Designation: F2147 – 01 (Reapproved 2016)

Standard Practice for Guinea Pig: Split Adjuvant and Closed Patch Testing for Contact Allergens[1]

This standard is issued under the fixed designation F2147; 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 intended to determine the potential for a substance, or material extract, to elicit contact dermal allergenicity.

1.2 This practice is intended as an alternative to the Guinea Pig Maximization Test (GPMT), given the limitations on dosage form and tendency for false positives associated with the latter test. See Rationale and References.

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

1.4 *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:*[2]

F619 Practice for Extraction of Medical Plastics

F720 Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test

2.2 *ISO Document:*

ISO 10993-10, 1995 Tests for Irritation and Sensitization[3]

3. Terminology

3.1 *Definitions:*

3.1.1 *2,4 dinitrochlorobenzene* (DNCB)—strong sensitizer, used as a positive control.

3.1.2 *Freund's Complete Adjuvant* (FCA)—a commercially-available mixture of oil and *Mycobacterium* that is known to elicit an immune response.

3.1.3 *Guinea Pig Maximization Test* (GPMT)—procedure described in Practice F720 accepted as a "worst case" assay for allergenic potential.

4. Summary of Practice

4.1 The split adjuvant method is used when topical application is considered relevant, and the dosage form is a solid, liquid, extract, paste, or gel. The method includes four induction doses applied over ten days to the same shaved or depilated site on guinea pigs, followed by occlusive patching. Freund's Complete Adjuvant (FCA) is injected near the dose site on the fourth day (second induction dose). Following a rest period, animals are challenged at a previously unexposed site, and the reaction evaluated at 24, 48, and 72 h.

4.2 The closed patch method is used when topical application is relevant, but the preferred dosage form does not permit injection under the skin or intradermally, and the discomfort involved with extended occlusive patching and adjuvant use is to be avoided. It involves repeated induction doses (3 to 6) over 14 days at the same shaved/depilated site, followed each time by 6 h of occlusive wrapping. After a rest period, animals are challenged at previously untreated sites, and their reactions evaluated at least 24 and 48 h later.

5. Significance and Use

5.1 In selecting a material for human contact in medical applications, it is important to ensure that the material will not stimulate the immune system to produce an allergic reaction under relevant exposure conditions. Extractable chemicals produced by skin contact or during physiological exposures may cause allergic reactions. Therefore, this practice provides for evaluations of solid or semisolid dosage forms using material extracts or direct evaluation of the test article. The rationale for this animal model is based on the fact that the guinea pig has been shown to be an appropriate animal model for predicting human contact dermatitis. Its tractable nature, its availability from reputable suppliers, the historical database of

[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] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

information already acquired using this species, and the correlation of such results to data on known human allergens, all contribute to its widespread use for allergenicity studies (**1-5**).[4]

5.2 The need for sensitization procedures other than the maximization test (Practice F720) is based on: (*1*) the need for a route of exposure more similar to use conditions; (*2*) concern over the use of adjuvant because of its recruitment of cell types to the test site which are not typically involved in immunologic reactions, and because of the discomfort this causes in the animals; (*3*) absence of a proper FCA-irritant control group in the traditional maximization design; and (*4*) the frequency of false positives often encountered with the GPMT. Both of these tests are internationally accepted (**1**).

6. Materials and Manufacturers

6.1 Hartley strain guinea pigs, either sex (but all in the test of the same sex), 300 to 500 g at the start of the test, should be from the same shipment and supplier, and should be healthy.

6.2 At least ten animals are used for each test material and five for each control group.

6.3 Freund's Complete Adjuvant (FCA) (split adjuvant test only).

6.4 Cotton gauze and occlusive bandage (examples, Elastopore from 3M) or Hilltop chambers (Hilltop, Cincinnati, OH) (optional for solid samples) and Vet wrap.

6.5 Positive control substance (0.1 to 1 % 2,4 DNCB is a strong sensitizer; to test method sensitivity, it may be advisable to use cinnamaldehyde (10 % induction, 1 % challenge) as a positive control. (**2**)

7. Preparation of Test Samples

NOTE 1—All steps are applicable to both methods.

