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Detection of Bacterial Canker Pathogen in Tomato Seeds and Latent Infections of Seedlings

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Seed Extraction

Source: Cull piles, water systems, soil

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Seed Assay
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Seed Assay

NO procedure is 100% correct

Use ***Negative*** and ***Positive*** samples for ***most correct results***

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Crop Losses due to Seed-borne Diseases

Immediate losses
- Reducing germination, poor seedling vigor,
- Abnormal seedlings, first crop losses from infected seedlings

Long term losses
- Pathogen surviving in soil
- Future inoculum source to new crops

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Seed Health Testing: A Balance Act

- Management tool for the control of seed-borne/seed-transmitted diseases

- Increased product liability
- Competitive pressure within the industry
- Seed health becomes an important quality trait

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Variation in Test Results

Sampling Error

Assay Sensitivity

Misdiagnosis & Saprophytes

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Challenges in Detecting Seed-borne Pathogens

Assay specificity/identification	Need high level of confidence
Contamination	Target pathogen vs saprophytes
Inoculum density	Low level of the pathogen in seeds
Sampling Error	Too small of a sample/not representative sample

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Challenges in detecting Seed-borne pathogens

1. A specific method/ condition may be ideal for a given sample but not for another
2. Percent recovery is dependent on the inoculum density
3. Naturally infected seeds must be used for final method evaluation

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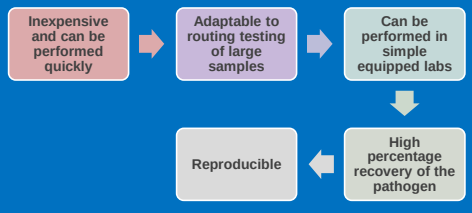
Characteristics of an Optimum Method



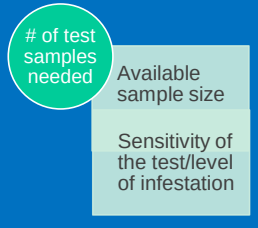
Challenges of a Seed Health Testing

Taking a representative sample → Assay performed on an adequate number of seeds (sample size)

Detection limit of the assay

- If detection limit is 1 contaminated seed in 10,000 healthy seeds: You need 10,000 seed replications
  (sample size is 30,000 seeds 3 X10,000 seeds reps)

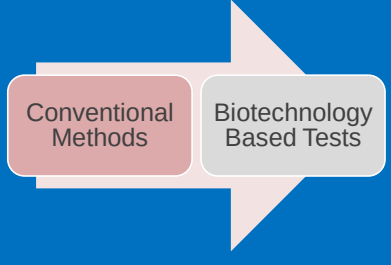
Challenges of a Seed health Testing



Standardized Sampling Procedures

Association of Seed Analysts (AOSA) → International Seed Testing Association (ISTA)

Protocols



Conventional methods

- Conventional: Grow-out test (seedling test); Inoculating tomato plants with ground seed filtrates
- Conventional: Selective/differential media test; General agar media test
- Conventional: Immunofluorescence staining (most commonly used)

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Biotechnology based tests

- Immunoassay based assays: ELISA; Poly/Monoclonal antibodies
- Nucleic acid based assay: DNA probes; Classic PCR and Real-time PCR/Bio-PCR
- Bacteriophage: Phage Plaque Counting

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PCR-Based Assay

Classic PCR: Culturing the pathogen from the infected seeds, extracting the DNA from the culture and subjecting it to PCR

Bio-PCR/Direct-PCR: Extraction of DNA from infected seeds and detection of the pathogen from the total population of bacteria on semi-selective media

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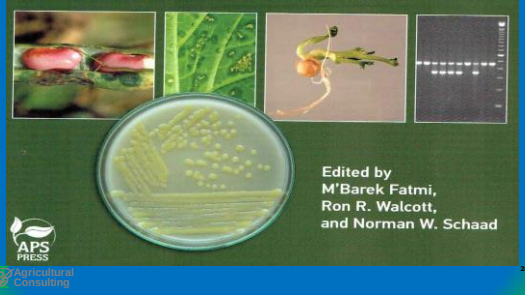
Detection of Plant-Pathogenic Bacteria in Seed and Other Planting Material

