# AmplifyRP® XRT for *Cms*
## Validation Report
## *Clavibacter michiganensis* subsp. *sepedonicus*
## Product No. XCS 70002

## Test Characteristics

| | | | |
|---|---|---|---|
| **Test Name** | Clavibacter michiganensis subsp. sepedonicus | **Test Label** | FAM-labeled target probe |
| **Catalog Number** | 70002 | **Internal Control** | N/A |
| **Acronym** | Cms | **Format** | XRT |
| **Genus** | Clavibacter | **Diluents** | AMP1/PD1 |
| | | **Sample Dilution** | 1:10 |

## Summary

AmplifyRP XRT for *Cms* is a rapid DNA amplification and detection platform designed for testing potato tubers for *Clavibacter michiganensis* subsp. *sepedonicus.* This kit includes lyophilized reaction pellets containing the necessary reagents to amplify *Cms* DNA at a single operating temperature (42 °C).

## Diagnostic Sensitivity

| | |
|---|---|
| **True Positives** | 104 |
| **Correct Diagnoses** | 104 |
| **Percent** | 100% |

## Analytical Sensitivity

| | |
|---|---|
| **Analytical Sensitivity:** | The assay is 95.0% sensitive between 100 fg/µL and 10 fg/µL. (n=20) |
| **Limit of Detection:** | The assay has a 100% detection rate at 100 fg/µL with DNA fragement. (n=10) |
| | The assay has a 90% detection rate at 10 fg/µL with DNA fragement. (n=10) |
| **Analytical Sensitivity:** | The assay is 87.5% sensitive between $10^5$ CFU and $10^4$ CFU. (n=8) |
| **Limit of Detection:** | The assay has a 100% detection rate at $10^5$ CFU with Cms bacteria. (n=4) |
| | The assay has a 75% detection rate at $10^4$ CFU with Cms bacteria. (n=4) |

## Analytical Specificity

### Inclusivity:

**Isolates and Geographic Regions Detected:**

| | |
|---|---|
| Cms-2 (INM) (ID, USA) | Cms-AS-1 (MN, USA) |
| Cms-BCP45 | Cms-BCP45 (RifR mutation) |
| Cms-CIC4 | Cms-CIC77 |
| Cms-CIC8 | Cms-CS R8 |
| Cms-CS101 (Canada) (ATCC® 33113™) | Cms-Cs2 |
| Cms-Cs2 (RifR mutation) | Cms-Cs3 (Canada) |
| Cms-Cs3 (RifR mutation) | Cms-Cs3-1 |
| Cms-Cs3-2 | Cms-Cs3M |
| Cms-Cs3NM (avirulent) | Cms-Cs3NM (virulent) |
| Cms-Cs3RC | Cms-Cs4 |
| Cms-Cs5 (NY, USA) (ATCC® 9850™) | Cms-Cs7 |
| Cms-Cs7 (RifR mutation) | Cms-Cs9 |
| Cms-CsR8 | Cms-DGBBC 235 |
| Cms-P45 (lacks pCS1) | Cms-R5 |

### Exclusivity:

**Cross-reacts With:**

| | |
|---|---|
| None Known | |

**Does Not Cross-react With:**

| | |
|---|---|
| Clavibacter michiganensis subsp. insidiosus (Cmi) | Clavibacter michiganensis subsp. michiganensis (Cmm) |
| Clavibacter michiganensis subsp. nebraskensis (Cmn) | Clavibacter michiganensis subsp. tessellarius (Cmt) |
| Curtobacterium flaccumfaciens pv. batae[1] | Curtobacterium flaccumfaciens pv. flaccumfaciens[1] |
| Curtobacterium flaccumfaciens pv. oorti[1] | Curtobacterium flaccumfaciens pv. poinsettiae |
| Dickeya chrysanthemi | Dickeya solani |
| Pectobacterium carotovorum subsp. atrosepticum | Pectobacterium carotovorum subsp. carotovorum |
| Pectobacterium wasabiae | |
| [1]Predicted non-detection by *in silico* analysis only | |

