Designation: E ~~-~~ 1536 – ~~93~~00

Standard Practice for ~~Large Volume Testing~~ Detection of ~~Serum for~~ 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 ~~problems,~~ 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:*

---

[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 ~~March 15, 1993. Published~~ May 10, 2000. Published June 2000. Originally published as E 1536 – 93. Last previous edition E 1536 – 93.

E 1531 Practice for ~~Direct~~ the Detection of Mycoplasma Contamination of Cell Cultures by ~~Broth Enrichment and Agar~~ Growth on Agarose Medium[2]

E 1532 Practice for ~~Indirect~~ the Detection of Mycoplasma Contamination of Cell Cultures by ~~DNA Binding with~~ the Use of the Bisbenzamide DNA-Binding Fluorochrome ~~Stain~~[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 ~~mycoplasma—detection of mycoplasma by cultivation in culture media.~~ characteristic colonial growth on axenic agar medium.

3.1.2 ~~*indirect detection of mycoplasma*—detection of mycoplasma by DNA staining or any method other than cultivation.~~ large volume testing, *n*—using a large volume inoculum in an enrichment culture.

3.1.3 ~~*large volume testing*—using a large volume of the material to be tested as an inoculum in direct detection of mycoplasma.~~

~~3.1.4 *mycoplasma*—the smallest~~mycoplasma (Mollicute), *n*—smallest prokaryotes capable of ~~living freely, lacking a cell wall, having a circular double-stranded DNA relatively rich in adenine and thymine, and containing 16s and 23s ribosomal RNAs. They can be found as contaminants in cell cultures and biological products such as serum.~~ self replication.

4. Significance and Use

~~4.1 Testing for mycoplasma contamination in serum used in cell culture media requires methods that ensure detection~~

4.1 Mycoplasmas of ~~a small number of~~ bovine origin are prevalent contaminants of cell cultures. Contamination can be detected by the large volume ~~of serum.~~ method.[3,4] ~~This is accomplished by using a large volume of~~

4.2 Heat inactivated serum ~~as an inoculum in a broth medium.~~

~~4.2 This practice is intended~~ need not be tested for mycoplasmas. Heating serum to 56°C for 30 minutes will kill mycoplasmas.

4.3 Mycoplasmas may be present in ~~testing sera (to~~ any particular lot of serum but may not be ~~used as a cell culture medium supplement) for the presence~~ detected because of ~~mycoplasma contamination.~~

~~4.3 This practice is~~ inadequate sample size; thus, negative test results do not ~~intended for use in testing cell cultures for mycoplasma contamination. For additional information, see Practices E 1531, E 1532, and E 1533.~~ provide absolute assurance that the test serum is free of mycoplasmas.

5. ~~Quality Control~~

~~5.1 Test the growth promoting properties of the~~Liquid Medium Preparation

5.1 Add 105-g mycoplasma ~~media by using *Acholeplasma laidlawii*, American Type Culture Collection, (ATCC) No. 23206, *Mycoplasma ovale*, *Mycoplasma arginini* ATCC No. 23838,~~ broth base, 5-g glucose, 5-g arginine, and ~~*Mycoplasma pneumoniae* ATCC No. 15531. Do not use control cultures beyond passage 15 in laboratory culture or above~~ 20 mL of a ~~titer~~ 0.5 % solution of ~~10^2 CFU/mL.~~ 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. ~~Procedure~~

~~6.1 *Preparation*~~*Quality Control*

6.1 Prior to testing large volumes of ~~Mycoplasma Broth Medium:~~

~~6.1.1 *Preparation of Stock Solution* :~~

~~6.1.1.1 Add dextrose (50 g), L-arginine HCl (10 g), thymic DNA (0.02 g), choline chloride (0.922 g), i-inositol (0.110 g), niacinamide (0.024 g), D-calcium pantothenate (0.024 g), pyridoxal HCl (0.020 g), folic acid (0.013 g), riboflavin (0.010 g), cyanocobalamin (0.003 g), D-biotin (0.002 g),~~ bovine serum, check sterility and ~~thiamine HCl (0.010 g) to 900 mL distilled water.~~

~~6.1.1.2 Mix at 37°C until dissolved and bring final volume to 1000 mL.~~

~~6.1.1.3 Sterilize the solution by filtration using a 0.22 µm filter.~~

~~6.1.2 *Preparation* *ability of Broth*:~~

~~6.1.2.1 Add 14.7 g~~ liquid medium to support mycoplasma ~~broth base (BBL 11458) and 0.2 g phenol red~~ growth.

