PCR MultiScan for quick disease diagnosis

By
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CSP Labs
California Seed and Plant Lab., Inc.

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Agenda

About CSP Labs

Easy diagnosis - symptoms

PCR MultiScan for difficult samples

About us

15 years in business
20+ Full time employees

Accredited

National Seed Health System - NSHS
California Crop Improvement Association - CCIA
California Dept Food and Agriculture - CDFA
Monterey County – Grower Shipper
USDA – Grain Inspection, Packers & Stockyards Administration
CGC – Canadian Grain Commission

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Facilities

10,000 sq ft lab space – New Building
5 on-site greenhouses
Walk-in growth rooms

State of the art equipment
- Real-time PCR
- Genetic analyzer
- epMotion Liquid handler
- Light Scanner – High resolution melt analysis
- KASP[New] – VID and hybrid purity platform.

State of the art greenhouses

Growth room

Easy ID

Crown gall of stone fruits

Easy ID …

Loose smut of wheat

Easy – Google can help

Strawberry anthracnose

Angular leaf spot

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Easy – Google can help

Grape powdery mildew

Soybean rust

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Difficult

Phytophthora

Verticillium

Macrophomina

Fusarium

Pallidosus

Difficult …

Some sort of virus?

Tomato yellow leaf curl Begomo virus

Another Begomo

Tomato spotted wilt (TOSPO)

