Designation: E 1531 – 00

Standard Practice for Detection of Mycoplasma Contamination of Cell Cultures by Growth on Agarose Medium[1]

This standard is issued under the fixed designation E 1531; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers the procedures used for detection of mycoplasma contamination by direct microbiological culture.

1.2 This practice does not cover indirect methods for detection of mycoplasma such as DNA staining, biochemical detection, or genetic probes.

1.3 This practice does not cover methods for identification of mycoplasma organisms.

1.4 This practice will not detect cultivar α strains **(1)**[2] of *Mycoplasma hyorhinis*.

1.5 This practice is not intended for use in detection of mycoplasma contamination in sera, culture media, vaccines, or other systems.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 1532 Practice for Detection of Mycoplasma Contamination of Cell Cultures by the Use of the Bisbenzamide DNA Binding Fluorochrome[3]

E 1533 Practice for Indirect Detection of Mycoplasma in Cell Culture by 4′–6–Diamidino–2–2 Phenylindole (DAPI) Staining[3]

E 1536 Practice for Detection of Mycoplasma Contamination of Bovine Serum by the Large Volume Method[3]

3. Terminology

3.1 *Definitions:*

3.1.1 *direct mycoplasma detection*, *n*—demonstration of characteristic colonial growth on axenic agar medium.

3.1.2 *indirect detection of mycoplasma*, *n*— detection of mycoplasma by DNA staining or any method other than cultivation.

3.1.3 *mycoplasma (Mollicute)*, *n*—smallest prokaryotes capable of self replication.

4. Significance and Use

4.1 The demonstration of characteristic colonial growth on axenic solid medium is a sensitive and specific method to detect mycoplasma infection of cell cultures and it is the standard detection method **(2)**.

4.2 When mycoplasmas contaminate cell cultures they usually grow to high titer (10^8 colony forming units/mL) and when inoculated onto agar medium they produce abundant and easily detectable growth **(3)**.

4.3 *M. hyorhinis* cultivar α strains do not grow on conventional mycoplasma media **(1)** but require an indicator cell culture system to detect their presence (see Practice E 1532). Alternatively, a specialized axenic medium is suitable for direct isolation of cultivar α from infected cell cultures **(4)**.

4.4 Immunofluorescent procedures are used to identify mycoplasma isolates **(5)**.

5. DM-1 Solid Medium Preparation

5.1 Dissolve CMRL-1066 powder (CMRL-1066 powder Formula No. 78–5156EF[4], packaged for 10L), in 5000 mL of distilled water. This is one-half the volume of water specified on the package. Add 47.6 g HEPES[5], and 9.35 g NaCl.

5.2 Adjust the pH to 7.3 and filter sterilize (450 nm). Store this 2X CMRL in the refrigerator in 500 mL amounts.

5.3 Dissolve 10.0 g of Myosate[6] and 12 g of agarose[7] in 400 mL of distilled water. Autoclave at 121°C for 15 minutes. Cool the autoclaved solution to about 50°C, and combine with 500 mL of 2X CMRL and 100 mL of sterile horse serum[8] (both ingredients also warmed to 50°C).

5.4 Aseptically dispense medium in 5 mL amounts in petri

[1] This practice is under the jurisdiction of ASTM Committee E-48 on Biotechnology and is the direct responsibility of Subcommittee E48.02 on Characterization and Identification of Biological Systems.

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[2] The boldface numbers in parenthesis refer to the list of references at the end of this standard.

[3] *Annual Book of ASTM Standards*, Vol 11.05.

[4] Available from Life Technologies, Gaithersburg, MD.

[5] Available from Research Organics, Cleveland, OH.

[6] Available from BBL Microbiology Systems, Cockeysville, MD.

[7] Available from SeaPlaque agarose, FMC Bioproducts, Rockland, MA.

[8] Available from Whittaker Bioproducts, Walkersville, MD.

dishes and allow to solidify. Store at 4°C and prevent drying. The DM-1 plates have a shelf-life of eight weeks.

6. Quality Control

6.1 It is important that solid medium used for isolation have high plating efficiency; therefore, quality control should include titration of mycoplasma suspensions onto the solid medium to determine the highest dilution to produce colonies.

6.2 Test mycoplasma strains should not be adapted to artificial media. Instead, cell culture grown mycoplasma strains should be used. Test strains should include: *M. hyorhinis*, BTS-7; *M. orale*, CH 19299; *M. pirum*, 70–159; *M. arginini*, G230; *M. fermentans*, PG-18; Mycoplasma infected BHK-21 cell cultures are stored at –70°C.

6.3 Prepare test batches of DM-1 medium with each new lot of agarose, myosate, horse serum, or CMRL-1066.

6.4 Inoculate ten-fold dilutions of the battery of quality control strains with known titers onto medium plates and compare to DM-1 prepared with previously tested components.

6.5 A new lot of material is acceptable if there is no more than a ten-fold difference between number of colonies on test and control media plates.

7. Mycoplasma Isolation

7.1 Samples include monolayer, suspension or frozen cell cultures that have been grown in antibiotic free medium. Monolayer cultures should be scraped with a pipette to provide a cell suspension.

7.2 Inoculate one DM-1 medium plate with 0.1 mL of the cell culture sample, and incubate anaerobically at 36°C.[6]

7.3 Microscopically examine the plate at 5 and 14 days. Most isolates develop colonies in 5 days.

8. Keywords

8.1 cell culture; cultivation; mycoplasma

REFERENCES

(**1**) Del Giudice, R. A., Gardella, R. S., and Hopps, H. E., "Cultivation of Formerly Noncultivable Strains of *Mycoplasma hyorhinis*," *Curr. Microbiol*., Vol 4, 1980, 75–80.

(**2**) Del Giudice, R. A., and Tully, J. G., "Isolation of Mycoplasmas from Cell Cultures by Axenic Cultivation Techniques," *Molecular and Diagnostic Procedures in Mycoplasmology*, Joseph G. Tully and Schmuel Razin, Eds., Academic Press, 1996, Vol II, 411–418.

(**3**) Del Giudice, R. A., and Gardella, R. S., "Mycoplasma Infection of Cell Culture: Effects, Incidence, and Detection," "Use and Standardization of Vertebrate Cell Cultures," *In Vitro Monograph* No. 5., pp. 104–115. Tissue Culture Assoc., Gaithersburg, MD, 1984.

(**4**) Del Giudice, R. A., "M-CMRL a New Axenic Medium to Replace Indicator Cell Cultures for the Isolation of All Strains of *M. hyorhinis*," *In Vitro Cell. Dev. Biol.-Animal*, Vol 34, 1998, pp. 88–89.

(**5**) Gardella, R. S., Del Giudice, R. A., and Tully, J. G., "Immunofluorescence," *Methods in Mycoplasmology*, Joseph G. Tully and Schmuel Razin, Eds., Vol 1, 1983, pp. 431–439.