Designation: F 813 – 01

Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices[1]

This standard is issued under the fixed designation F 813; 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 a reference method of direct contact cell culture testing which may be used in evaluating the cytotoxic potential of materials for use in the construction of medical materials and devices.

1.2 This method may be used either directly to evaluate materials or as a reference against which other cytotoxicity test methods may be compared.

1.3 This is one of a series of reference test methods for the assessment of cytotoxic potential, employing different techniques.

1.4 Assessment of cytotoxicity is one of several tests employed in determining the biological response to a material, as recommended in Practice F 748.

1.5 The L-929 cell line was chosen because it has a significant history of use in assays of this type. This is not intended to imply that its use is preferred; only that the L-929 is an established cell line, well-characterized and readily available, that has demonstrated reproducible results in several laboratories.

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:*

F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices[2]

F 1027 Practice for Assessment of Tissue and Cell Compatibility of Orofacial Prosthetic Materials and Devices[2]

2.2 *Other Documents:*

The American Type Culture Collection (ATCC), Catalogue of Strains II[3]

USP Negative Control Plastic Reference Standard[4]

3. Summary of Practice

3.1 Cell cultures are grown to a confluent monolayer in culture dishes. The growth medium is aspirated and replenished to provide a resting, confluent cell layer. Test and control specimens are placed in direct contact with the cell layer to provide an accelerated assessment of the presence or absence of a cytotoxic effect from a given material or device. See Practice F 1027 for definitions.

4. Significance and Use

4.1 This practice is useful for assessing cytotoxic potential both when evaluating new materials or formulations for possible use in medical applications, and as part of a quality control program for established medical materials and medical devices.

4.2 This practice assumes that assessment of cytotoxicity potential provides one method for predicting the potential for cytotoxic or necrotic reactions to medical materials and devices during clinical applications to humans. In general, cell culture testing methods have shown good correlation with animal assays and are frequently more sensitive to toxic moieties.

4.3 This cell culture test method is suitable for adoption in specifications and standards for materials for use in the construction of medical devices that are intended to be implanted in the human body or placed in contact with tissue, tissue fluids, or blood on a long-term basis. However, care should be taken when testing materials that are resorbable to be sure the method is applicable.

5. Apparatus

5.1 The following apparatus shall be used:

5.2 *Incubator*, to maintain 37 ± 2°C and 4 to 6 % CO_2 with greater than 90 % relative humidity.

5.3 *Tissue Culture Grade Culture Dishes*, that are sterile and 35 mm in diameter by 10 mm deep.

NOTE 1—Plastic dishes are recommended because they provide a flat surface that contributes to the formation of a uniform cell monolayer.

[1] This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

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[2] *Annual Book of ASTM Standards,* Vol 13.01.

[3] American Type Culture Collection, 12031 Parklawn Drive, Rockville, MD 10852.

[4] *U.S. Pharmacopeia*, Vol 24, Rand McNally, Taunton, MA, 1994, pp. 1652–1653. Use latest publication to ensure current cumulative revisions are used.

5.4 *Disposable, Sterile, Centrifuge Tubes*.

5.5 *Inverted Optical Microscope*, with magnifications of 40×, 100×, and 200×.

5.6 *Clinical Centrifuge*, capable of attaining 1300xg.

5.7 *Filter Disks*—10 mm in diameter (for evaluation of liquids).

NOTE 2—Millipore AP2501000 filter disks have been found satisfactory for use in cytotoxicity evaluations because they elicit no cytopathic effect. Other filter disks that do not elicit a cytopathic effect may also be used.

5.8 *Water Bath*, capable of maintaining a temperature of 37 ± 2°C.

NOTE 3—A laminar flow work area capable of filtering out 99.99 % of all particles greater than 0.5 μm in diameter, or a class 100 clean room may be necessary to prevent contamination of cultures.

6. Reagents

6.1 The following reagents shall be used:

6.1.1 *Minimum Essential Medium* (MEM), prepared without L-glutamine and augmented by the addition of Earle's salts and 5–10 % fetal bovine serum.

NOTE 4—Glutamine is omitted from this formulation in order to maximize the shelf life of the medium. Immediately before use, 5 mL of L-glutamine solution (see 6.1.2) are added to each 500 mL of MEM.

NOTE 5—Opened containers of MEM may be stored at a temperature of 2 to 8°C for periods of not more than one week.