Tobacco mosaic

Tomato mosaic

Cucumber mosaic

Poty viruses

Impossible

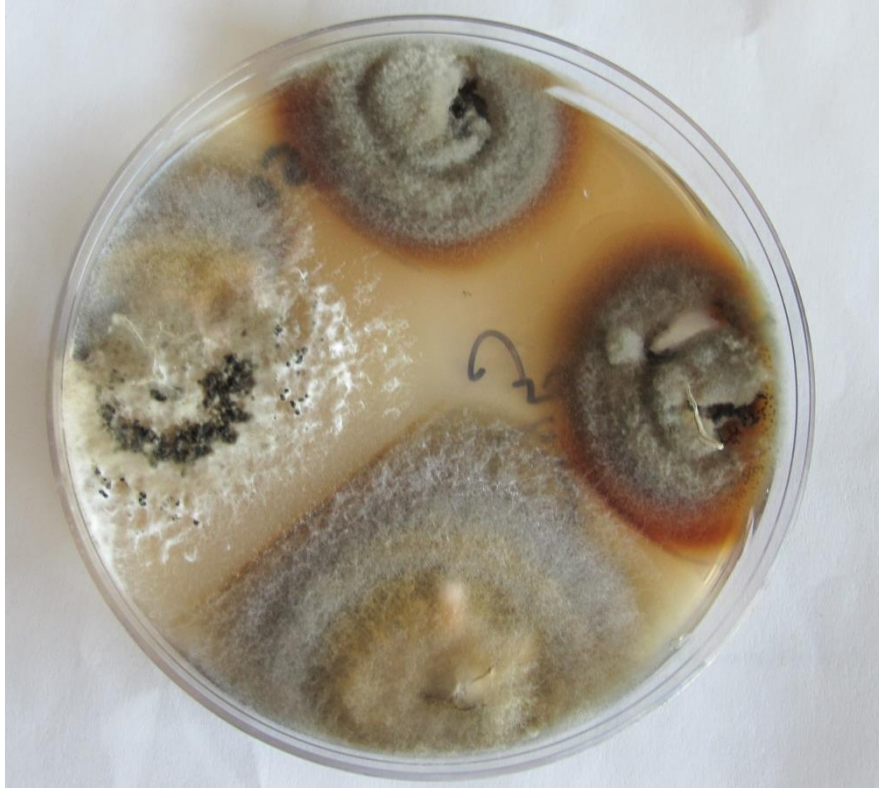Atypical symptoms
Latent infections

Impossible

Air samples

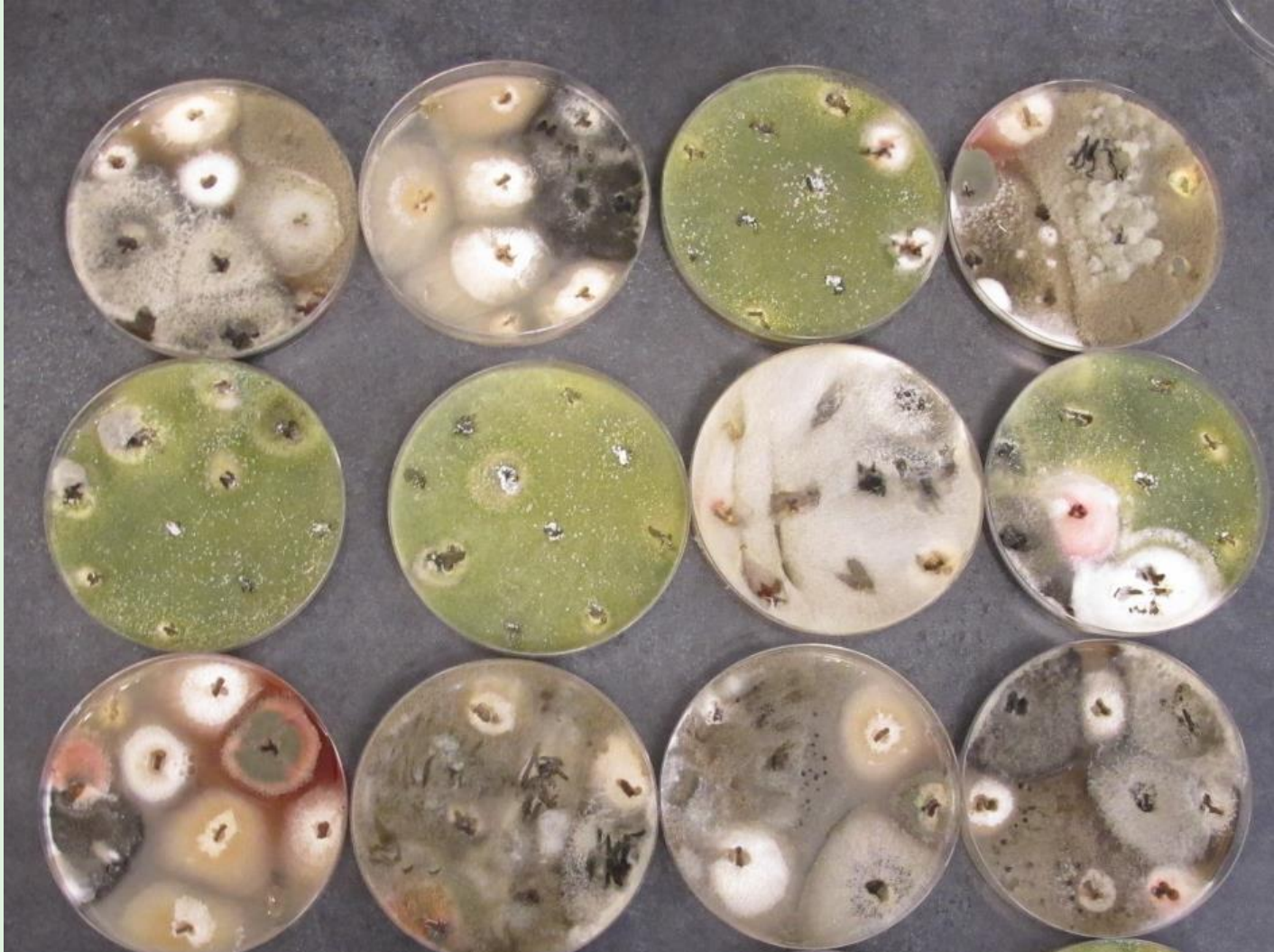# Candidate for Virus MultiScan

Culture tests

4 fungi

1 fungus

Root cultures – where is the pathogen?

Almond fruit gummosis

Bacteria and fungal MultiScan

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Culture test - downside

False negative

Other fungi and bacteria interfere with readability of agar plates

Culture Technique

Gold standard as it provides a positive proof of pathogen

But

1. Requires 1-3 weeks
2. False negatives if plates are not readable

Alternative test: ELISA

Good for viruses
Less effective for fungi / bacteria
Not good for Viroids

Alternative test: PCR

1. Modern method based on genetic code (DNA/RNA)

2. Works for all pathogens (Bacteria, fungi, obligates, viruses, viroids, phytoplasma)

PCR MultiScan approach

1. Quick diagnosis (1 day)

2. Screens sample for all possible pathogens

3. Cost effective

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Building a MultiScan
500+ primers to choose from

Step 1
List all pathogens for a crop
(Index, Google)

Step 2
Narrow down list by looking at
symptoms

Step 3
Select primers for multiplex or
uniplex PCR test

Equivalent to

crop specific chips

Tomato full scan (all pathogens)

Tomato bacterial scan

Tomato virus scan

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Grapevine trunk disease
5-fungi MultiScan

- Eutypa lata
- Phaeomoniella
- Togninia minima
- Cylindrocarpon
- Botryosphaeria sp.

No need to add viruses to this MultiScan

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Grapevine
23 virus MultiScan

- Fan leaf
- Leaf roll 1-11
- Vitivirus A, B, D
- Fleck
- Rupestris stem pitting
- … More

No need to add fungi to this MultiScan

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Strawberry decline
7 pathogen MultiScan

Surendra Dara, UCCE

- Pallidosus virus
- Beet pseudo yellow virus
- Macrophomina
- Verticillium dahliae
- Rhizoctonia
- Fusarium oxy. fragariae
- Phytophthora cactorum
- …More

Tomato wilt
10 pathogen MultiScan

- Fusarium 1, 2, 3
- Verticillium, 1, 2
- Ralstonia
- Pseudomonas corugata
- Erwinia carotovora
- Clavibacter
- Phytophthora

Water Scan

- Water is tested for pathogens specific for crop to be irrigated.

