OECD Quantitative Method
Fungicidal Activity

Stephen Tomasino, Ph.D.
Senior Scientist
Microbiology Laboratory Branch
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OECD Guidance Document TOC

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Preparation of Conidial Stocks

- **Microbe:** *Aspergillus niger* (ATCC 64958)
- **Annex 2** provides the procedures for maintenance of the fungus – frozen stocks
- For this test organism, the culture medium is prepared as follows:
  - Growth and recovery media – plates of Sabouraud's Dextrose Agar.
  - The stock culture of *A. niger* is maintained on a Sabouraud's Dextrose Agar plate at 4 ± 2°C. At one-month intervals, fresh agar plates are inoculated and incubated for ten days at 30 ± 2°C.
  - Conidial suspensions are prepared from these stocks.

Preparation of Conidial Suspension

- To prepare a conidial suspension, a loop of the test fungus is inoculated at the centre of each of four or more Sabouraud's Dextrose plates and then incubated at 30 ± 2°C for ten days. The mycelial matt is removed from the plates with a sterile spatula and transferred to a 250 mL conical flask containing 25-50 mL PBS and 5-7 glass beads. The flask is shaken vigorously enough to break off the conidia from the hyphae.

- The resulting suspension is filtered through sterile absorbent cotton and conidia are collected in the filtrate. The suspension is standardized as needed for testing by diluting it with PBS. The conidial concentration in the suspension may be estimated by using any suitable means (e.g. haemocytometer, microscopic evaluation, plate count, spectrophotometrically). Conidial suspensions stored at 4 ± 2°C can be used for up to four weeks to inoculate carriers.

Preparation of Test Suspension

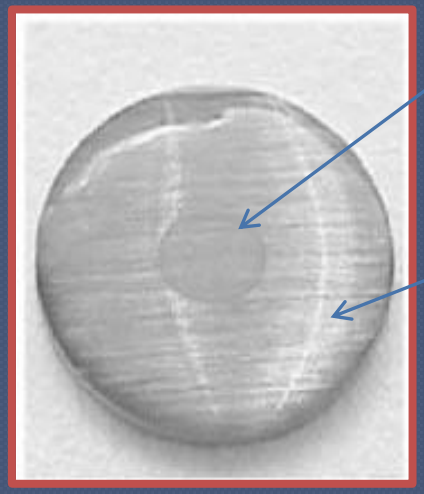
- Following storage, use the conidial suspension to prepare a test suspension of the test organism. The conidial suspension should be centrifuged as described below to achieve the desired level of viable organisms on the dried carrier count.

- Test organism requires centrifugation of the broth culture to obtain the required number of viable conidia. The product of centrifugation (g force) and time for which it is applied (t minutes) determines the organism's sedimentation rate. The centrifugation should be between 5000 and 10000 gN for 20 ± 5 minutes and resuspend the pellets in PBS. Centrifugation for less than 5000 gN may result in incomplete sedimentation of the test fungi. If multiple tubes are centrifuged, pool the resuspended pellets.

Next Steps

- Seeking stakeholder involvement with expertise in fungicidal testing to:
  - Confirm test culture procedures
  - Establish/demonstrate control carrier counts
  - Develop a reference standard
  - Confirm neutralization assay
  - Conduct method performance assay
  - Conduct a collaborative study – limited in scope
- EPA will assist in prioritization and facilitate the process

Questions/Comments

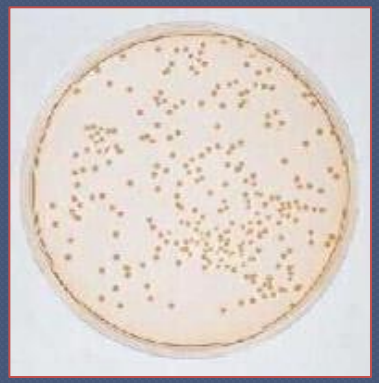


10 µL dried inoculum

50 µL disinfectant

1 cm brushed stainless steel disk

Vial with inoculated carrier



Count colonies and determine log density for control and treated carriers.