NSHS

National Seed Health System

DIRECT VS INDIRECT TESTING

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Seed Health Testing

- Goal – to accurately determine if pathogens are present in a representative samples of seed
  - Reduced risk for phytosanitary movement and to growers
- Faster, higher throughput and cheaper testing always needed

Possible testing outcomes

	Pathogen is present	**Pathogen not present**
Test result negative	False Negative	True Negative
Test results positive	True Positive	False Positive

Types of seed health testing

TEST TYPE	FUNGI	BACTERIA	VIRUS/ VIROID	NEMATODES
Visual Examination	yes	no	no	no
Seedling Grow-out	no	yes	maybe	no
Virus Indicator Test	no	no	yes	no
Wash/Soak –microscope exam	yes	no	no	yes
Non-selective agar/Blotter Tests	yes	no	no	no
Selective Agar Media Tests	no	yes	no	no
ELISA (antibody based) Tests	no	yes	yes	no
PCR / DNA or RNA Based Tests	no	yes	yes	no

Visual Examination

- Limited to pathogens that you can see on the seed
- Examples – downy mildew of soybeans or ergot in cereal

Seedling grow out

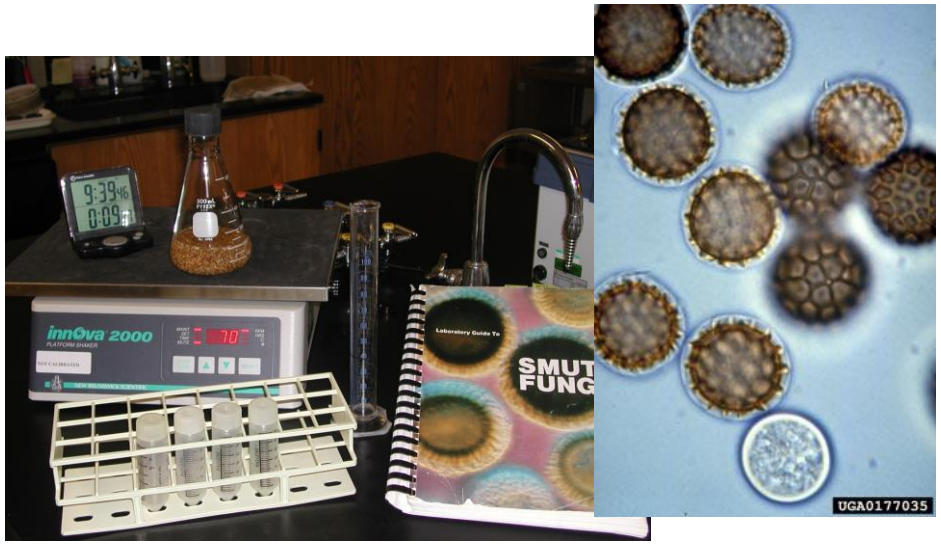
- Growing plants to a time where disease symptoms can be reliably observed
- Example – Bacterial fruit blotch testing of cucurbits

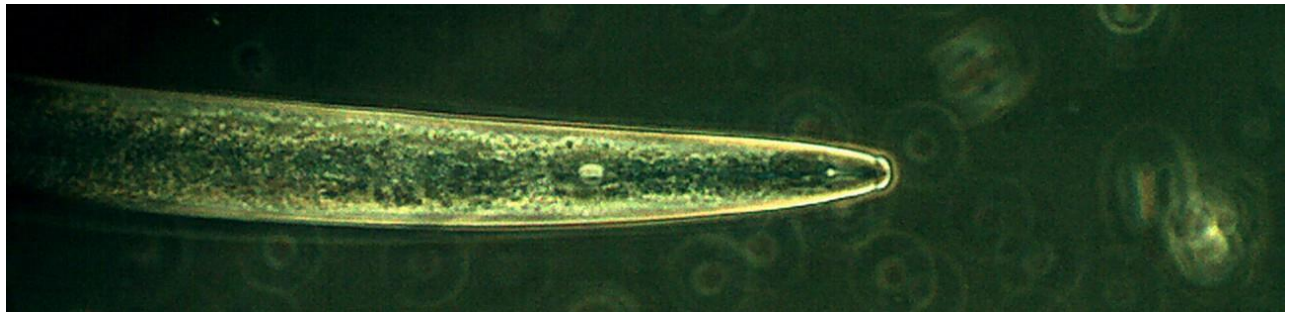
Virus indicator tests

- Usually done to prove that a virus is viable and able to cause disease
- Example – tobamoviruses on tomato/peppers

