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Revisions to the AOAC Germicidal Spray Products and Tuberculocidal Test Methods

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Germicidal Spray Test Revisions

- ◉ AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants
 - Posted March 2013
 - MLB SOP MB-06-07. Germicidal Spray Products as Disinfectants: Testing of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella enterica*
- ◉ Most revisions (for microbe culture) are in-line with the Use-dilution method

Germicidal Spray Test Revisions

- *Synthetic broth (SB)*.—Use for (10mL) daily transfers and (10mL) test cultures for *S. enterica*, *S. aureus*, and *P. aeruginosa*.
- Alternatively, *nutrient broth*.—Use for (10 mL) daily transfers and (10 mL) test cultures for *P. aeruginosa*.
- Adjusted concentrations of listed neutralizing constituents and/or alternate ingredients (e.g., sodium thiosulfate) may be added to the base subculture media or alternate subculture media (e.g., Dey/Engley broth) may be used to allow for adequate test substance neutralization, as necessary.

Germicidal Spray Test Revisions

- (v) Tryptic Soy Agar (TSA) – For use in propagation of the test organism to generate frozen cultures.
Alternately, TSA with 5% sheep blood may be used.
- (c) Preparation of frozen stock cultures for *S. enterica*, *S. aureus*, and *P. aeruginosa*.—Using a tube containing 5–6 mL TSB (for *S. aureus* and *P. aeruginosa*) or **NB** (for *S. enterica*), aseptically withdraw 0.5 to 1.0 mL and rehydrate the lyophilized culture.
- Note: For each bacterium, **one** daily transfer is required prior to the inoculation of a final test culture. Daily cultures may be **subcultured** for up to 5 days; each daily may be used to generate a test culture.

Germicidal Spray Test Revisions

- Note: Specialized neutralizer/subculture media such as Dey/Engley broth **will not show turbidity**; rather, a color change to the medium (yellow for growth of *S. aureus* and *S. enterica*) or the presence of pellicle at the surface of the medium (for *P. aeruginosa*) must be used to assess the results as a positive or negative outcome. Use viability controls ((h), Sterility and Viability controls) for comparative determination of a positive tube.
- If the product passes the performance standard, a minimum of 20% of the **remaining negative tubes** will be assayed for the presence of the test microbe using isolation streaks on TSA or TSA with 5% sheep blood.
- Alternatively, the letheen broth tubes may be **pooled** after vortexing for each set of 3 carriers. An aliquot of the pooled media (60 mL) will be serially diluted and plated, and the average carrier count per set will be calculated.

Germicidal Spray Test Revisions



Germicidal Spray Test Revisions

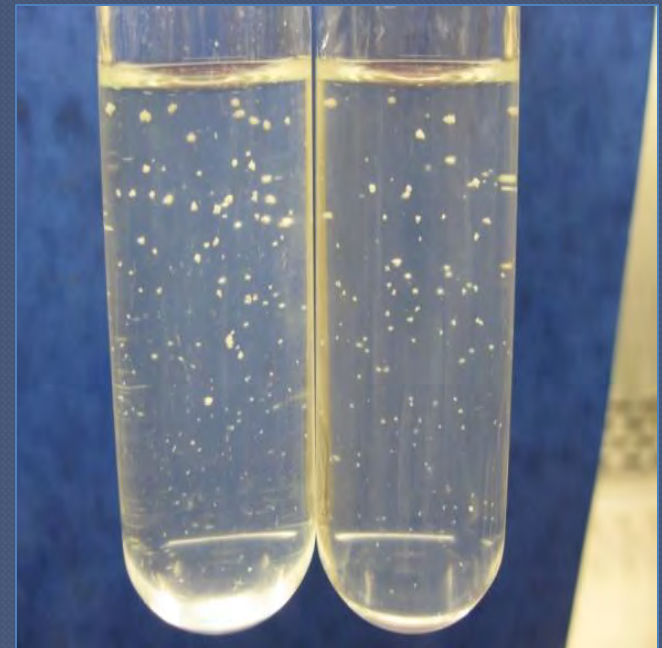
- ⦿ (c) *Test culture preparation.*—For *P. aeruginosa*, defrost a single cryovial at room temperature and briefly vortex to mix. Each cryovial should be single use only. Add 10 μ L of the thawed frozen stock to a tube containing 10 mL broth (synthetic **or nutrient broth**) and then vortex to mix.
- ⦿ Alternatively, **Petrifilm** may be used for enumeration of bacterial organisms. Follow manufacturer's instructions for preparation and incubation of Petrifilm cards. *Note:* A culture purity check should be conducted on one dilution of one carrier.
- ⦿ (g) *Test procedure.*— the slides are sequentially sprayed in a **horizontal** position

