Assessment of *Mycobacterium bovis* (BCG) grown with and without agitation using the AOAC *In Vitro* Test for Determining Tuberculocidal Activity

2014 collaborative study

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Background

- The AOAC *In vitro* Test for Determining Tuberculocidal Activity (method 965.12 , 2012) measures the efficacy of tuberculocides on hard inanimate surfaces.
- The EPA is interested in enhancing method 965.12 by introducing a procedural modification for the use of test cultures grown with agitation.
- This study will assess the impact of the proposed modification on the outcome of the control carrier counts and the efficacy test.

Benefits of Culture Grown with Agitation

- Shorter incubation time
  - Approximately 11-18 total days for the agitated culture
  - 19-23 days for the statically grown culture
- More uniform culture requiring less manipulation
  - Agitated culture is grown in one flask
  - No homogenization
  - Over/under inoculation is avoided
- Reduces biosafety concerns associated with homogenizing the statically grown culture.

Study Phases

1) Grow the agitated culture and generate carrier counts for both the static and agitated cultures within the appropriate range
2) Conduct efficacy testing to compare the two methods of culture preparation

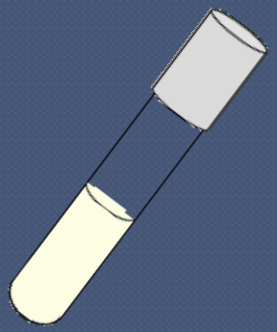
Study Attributes

- In this study, trained analysts will conduct AOAC method 965.12 and evaluate a set of EPA-registered hospital disinfectants (liquids) against *Mycobacterium bovis* (BCG)
- 3 classes of active ingredients
  - Quaternary ammonium compounds
  - Phenol
  - Sodium hypochlorite
- The number of carrier sets with growth following exposure to a disinfectant treatment is the main test variable.
- Required carrier count range: 1.0×10^4 to 1.0×10^6 CFU/carrier

Study Attributes

- 3 participating laboratories
- 2 culture preparation procedures
- 3 products tested per day
- Replicate testing
- Media performance assessments

Current (Static) Culture Preparation Procedure

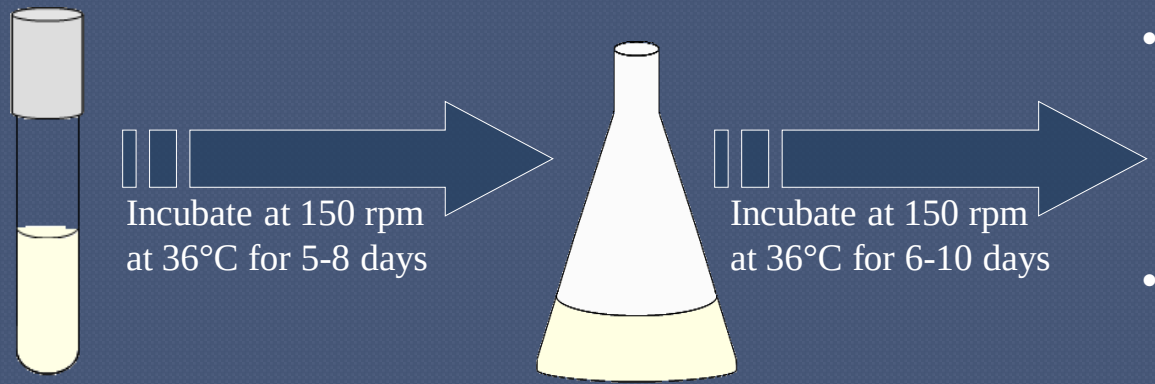


20 mL Modified Proskauer Beck (MPB) medium inoculated with *M.b.* (from M7H9 stock slant)

Incubate slanted without agitation at 36°C for 21±2 days

- Transfer culture to a tissue grinder
- Add 1.0 mL saline with 0.1% (v/v) polysorbate 80 to grinder
- Grind to break up large aggregates of the test organism
- Dilute the homogenized culture with 9 mL MPB
- Transfer suspension to a sterile test tube and allow culture to settle for 10-15 min.
- After settling, remove the top ¾ and pool culture
- Dilute pooled culture using MPB to 20.0% ± 1% transmittance at 650 nm

Proposed Agitated Culture Preparation Procedure



1.) Inoculate 10 mL M7H9 plus 0.1% (v/v) polysorbate 80 (M7H9/P80) with a loopful of *M.b.* (from M7H9 stock slant)

1° Culture

2.) Inoculate 50 mL M7H9/P80 with 1 mL of *M.b.* (from 1° culture)

2° Culture
(Test Culture)

- Transfer the suspension to sterile test tubes and allow culture to settle for 10-15 min.
- After settling, remove the top ¾ and pool culture.
- Dilute pooled culture using saline + 0.1% (v/v) polysorbate 80 to 20.0% ± 1% transmittance at 650 nm

Evaluating Outcomes

- Control carrier counts
  - Mean log densities
- Equivalency testing
  - Comparison of the mean number of positive carriers for the Modified and Current methods

Timeline

- Protocol submitted to labs: January 2014
- Initiate testing: February/March 2014
- Data submission: August 2014

Acknowledgements

- Collaborating Laboratories
  - EPA, Microbiology Laboratory Branch
  - MICROBIOTEST
  - ATS Labs

Questions?