Designation: F 749 – 98 (Reapproved 2002)ε1

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

This standard is issued under the fixed designation F 749; 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.

ε[1] NOTE—Footnote 3 was editorially corrected in November 2002.

1. Scope

1.1 This practice is a nonspecific, acute toxicity test used to help determine the biocompatibility of materials used in medical devices.

1.2 The liquids injected in the rabbits are those obtained by Practice F 619 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 F 748 may provide guidance for the selection of appropriate methods for testing materials for a specific application.

1.4 The values stated in SI units are to be regarded as the standard.

2. Referenced Documents

2.1 *ASTM Standards:*

F 619 Practice for Extraction of Medical Plastics[2]

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

3. Summary of Practice

3.1 The extract liquid is prepared in accordance with Practice F 619. The extraction vehicles are saline and vegetable oil, or other extraction vehicles can be used, as described in Practice F 619. The extract liquid is injected into rabbits and the animals are observed at regular intervals for 72 h for erythema, edema, and 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.

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 in view of the materials being tested, their potential applications, and the recommendations contained in Practice F 748.

4.3 The only limitation applicable is the extract preparation. Refer to Sections 4.3 and 4.4 of Practice F 619 for a description of this limitation.

5. Apparatus

5.1 *Cages*—There shall be one cage for each rabbit exposed to one extract liquid. Each rabbit will 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 gage shall be used.

6. Test Animals

6.1 *Rabbits*—The rabbits shall be thin-skinned albino type, healthy, and not previously used for any test. Animal care shall be in accordance with 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 minimum of two rabbits are used in the test. If the results of the first test are inconclusive, three more rabbits are needed to complete the test with that extraction vehicle for one material.

6.1.1 During the test the rabbits shall be fed normally, with commercially available feed and tap water.

7. Sampling

7.1 Sample in accordance with Practice F 619.

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 F 619, for each extraction vehicle there are available: (*1*) sample extract liquid, and (*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

[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] *The Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Research Publication. Available from National Academy Press, 500 Fifth St., NW, Lockbox 285, Washington, DC 20055.

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 F 619, or as required by the standard for the medical device.

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.

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 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 and consider in reporting results.

9.3 Inject intracutaneously 0.2 mL of the sample extract liquid at five sites on one side of each of two rabbits. Similarly, at five other sites on the other side of each rabbit, inject 0.2 mL of the corresponding blank extract liquid.

9.4 Examine the injected sites 24, 48, and 72 h after the injection for gross evidence of tissue reaction, such as erythema, edema, and necrosis. To facilitate the examination, swab the skin lightly with diluted alcohol, and clip the fur, if necessary. Rate the tissue reaction for all ten sites of the sample and for the five sites of the blank extract 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.

TABLE 1 Severity Rating for Erythema

Severity of Erythema	Numerical Rating
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

TABLE 2 Severity Rating for Edema

Severity of Edema[A]	Numerical Rating
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area defined by definite raising)	2
Moderate edema (area raised approximately 1 mm)	3
Severe edema (area raised more than 1 mm and extending beyond area of injection)	4

[A] Edema is tissue swelling. Apparent swelling attributable to the injection vehicle is not considered edema.

10.2 *Retest*—If either rabbit has a sample extract tissue reaction of moderate or severe but the other does not, repeat the test using fresh extracts in three more rabbits. The sample extract passes if in this retest the tissue responses for the sample extracts are not biologically different from those for the blank extract liquid.[4]

10.3 A retest (see 10.2) requires that the extraction procedure be done a second time, since the extraction fluids must be used within 24 h of the end of the extraction.

11. Report

11.1 Describe the sample that was extracted, including generic name, trade name, manufacturer's code, catalog number, date of manufacture, formulation, fabrication procedures or processes, etc., as appropriate. Describe the extraction vehicle and the conditions of the extraction (temperature and time).

11.2 Report the scores for each type of tissue reaction at each observation period as described in 10.1 for the test and control extracts.

11.3 If a retest was made, report the data for that test, as in 11.2.

12. Precision and Bias

12.1 Intralaboratory and interlaboratory reproducibility have not been systematically determined. Reproducibility may be inferred from previous round robin studies.[5,6]

13. Keywords

13.1 acute toxicity tests; biocompatibility; intracutaneous injection; rabbits; test animals

[4] *U. S. Pharmacopeia*, Vol 23, Rand McNally, Taunton, MA, 1994, pp. 1699–1703.

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

[6] Materials Science Toxicology Laboratories, University of Tennessee Center for the Health Sciences, Memphis, Tenn., "Determination of Levels of Chemical Purity for Biomaterials Used as Surgical Implants," *Round Robin Evaluation of Primary Acute Toxicity Screening Protocols*, Quarterly Report No. 15–16, Part II, Contract No. FDA 223-73-5231, 1978.