7.1 *Solid Samples*—Cut flat sheet-like samples into 1- by 1-cm squares. These can be used for direct contact testing as long as the sample thickness does not exceed 1.0 mm.

NOTE 2—Pressure exerted by bandaging thick samples causes mechanical irritation. The cotton pad may be removed from the Hilltop chamber (or the chamber need not be used) to reduce pressure on thick solid test articles. Further cutting should be considered if test articles are still causing pressure without the chamber or chamber pad.

7.2 *Gels, Pastes, Ointments*—Semisolid test articles can be used directly, applied at 0.2 mL/site.

7.3 *Extracts*—Prepare extracts in accordance with Practice F619, at the highest temperature tolerated by the material without physical melting or decomposition. Both aqueous and nonaqueous extracts are recommended. Extracts should be decanted upon cooling, stored at room temperature (22 to 30°C), and used within 24 h. Extracts should be prepared fresh for each treatment, preferably using a solvent which does not give background reactions (ethanol is sometimes a problem in this regard), and is known to produce measurable extractables (determined by a technique such as a nonvolatile residue test) without dissolving the test article.

7.4 *Negative Controls*—Prepare solvent sham controls ("blanks") under the same conditions as test article extracts. Saline controls may be eliminated if sufficient data to predict their results are available.

7.5 *Positive Controls*—Positive controls should be prepared fresh before induction in the same solvent used for extraction if possible. If the solvent is volatile, a fresh solution may be needed for challenge. The use of amber bottles with minimum headspace should also be considered. Alternatively, positive control testing may be performed quarterly or at another reasonable frequency if the laboratory performs significant numbers of these tests and results are consistent. The latter practice reduces animal usage.

8. Trial and Naive Challenge Tests

8.1 It is recommended that at least two guinea pigs be used to assess the ability of the test article or undiluted extract to irritate. Each flank of each animal can be used to patch two sites (upper and lower) of samples such as the test article, 100 % extract, 75 % extract, and 50 % extract. Animals should be shaved and wrapped as in the complete test (see Section 9), and the sites evaluated after 24 to 72 h. Scoring should also be performed as in the complete test.

8.2 It is also advisable to determine the difference between irritation and sensitization under full test conditions for the positive control by including in at least one test per laboratory a "naive challenge" group which is exposed to controls only for the challenge period. DNCB, for example, can be an irritant, and it is important that erythema and edema reactions seen after challenge be true sensitization responses.

9. Procedure

NOTE 3—This procedure is applicable to both methods except as noted.

9.1 Table 1 shows the timing of animal preparation, induction dosing, challenge, and evaluation.

9.2 *Animal Preparation:*

9.2.1 Weigh and shave or depilate animals within 24 h of test start. Depilatories should be used carefully and tested beforehand to understand proper use regimen so as not to produce background irritation. Shave or depilate a site on the left flank or shoulder area (use one or the other consistently) approximately a 2-in. square to expose bare skin, avoiding any abrasions or other abnormalities. Check animal health daily throughout the test.

9.2.2 Apply 0.3 mL of extract or semisolid (or less, if the amount has been validated, or 1 cm^2 of a solid sample (less than 1.0 mm thick) to the cotton pad of a Hilltop chamber. (A padless chamber can be used to dose gels or thicker samples). Stick the chamber to the skin and wrap with an appropriate elastic bandage. If a Hilltop chamber is not used, apply the test sample to gauze and cover with occlusive wrap. Follow the unwrap/evaluate schedule for the particular procedure as in Table 1.

9.2.3 After unwrapping, wait about 30 min before evaluation. The test article may be removed by gentle wiping with gauze soaked with purified water or isopropyl alcohol (IPA)

[4] The boldface numbers in parentheses refer to the list of references at the end of this standard.