TB Test revisions

- ◉ AOAC Official Method 965.12 Tuberculocidal Activity of Disinfectants
- ◉ Revised in 2012
 - (i) ***Spectrophotometer***.—Calibrated; for preparing standardized test culture.
 - Inoculated carriers should be **used for testing within 2 h of drying**.
 - (f) ***Enumeration of viable bacteria from carriers (carrier counts)***.—After inoculated carriers have dried, randomly select 3 carriers for assay. **Assay 1 carrier immediately prior to conducting the efficacy test and 2 carriers following the test.**

TB Test revisions

- (g) *Media quality assessment.*—An assessment of media quality (performance) is necessary to ensure the validity of the tuberculocidal efficacy results. An example of an appropriate method for assessing media quality is provided under “Additional Guidance, D.”



TB Test revisions

◉ Additional Guidance Section

- A. Neutralization Confirmation
 - Compared to the corresponding inoculated control tube (with low level CFU/tube), *M. bovis* (BCG) may not exhibit the same amount of growth in each subculture medium tube **or may not grow** when neutralizer (2 mL) is added; however, the primary tube (with carrier) **should** show growth. Untreated inoculated control tubes are used for baseline media performance.

TB Test revisions

- D.(b) *Performance of solid media.*—(1) *For media in plates*, spread or pour plate 100 μL of the 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} dilutions of the standardized culture, in duplicate. The dilutions should result in plate counts which are TNTC through extinction or near extinction levels.