Second Edition 2018



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CHAPTER 17

Detection of *Clavibacter michiganensis* subsp. *michiganensis* in Tomato Seeds

M'Barek Fatmi, Hasan Bolkan, and Norman W. Schaad

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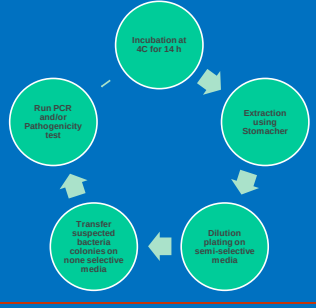
U.S. National Seed Health System and ISHI Approved Standard Method



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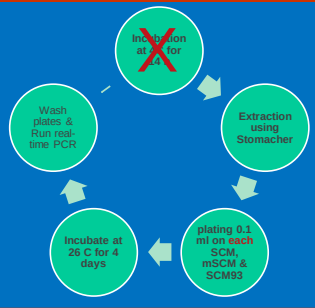
The BIO/Real-Time PCR Protocol



Time needed 4-5 Days

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SCM

- **Semi-selective Agar Medium**
- ***Ref:*** *Fatmi & Schaad. 1988. Phtopathology 78: 121-126*

MSCM

- **Modified Semi-selective Medium**
- *Ref: Waters & Bolkan. 1992. Phytopathology 82: 1072*

SCM93

- **Modified Semi-selective Medium 93**
- ***Ref:*** *Fatmi &Atik.2000. Quqtrieme congres de l'AMPP, Rabat, Morocco*

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Sterilized bag

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The Stomacher

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To Spike or not to Spike

Spiking of seed extracts with a known CMM culture to improve reliability of Cmm test

Courtesy of: Dr Harrie Koenraadt

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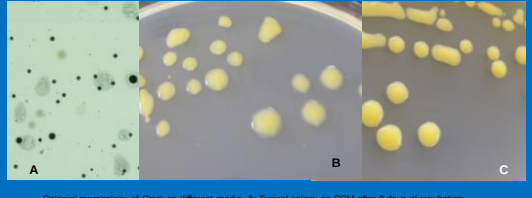
CMM colonies on different media



Colonial morphology of *Cmm* on different media. **A**: Typical colony on SCM after 9 days of incubation; **B**: Typical colony on mSCM after 5 days of incubation; **C**: Typical colony on NBY after 4 days of incubation.

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Problems with PCR

- High sensitivity (contamination issues can give false positives)
- Lab must be very clean
- Operator very careful
- Chemicals of high purity (especially the tag polymarase)
- DNA, Primer & Mg concentrations
- Detection levels may not correlate to disease development

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Pathogenicity Test

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Conclusions

Molecular based assays are reliable and specific; but are expensive

Conventional methods for detecting seed-associated pathogens are cheap and easy to perform but not always reliable

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Detection of Latent Infections of Seedlings

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LATEN INFECTION

- A stage in which a host is infected with a pathogen but does not show symptoms
- The interval from infection to display of symptoms (*incubation period*)

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Detection Methods

Bioassay	• Moist Blotters • Selective media
Histological	• Microscopic examination
Serological & Molecular Techniques	• ELISA • Real-time PCR/Classic PCR

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Innovative Detection Methods

Spectroscopy-based methodology	• Analysis of volatile compounds as biomarkers
Remote sensing technologies	• Use of high-resolution portable spectral sensors to detect diseased leaves of tomato
Biosensors & Biophottonics	• devices that convert a biological response into an electrical signal • detection of photons (quantum units of light)

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Confirmation of *CMM* in the field by ImmunoStrips

Use of immunoStrips specific to *CMM* is a quick way do confirm the presence of bacterial canker disease in the field. However, be aware of false positives; confirmation of the disease by a laboratory may be needed. Sample preparation A, B, C

A - ImmunoStrips

B – Sample bag with buffer

C– Add suspected tissue sample and grind

(Pictures copied from Agdia's brochure)

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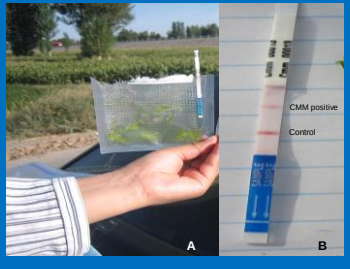
ImmunoStrip Assay

ImmunoStrip immersed in suspected ground tissue in buffer (A), positive results (B).



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Latent infection

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THANK YOU

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