TABLE 1 Timing of Animal Preparation, Induction Dosing, Challenge, and Evaluation

Day(s) of Study	Test Dose(s)	Activity	
		Modified Split Adjuvant	Closed Patch
–1	NA	randomize/shave	randomize/shave
1	0.3 mL of liquid or a 1-cm^2 solid piece (thickness <1 mm)	apply dose to upper flank; bandage occlusively	apply dose to upper flank; bandage occlusively for 6 h, then evaluate
3	0.3 mL of liquid or a 1-cm^2 solid piece (thickness <1 mm)	unwrap[A] ; evaluate after stabilization period (~30 min). apply new samples	NA
5	0.3 mL of liquid or a 1-cm^2 solid piece (thickness <1 mm)	unwrap and evaluate. apply samples and wrap; inject 1:1 FCA in water around test sites (0.05 mL per injection; 0.2-mL total)	NA
7–8	0.3 mL of liquid or a 1-cm^2 solid piece (thickness <1 mm)	unwrap; evaluate. apply samples	apply samples; wrap occlusively for 6 h, then unwrap and evaluate
9–10	0.3 mL of liquid or a 1-cm^2 solid piece (thickness <1 mm)	unwrap; evaluate	NA
9–23 or 10–24	NA	rest period	NA
14	0.3 mL of liquid or a 1-cm^2 solid piece (thickness <1 mm)	NA	apply samples; wrap for 6 h, then unwrap and evaluate
14–28	0.3 mL of liquid or a 1-cm^2 solid piece (thickness <1 mm)	NA	rest period
23 or 24	0.3 mL of liquid or a 1-cm^2 solid piece (thickness <1 mm)	Shave new site on opposite upper flank ~2 h before treatment; apply sample; wrap	NA
24/25	NA	unwrap; evaluate	NA
25/26	NA	evaluate	NA
26/27	NA	evaluate	NA
28	0.3 mL of liquid or a 1-cm^2 solid piece (thickness <1 mm)	evaluate; do not redose	shave new site on opposite flank ~2 h before treatment; apply sample, wrap for 6 h
29	NA	NA	evaluate
30	NA	NA	evaluate
31	NA	NA	evaluate

[A] Wrapping of a shorter duration may be used if validated.

that has been diluted such that it will not dry the skin. Evaluate the site using the criteria in Table 2. Rewrap if required (split adjuvant.)

TABLE 2 Evaluation Criteria

Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible and patchy)	1
Well-defined erythema (slight but confluent, or moderate patchy)	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Necrosis	N
Scab	S
Edema Formation	**Value**
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

9.2.4 Repeat doses as outlined in Table 1. At the second dose of the split adjuvant procedure, inject 0.05 mL of FCA emulsified 1:1 with water for injection at four locations bordering every test and control site (0.2 mL total).

9.2.5 At the end of the induction period, allow the animals to rest unwrapped for 10 to 14 days.

9.2.6 Challenge using the same procedures as for induction, but at a site on the right shaved flank/shoulder.

9.2.7 Unwrap and evaluate as described in Table 2. It is recommended that the reader be experienced, and unfamiliar with the site treatment during reading.

10. Interpretation and Results

10.1 On the same day post-challenge all of the positive control animals shall have scores ≥1 (one level above the highest negative control score), or at least 60 % of these animals must have scores ≥2 (at least one level higher than the highest negative control score). A majority of the negative control group should have scores of 0, and no score should be above 1.

10.2 Response frequency is calculated by dividing the number of animals in each group with a positive response

(scores at least one level higher than the highest negative control score) by the total number of animals treated in that group.

10.3 For a material to be considered a sensitizer, a majority (>50 %) of the animals in a treatment group must be considered sensitized. The level and frequency of scores determine the degree of the sensitization, with frequency being the more important. A low frequency of high scores is unusual and may suggest a retest or another type of evaluation/investigation is needed. A high frequency of low scores may also require a reassay for clarification. Classification of materials by assay results is not provided, as it is up to the device manufacturer to determine the acceptability of test results.