Wash or soak test

- Done for fungal spores on the outside of the seed or nematodes
- Examples – *Tilletia controversa* on wheat and *Aphelencoides* on rice



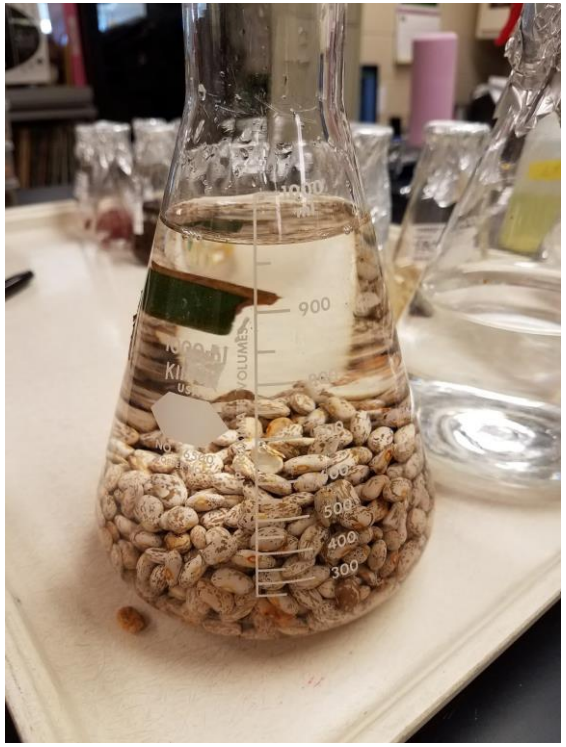


Blotter testing

- Used to ID fungi on seed
- Example – *Diplodia maydis* (*Stenocarpella*) on corn