## Diagnostic Specificity

**True Negatives** 83
**Correct Diagnoses** 83
**Percent** 100%

### Selectivity:

| **No Matrix Effect Observed With:** | | | |
|---|---|---|---|
| Potato leaves | Potato petioles | Potato stems | Potato tissue culture plantlets |
| Potato tubers | | | |
| The hosts on the above list have been chosen to represent those which historically cause a range of matrix effects, in addition to those expected to be screened for this pathogen. Not all plant species susceptible to this pathogen have been screened, but may still be used with this assay unless otherwise noted below. As with all diagnostic tools, Agdia recommends confirming all results with a secondary detection method before making any economic decisions (ex: discarding plants due to positive test results, etc.). | | | |

| **Matrix Effect Observed With:** | | | |
|---|---|---|---|
| None Known | | | |

## Repeatability

**Number of Samples** 183
**Replicates per Sample** 2 - 8
**Total Replicates** 448
**Replicates in Agreement** 436
**Percent Agreement** 97.3%

## Reproducibility

**Number of Samples** 24
**Replicates per Sample** 3
**Number of Operators** 4
**Total Replicates** 288
**Replicates in Agreement** 279
**Percent Agreement** 96.9%

## Robustness

**Planned deviation analysis:**
No deviations from the user guide protocol were validated.

**Stability:**

| | 1-year stability (accelerated) | Real-time Stability Verification |
|---|---|---|
| Positive Sample (High) | Pass | Monitoring |
| Positive Sample (High) | Pass | Monitoring |
| Positive Sample (Low) | Pass | Monitoring |
| Positive Sample (Low) | Pass | Monitoring |
| Positive Sample (Low) | Pass | Monitoring |
| Positive Sample (Low) | Pass | Monitoring |
| Negative Sample | Pass | Monitoring |
| Negative Sample | Pass | Monitoring |

## Glossary

**Diagnostic sensitivity[1]:** The percentage of positive samples correctly identified in an experiment with known positive controls.

**Diagnostic specificity[1]:** The percentage of negative samples correctly identified in an experiment with known negative controls.

**Analytical sensitivity[3]:** The smallest amount of target that can be detected reliably (this is sometimes referred to as the 'limit of detection')

**Analytical specificity[3]:** (comprises inclusivity and exclusivity)

**Inclusivity[3]:** The performance of a test with a range of target isolates covering genetic diversity, different geographical origin and/or hosts associated with the target organism.

**Exclusivity[3]:** The performance of a test with a range of non-targets (e.g. cross-reaction with closely related organisms, contaminants)

**Selectivity[2]:** The level of effect that matrices and relevant plant parts have on the performance of the assay.

**Repeatability[2]:** The agreement between test replicates of the same sample tested by the same operator.

**Reproducibility[3]:** The ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g. time, users, equipment, location)

**Robustness[1,3]:** The extent to which varying test conditions (e.g. temperature, volume, change of buffers) affect the established test performance values. May also be referred to as planned deviation analysis.

**Stability[1]:** The performance of test reagents or controls over time.

**References:**
[1]Groth-Helms, D., Rivera, Y., Martin, F. N., Arif, M., Sharma, P., Castlebury, L. A. (in press). Terminology and Guidelines for Diagnostic Assay Development and Validation: Best Practices for Molecular Tests. PhytoFrontiers.
[2]Eads, A., Groth-Helms, D., Davenport, B., Cha, X., Li, R., Walsh, C., Schuetz, K., (in press). The Commercial Validation of Three Tomato Brown Rugose Fruit Virus Assays. PhytoFrontiers.
[3]EPPO (2018) PM 7/76 (5) Use of EPPO Diagnostic Standards, EPPO Bulletin 48, 373– 377.

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