 100 µL of spiked control is plated on media.

Plates should be incubated at 28°C (±3°C) for 3 to 6 days.

3.3.3.3 | *Interpretation of the results of the dilution plating test*

After 3-6 days of incubation at 28°C (±3°C), growth of bacterial colonies can be recorded. On MT, XCP1 and PTSCA, *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* colonies are yellow, domed and mucoid. The colonies are also glistening on XCP1 medium. On XCP1 and PTSCA media colonies are surrounded by a halo of starch hydrolysis (Figures 4 and 5) while on MT, colonies are surrounded by two zones of hydrolysis; a large clear zone (corresponding to casein hydrolysis) and a smaller milky zone (corresponding to Tween™ 80 lysis). The diffusion of a brown pigment is observed for the fuscous strains of *X. citri* pv. *fuscans* (Figure 4).

The recovery of *X. phaseoli* pv. *phaseoli* and/or *X. citri* pv. *fuscans* colonies with appropriate features should be observed on the positive control and spiked control plates whereas no colonies resembling *X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans* should be observed on the negative control plates.

If no typical colonies for these 2 taxa are observed for all the subsamples, neither *X. phaseoli* pv. *phaseoli* nor *X. citri* pv. *fuscans* are detected in the sample. For treated seeds, if no suspect colonies are observed on the sub-sample plates and if the number of colonies on the spiked control are not as expected, the test result is inconclusive.

If presumptive colonies that look like the positive control are observed on the plates of at least one sub-sample, these colonies and one colony of the positive control should be transferred onto a non-selective medium (e.g. Wilbrink's and, King's B, YPGA or YDC) and incubated at 28°C (±3°C) for 1 to 3 days. On YDC (Figure 4d), *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* colonies are yellow and mucous. The

diffusion of a brown pigment is observed for the fuscous strains of *X. citri* pv. *fuscans*. Tests for identification of presumptive isolates of *X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans* are described in Section 4 Identification.

3.3.4 | Screening tests

Screening tests can be used for the detection of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* in seeds.

3.3.4.1 | *Molecular tests*

A Loop-Mediated Isothermal Amplification (LAMP) test was developed for the detection of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* in seeds (De Paiva et al., 2020) but further experience in the EPPO region and further validation data are needed (e.g. inclusion of strains from various geographical origins) before it can be recommended as a screening test.

A seed extract (SE) real-time PCR with enrichment in liquid media was developed for the detection of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* in seeds (GEVES, Vilmorin-Mikado & HM Clause, 2023) and is described in Appendix 2. In the case of positive results, isolation on PTSCA medium (see Section 3.3.3) and identification (see Section 4) should be performed.

4 | IDENTIFICATION

Pure cultures of presumptive *X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans* isolates should be identified using one or two of the molecular tests listed in Section 4.1. A rapid screening with MALDI-TOF MS can be performed prior to molecular testing as described in Section 4.3.1. For critical cases, a confirmative pathogenicity test is recommended (Section 4.1.2).

4.1 | Molecular tests

4.1.1 | PCR tests

The following tests are recommended for the identification of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans:*

- Conventional PCR (Audy et al., 1994), described in Appendix 3.
- Real-time PCR (Baldwin, 2016, 2019; ISF-ISHI, 2019), described in Appendix 4.
- Real-time PCR (He, 2010), described in Appendix 5.

4.1.2 | DNA barcoding

DNA barcoding can be used to identify *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*. It is the only suitable molecular test for identification of *X. citri* pv. *fuscans* from lablab bean samples. Protocols for routine barcoding using *16S rDNA*, *gyrB* and *avrBs2* sequences are described in Appendix 2 of the EPPO Standard PM 7/129 (2) *DNA barcoding as an identification tool for a number of regulated pests* (EPPO, 2021, under revision). Reference sequences for those loci from strains are available at https://qbank.eppo.int/bacteria/. Guidance for sequence analysis is given in Appendices 7 and 8 of the EPPO Standard PM 7/129 (EPPO, 2021, under revision).

4.2 | Pathogenicity tests

Pathogenicity tests may be used for the identification of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*. In critical cases, pathogenicity testing should be done as a confirmation in the diagnosis of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*. The procedures for pathogenicity testing are presented in Appendix 6.

4.3 | Other tests

4.3.1 | Proteomic analysis based on MALDI-TOF-MS

Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF-MS) for proteomic analysis allows rapid, reliable and robust identification at the genus level and can be used to screen colonies prior to molecular testing. Guidelines on how to perform MALDI-TOF MS will be provided in PM 7/XXX *Matrix assisted laser desorption/ionization time-of-flight* (*MALDI-TOF*) *mass spectrometry* (*MS*) *for the identification of plant pathogenic bacteria* (EPPO, in preparation).

5 | REFERENCE MATERIAL

X. phaseoli* pv. *phaseoli (Smith, 1897) Constantin et al. (2016).

Pathotype strain: LMG 7455; NCPPB 3035; CFBP 8479; CFBP 2534; ATCC 9563; ICMP 5834. The pathotype strain of *X. phaseoli* pv. *phaseoli* (LMG 7455) and the type strain of *X. phaseoli* (LMG 29033) are members of the same taxon. Future pathogenicity studies should clarify if the type strain of *X. phaseoli* can be classified in this pathovar. This pathovar includes only the strains pathogenic to bean classified in *X. phaseoli* (subgroup 9.4 of Rademaker et al., 2005).

X. citri* pv. *fuscans (Schaad et al., 2005) Constantin et al. (2016).

Pathotype strain (fuscous): LMG 826; NCPPB 381; CFBP 6165; ATCC 19315; ICMP 239. The pathovar contains both the non-fuscous strains (GL2 & GL3)

and the fuscous strains pathogenic to bean classified in *X. citri* (subgroup 9.6 of Rademaker et al., 2005). As well as, awaiting their reclassification as a new pathovar, the non-fuscous strains isolated from lab-lab classified in *X. citri* (subgroup 9.5 of Rademaker et al., 2005).

Reference sequences for the DNA barcoding loci are available at https://qbank.eppo.int/bacteria/.

6 | REPORTING AND DOCUMENTATION

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis*.

7 | PERFORMANCE CHARACTERISTICS

When performance characteristics are available, these are provided with the description of the test. Validation data are also available in the EPPO Database on Diagnostic Expertise (http://dc.eppo.int), and it is recommended to consult this database as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports, etc.).

8 | FURTHER INFORMATION

Further information on this organism can be obtained from:

Ms. Foucher, GEVES, 25 rue Georges Morel, CS 90024, 49071 Beaucouzé cedex, France; justine.foucher@geves.fr.

Ms. Pirc, National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 121, SI-1000 Ljubljana, Slovenia; manca.pirc@nib.si.

9 | FEEDBACK ON THIS DIAGNOSTIC STANDARD

If you have any feedback concerning this Diagnostic Standard, or any of the tests included, or if you can provide additional validation data for tests included in this Standard that you wish to share please contact diagnostics@eppo.int.

10 | STANDARD REVISION

A regular review process is in place to identify the need for revision of Diagnostic Standards. Standards identified as needing revision are marked as such on the EPPO website.

When errata and corrigenda are in press, this will also be marked on the website.