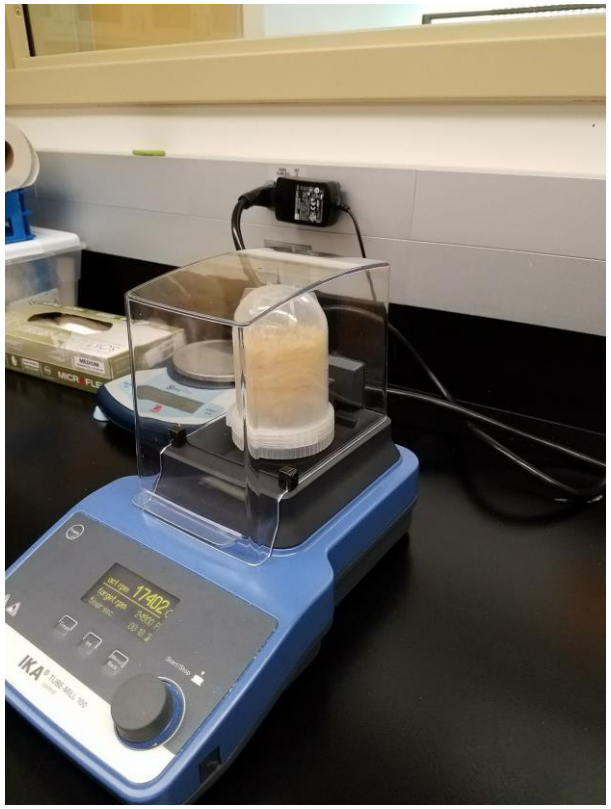
Selective Media tests

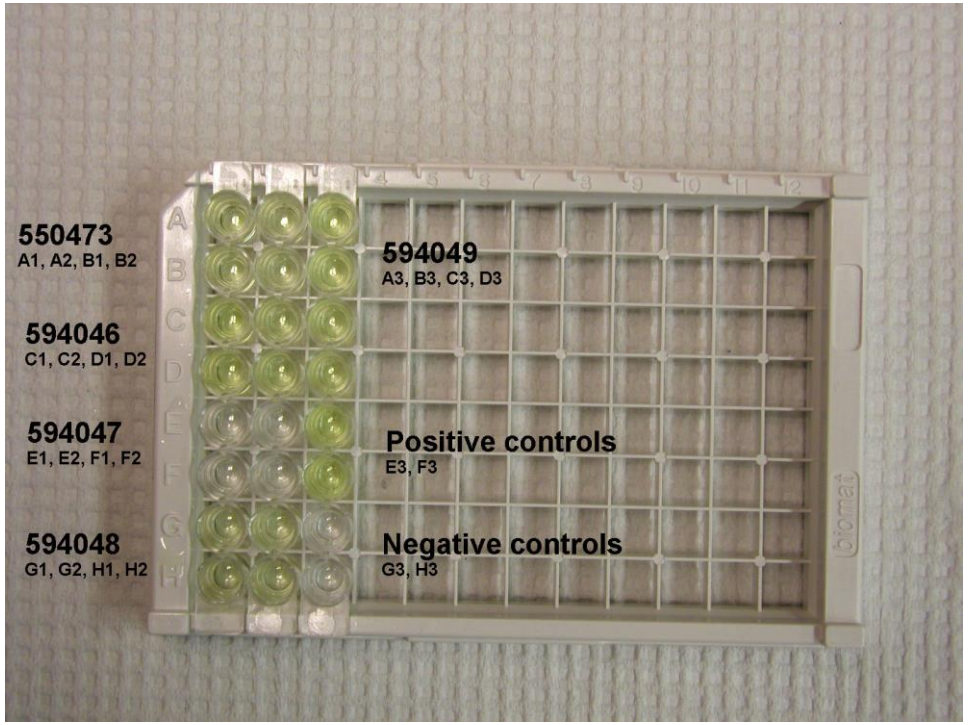
- Used mostly for bacteria
- Curtobacteria on beans



ELISA tests

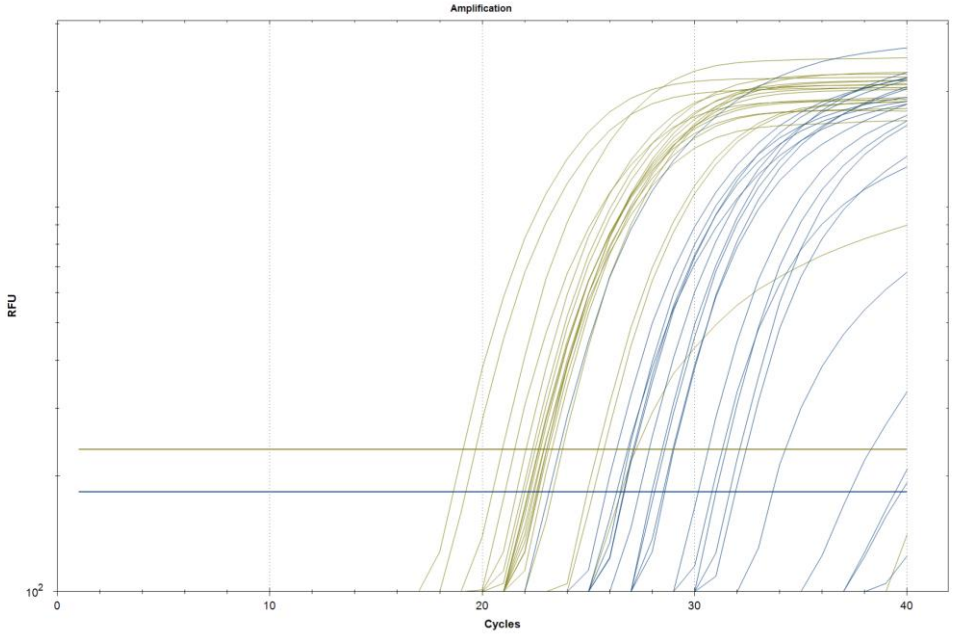
- Used for some bacteria and many viruses
- Example – Wheat streak mosaic virus