There is no need to test for all the pathogens.

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Soil Scan

- Soil should be tested for pathogens specific for crop to be planted.

There is no need to test for all the pathogens.

A PCR test is as good as the primer used

"Primers and primers are everywhere but good primers are very rare"

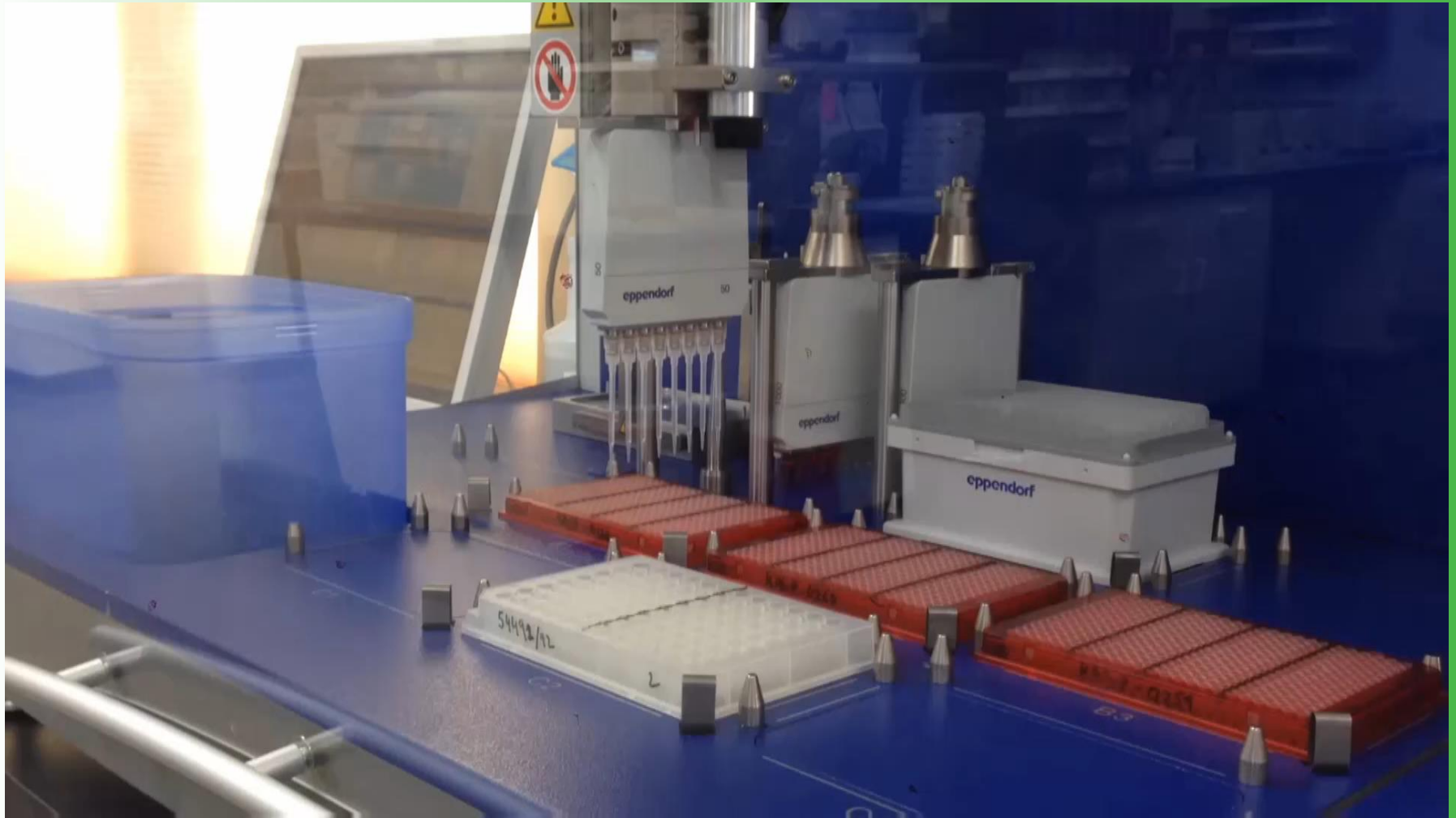**We use robust and validated primers**

Our MultiScans

- High throughput
  - Our capacity is 5000 PCR reactions per day
- Quantitative
  - Most assays are qPCR
- Specific
  - Primers are of very high quality

We collaborate with UC Davis, USDA scientists in developing and validating primer sets

Robotic liquid handling

Robotic qPCR equipment

Questions?

Thank you