Designation: E1533 – 00(Reapproved 2006)

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 E1533; 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 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:*[2]

E1531 Practice for Detection of Mycoplasma Contamination of Cell Cultures by Growth on Agarose Medium

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

E1536 Practice for Detection of Mycoplasma Contamination of Bovine Serum by Large Volume Method

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 E1531, E1532, and E1536. 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.

[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.

Current edition approved Nov. 1, 2006. Published December 2006. Originally approved in 1993. Last previous edition approved in 2000 as E1533 – 00. DOI: 10.1520/E1533-00R06.

[2] 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.