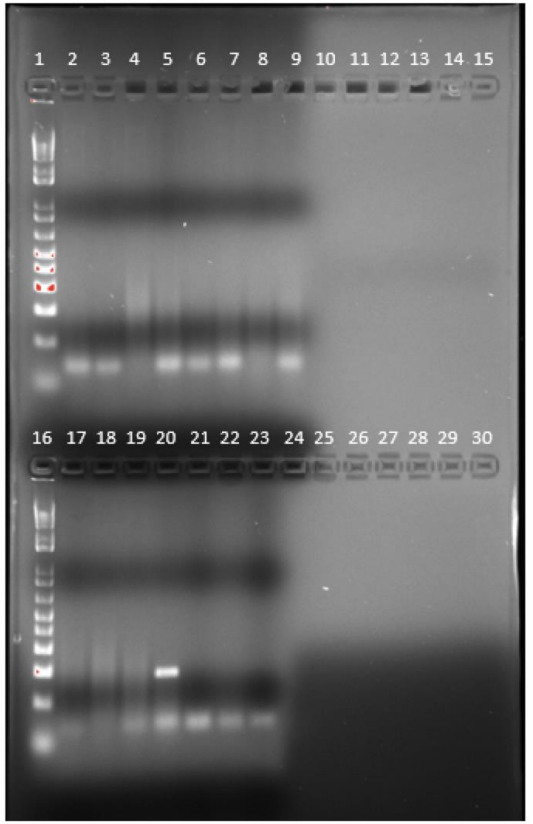




Molecular tests

- DNA or RNA based
- Can be used for many pathogens
- Example – viroids on tomato seed

