Designation: F813 – 20

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

This standard is issued under the fixed designation F813; 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 practice 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 F748.

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 a well characterized, readily available, established cell line that has demonstrated reproducible results in several laboratories.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.7 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.8 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

F895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity

F1027 Practice for Assessment of Tissue and Cell Compatibility of Orofacial Prosthetic Materials and Devices

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 F1027 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 when only chemical toxicities are being considered.

[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] 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.

[3] American Type Culture Collection, P.O. Box 1549, Manassas, VA 20108..

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

NOTE 1—The results obtained using this method may not predict *in vivo* behavior which can be influenced by multiple factors such as those arising from site of application or physical properties that may result from design and fabrication.

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 have direct contact with tissue, tissue fluids, or blood. However, care should be taken when testing materials that are absorbable, include an eluting or degradable coating, are liquid or gelatinous in nature, are irregularly shaped solid materials, or have a high density or mass, to make sure that the method is applicable. If leachables from the test sample are capable of diffusing through the agar layer, agarose-based methods such as Test Method F895 may be considered as an alternate method, depending on sample characteristics, or in cases where investigators wish to further evaluate the cytotoxic response of cells underlying the test sample.

5. Apparatus

5.1 The following apparatus shall be used:

5.2 *Incubator,* to maintain a temperature of 37 ± 1°C with a humidified atmosphere of 4 to 6 % CO_2 in air.

5.3 *Tissue Culture Grade Culture Dishes,* that are sterile and 60 mm in diameter by 10 mm deep. Alternative sizes may be suitable if the grading recommended in Table 1 can be performed adequately.

NOTE 2—Plastic dishes are recommended because they provide a flat surface that contributes to the formation of a uniform cell monolayer.

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 3—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 4—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 with L-glutamine and augmented by the addition of Earle's salts and 5–10 % fetal bovine serum.

NOTE 5—Glutamine may be omitted from this formulation in order to maximize the shelf life of the medium. Prior to use, 5 mL of L-glutamine solution (see 6.1.2) should be added to each 500 mL of MEM.

NOTE 6—Opened containers of MEM may be stored at a temperature of 2 to 8°C for periods of not more than one week.

NOTE 7—Antibiotics, such as penicillin G10,000 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. A passage or doubling limit should be established within the laboratory with supportive data to prevent the use of aged cells in this practice, which may exhibit phenotypic abnormalities that could affect morphologic assessment.

8. Control Materials

8.1 Prepare negative control specimens in accordance with Section 10 from a material that consistently elicits 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 reactivity.

9. General Technique

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

NOTE 8—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.