Designation: ~~F749 – 13~~ F749 – 20

Standard Practice for Evaluating Material Extracts by Intracutaneous Injection in the Rabbit[1]

This standard is issued under the fixed designation F749; 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 is ~~a nonspecific, acute toxicity~~ an intracutaneous reactivity test used to ~~help determine~~ assess the ~~biocompatibility of materials used in medical devices.~~potential of the material under test to produce irritation following intradermal injections of extracts of the material.

1.2 The liquids injected into the rabbits are those obtained by Practice F619 where the extraction vehicles are saline, vegetable oil, or other liquids simulating human body fluids.

1.3 This practice is one of several developed for the assessment of the biocompatibility of materials. Practice F748 may provide guidance for the selection of appropriate methods for testing materials for a specific application.

1.4 The values stated in SI ~~units~~ units, including units officially accepted for use with the SI, are to be regarded as standard. No other ~~units~~systems of measurement are included in this standard.

1.5 *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]

F619 Practice for Extraction of Medical Plastics

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

3. Summary of Practice

3.1 The extract liquid is prepared in accordance with Practice F619. The extraction vehicles are saline and vegetable oil, or other extraction vehicles can be used, as described in Practice F619. The extract liquid is injected into rabbits and the animals are observed at regular intervals for 72 h for erythema, edema, eschar formation or necrosis.

4. Significance and Use

4.1 This practice is to be used to help assess the biocompatibility of materials used in medical devices. It is an acute toxicological test designed to ~~detect the presence of injurious leachable substances.~~evaluate any irritation caused by device materials by gross assessment.

4.2 This practice may not be appropriate for all types of implant applications. The user is cautioned to consider the appropriateness of ~~the method~~this practice in view of the materials being tested, their potential applications, and the recommendations contained in Practice F748.

NOTE 1—Some materials (e.g., absorbables) may result in an extract pH (e.g., ≤2.0 or ≥11.5) that cannot be used with this practice.

4.3 The only applicable limitation is the extract preparation. Refer to ~~Sections~~Section 4.3 ~~and 4.4~~ of Practice F619 for a description of this limitation.

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[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.

5. Apparatus

5.1 *Cages*—There shall be one cage for each rabbit exposed to one extract liquid. Each rabbit shall be uniquely identified with this identity recorded.

5.2 *Syringes*—Sterile syringes, not greater than 2 mL in volume, with a precision of no less than ±0.10 mL shall be used. Sterile needles of 21 to 26 gauge shall be used.

6. Test Animals

6.1 *Rabbits*—The rabbits shall be healthy thin-skinned albino type, not previously used for any test. Animal care shall be in accordance with the Guide for Care and Use of Laboratory Animals.[3] Rabbits with significant scars or wounds are not suitable for this test. ~~For each extraction vehicle, a~~ A minimum of three rabbits are used in the test. If the results of the first test are inconclusive, three more rabbits will be needed to complete the ~~test with that extraction vehicle for one material.~~test.

6.1.1 During the test, the rabbits shall be fed ~~normally, with commercially available feed and tap~~ appropriate laboratory animal diet and potable, uncontaminated drinking water.

7. Sampling

7.1 Sample in accordance with Practice F619.

8. Sample and Test Specimen

8.1 The sample is the extract of the test article (that is, plastic or other material) exposed to the extraction procedure. As a result of the extraction in Practice F619, for each extraction vehicle there are available: ~~(1~~*(1)*~~)~~ sample extract liquid, and ~~(2~~*(2)*~~)~~ a blank extract liquid. These extract liquids are to be injected into the test animals within 24 h of the end of the extraction procedure. Record storage conditions if not used immediately after preparation.

8.1.1 There are usually four extract liquids prepared from two extraction vehicles available for test, those based on saline and vegetable oil. Samples based on other extraction vehicles may be available, as described in Practice F619, or as required by the standard for the medical device. Both extracts and blank controls shall be subjected to the same extraction temperature and time prior to use in the test animals.

8.2 The test specimen is the combination of the test site and 0.2 mL of the injected extract liquid. A total of 10 sites are to be injected with the sample extract liquid ~~and 10 sites with the blank extract liquid.~~(i.e., 5 sites with saline and 5 sites with vegetable oil extracts) and 10 sites (i.e., 5 each) with the corresponding blank extract liquids.

9. Procedure

9.1 *Preparation of Rabbits*—On the day (no more than 24 h) before the test, closely clip the fur on the animal's back on both sides of the spinal column over a sufficiently large test area. Avoid mechanical irritation and trauma. Remove loose hair by means of a vacuum. The use of a depilatory agent that does not cause skin irritation in place of or in addition to clipping may be desirable. Swab the skin ~~slightly~~gently with diluted alcohol, and dry the skin prior to injection.

9.2 Agitate each extract liquid vigorously prior to withdrawal of each injection dose to ensure even distribution of the extracted matter. If the extract liquid appears to contain particulates, record this and consider it when reporting the results.

9.3 Inject intracutaneously 0.2 mL of the sample extract liquid per site at five sites on one side of the dorsal midline of each of ~~two~~three rabbits. Similarly, at five other sites on the other side of the dorsal midline of each rabbit, inject 0.2 mL per site of the corresponding blank extract liquid. See Fig. 1.

9.4 Examine the injected sites 24, 48, and 72 h after the injection for gross evidence of tissue reaction, such as erythema, edema, eschar, or necrosis. To facilitate the examination, swab the skin lightly with diluted alcohol, and clip the fur, if necessary. ~~Rate~~ On each animal, rate the tissue reaction for all ten sites of the sample (5 per extract) and for ~~the five~~all ten sites of the blank ~~extract~~ extracts (5 per extract liquid) at each observation period for each type of tissue reaction. The rating scales for erythema and edema are given in Tables 1 and 2.

10. Interpretation of Results

~~10.1 A sample extract passes the test if each type of tissue reaction of the test and blank extracts are similar for all observation periods.~~

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[3] ~~The~~ National Research Council Guide for the Care and Use of Laboratory Animals, 8th ed. (2011), Institute of Laboratory Animal Research ~~Publication. Available from National Academy Press, 500 Fifth St., NW, Lockbox 285, Washington, DC 20055.~~Division on Earth and Life Sciences, Washington, D.C., National Academies of Science Press (http://www.nap.edu/catalog.php?record_id=12910).

[4] ~~"<88> Biological Reactivity Tests, *in Vivo*," *U. S. Pharmacopeia*, Vol 26, Rockville, MD, 2002.~~

[5] ~~Brewer, John H., "Toxicity Standards for Plastics," Bulletin of Parenteral Drug Association, Vol 19, 1965, pp. 22–28.~~