Designation: E 1536 – 00

Standard Practice for Detection of Mycoplasma Contamination of Bovine Serum by the Large Volume Method[1]

This standard is issued under the fixed designation E 1536; 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 in serum by direct microbiological culture.

1.2 This practice does not cover procedures used for detection of mycoplasma in cell cultures.

1.3 This practice does not cover indirect methods for detection of mycoplasma contamination.

1.4 This practice does not cover methods for identification of mycoplasma cultures.

1.5 *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 1531 Practice for the Detection of Mycoplasma Contamination of Cell Cultures by Growth on Agarose Medium[2]

E 1532 Practice for the Detection of Mycoplasma Contamination of Cell Cultures by the Use of the Bisbenzamide DNA-Binding Fluorochrome[2]

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

3. Terminology

3.1 *Definitions:*

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

3.1.2 *large volume testing*, *n*—using a large volume inoculum in an enrichment culture.

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

4. Significance and Use

4.1 Mycoplasmas of bovine origin are prevalent contaminants of cell cultures. Contamination can be detected by the large volume method.[3,4]

4.2 Heat inactivated serum need not be tested for mycoplasmas. Heating serum to 56°C for 30 minutes will kill mycoplasmas.

4.3 Mycoplasmas may be present in any particular lot of serum but may not be detected because of inadequate sample size; thus, negative test results do not provide absolute assurance that the test serum is free of mycoplasmas.

5. Liquid Medium Preparation

5.1 Add 105-g mycoplasma broth base, 5-g glucose, 5-g arginine, and 20 mL of a 0.5 % solution of phenol red to 4080 mL of distilled water. Mix to dissolve ingredients.

5.2 Dispense medium, in 400-mL amounts into 500-mL screw-capped bottles.

5.3 Autoclave.

5.4 Sterile refrigerated medium is stable for four months.

6. Quality Control

6.1 Prior to testing large volumes of bovine serum, check sterility and ability of liquid medium to support mycoplasma growth.

6.2 Strains used to test for growth support: *M. arginini*, G230, *M. bovis*, Donetta; *A. laidlawii*, PG8.

6.3 For quality control, a portion of the base liquid medium is supplemented with 20 % of newborn calf serum. This batch of serum must be extensively tested to ensure that it is free of mycoplasma contamination and it should be in sufficient quantity to last for an extended period of time. Challenge mycoplasma strains for the quality control test should be diluted so that approximately 100 colony-forming units are contained in the inoculum.

7. Test Procedure

7.1 The sample is 100 mL of uninactivated bovine or equine serum. Multiple samples will increase the probability of mycoplasma detection.

---

[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.

Current edition approved May 10, 2000. Published June 2000. Originally published as E 1536 – 93. Last previous edition E 1536 – 93.

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

[3] Barile, M. F., and Kern, J., "Isolation of *Mycoplasma arginini* from Commercial Bovine Sera and Its Implication in Contaminated Cell Cultures," *Proceeding of the Society for Experimental Biology and Medicine*, 138, 1971, pp. 432–437.

[4] Del Giudice, R. A., 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, pp. 411–418.