Designation: F 719 – 81 (Reapproved 2002)ε1

Standard Practice for Testing Biomaterials in Rabbits for Primary Skin Irritation[1]

This standard is issued under the fixed designation F 719; 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—Editorial changes were made throughout in June 2002.

1. Scope

1.1 This practice covers a procedure by which the irritancy of a biomaterial may be assessed through contact with abraded and intact skin of rabbits.

1.2 The results of this practice depend upon the effectiveness with which contact between skin and the test material is established and maintained. Because of the operator technique included in performing this test, it is important that the test be performed by personnel with appropriate training.

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 619 Practice for Extraction of Medical Plastics[2]

3. Summary of Practice

3.1 Exposure of skin to the test material is accomplished by means of a patch test technique employing two intact and two abraded sites on the back of each of six albino rabbits. The skin is clipped free of hair one day prior to testing. The test substance is applied using 0.5 mL for liquids, 0.5 g for solids or semisolids, and a 2.5 by 2.5-cm square patch for films. After application, each test site is covered with a 2.5 by 2.5-cm gauze flat, and the entire trunk is occluded with a polyethylene sleeve. After 24 h, the sleeve, flat, and test material are removed, and test sites are evaluated for erythema and edema.

4. Significance and Use

4.1 Materials that are to be in contact with the skin should not cause irritation to the skin. Since it is probably the substances leached from a material that cause the irritation, this practice provides for direct material-skin contact testing or for skin exposure to the liquid extract of the test material. The rationale for this rabbit test is that it is a comparatively quick and inexpensive method which, through use over the years, has become a generally accepted method.

5. Materials and Manufacture

5.1 *Young New Zealand Albino Rabbits*,

5.2 *Gauze Flats*, 2.5 by 2.5-cm,

5.3 *Polyethylene Sleeves*, extra clear, and

5.4 *Adhesive Tape*, ½-in.

6. Test Specimen

6.1 The test specimen may be one of three forms:

6.1.1 Test 0.5 mL of liquids or saline extract liquids obtained in accordance with Practice F 619.

6.1.2 Test 0.5 g of solids or semisolids.

6.1.3 Test films 2.5 by 2.5 cm.

NOTE 1—A vehicle control for liquids is required because of the potential for false positive due to skin temperature changes when handling rabbits. Positive controls may be used to validate the test method. The use of 5 % procaine HCl as a positive control is suggested.[3]

6.2 The pH of the solutions should be measured and reported, if appropriate.

7. Procedure

7.1 *Preparation of Test Animals*:

7.1.1 Twenty-four hours before the test, clip the hair from the backs of the animals so as to expose two test areas on each side of the spine, which are 10 cm apart.

7.1.2 To obtain more effective contact between the skin and the test substance, it may be necessary to use a nonirritating depilatory agent. This test method may be used to ensure that the depilatory agent is nonirritating.

7.1.3 Test sites may be designated as two on each side of the spine. Alternatively, the area may be divided into quadrants with test and control substances applied to each quadrant.

7.2 *Test Procedure*:

7.2.1 Wipe the exposed area of the back with alcohol.

7.2.2 Using a sterile blade, abrade two of the four sites by moving the blade at right angles to the cutting surface in a

[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.

Current edition approved April 24, 1981. Published July 1981. Originally published as F 791 – 81.

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

[3] H. H. Draize, *Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics*, 1965, p. 46.

Copyright © ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States.

scraping motion to create a denuded area of skin. Alternatively, make four epidermal incisions (which penetrate the stratum corneum but not the dermis), with two perpendicular to the other two.

7.2.3 Place the appropriate amount of the test material, as described in 6.1, on one intact and one abraded or incised site. Place the control material on the other abraded and intact sites.

7.2.4 Immediately occlude the sites by placing gauze flats on the test and control sites. Secure the patches with adhesive tape.

7.2.5 Tightly wrap the animal's trunk with a clear polyethylene sleeve.

7.3 *Test Site Examination and Scoring*:

7.3.1 Remove the polyethylene sleeve, gauze flats, test and control materials, and any excess test or control liquids 24 h after applying the test and control substances. Use alcohol solutions for removing excess liquids.

7.3.2 Using the criteria of Table 1, score test sites for erythema and edema 1 h after removal.

7.3.3 Rescore test sites for erythema and edema at 24 and 48 h after removal in accordance with Table 1. Take care to distinguish test site erythema from minor skin temperature changes.

NOTE 2—There is a possibility of infection associated with skin abrasion. Since infection would cause the same symptoms (erythema and edema) as would primary irritation, it is essential to ensure that the reaction is not due to infection. Any reaction at the control sites would be an indication of infection. If infection is suspected, the test should be repeated with a new animal, and the test substance cultured.

TABLE 1 Scoring Criteria for Test Reactions

Reaction	Description	Score
Erythema (ER)	Erythema and Eschar	
	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well-defined erythema (pale red in color)	2
	Moderate to severe erythema (red and area well defined)	3
	Severe erythema (beet redness to slight eschar formation)	4
Edema (ED)	Edema Formation	
	No edema	0
	Very slight (barely perceptible)	1
	Slight edema (edges of area well defined by definite raising)	2
	Moderate edema (edges raised approximately 1 mm)	3
	Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

8. Interpretation and Calculation of Results

8.1 Score abraded and intact test sites for erythema and edema in accordance with Table 1.

8.2 The primary irritation index (PII) for each test animal and substance is the total of the abraded (incised) and intact skin scores for erythema and edema at 1, 24, and 48 h after removal, divided by six. The average primary irritation index is equal to the sum of the PIIs for each substance for all test animals divided by the number of test animals.

9. Report

9.1 The report should contain a description of the test material, batch or lot number, vehicles, and extraction procedure used.

9.2 Report the pH value of test solution and vehicle solution if appropriate.

9.3 Report test data for erythema and edema in accordance with Fig. 1.

10. Keywords

10.1 acute toxicity tests; biocompatibility; rabbits; skin irritation; test animals

Primary Skin Irritation Test for Albino Rabbits
Test Material _____
Control Material _____

Results

Animal Number	Irritation Scores for Abraded Skin Sites after Removal						Irritation Scores for Intact Skin Sites after Removal						PII
	1 h		24 h		48 h		1 h		24 h		48 h		
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	
1													
2													
3													
4													
5													
6													

Subtotal (PII sum) _____
Average Primary Irritation Index (sum/6) _____

FIG. 1 Sample Report Form

ASTM International takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, at the address shown below.

This standard is copyrighted by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States. Individual reprints (single or multiple copies) of this standard may be obtained by contacting ASTM at the above address or at 610-832-9585 (phone), 610-832-9555 (fax), or service@astm.org (e-mail); or through the ASTM website (www.astm.org).