10.4 If there is any question about the frequency, relevance, or reproducibility of scores, rechallenge the questionable group (along with appropriate controls) at new sites seven to nine days after the last challenge observation.

11. Report

11.1 Report the following information:

11.1.1 Test and control material descriptions, generic names, product names, manufacturers' names and addresses, and lot numbers;

11.1.2 Method of preparation of each extract;

11.1.3 General conditions of animal health;

11.1.4 Scoring of erythema and edema for each animal at each scoring period (see Tables 1 and 2);

11.1.5 Overall assessment of sensitization response.

12. Precision and Bias

12.1 Precision and bias for this practice has not been determined because the required studies would be time-consuming, expensive, and an inappropriate use of animals.

13. Keywords

13.1 acute toxicity; allergenicity; biocompatibility; guinea pigs; immunotoxicity; sensitization

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 The maximization test is a "worst case" evaluation of potential allergenicity under exposure and physiological conditions which do not mimic most medical applications. Materials are injected into the skin, and therefore, dosage forms are limited to liquids, or extracts prepared in physiologically acceptable solvents, for example, saline, which often is a poor polymer solvent. The use of Freund's Adjuvant, although included here for a modified split adjuvant test, can be criticized as recruiting types of immune cells to the exposure site that would not be involved under typical exposure conditions. The FCA and extensive wrapping can cause significant discomfort to the animals; FCA use requires the animals be reported in the U.S. Department of Agriculture Category E for pain and suffering.

X1.2 The original GPMT design has been criticized by its own originators (**6**) as lacking a necessary control group, and careful interpretation is required because of its higher frequency of false positives. Although the closed patch technique has been criticized by some, it is accepted internationally (**1**), and can be tested using cinamaldehyde as a positive control. The 5/6/99 FDA immunotoxicity guidance allows for GPMT and other sensitization test procedures, as long as the choice of procedure can be justified.

X1.3 Table X1.1 has been provided to compare the various aspects of the methods here, along with the GPMT. See details of procedures in Tables 1 and 2.

TABLE X1.1 Comparisons of Three GPSS Methodologies

Parameter/Consideration	Method		
	GPMT (Practice F720)	Split Adjuvant	Closed Patch
Use of adjuvant (considered by some an animal welfare concern and responsible for artifacts)	X (up to 0.1 mL per injection; mixed with test material 1:1, or given alone)	X (0.2 mL in 0.05-mL doses around site)	NA
Length of assay	up to 26 days	28 days	31 days
Involves doses that cross the skin barrier	X	NA	NA
Tests extracts and solids	solids or semisolids may be problematic	X	X
Sensitivity	high (may result in false positives)	good, per literature references	good, per literature references
Animal trauma	adjuvant and wrapping	adjuvant and wrapping	wrapping of only 6 h required
Uses irritant to heighten response	X	NA	NA
Doses	0.1 mL	up to 0.3 mL or 1 cm^2	up to 0.3 mL or 1 cm^2
Wrapping	occlusive	occlusive	occlusive
Uses a classification system based on results	X	NA	NA

REFERENCES

(1) Immunotoxicity Testing Guidance 5/6/99 http://www.fda.gov/cdrh/ost/ostggp/immunotox.html.

(2) Ritz, H.L., and Buehler, E.V., "Planning, Conduct, and Interpretation of Guinea Pig Skin Sensitization Patch Tests," in *Current Concepts in Cutaneous Toxicity*, Academic Press, NY, 1980, pp. 25-42.

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(4) Maguire, H.C., "The Bioassay of Contact Allergens in the Guinea Pig," *J. Soc. Cosmet. Chem.*, Vol 24, 1973, p. 151.

(5) Marzulli, F.N., and Maibach, H.I., Eds., *Dermatoxicology*, third edition, Hemisphere Publishing, Washington, DC, 1987 , pp. 227-275.

(6) Kligman, A.M., and Basketer, D.A., "A Critical Commentary and Updating of the Guinea Pig Maximization Test," *Contact Dermatitis*, Vol 32, 1995, pp. 129-134.