ACKNOWLEDGEMENTS

This Standard was originally drafted by Mr. Cottyn (ILVO, BE), Ms. Foucher (GEVES, FR) and Ms. Pirc (NIB, SI). The Standard was reviewed by the Panel on Diagnostics in Bacteriology.

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APPENDIX 1 - BUFFERS AND MEDIA

All buffers and media are sterilized by autoclaving at 121°C for 15 min, except when stated otherwise.

1. Buffers

10 mM Phosphate buffered saline (*PBS*), pH 7.2 for extraction from symptomatic plants

Na_2HPO_4	1.07 g
$NaH_2PO_4 \times 2H_2O$	0.4 g
NaCl	8 g
Distilled water	1 L

Sterile saline plus Tween™ 20 (0.02 % v/v), for soaking seeds

Sodium chloride (NaCl)	8.5 g
Distilled water	1 L
After autoclaving add 0.2 mL of sterile Tween™ 20 per 1 L.	

Sterile saline water

Sodium chloride (NaCl)	8.5 g
Distilled water to	1 L

2. Media

2.1 Non-selective media

Nutrient dextrose (*ND*) *agar* (Lelliott & Stead, 1987). Add 1% (w/v) d-glucose to nutrient agar (such as Oxoid CM3 or Difco).

Wilbrink's medium (Koike, 1965)

Peptone	5.0 g
K_2HPO_4	0.5 g
$MgSO_4 \times 7H_2O$	0.25 g
Sucrose	10.0 g
Microbiological grade agar	18.0 g
Distilled water	1 L
Adjust pH to 7.0 before adding agar	

King's B medium (King et al., 1954)

Proteose peptone	20.0 g
Glycerol	15.0 mL
K_2HPO_4	1.5 g
$MgSO_4 \times 7H_2O$	1.5 g
Microbiological grade agar	15.0 g
Distilled water	1 L
Adjust pH to 7.2 before adding agar	

Yeast peptone glucose agar (*YPGA*)

Yeast extract	5.0 g
Bacto peptone	5.0 g
D(+) glucose	10.0 g
Difco bacto agar	15.0 g
Distilled water	1 L

If needed, supplement with 50 mg/L adenine hemisulphate for faster growth of the bacteria.
If needed, 50 mg/L cycloheximide can be added after autoclaving to inhibit fungal growth.

Yeast extract-dextrose-$CaCO_3$ (YDC):

Yeast extract	10.0 g
Glucose	20.0 g
$CaCO_3$	20.0 g
Microbiological grade agar	15.0 g
Distilled water to	1 L
Adjust pH to 6.8–7.0	

2.2 Semi-specific media

Milk Tween agar medium (MT) (ISTA, 2024a adapted from Goszczynska & Serfontein, 1998):

Section A	Proteose peptone no. 3	10.0 g
	$CaCl_2$	0.25 g
	Tyrosine	0.5 g
	Microbiological grade agar	15 g
	Distilled water	0.5 L
Section B	Skimmed milk powder	10.0 g
	Distilled water	0.5 L
Section C	Tween™ 80	10.0 mL
Section D	Nystatin*	40 mg/L
	Cephalexin	80 mg/L
	Vancomycin	10 mg/L

Weigh out all ingredients listed in section A and add them to a suitable container. Then, add 500 mL of distilled water and dissolve the ingredients. In a separate container, dissolve the skimmed milk powder in 500 mL of distilled water. Next, prepare 10 mL of Tween™ 80 separately. Sterilize the preparations from Section A, the skimmed milk solution from Section B, and the Tween™ 80 from Section C separately at 121°C for 15 min. After sterilizing, aseptically add a sterilized skimmed milk preparation and sterilized Tween™ 80 to the sterilized ingredients in Section A.

Prepare antibiotic solutions (Section D). Allow medium to cool to approximately 50°C and add antibiotics.

*Cycloheximide can be used as an alternative to nystatin to control fungi. Dissolve 500 mg of cycloheximide in 10 mL 70 % ethanol, add 1 mL to cooled medium.

XCP1 medium (ISTA, 2024a, adapted from McGuire et al., 1986):

Section A	KBr	10.0 g
	$CaCl_2$	0.25 g
	Soluble potato starch	10.0 g
	Peptone	10.0 g
	Microbiological grade agar	15 g
	Crystal Violet (1% aqueous)	0.15 mL
	Distilled water	1 L
Section B	Tween™ 80	10.0 mL
Section C	Nystatin*	40 mg/L
	Cephalexin	10 mg/L
	Fluorouracil	3 mg/L
	Tobramycin	0.16 mg/L

Weigh all the ingredients in Section A into a suitable container. Add 1000 mL of distilled water to dissolve the ingredients. Add crystal violet. Sterilize at 121°C for 15 min. Sterilize 10 mL of Tween™ 80 separately (Section B) at 121°C for 15 min. Aseptically add Tween™ 80 to the ingredients in Section A.

Prepare antibiotic solutions (Section C). Allow medium to cool to approximately 50°C and add antibiotics.

*Cycloheximide can be used as an alternative to nystatin to control fungi. Dissolve 500 mg of cycloheximide in 10 mL 70 % ethanol, add 1 mL to cooled medium.

Peptone Tyrosine Sodium chloride Agar (*PTSCA*) (ANSES, 2013)

Bactopeptone	10.0 g
L-tyrosine	1.0 g
Insoluble (or soluble) potato starch	4.0 g (15.0 g)
Sodium chloride (NaCl)	5.0 g
Cephalexin*	40.0 mg
Cycloheximide (Actidione)*	50.0 mg
Microbiological grade agar	15.0 g
Distilled water to	1 L
Adjust pH to 6.8–7.0	

*Added after autoclaving.

YP+ATB+ACAT broth

YP, Yeast and Bactopeptone; ATB, antibiotics; ACAT, *Acidovorax cattleyae*.

Section A	Yeast extract	7.0 g
	Bactopeptone	7.0 g
	Distilled water	1 L
Section B	Cephalexin	40.0 mg
Section C	Suspension of boiled *Acidovorax cattleyae** (approximatively 10^8 CFU/mL)	10 mL
Section D	Nystatin	217 175 UI

Cephalexin is added after autoclaving Section A and cooling to approximately 50°C. This solution can be stored for 2 months at 4°C. Prepare a suspension of boiled *Acidovorax cattleyae* at approximatively 10^8 CFU/mL (Section C). This *A. cattleyae* suspension can be stored at approximately −20°C. Add 10 mL of this suspension to the broth.

*The quantity of *A. cattleyae* suspension may be reduced to be closer to the detection limit. Because of its short shelf life, the antibiotic nystatin should be added to the medium no more than 3 days before analysis.

APPENDIX 2 - SEED EXTRACT REAL-TIME PCR WITH ENRICHMENT IN LIQUID MEDIA (GEVES, VILMORIN-MIKADO & HM CLAUSE, 2023)

The test below is described as it was carried out to generate the validation data. Other equipment, kits or reagents may be used provided that a verification (*see PM 7/98*) *is carried out.*

1. General information

1.1 This test can be used for the detection of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* in seed extract after a bacterial enrichment step in liquid media.

1.2 This test was developed during a French collaborative project between GEVES (FR), Vilmorin-Mikado (FR) and HM Clause (FR) (GEVES, Vilmorin-Mikado & HM Clause, 2023).

