Designation: E 1533 – 00

Standard Practice for Indirect Detection of Mycoplasma in Cell Culture by 4′-6-Diamidino-2-2 Phenylindole (DAPI) Staining[1]

This standard is issued under the fixed designation E 1533; 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 procedures used for the detection of mycoplasma contamination by indirect DNA staining.

1.2 This practice does not cover direct methods for the detection of mycoplasma or other indirect methods such as enzymatical detection or DNA probes.

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

1.4 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.5 *This standard does not purport to address all of the safety problems, 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 Detection of Mycoplasma Contamination of Cell Cultures by Growth on Agrose Medium[2]

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

E 1536 Practice for Detection of Mycoplasma Contamination of Bovine Serum by the Large Volume Method[2]

3. Terminology

3.1 *Definitions:*

3.1.1 *DAPI staining*—staining of DNA in particular by using DAPI fluorochrome stain.

3.1.2 *direct detection of mycoplasma*—detection of mycoplasma by cultivation in culture media.

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

3.1.4 *mycoplasma*—the 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.

4. Significance and Use

4.1 Mycoplasma contamination of cell cultures is a common problem that can affect the growth, metabolism, and function of cultured animal cells. The ability to detect mycoplasma in cell cultures provides an opportunity to ensure that cells are free of contamination, and to replace those that are not. For additional information, see Practices E 1531, E 1532, and E 1536. Strict adherence to established, well-tested procedures is necessary. This practice was developed by Task Group E48.01.02 to assist in developing and maintaining an established regimen for mycoplasma detection by indirect 4′-6-Diamidino-2-Phenylindole (DAPI) fluorochrome staining.

4.2 This practice is intended for use in examining cultured animal cells for the presence of mycoplasma contamination.

4.3 This practice is not intended for use in the detection of mycoplasma contamination in serum, culture media, or systems other than cultures of animal cells.

4.4 All cell cultures to be examined for mycoplasma should undergo a minimum of two passages in antibiotic-free tissue culture medium before testing.

5. Quality Control

5.1 Visually examine the DAPI stain concentrate routinely for contamination. Fresh stock should be prepared periodically.

5.2 *Indicator cells*:

5.2.1 Indicator cells support the growth of mycoplasma species and provide positive and negative controls.

5.2.2 Use continuous cell lines such as the African green monkey kidney cell line, Vero, American Type Culture Collection (ATCC CCL81) as indicator cells as described in this practice; 3T6 mouse fibroblast (ATCC CCL 96) may also be used.

5.2.3 Do not use transformed cells as indicators since they produce large amounts of extra nuclear fluorescence.

6. Procedure

6.1 *Preparation of DAPI Stain Concentrate*:

6.1.1 Add 1.0 mg DAPI stain to 100 mL sterilized distilled water and mix thoroughly at room temperature.

6.1.2 The stain is heat and light sensitive. Prepare the concentrate in a bottle wrapped completely in aluminum foil,

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