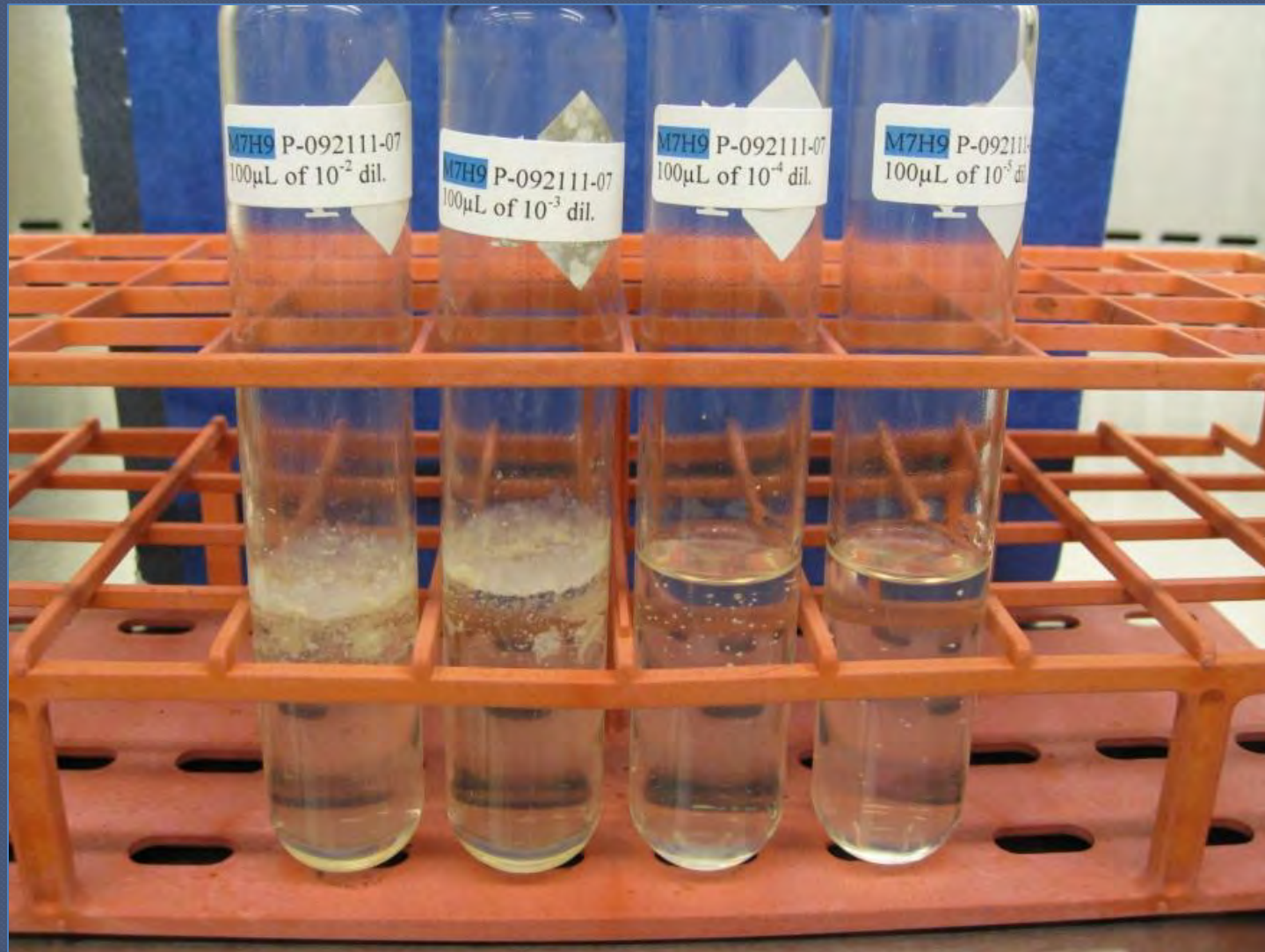


TB test revisions

● D.(c) *Performance of liquid media.*

- If possible, conduct concurrently with assessment of solid media. For each liquid medium, inoculate each tube with 100 μL of the standardized culture, in duplicate, from the 10^{-3} to the 10^{-6} dilution. Thus, 8 total tubes of each medium will be inoculated.
- Verify CFU/tube by spread or pour plating in duplicate the diluted inoculum on M7H9 or M7H11 agar.
- Incubate liquid media at $36 \pm 1^\circ\text{C}$ for up to 60 days. Preliminary results (growth/no growth) may be recorded between 10–21 days of incubation; visual evidence of growth in the **lower dilutions** with high inoculum levels may be used for planning purposes to initiate efficacy testing.
- Continue to incubate for up to 60 days. Tubes may be monitored for growth during this time. Final results are read and recorded at 60 days of incubation.

TB test revisions



Other TB test revisions

- Specific to MB SOP-07-06
 - See “footnotes” for edits to the method – “MLB proposed revision, not currently in AOAC Method 965.12.”
 - *Inoculating loop*. For culture inoculation, 1 μL sterile disposable loops (Fisher Scientific).
 - Initiate test culture by inoculating a sufficient number of 25 \times 150 mm tubes containing 20 mL MPB (approximately 10) from stock culture slant(s) (M7H9 or M7H11 agar slants) by transferring 1-2 1 μL loopfuls from the stock culture onto the surface of the broth.



Other TB test revisions

- After 15 ± 1 min contact period, remove cylinders using flamed wire hook and shake carriers vigorously against side of the tube to remove excess culture. (Alternatively, drain the inoculum from the carriers with a pipette; avoid direct contact of the carrier with the tip of the pipette. Briefly tap each carrier against the side of the tube to remove excess culture.)

Questions/Comments