1.3 The Trb1 primers' target sequence is located on the pA plasmid of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*. The Audy primers' target sequence is located on the 3.4-kb plasmid DNA fragment (p7) of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*. The Acat primers' targeting the chromosome of *A. cattleyae* are used as an internal positive control (IPC).

1.4 Oligonucleotides:

1.4.1 Audy primers and probe

	Name	Sequence	Amplicon size*
Forward primer	Au-F1	5′-ACG GCC GGC GTC TTG TCT CT-3′	149 bp
Reverse primer	Au-R1	5′-GCC GAG GTC CGC GAG ATT CT-3′	
Probe	Au-1FAM	5′-**FAM**-CGT CTC TGG CTT GAC TGC GGT CGC-**BHQ-1**-3′	

*Including primer sequences.

1.4.2 Trb1 primers and probe

	Name	Sequence	Amplicon size*
Forward primer	Trb1F	5′-AGC AGC ACG CTC GAC AAG A-3′	112 bp
Reverse primer	Trb1R	5′-ACG GGT CTA GTC TGG ATC TGG A-3′	
Probe	Trb1Pr	5′-**Yakima Yellow**- ACA GGT CAA CAC CTG GCA TGC CC–**BHQ-1**-3′	

*Including primer sequences.

1.4.3 Acat primers and probe

	Name	Sequence	Amplicon size*
Forward primer	Acat2-F	5′-TGT AGC GAT CCT TCA CAA G-3′	152 bp
Reverse primer	Acat2-R	5′-TGT CGA TAG ATG CTC ACA AT-3′	
Probe	Acat1-Pr	5′-**Texas Red** – CTT GCT CTG CTT CTC TAT CAC G – **BHQ-1**-3′	

*Including primer sequences.

2. Methods

2.1 Extraction and enrichment of bacteria

2.1.1 The bacteria are extracted from the seeds by soaking seeds as described in Section 3.3.2.

2.1.2 Transfer 1.5 mL of the extract into a tube. Centrifuge for 15 min at 2250 *g*.

2.1.3 Discard 1.4 mL of the supernatant. It is recommended to remove the supernatant with a 1 mL micropipette (taking 700 µL twice) to avoid resuspension of the pellet.

2.1.4 Add 1 mL of YP+ATB+ACAT broth (Appendix 1) containing the IPC, resuspend the pellet and incubate at 28°C (±3°C) for 24 h (±1h) at 150–200 rpm.

2.2 Nucleic acid extraction

2.2.1 Centrifuge the tube for 10 min at 2250 *g*.

2.2.2 Discard the supernatant and resuspend the pellet in 200 µL NaOH (0.5 N).

2.2.3 Incubate the tube for 10 min at 65°C.

2.2.4 Mix 20 µL of extract and 180 µL of Tris-HCl (20 mM). The extract can be stored at approximately −20°C.

2.3 Real-time PCR

2.3.1 The real-time PCR can be performed as a duplex (Trb1 and Acat primers) or as a triplex (Audy, Trb1 and Acat primers).

2.3.2 Master Mix for the triplex PCR

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular grade water	N.A.	9.85	N.A.
PerfeCTa Multiplex qPCR Toughmix (*Quantabio*)	5×	5.0	1×
Forward primer (Primer Au-F1)	10 µM	1.0	0.4 µM
Reverse primer (Primer Au-R1)	10 µM	1.0	0.4 µM
Probe (Probe Au-1FAM)	10 µM	0.20	0.08 µM
Forward primer (Primer Trb1F)	10 µM	1.0	0.4 µM
Reverse primer (Primer Trb1R)	10 µM	1.0	0.4 µM
Probe (Probe Trb1Pr)	10 µM	0.20	0.08 µM
Forward primer (Primer Acat2-F)	20 µM	0.25	0.2 µM
Reverse primer (Primer Acat2-R)	20 µM	0.25	0.2 µM
Probe (Probe Acat1-Pr)	10 µM	0.25	0.1 µM
Subtotal		20	
Nucleic acid extract		5	
Total		25	

2.3.3 Master Mix for the duplex PCR

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular grade water	N.A.	11.75	N.A.
PerfeCTa Multiplex qPCR Toughmix (*Quantabio*)	5×	5.0	1×
Forward primer (Primer Trb1F)	10 µM	1.0	0.4 µM
Reverse primer (Primer Trb1R)	10 µM	1.0	0.4 µM
Probe (Probe Trb1Pr)	10 µM	0.50	0.2 µM
Forward primer (Primer Acat2-F)	20 µM	0.25	0.2 µM
Reverse primer (P Primer Acat2-R)	20 µM	0.25	0.2 µM
Probe (Probe Acat1-Pr)	10 µM	0.25	0.1 µM
Subtotal		20	
Nucleic acid extract		5	
Total		25	

2.4 PCR conditions: minimum 3 min at 95°C, 40 cycles of 15 s at 95°C and 1 min at 60°C.

3. Essential Procedure Information

3.1 Controls

For a reliable test result to be obtained, the following controls should be included for each series of samples.

- Negative isolation control (NIC) to monitor the contamination during the extraction and enrichment step: sterile purified water used for soaking seeds. This control is included in the first step of the analysis.
- Positive isolation control (PIC) to ensure that enrichment steps have worked correctly: a target strain prepared at a final concentration higher than the LOD in sterile purified water and that has followed the process from point 2.1.2. onwards.
- Positive amplification control (PAC) to ensure that the real-time PCR mix used amplifies the target DNA of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*: DNA extracted from a reference strain (*X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans*).
- Negative amplification control (NAC) to rule out false positives due to contamination during the preparation of the reaction mix: application of the amplification procedure to molecular grade water that was used to prepare the reaction mix.

- Spiked control (SC) consisting of removing part of the extract from each sample and inoculating it with a suspension of *X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans* in order to obtain a final concentration higher than the LOD (e.g. prepare a suspension of *X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans* at 10^8 CFU/mL, prepare a series of dilutions to obtain a 10^5 CFU/mL suspension and add 150 µL of the 10^5 CFU/mL suspension to 1350 µL of extract to obtain a final concentration of 10^4 CFU/mL). This spiked extract follows the process from point 2.1.2.

An internal positive control (IPC) is used to monitor each individual sample separately (Acat primers, see 1.3.).

3.2 Interpretation of results: in order to assign results from this PCR-based test the following criteria should be followed:

The Ct cut-off value given below is as established in the validation data. As a Ct cut-off value is equipment, material and chemistry dependent, it needs to be verified in each laboratory when implementing the test.

Verification of the controls

- The Ct value for the Acat test (IPC) should be equal or higher than 35 for the NAC.
- The amplification curves for Trb1 (and Audy if used) should be exponential with a Ct value below 35 for the PIC, PAC and SC. It should be equal or higher than 35 for the NIC and NAC.

When these conditions are met:

- A test will be considered positive if it produces an exponential amplification curve for Trb1 primers (and Audy primers if used) with a Ct value below 35.
- A test will be considered negative, if it does not produce an amplification curve for Trb1 primers (and Audy primers if used) or if it produces a curve which is not exponential or with a Ct value equal or above 35. The amplification curves for Acat (IPC) should be exponential with a Ct value below 35.
- Tests should be repeated if any contradictory or unclear results are obtained, such as negative samples with a Ct value above 35 for the IPC (Acat) or if the SC control has a Ct equal or higher than 35 and/or presents a non-exponential amplification curve.