6.2 Strains used to ~~600 mL distilled water and heat to dissolve.~~

~~6.1.2.2 Sterilize the solution by autoclaving~~ test for ~~15 min at 121°C and allow to cool to room temperature.~~

---

[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~~ Medicine*, 138, 1971, pp. 432–437.

[4] ~~Hayflick, L. "Screening Tissue~~

[4] Del Giudice, R. A., Tully, J. G., "Isolation of Mycoplasmas from Cell Cultures ~~for Mycoplasma Infections," *Tissue Culture Methods*~~ *by Axenic Cultivation Techniques," Molecular and ~~Applications~~*, Diagnostic Procedures in Mycoplasmology, Joseph G. Tully and Schmuel Razin, Eds., Academic Press, ~~1973.~~ 1996, Vol II, pp. 411–418.

~~6.1.2.3 Aseptically add 100 mL fresh yeast extract and 100 mL thawed stock solution (6.1.1) and mix thoroughly.~~

~~6.2 *Inoculation of Test Sample*~~ *growth support:* ~~:~~

~~6.2.1 Add 50 mL~~*M. arginini*, G230, *M. bovis*, Donetta; *A. laidlawii*, PG8.

6.3 For quality control, a portion of the ~~serum to be tested to 200 mL~~ base liquid medium is supplemented with 20 % of ~~the mycoplasma broth, or 100 mL~~ newborn calf serum. This batch of ~~the~~ serum ~~to~~ must be extensively tested to ~~400 mL~~ ensure that it is free of ~~the~~ mycoplasma ~~broth medium.~~

~~6.2.2 Incubate broth cultures aerobically~~ contamination and ~~anaero-bically at 37°C for four weeks and observe for turbidity and change~~ it should be in ~~pH.~~

~~6.2.3 Inoculate mycoplasma agar medium with 0.1 mL of the test broth cultures at 5 days, 14 days, and 21 days.~~

~~6.2.4 Incubate agar cultures at 37°C aerobically and in a 5 % CO_2-95 % nitrogen atmosphere.~~

~~6.2.5 Microscopically examine the agar plates weekly~~ sufficient quantity to last for ~~at least 3 weeks for mycoplasma colony formation and growth before scoring them as negative. Observe the plates at 40, 100, and 300 magnification using~~ an ~~inverted microscope, or a standard microscope by inverting the plates. Mycoplasma colonies can be positively identified directly by subculturing or indirectly by staining.~~

~~6.2.5.1 Subculture a small section (1 cm^2)~~ extended period of ~~the suspicious area of the agar culture into a new broth culture and observe~~ time. Challenge mycoplasma strains for ~~turbidity and pH changes.~~

~~6.2.5.2 Use~~ the ~~Dienes stain (Hayflick, 1973) to stain colonies. True mycoplasma colonies will absorb~~ quality control test should be diluted so that approximately 100 colony-forming units are contained in the ~~stain.~~ 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.

7.2 Inoculate one 100-mL sample of fetal bovine serum into 400 mL of medium, and incubate for 21 days at 37°C.

7.3 After incubation for 5, 10, and 21 days, 0.1 mL is subcultured to each of two agar plates (see Practice E 1531). Incubate one plate anaerobically and one plate aerobically. Examine all plates after incubation for 5 and 14 days.

7.4 Serum is considered contaminated if typical mycoplasma colonies grow on the agar medium.

8. Keywords

~~7~~8.1 mycoplasma; serum