Designation: F2147 – 01 (Reapproved 2010)

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 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 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]

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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.

Current edition approved Sept. 1, 2010. Published November 2010. Originally approved in 2001. Last previous edition approved in 2006 as F2147 – 01 (2006). DOI: 10.1520/F2147-01R10.

[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.

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

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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 start of test, should be from the same shipment, same 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 there are sufficient data available to predict their results.

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 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) 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.)

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.