4. Performance characteristics available

Validation data were obtained by GEVES (FR) and Vilmorin-Mikado (FR) and HM Clause (FR) using a slightly modified procedure (GEVES, Vilmorin-Mikado & HM Clause, 2023). Validation was carried out in accordance with PM 7/98.

4.1 Analytical sensitivity

Triplex real-time PCR (Trb1/Audy/Acat): Analytical sensitivity was evaluated by GEVES (FR) by spiking a target strain (CFBP 6473 identified as *Xanthomonas fuscans* subsp. *fuscans*) in a seed extract. Three different seed lots were tested, with low medium and high concentrations of saprophytic flora respectively. Different dilutions of the strain were tested in triplicate. The LOD was 6.67 CFU/mL for the Audy primers and for the Trb1 primers. The analytical sensitivity evaluated here corresponds to the detectable concentration present in the macerate (before enrichment).

Duplex real-time PCR (Trb1/Acat): Analytical sensitivity was evaluated by Vilmorin-Mikado (FR) by spiking the extract of 3 healthy seeds lots with different concentration of saprophytic flora (low, medium and highly concentrated) with a suspension of a target strain (CFBP 6546 identified as *Xanthomonas axonopodis* pv. *phaseoli*). Different dilutions of the target strain were tested. The results showed a detection threshold of 10 CFU/mL. The analytical sensitivity evaluated here corresponds to the detectable concentration present in the macerate (before enrichment).

4.2 Analytical specificity

The analytical specificity was evaluated by GEVES (FR).

For Trb1 primers (evaluated in simplex reactions on isolated strains):

Inclusivity: evaluated on 30 target strains in total of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* (14 strains were non-fuscous, 12 strains were fuscous, and 4 other strains (fuscousity unknown)): 100 %.

Exclusivity: evaluated on 40 non-target saprophytic isolates obtained from bean seeds: 100 %. The isolates were not identified but they do not correspond to *X. phaseoli* pv. *phaseoli* nor *X. citri* pv. *fuscans* based on the characteristics of the colonies on PTSCA media.

For Audy primers (evaluated in duplex with IPC primers Wu (see Appendix 4)):

Inclusivity: evaluated on 28 target strains of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*: 100 %.

Exclusivity: evaluated on 30 non-target strains (1 *X. hortorum* pv. *carotae*, 1 *X. vesicatoria* and 28 saprophytic isolates obtained from bean seeds): 93.3 %. The 2 saprophytic isolates that cross reacted were not identified but they do not correspond to *X. phaseoli* pv.

phaseoli nor *X. citri* pv. *fuscans* based on the characteristics of the colonies on PTSCA media.

4.3 Diagnostic sensitivity, diagnostic specificity, repeatability, and reproducibility

For the duplex real-time PCR test (Trb1/Acat):

A test was performed by Vilmorin-Mikado (FR) on three different dates with healthy and infected (high and low levels of infection) samples. Tests were done by different analysts. A total of 28 healthy samples, 30 infected samples with low level of infection and 51 infected samples with high level of infection were tested. Each seed lot (healthy, low level of infection or high level of infection) was tested 5 times on different dates by different operators. Samples from the same lot tested on the same date by the same operator were considered as replicates. Depending on the test, between 2 and 15 replicates were tested. The results showed a level of 100% for the repeatability and reproducibility.

The diagnostic sensitivity and specificity were also evaluated by Vilmorin-Mikado (FR) by analysing 34 healthy seed lots and 10 infected lots. Each sample was tested by a real-time PCR test following enrichment on medium or by a dilution plating test and were tested by seed extract real time PCR with enrichment in liquid media. Diagnostic sensitivity and diagnostic specificity were 100%.

An interlaboratory comparison (ILC) was organized between three laboratories (GEVES, HM Clause and Vilmorin-Mikado (all FR)). 19 samples from healthy and naturally infected lots were analysed in each laboratory. For each lot, between 5 and 9 replicates were tested in each laboratory. The repeatability and reproducibility were 93% for the healthy samples and 100% for the infected samples. The diagnostic specificity was 96% and diagnostic sensitivity was 100%.

For the triplex real-time PCR (Trb1/Audy/Acat):

The panel of 19 samples used during the ILC was also analysed by GEVES (FR) using the triplex real-time PCR. Diagnostic sensitivity, diagnostic specificity, repeatability, and reproducibility were 100%. Reproducibility was evaluated by performing the test by two different operators on different dates.

4.4 Additional information

The possibility of pooling extracts from 5 samples was evaluated by Vilmorin-Mikado (FR) by testing 6 lots of infected seeds and 2 lots of healthy seeds. For each lot, 5 samples of 1000 seeds were soaked, and the enrichment step was carried out on 1.5 mL of each extract or on a pool extract consisting of 300 µL of each extract. The results of each sample were always concordant with the results of the pool. The delta Ct between the Ct obtained on the positive sample and the positive pool was 0.83 on average (evaluated for Ct ranging from 24.03 to 34.73). The maximum delta Ct observed was 1.35. Those results show that 5 extracts of 1000 seeds can be pooled in a tube (300 µL per sample, 1.5 mL in total).

APPENDIX 3 - CONVENTIONAL PCR (AUDY ET AL., 1994)

The test below differs from the one described in the original publication (*see 1.2*).

The test below is described as it was carried out to generate the validation data provided in section 4 (*ISTA*). *Other equipment, kits or reagents may be used provided that a verification* (*see PM 7/98*) *is carried out.*

1. General information

1.1 This test can be used for the identification of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* colonies. It amplifies a fragment of the plasmid that is commonly carried in strains of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*, but not all strains of *X. citri* pv. *fuscans*, including the pathotype strain, and the lablab strains.

1.2 The test was developed by Audy et al. (1994) and adapted by ISTA in 2014 (ISTA, 2024a).

1.3 The target sequence is located on the 3.4-kb plasmid DNA fragment (p7).

1.4 Oligonucleotides:

	Name	Sequence	Amplicon size*
Forward primer	p7X4c	5′-GGC AAC ACC CGA TCC CTA AAC AGG-3′	813 bp
Reverse primer	p7X4e	5′-CGC CGG AAG CAC GAT CCT CGA AG-3′	

*Including primer sequences.

2. Methods

2.1 Nucleic Acid Extraction and Purification

2.1.1 Suspend approximately 1 µL of cell culture (e.g. using a 1-µL loop) or one colony in 500 µL of sterile purified water.

2.1.2 Heat in closed tubes at approximately 95–100°C for approximately 5 to 10 min and cool down.

2.1.3 DNA should preferably be stored at approximately −20°C.

2.2 Conventional PCR

2.2.1 Master Mix

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular grade water	N.A.	10.02	N.A.
GoTaq® Reaction Buffer (with $MgCl_2$) * (*Promega*)	5×	4	1×
dNTP	2.5 mM each	1.6	0.2 mM
Forward primer (p7X4c)	20 µM	0.15	0.15 µM
Reverse primer (p7X4e)	20 µM	0.15	0.15 µM
GoTaq DNA polymerase (*Promega*)	5 U/µL	0.08	0.4 U
Subtotal		16	
Nucleic acid extract		4	
Total		20	

*Laboratories using a buffer without $MgCl_2$ will have to add this salt to a final concentration of 1.5 mM.

