Designation: E1531 – 00(Reapproved 2006)

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

This standard is issued under the fixed designation E1531; 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:*[3]

E1532 Practice for Detection of Mycoplasma Contamination of Cell Cultures by Use of Bisbenzamide DNA-Binding Fluorochrome

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 E1532). 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,[4,5] 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,[5,6] 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[5,7] and 12 g of agarose[5,8] in 400 mL of distilled water. Autoclave at 121°C for 15 minutes.

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[1] This practice is under the jurisdiction of ASTM Committee E55 on Manufacture of Pharmaceutical Products and is the direct responsibility of Subcommittee E55.04 on General Biopharmaceutical Standards.

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

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[4] The sole source of supply of the apparatus known to the committee at this time is Life Technologies, Gaithersburg, MD.

[5] If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[6] The sole source of supply of the apparatus known to the committee at this time is Research Organics, Cleveland, OH.

[7] The sole source of supply of the apparatus known to the committee at this time is BBL Microbiology Systems, Cockeysville, MD.