NOTE 6—Antibiotics, such as penicillin G 10,000 I.U./ml and streptomycin 10,000 I.U./ml, may be added to the medium (1 ml of antibiotic per 100 ml of media) to reduce the incidence of bacterial contamination. This may, however, have an adverse effect on the viability of the cell cultures.

6.1.2 *L-glutamine Solution*, 29.2 mg/mL of sterile water.

6.1.3 *Hanks' Solution*, calcium-and magnesium-free (store at room temperature).

6.1.4 *Trypsin*, 0.1 % solution in Hanks' solution or calcium-and magnesium-free, phosphate-buffered saline (store frozen).

6.1.5 *Water*, distilled, deionized, and sterile, with a minimum resistivity of 1 MΩ·cm.

6.2 All reagents shall be tissue-culture grade or equivalent.

6.3 Reagents shall be reconstituted in accordance with the manufacturer's directions, using aseptic technique.

6.4 Reagents shall be stored in accordance with the manufacturer's directions unless otherwise indicated in 6.1.

7. Cell Cultures

7.1 Cell cultures used in this assay should be the ATCC, CCL 1 NCTC clone 929 strain (clone of Strain L, mouse connective tissue) designated L-929. Other suitable validated cell lines may be considered. Cells should be tested periodically for Mycoplasma contamination.

8. Control Materials

8.1 Prepare negative control specimens in accordance with Section 10 from a material that consistently ellicits negligible cellular response in this assay (for example, USP Negative Control Plastic Reference Standard).

8.2 Prepare positive control specimens in accordance with Section 10 from a material that consistently elicits a predictable, moderate degree of cytotoxicity.

8.2.1 Use aqueous phenol (0.45 ± 0.05 % by volume) as a positive control for a diffuse reaction of cellular degeneration and sloughing. Take care to ensure that the preparation is homogenous.

8.2.2 Latex rubber has been used as a positive polymeric control for a zone of inhibition.

9. General Technique

9.1 Use the aseptic technique throughout this assay to minimize microbial contamination.

NOTE 7—Mouth pipetting should not be employed to transfer cells, medium, or reagents.

9.2 Warm all solutions and materials to a temperature of 37 ± 2°C before placing in contact with cells.

10. Preparation of Specimens

10.1 Sterilize all specimens by a method appropriate to the end use of the device.

10.2 Where a device is sufficiently small (see 10.3 and 10.4) to fit into the culture dish leaving an adequate margin of cells for evaluation, use the entire device as a specimen.

10.3 Cut large solid materials and devices in cross-section to obtain a flat surface having an area of 100 to 250 mm^2 to be placed in direct contact with the cell monolayer.

10.4 Prepare specimens of rod or tubing or of rod- or tube-shaped devices as follows:

10.4.1 Where the diameter is less than 6.4 mm, cut 5 to 15 mm in length.

10.4.2 Where the diameter is 6.4 to 15 mm, cut 2 to 8 mm in length.

10.4.3 Where the diameter exceeds 15 mm, prepare cross-sections as described in 10.3.

10.5 Obtain specimens from larger medical items from locations with relatively large cross-sections in order to expose interior material.

10.6 If a device is constructed of two or more materials, cut either the test specimen from the materials' interface or test separate specimens from each material.

10.7 Prepare specimens for evaluating the cytotoxicity of liquids or extracts by saturating a sterile filter disk and allowing the excess liquid to drain off while maintaining asepsis. Use the saturated filter disk as a test specimen.

NOTE 8—When ethylene oxide or other chemical sterilants are used, adequate aeration time should be allowed, to permit dissipation of residues which may adversely affect the results recorded in this assay.

NOTE 9—In general, specimens should be cleaned to remove any residues from specimen preparation, and sterilized after they have been cut to size. If the large solid materials are very hard, like ceramics, which require cutting with metal or diamond saws, care should be taken to remove any contamination from the metal blade or from the metal bonding the diamonds to the blade. When evaluating the cytotoxicity potential of medical materials or devices that are contained in the final sterile package, resterilization, further processing, or delay between the time of opening the package and starting the test must be avoided. With small items the entire content of the sterile package may be used as the test specimen. When the size of the sterile packaged item is too large, an appropriate, representative, small-sized specimen must be obtained. The application of this assay to items in the final sterile package is limited to items that are small or can be cut and reshaped with aseptic technique.

NOTE 10—The size and shape of test specimens may vary considerably,