2.2.2 PCR conditions: 3 min at 94°C, 35 cycles of 1 min at 94°C and 2 min at 72°C, a final step for 10 min at 72°C.

3. Essential Procedural Information

3.1 Controls

For a reliable test result to be obtained, the following controls should be included for each series of nucleic acid extraction and amplification of the target organism and target nucleic acid, respectively.

- Negative isolation control (NIC) to monitor contamination during nucleic acid extraction: for the NIC the same volume of purified distilled water as for preparation of suspension, is used.
- Positive isolation control (PIC) to ensure that nucleic acid of sufficient quantity and quality is isolated: a suspension of reference cultures of *X. phaseoli* pv. *phaseoli* and/or *X. citri* pv. *fuscans* should be used and prepared as for the tested strains.
- Negative amplification control (NAC) to rule out false positives due to contamination during the preparation of the reaction mix: application of the amplification procedure to molecular grade water that was used to prepare the reaction mix.
- Positive amplification control (PAC) to monitor the efficiency of the amplification: amplification of nucleic acid of the target organism. This includes nucleic acid extracted from *X. phaseoli* pv. *phaseoli* and/or *X. citri* pv. *fuscans*.

3.2 Interpretation of results: in order to assign results from this PCR-based test the following criteria should be followed:

Verification of the controls
- NIC and NAC: no band is visualized.
- PIC, PAC a band of the expected size 813 bp is visualized.

When these conditions are met:
- A test will be considered positive if a band of the expected size 813 bp is visualized.
- A test will be considered negative, if no band or a band of a different size than expected is visualized.
- Tests should be repeated if any contradictory or unclear results are obtained.

4. Performance characteristics available

Validation data were obtained by ISTA (GM13-06 pages 45 to 54, Grimault, 2012) and by the NIB. The test may have been adapted further and validated or verified using other critical reagents, instruments and/or other modifications. If so, the corresponding test descriptions and validation data can be found in the EPPO database on diagnostic expertise (section validation data https://dc.eppo.int/validation_data/validationlist).

4.1 Analytical sensitivity

Not evaluated. Pure bacterial cultures (concentration 10^6–10^9 CFU/mL) were used to evaluate the performance characteristics of the tests.

4.2 Analytical specificity

ISTA:

Inclusivity: evaluated on 28 target strains of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*: 100 %; Type strain of *X. citri* pv. *fuscans* (NCPPB 381) was not included because of DNA extraction issues.

Exclusivity: evaluated on 30 non-target strains: 96.65% (cross reaction with *X. axonopodis* pv. *dieffenbachiae*).

NIB (validation performed with polymerase GoTaq® G2 Hot Start Polymerase (Promega), Grujić et al., in preparation):

Inclusivity: evaluated on 18 target strains of *X. phaseoli* pv. *phaseoli and X. citri* pv. *fuscans*: 77%; False negative results were obtained with one *X. citri* pv. *fuscans* strain from two different sources (type strain NCPPB 381 and LMG 8132). The type strain was previously reported as giving positive results using this test. As a consequence, it is assumed that it may have lost the target sequence of the test. False negative results were obtained with two *X. phaseoli* pv. *phaseoli* strains from lablab bean (LMG 829 and LMG 8013).

Exclusivity: evaluated on 17 non-target strains: 100% (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (6), *Pseudomonas syringae* pv. *syringae* (1), *Pseudomonas viridiflava* (1), *Xanthomonas vesicatoria* (2), *Xanthomonas citri* pv. *glycines* (2), *Stenotrophomonas maltophilia* (5)).

4.3 Repeatability
ISTA: 100%.

4.4 Reproducibility
ISTA: 100%.

APPENDIX 4 - REAL-TIME PCR (ISF-ISHI, 2019)

The test below differs from the one described in the original publication (*see 1.2*).

The test below is described as it was carried out to generate the validation data provided in section 4. Other equipment, kits or reagents may be used provided that a verification (*see PM 7/98*) *is carried out.*

1. General information

1.1 This test can be used for the identification of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* colonies except for those isolated from lablab beans.

1.2 This TaqMan real-time PCR test (ISF-ISHI, 2019) was developed by Baldwin (2016) and validated by Baldwin (2019), based on the work done by Wu et al. (2008) and Audy et al. (1994). The test was further adapted by GEVES (FR).

1.3 The target sequence of Audy primers is located on the 3.4-kb plasmid DNA fragment (p7). Wu primers target the 16S rRNA present in all gram-negative bacteria and are used as internal positive control (IPC).

1.4 Oligonucleotides:

1.4.1 Audy primers.

	Name	Sequence	Amplicon size*
Forward primer	Au-F1	5′-ACG GCC GGC GTC TTG TCT CT-3′	149 bp
Reverse primer	Au-R1	5′-GCC GAG GTC CGC GAG ATT CT-3′	
Probe	Au-1FAM	5′-**FAM**-CGT CTC TGG CTT GAC TGC GGT CGC-**BHQ-1**-3′	

*Including primer sequences.

1.4.2 Wu primers.

	Name	Sequence	Amplicon size*
Forward primer	Wu-F	5′-CAA CGC GAA GAA CCT TAC C-3′	227 bp
Reverse primer	Wu-R	5′-ACG TCA TCC CCA CCT TCC-3′	
Probe	Wu-Pr2	5′-**VIC**-ACG ACA GCC ATG CAG CAC CT-**QSY**-3′	

*Including primer sequences.

2. Methods

2.1 Nucleic acid extraction and purification

2.1.1 Prepare a suspension at approximately 10^7 CFU/mL (OD_{600nm} ≈0.05) of each suspect colony in sterile water. Bacterial cultures should be <5 days old.

2.1.2 Heat in closed tubes at approximately 95–100°C for approximately 5 to 10 min and cool down.

2.1.3 DNA should preferably be stored at approximately −20°C.

2.2 Duplex real-time PCR

2.2.1 Master mix

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular grade water	NA	10.55	NA
PerfeCTa Multiplex qPCR Toughmix (*Quantabio*)	5×	5.0	1×
Forward primer (Primer Au-F1)	10 µM	1.0	0.4 µM
Reverse primer (Primer Au-R1)	10 µM	1.0	0.4 µM
Probe (Probe Au-1FAM)	10 µM	0.20	0.08 µM
Forward primer (Primer Wu-F)	10 µM	1.0	0.4 µM
Reverse primer (P Primer Wu-R)	10 µM	1.0	0.4 µM
Probe (Probe Wu-Pr2)	10 µM	0.25	0.1 µM
Subtotal		20	
Nucleic acid extract		5	
Total		25	

2.2.2 PCR conditions: 10 min at 94°C, 40 cycles of 15 s at 95°C and 1 min at 60°C.

3. Essential procedural information

3.1 Controls

For a reliable test result to be obtained, the following controls should be included for each series of nucleic acid extraction and amplification of the target organism and target nucleic acid, respectively.

- Negative isolation control (NIC) to monitor contamination during nucleic acid extraction: nucleic acid extraction and subsequent amplification of sterile purified water. The suspension of another non-target strain can be used as additional NIC.