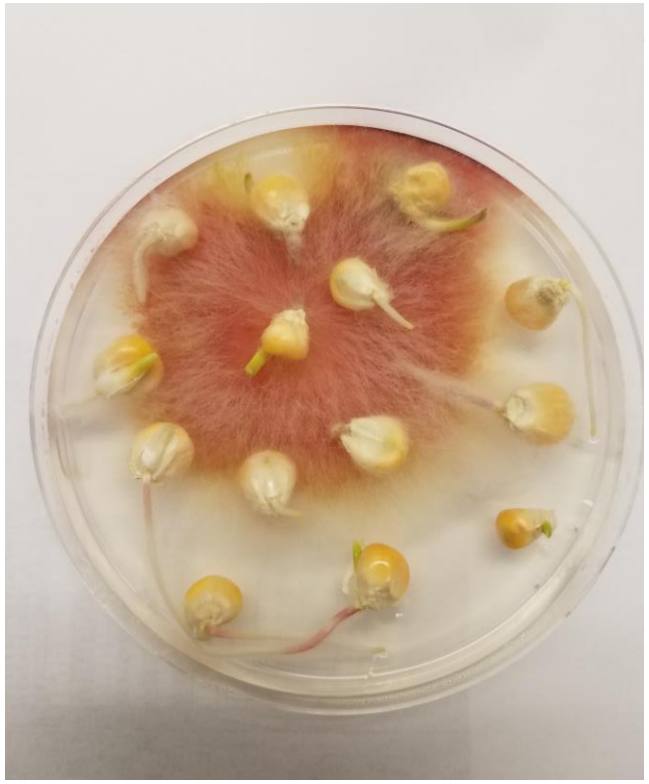




Direct vs Indirect Tests

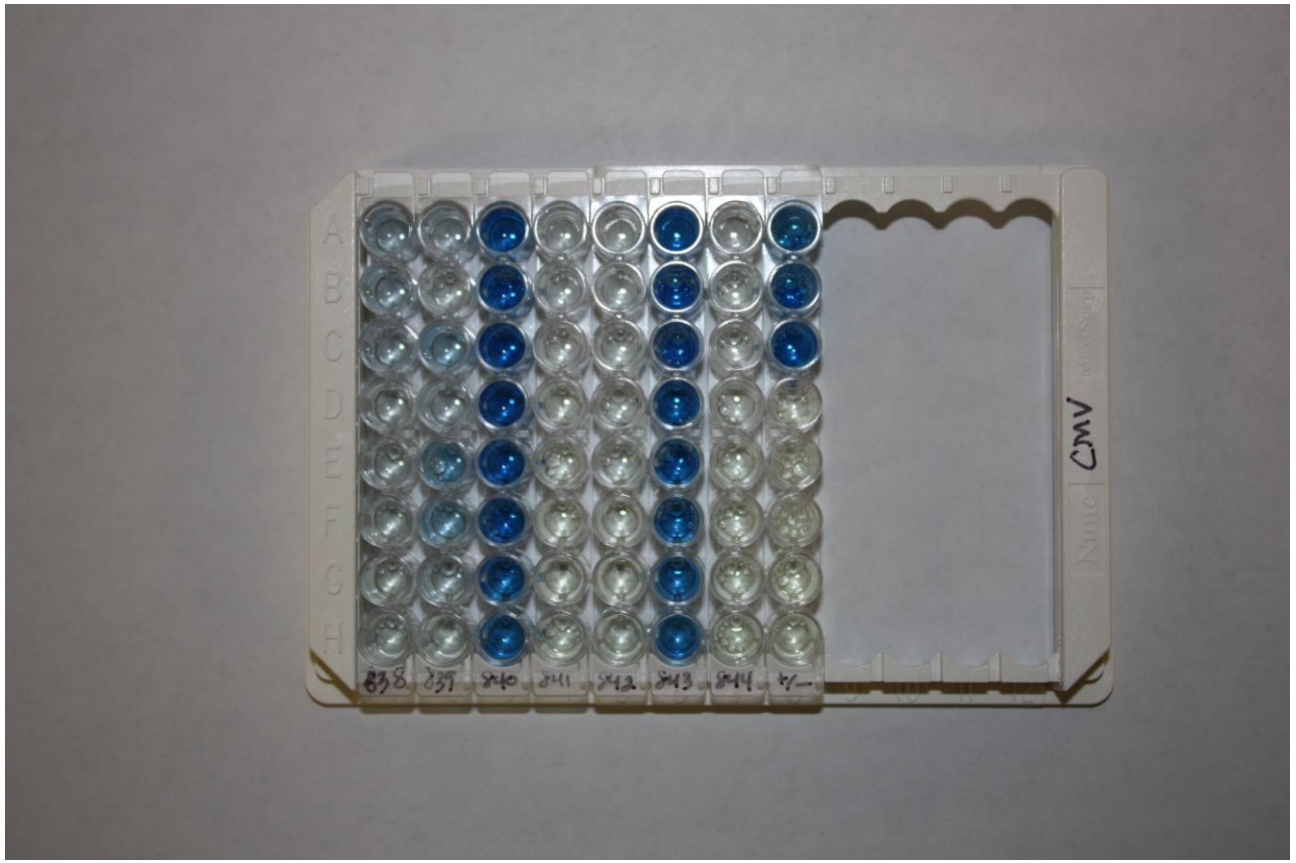
- **In ISPM 38 section 4.3**
- **Direct Testing** – a test which demonstrates the presence, viability and pathogenicity of a pathogen on or in the seed
  - Example – blotter testing for fungi where you can identify the spores of fungus growing on a seed/seedling and causing symptoms on the seedling
- **Indirect Testing** – A test that demonstrates the presence of proteins or nucleic acid of a pathogen but not viability or pathogenicity
  - Example – ELISA testing for a virus indicated the coat proteins of the virus is present but not if the intact and infectious virus is present

Direct Testing – fungal blotters/media



Indirect Testing – Virus ELISA

- Detects the coat protein of the virus



- Cannot isolate the virus at the end of the test.
- Does not indicate the viability of the virus
- What if there is no indicator test for this virus?
- Accepting positive test results may restrict the movement of seed that poses no risk

Seed treatments

- Treatment of seed by thermal or chemical methods to remove or destroy pathogens that might be present in or on seed
  - Example – Pepper seed treated to inactivate viruses using TSP or hypochlorite
    - Indirect test - ELISA or PCR to detect tobamoviruses will be positive due to coat proteins or nucleic acids
    - Direct test – Viral indicator test using resistant tobacco plants demonstrates if any viable virus particles remain

Workflow of pepper testing

Indirect Test for PMMoV (ELISA)

- Positive – move to direct test
- Negative – no further testing needed

Direct Test for PMMoV (Viral Indicator)

- Positive - Infectious pathogen present
- Negative – seed contains NO infectious virus = No Risk

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Direct Tests

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Thank you!