



Standard Practice for Evaluation of Delayed Contact Hypersensitivity Using the Murine Local Lymph Node Assay (LLNA)¹

This standard is issued under the fixed designation F 2148; 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 provides a methodology to use an in situ procedure for the evaluation of delayed contact hypersensitivity reactions.

1.2 This practice is intended to provide an alternative to the use of guinea pigs for evaluation of the ability of a device material to stimulate delayed contact hypersensitivity reactions. This alternative is particularly applicable for materials used in devices that contact only intact skin. However, the guinea pig maximization test is still the recommended method when assessing the delayed hypersensitivity response to metals or when testing substances that do not penetrate the skin but are used in devices that contact deep tissues or breached surfaces. The guinea pig maximization test should be used for these substances.

1.3 This practice consists of a protocol for assessing an increase in lymphocyte proliferation within the nodes draining the site of administration on the ears of mice.

1.4 The LLNA has been validated only for low molecular weight chemicals that can penetrate the skin. The absorbed chemical or metabolite must bind to macromolecules, such as proteins, to form immunogenic conjugates.

1.5 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.

1.6 *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.*

1.7 Identification of a supplier of materials or reagents is for the convenience of the user and does not imply single source. Appropriate materials and reagents may be obtained from many commercial supply houses.

2. Referenced Documents

2.1 ASTM Standards:

F 619 Practice for Extraction of Medical Plastics²

F 720 Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test²

F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices²

F 750 Practice for Evaluating Material Extracts by Systemic Injection in the Mouse²

2.2 Other Document:

ICCVAM NIH Publication No: 99-4494 The Murine Local Lymph Node Assay, 1999³

3. Terminology

3.1 Definitions:

3.1.1 *AOO*—acetone olive oil solution (4:1 v/v) is a suitable nonpolar solvent.

3.1.2 *aqueous solvent*—in this assay refers to the polar solvent, saline.

3.1.3 *DMSO*—dimethylsulfoxide (nonaqueous, suitable organic solvent).

3.1.4 *DNCB*—2,4-dinitrochlorobenzene.

3.1.5 *formalin*—a 1/10 dilution of 37 to 39 % formaldehyde solution (formaldehyde) in PBS.

3.1.6 *ICCVAM*—Interagency Coordinating Committee on the Validation of Alternative Methods.

3.1.7 *nonaqueous solvent*—in this assay refers to the organic or nonpolar solvent, which shall be dimethylsulfoxide (DMSO) or acetone olive oil (AOO).

3.1.8 *PBS*—phosphate buffered saline pH 7.2.

3.1.9 *positive control*—a substance capable of consistently stimulating lymphocyte proliferation.

3.1.10 *saline*—0.9 % sodium chloride (aqueous, polar solvent).

3.1.11 *TCA*—5 % trichloroacetic acid.

3.1.12 *tritiated thymidine*—³H methyl thymidine, specific activity 2 Ci/mM (in PBS) I¹²⁵ IUDR-radioactive uridine.

3.1.13 *vehicle controls*—an aqueous, polar solvent and a non-aqueous, nonpolar solvent.

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 Oct. 10, 2001. Published January 2002.

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

³ Available from NICEATM, NIEHS, 79 Alexander Dr., Mail Drop EC-17, Research Triangle Park, NC 27709.