- Positive isolation control (PIC) to ensure that nucleic acid of sufficient quantity and quality is isolated: nucleic acid extraction and subsequent amplification of a suspension of a reference strain (*X. phaseoli* pv. *phaseoli* and/or *X. citri* pv. *fuscans*) in sterile purified water (approximately 10^7 CFU/mL).
- Negative amplification control (NAC) to rule out false positives due to contamination during the preparation of the reaction mix: application of the amplification procedure to molecular grade water that was used to prepare the reaction mix.
- Positive amplification control (PAC) to monitor the efficiency of the amplification: amplification of nucleic acid of the target organism (*X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans*).

As an alternative (or in addition) to the PIC, an internal positive control (IPC) is used to monitor each individual sample separately (Wu primers, see 1.4.2.).

3.2 Interpretation of results: in order to assign results from this PCR-based test the following criteria should be followed:

The Ct cut-off value given below is as established in GEVES (FR). As a Ct cut-off value is equipment, material and chemistry dependent, it needs to be verified in each laboratory when implementing the test.

Verification of the controls
- The PIC and PAC amplification curves for Audy should be exponential with a Ct value below 30.
- NIC(s) and NAC should give a Ct value above 30 or no amplification for Audy primers.

When these conditions are met:
- A test will be considered positive if it produces an exponential amplification curve for Audy primers with a Ct value below 30.
- A test will be considered negative, if it does not produce an amplification curve for Audy primers or if it produces a curve which is not exponential or with a Ct value above 30. The Ct value of the IPC (Wu) for the sample should be below 30 and at least 3.3 cycles below the Ct value of the NAC.
- Tests should be repeated if any contradictory or unclear results are obtained, such as negative samples with a Ct value above 30 for the IPC (Wu).

4. Performance characteristics available

Validation data for the tests described above were obtained by GEVES (FR). Validation was carried out in accordance with PM7/98. The test was originally validated by Baldwin (2019) using a slightly different procedure (different reaction mix and an Audy Ct threshold of 35 instead of 30). Some additional validation data from NIB for the Audy primers/probe using a slightly different procedure (simplex reaction, different Master Mix) is also provided.

The test may have been adapted further and validated or verified using other critical reagents, instruments and/or other modifications. If so, the corresponding test descriptions and validation data can be found in the EPPO database on diagnostic expertise (section validation data https://dc.eppo.int/validation_data/validationlist).

4.1 Analytical sensitivity

Evaluated by GEVES and Baldwin T in 2019 (ISF):

Diluted suspensions of two strains with an OD_{600nm} between 0.001 to 1.730 were tested with the real-time PCR test. This result showed that the real-time PCR works for DNA extracted from bacterial suspensions at an $OD_{600nm} \approx 0.05$ as recommended in the test.

4.2 Analytical specificity

Evaluated by GEVES (Audy Ct threshold: 30):

Inclusivity: evaluated on 28 target strains of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*: 100%.

Exclusivity: evaluated on 30 non-target saprophytic isolates isolated from bean seeds: 100 %. The isolates were not identified but they did not correspond to *X. phaseoli* pv. *phaseoli* nor *X. citri* pv. *fuscans* based on the characteristics of the colonies on PTSCA media.

Evaluated by Baldwin T. 2019 (ISF) (Audy Ct threshold: 35):

A first evaluation of the analytical specificity of the test was performed without the IPC (Wu) primers. During this first evaluation the inclusivity and exclusivity were at 100%. The test was performed on 25 target strains (identified as *X. axonopodis* pv. *phaseoli*) and 22 non-target strains (15 *X. axonopodis* pv. *phaseoli* look-alike isolates from bean seeds, 1 *X. euvesicatoria* (CFBP 6869), 1 *X. perforans* (CFBP 7293), 1 *X. vesicatoria* (CFBP 4645), 1 *X. gardneri* (CFBP 6822), 1 *X. campestris* pv. *raphani* (CFBP 5829), 1 *X. hortorum* pv. *carotae* and 1 *X. campestris* pv. *campestris*).

A second evaluation of the analytical specificity of the test was performed with the IPC (Wu) primers. During this second evaluation:

Inclusivity was evaluated on 29 target strains of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* (15 *X. axonopodis* pv. *phaseoli* and 14 *X. axonopodis* pv. *phaseoli* var *fuscans*): 100%.

Exclusivity: evaluated on 30 non-target strains (1 *X. axonopodis* pv. *dieffenbachiae* (CFBP 3132), 1 *X. axonopodis* pv. *vesicatoria* (CFBP 5618), 1 *X. axonopodis* pv. *manihotis* (CFBP 2624), 1 *X. axonopodis* pv. *citrumelo* (CFBP 3371), 1 *X. axonopodis* pv. *malvacearum* (CFBP 2530), 1 *X. axonopodis* pv. *vasculorum* (CFBP 5696), 1 *X. axonopodis* pv. *vignicola* (CFBP 7113), 1 *X.* sp. pv. *glycines* (CFBP 1559), 1 *X. axonopodis* pv. *axonopodis* (CFBP 4151), 1 *X. campestris* pv. *campestris*, 3

Stenotrophomonas spp., 2 *X. campestris*, 3 *X. arboricola*, 2 *X. axonopodis*, 10 non-target saprophytic isolates isolated from common bean): 96.6 %. One cross reaction was observed with a *X. phaseoli* pv. *dieffenbachiae* strain.

Evaluated by NIB (simplex reaction).

False negative results were obtained with two *X. phaseoli* pv. *phaseoli* strains from lablab bean (LMG 829 and LMG 8013).

4.3 Repeatability and reproducibility

Evaluated by GEVES:

Repeatability was evaluated on 4 target strains and 4 non-target strains. Each strain was tested in triplicate and gave the same results. The repeatability is 100%.

Reproducibility was evaluated by repeating the repeatability test twice with two different operators. Each strain gave the same results in both tests. The reproducibility is 100%.

Evaluated by Baldwin T. 2019 (ISF):

Repeatability and reproducibility were evaluated during a comparative test between 8 international laboratories. 30 target strains and 28 non-target strains were tested by each laboratory. The repeatability is 100% and the reproducibility is 100% for target strains and at 99.1% for non-target strains (one laboratory obtained 1 false positive result).

4.4 Robustness

Evaluated by Baldwin T. 2019 (ISF):

The robustness of the real-time PCR mix used was validated by performing analyses in different laboratories using different real-time PCR mixes (TaqMan Fast Universal PCR, Gene expression, Light cycler 480 probe, Quantabio PerfeCTa Multiplex qPCR ToughMix, TaqMan Universal MasterMix II, Sso Advance Universal Probes Supermix, IDT PrimeTime Gene Expression). The evaluation was performed on 3 targets and 3 non-target strains and revealed no effect of the mix.

APPENDIX 5 - REAL-TIME PCR (HE, 2010)

The test below differs from the one described in the original publication (*see 1.2*).

The test below is described as it was carried out to generate the validation data provided in section 4. Other equipment, kits or reagents may be used provided that a verification (*see PM 7/98*) *is carried out*.

1. General information

1.1 This test can be used for the identification of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* colonies except for those isolated from lablab beans.

1.2 The TaqMan real-time PCR test was developed by (He, 2010) and was adapted by NIB (SI).

1.3 Real-time PCR primers (XAP-F, XAP-R, XAP probe) target *X. phaseoli* pv. *phaseoli* strains. The target sequence is located on Xanthan biosynthesis acetyltransferase gumF gene. Real time PCR primers (XAPF-F, XAPF-R, XAPF probe) target *X. citri* pv. *fuscans* strains. The target sequence is located on a hypothetical protein.

