



Designation: F 895 – 84 (Reapproved 2001)

Standard Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity¹

This standard is issued under the fixed designation F 895; 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.

This standard has been approved for use by agencies of the Department of Defense.

1. Scope

1.1 This test method is appropriate for materials in a variety of shapes and for materials which are not necessarily sterile. This test method would be appropriate in situations in which the amount of material is limited. For example, small devices or powders could be placed on the agar and the presence of a zone of inhibition of cell growth could be examined.

1.1.1 This test method is not appropriate for leachables that do not diffuse through agar or agarose.

1.1.2 While the agar layer can act as a cushion to protect the cells from the specimen, there may be materials that are sufficiently heavy to compress the agar and prevent diffusion or to cause mechanical damage to the cells. This test method would not be appropriate for these materials.

1.2 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.

1.3 *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 Devices²

2.2 ATCC Document:

American Type Culture Collection, (ATCC) Catalogue of Strains II³

USP Negative Control Plastic Reference Standard⁴

¹ This test method 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 Fourth edition, 1983, is available from American Type Culture Collection, 12031 Parklawn Dr. Rockville, MD 10892. Library of Congress No. 76-640122.

⁴ U.S. Pharmacopeia, Vol XXIII, Mack Publishing Co., Easton, PA, 1995, p. 1652–1653. Use latest supplement to ensure current cumulative revisions are used.

TABLE 1 Zone Description

Zone Index	Description of Zone
0	No detectable zone around or under specimen
1	Zone limited to area under specimen
2	Zone extends less than 0.5 cm beyond specimen
3	Zone extends 0.5 to 1.0 cm beyond specimen
4	Zone extends greater than 1.0 cm beyond specimen but does not involve entire dish
5	Zone involves entire dish

3. Summary of Test Method

3.1 Cell cultures are grown to a confluent monolayer in culture dishes. The medium is aspirated and replaced with an agar-containing medium that is allowed to solidify. Test control articles are placed on the agar surface to evaluate the cytotoxic properties of a given material or device. Toxic components in the test article can diffuse into the culture medium forming a concentration gradient and adversely affecting cells at varying distances from the test article. This method is well suited for low-density materials (film, paper, and so forth), powders, liquids, and high-density materials that could physically damage the cells if placed in direct contact with the cell monolayer.

4. Significance and Use

4.1 This test method is useful for assessing the cytotoxic potential of new materials and formulations and as part of a quality control program for established medical devices and components.

4.2 This test method assumes that assessment of cytotoxicity provides useful information to aid in predicting the potential clinical applications in humans. Cell culture methods have shown good correlation with animal assays and are frequently more sensitive to cytotoxic agents.

4.3 This cell culture test method is suitable for incorporation into specifications and standards for materials to be used in the

TABLE 2 Lysis Description

Lysis Index	Description of Zone
0	No observable cytotoxicity
1	Less than 20 % of zone affected
2	20 to 39 % of zone affected
3	40 to 59 % of zone affected
4	60 to 80 % of zone affected
5	Greater than 80 % of zone affected