1.4 Oligonucleotides:

Primers for *X. phaseoli* pv. *phaseoli:*

	Name	Sequence	Amplicon size*
Forward primer	XAP-F	5′-CGC AGA TCA CCA TCA ACG AA-3′	56 bp
Reverse primer	XAP-R	5′-CAA CCC CGC GCT GTT C-3′	
Probe	XAP probe	5′-**FAM**-CAA GCA ACG CGC TCA-**MGB**-3′	

*Including primer sequences.

Primers for *X. citri* pv. *fuscans:*

	Name	Sequence	Amplicon size*
Forward primer	XAPF-F	5′-CCT TGT GGA ACA CGC TAG CA-3′	67 bp
Reverse primer	XAPF-R	5′-GCG AAG CAT CAG CTT GAT TG-3′	
Probe	XAPF probe	5′-**HEX**- CGC CTG CCT CAA CAG AAA ATG TGC A- **BHQ-1**-3′	

*Including primer sequences.

1.5 Validation data below (NIB) has been generated using ViiA 7 and QuantStudio™ 7 Flex Real-Time PCR System (both Applied Biosystems by Life Technologies).

2. Methods

2.1 Nucleic acid extraction and purification

2.1.1 Suspend approximately 1 µL of cell culture (e.g. using a 1-µL loop) or one colony in 500 µL of sterile purified water.

2.1.2 Heat in closed tubes at approximately 95°C or 100°C for approximately of 10 min and cool on ice.

2.1.3 DNA should preferably be stored at approximately -20°C.

2.2 Real-time PCR

2.2.1 Master mix (separate reactions)

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
TaqMan™ Universal PCR Master Mix (*Applied Biosystems*™)	2×	5.0	1×
Forward primer (XAPF or XAPF-F)	10 µM	0.9	0.9 µM
Reverse primer (XAPR or XAPF-R)	10 µM	0.9	0.9 µM
Probe (XAP probe or XAPF probe)	10 µM	0.2	0.2 µM
Molecular grade water	N.A.	1	N.A.
Subtotal		8	
Nucleic acid extract		2	
Total		10	

2.2.2 PCR conditions: 2 min at 50°C, 10 min at 95°C, 45 cycles of 15 s at 95°C and 1 min at 60°C.

3. Essential procedural information

3.1 Controls

For a reliable test result to be obtained, the following controls should be included for each series of nucleic acid extraction and amplification of the target organism and target nucleic acid, respectively.

- Negative isolation control (NIC) to monitor contamination during nucleic acid extraction: for the NIC the same volume of sterile purified water as for preparation of suspension, is used.
- Positive isolation control (PIC) to ensure that nucleic acid of sufficient quantity and quality is isolated: For the PIC, a suspension of reference cultures of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* should be used and prepared as for the tested strains.
- Negative amplification control (NAC) to rule out false positives due to contamination during the preparation of the reaction mix: application of the amplification procedure to molecular grade water that was used to prepare the reaction mix.
- Positive amplification control (PAC) to monitor the efficiency of the amplification: amplification of nucleic acid of the target organism. This includes nucleic acid extracted from the *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* organism.

3.2 Interpretation of results: in order to assign results from this PCR-based test the following criteria should be followed:

Verification of the controls

- The PIC and PAC amplification curves should be exponential.
- NIC and NAC should give no amplification.

When these conditions are met:

- A test will be considered positive if it produces an exponential amplification curve.
- A test will be considered negative, if it does not produce an amplification curve.
- Tests should be repeated if any contradictory or unclear results are obtained.

4. Performance characteristics available

The validation data were obtained by NIB (Grujić et al. in preparation). Additional validation data are reported in He (2010) using a slightly different procedure.

The test may have been adapted further and validated or verified using other critical reagents, instruments and/or other modifications. If so, the corresponding test descriptions and validation data can be found in the EPPO database on diagnostic expertise (section validation data https://dc.eppo.int/validation_data/validationlist).

4.1 Analytical sensitivity (NIB, Grujić et al. in preparation)

Not evaluated. Pure bacterial cultures (concentration 10^6–10^9 CFU/mL) were used to evaluate the performance characteristics of the tests.

4.2 Analytical specificity

He (2010):

Inclusivity:

Test for *X. citri* pv. *fuscans*: evaluated on 8 target strains of *X. citri* pv. *fuscans*: 100%;

Test for *X. phaseoli* pv. *phaseoli*: evaluated on 24 target strains of *X. phaseoli* pv. *phaseoli*: 100%;

Exclusivity:

Test for *X. citri* pv. *fuscans*: 95.6% evaluated on 45 non-target strains (*X. phaseoli* pv. *phaseoli* (24), *Pseudomonas syringae* pv. *syringae* (4), *Pseudomonas syringae* pv. *phaseolicola* (3), *Xanthomonas oryzae* pv. *oryzae* (1), *Xanthomonas campestris* pv. *vesicatoria* (1), *Xanthomonas campestris* pv. *campestris* (1), *Acidovorax avenae* subsp. *citrulli* (2), *Pseudomonas syringae* pv. *glycinea* (2), *Pantoea stewartii* subsp. *stewartii* (4), *Pantoea ananatis* (2), *Erwinia amylovora* (1). Two *X. phaseoli* pv. *phaseoli* strains cross reacted with this test).

Test for *X. phaseoli* pv. *phaseoli*: 96.5% evaluated on 29 non-target strains (the same bacteria as listed above but with *X. citri* pv. *fuscans* (8) instead of *X. phaseoli* pv. *phaseoli* (24)). One *X. citri* pv. *fuscans* strain cross reacted with this test.

NIB:

Inclusivity:

Test for *X. citri* pv. *fuscans*: evaluated on 7 target strains of *X. citri* pv. *fuscans*: 86%; False negative results were obtained with *X. citri* pv. *fuscans* strain CFBP 6988.

Test for *X. phaseoli* pv. *phaseoli*: evaluated on 10 target strains of *X. phaseoli* pv. *phaseoli*: 80%; False negative results were obtained with two *X. phaseoli* pv. *phaseoli* strains from lablab bean (LMG 829 and LMG 8013).

Exclusivity:

Test for *X. citri* pv. *fuscans*: 100% evaluated on 27 non-target strains (*X. phaseoli* pv. *phaseoli* (10), *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (6), *Pseudomonas syringae* pv. *syringae* (1), *Pseudomonas viridiflava* (1), *Xanthomonas vesicatoria* (2), *Xanthomonas citri pv. glycines* (2), *Stenotrophomonas maltophilia* (5)).

Test *X. phaseoli* pv. *phaseoli*: 91.6% evaluated on 24 non-target strains (the same bacteria as listed above instead *X. phaseoli* pv. *phaseoli* (10) the strains *X. citri* pv. *fuscans* (7)). Two strains of *Xanthomonas citri* pv. *glycines* (2) cross reacted with these primers, but Ct values were above 35.

The test for *X. phaseoli* pv. *phaseoli* also cross reacted with strains of *X. citri* pv. *fuscans*. However, the mean Ct value obtained with this test was 10 to 11 Ct higher than the one obtained for the same strains with the test for *X. citri pv. fuscans*. Therefore, the *X. citri* pv. *fuscans* and *X. phaseoli* pv. *phaseoli* strains can be easily correctly identified from this difference in Ct values.

APPENDIX 6 - PATHOGENICITY TEST

The pathogenicity test described below is based on the GEVES test (GEVES, 2024), ISTA test (ISTA, 2024a) and ANSES test (ANSES, 2013), based on the work done by Darsonval et al. (2009) and Grimault (2012).

This test is performed on suspect bacterial isolates to determine their pathogenicity.

The accuracy and reliability of this test is highly dependent on humidity conditions and inoculum concentrations. For this reason, it is essential to use the controls listed in Table A1. The pathogenicity test should be done at high humidity conditions. The validation data produced below were performed at 95% relative humidity however experience in laboratories show that the test works at relative humidities between approximately 70% to 95%.

TABLE A1 Controls required for pathogenicity test.

Name	Description
Positive control	Reference strain (*X. phaseoli* pv. *phaseoli* - *X. citri* pv. *fuscans*)
Negative process control*	Strain of *Xanthomonas* sp. non-pathogenic on bean
Non template control	Sterile water used for suspensions

*Optional for the confirmation of pathogenicity.

FIGURE A1 First trifoliate leaf soaking step. Courtesy: © GEVES (FR).

1. Bean plants of a cultivar susceptible to *X. phaseoli* pv. *phaseoli* - *X. citri* pv. *fuscans* (e.g. Michelet, Contender or Flavert) are grown at 20–30°C until the first trifoliate leaf stage (around 16 days after sowing). At least 3 plants per suspected isolate and per control should be inoculated.
2. The day before inoculation, plants are placed in the following conditions: 16 h under light at 28°C and 95% relative humidity, followed by 8 h without light at 25°C and 95% relative humidity.
3. A suspension of each suspected isolate and controls at approximately 10^7 CFU/mL (OD_{600nm} approximately 0.05) is prepared in sterile purified water from a fresh culture on a plate (24 to 48 h). Note that a too low concentration can lead to a false negative result, whereas a too high concentration can lead to a false positive result.
4. For each suspension (suspected isolates and controls), 3 plants are inoculated by dipping the first trifoliate leaf for 30 s in a recipient containing the inoculum (Figure A1). The volume of the suspension should allow the soaking of the leaflets to be inoculated (Figure A1).
5. Inoculated plants are incubated under the following conditions: 16 h under light at 28°C and 95% relative humidity, then 8 h without light at 25°C and 95% relative humidity. A minimum of 95% humidity is required for the development of typical symptoms.

Symptoms should appear 5 to 11 days after inoculation in the plants inoculated with the positive control but not in those inoculated with the negative control.

Typical symptoms of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* are (Figure A2):

- Small water soaked spots which may spread in areas.
- Necrotic lesions that can lead to tissue death.

6. Compare plants inoculated with suspected isolates with those inoculated with the controls. If a suspected isolate produces typical symptoms on a plant (similar

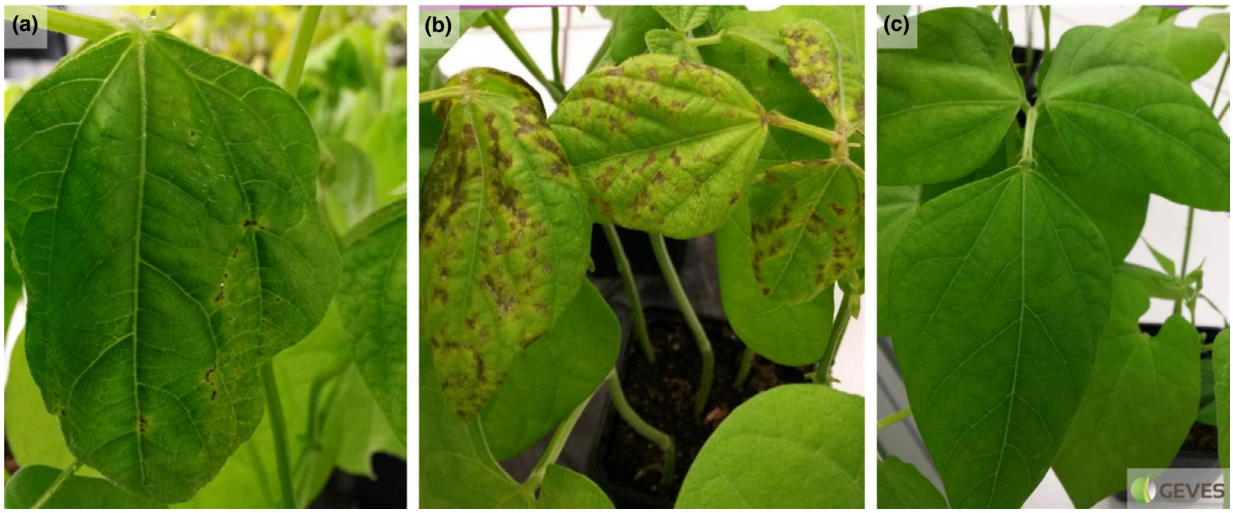

FIGURE A2 Typical symptoms caused by *X. phaseoli* pv. *phaseoli* or *X. citri* pv. *fuscans* on positive control (a, b) and absence of symptoms on negative control (c). Courtesy: © GEVES (FR).

to the positive control), the test is considered positive. If there are no symptoms after 11 days following inoculation, the test is considered negative. However, if desired, the test duration can be extended to 21 days after inoculation.

Performance characteristics available

Validation data were obtained by ISTA (GM13-06 pages 45 to 54, Grimault, 2012).

The test was evaluated during an inter-laboratory comparison (ILC) performed by ISTA, with 3 laboratories. All tests were performed with bacterial suspensions at 10^7 CFU/mL. Thirty target strains (9 *X. axonopodis* pv. *phaseoli* Non Fuscans (NF), 6 *X. axonopodis* pv. *phaseoli* NF1, 3 *X. axonopodis* pv. *phaseoli* NF2, 2 *X. axonopodis* pv. *phaseoli* NF3, 10 *X. axonopodis* pv. *phaseoli* var fuscans) and 30 non-target strains (1 *X. axonopodis* pv. *dieffenbachiae* (CFBP 3132), 1 *X. axonopodis* pv. *vesicatoria* (CFBP 5618), 1 *X. axonopodis* pv. *manihotis* (CFBP 2624), 1 *X. axonopodis* pv. *citrumelo* (CFBP 3371), 1 *X. axonopodis* pv. *malvacearum* (CFBP 2530), 1 *X. axonopodis* pv. *vasculorum* (CFBP 5696), 1 *X. axonopodis* pv. *vignicola* (CFBP 7113), 1 *X. citri* pv. *glycines* (CFBP 1559), 1 *X. axonopodis* pv. *axonopodis* (CFBP 4151), 1 *X. campestris* pv. *campestris*, 3 *Stenotrophomonas* spp., 2 *X. campestris*, 3 *X. arboricola*, 2 *X. axonopodis*, 10 non-target strains isolated from common bean) were tested. 2 plants were inoculated per strain.

During this ILC, analytical specificity was evaluated with an inclusivity of 97.6% (two false-negative results out of 30 target strains tested) and 98.9% exclusivity (one false positive result out of 30 non-target strains tested).

Reproducibility was evaluated at 96.2 % for target strains and 97.8 % for non-target strains.