Designation: D6355 – 07 (Reapproved 2022)

Standard Test Method for Human Repeat Insult Patch Testing of Medical Gloves[1]

This standard is issued under the fixed designation D6355; 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 test method is designed to evaluate the potential of glove materials under test to induce and elicit Type IV skin sensitization reactions (that is, allergic contact dermatitis) in humans.

1.2 This test method should be used by individuals experienced in or under the supervision of those experienced in the use of good clinical practice procedures.

1.3 During the performance of the Human Repeat Insult Patch Test (RIPT) for determining sensitization, investigators are confronted with skin responses that represent skin irritation (non-immunologic responses) or allergic contact dermatitis (ACD). The numerical scoring system for grading the intensity of both are similar and test facilities may vary in their scores that describe intensities of allergic and irritant skin responses. The hallmark of a mild allergic contact dermatitis is a sustained palpable erythematous reaction. Delayed-type allergic contact reactions from patch tests have intensity characteristics that favor scores of higher values for longer periods of time and typically do not produce a minimal score (score of 1, a just-perceptible erythema) for short durations (less than 48 h). It is the responsibility of the investigator to evaluate the scores in light of irritant reactions so that the responses are allergic in nature and not irritant. The investigator should denote a final score as either due to contact allergy or irritation. Paragraphs 9.5 – 9.5.5 describe a commonly used scoring system and discuss allergic and irritant responses in detail.

1.4 The Draize RIPT was published in 1944 as an attempt to decrease the frequency ACD.[2] The test techniques at that time were just being validated and this experimental design was largely empiric.[3] The principle of the test is as follows:

1.4.1 Multiple inductions of the study material at relatively non or low irritancy levels,

1.4.2 Approximately a two-week rest period, and

1.4.3 A standard diagnostic challenge of approximately 48 h and a delayed reading at approximately 96 h after patch application.

1.5 In the intervening years, with further experimentation added to this empiric approach, three additional principles have been learned:

1.5.1 Increasing the concentration of the study material,

1.5.2 Defining a no effect level (this is possible with only individual ingredients and not the final study material), and

1.5.3 The enhanced sensitivity and the use of occlusion (where occlusion would not ordinarily be present).

1.6 In 1945, Henderson and Riley[4] demonstrated that a test panel sample size of 30 000 subjects would have to be employed to ensure statistically that there would be no more than 0.1 % sensitization. If there are no allergic responses in a test panel of 200 subjects with exposures comparable to those of the population, then there could be as many as 1.5 allergic reactions per 100 users.

1.7 All medical devices must be safe and effective for their intended use. Since medical devices such as gloves come in contact with human tissue, they should be tested for biocompatibility in animals first. The human repeat insult patch test (RIPT) is one test that can be used to test rubber gloves for skin sensitization to chemicals used in the manufacture of gloves.

1.7.1 Since various forms of the RIPT exist, a single standardized test method that outlines the testing protocol, scoring system, and the criteria for skin sensitization should be developed.

1.8 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.9 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the*

[1] This test method is under the jurisdiction of ASTM Committee D11 on Rubber and Rubber-like Materials and is the direct responsibility of Subcommittee D11.40 on Consumer Rubber Products.

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[2] Draize, J.H., Woodward, G., and Calvery, H.O., "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes," *Journal of Pharmacology and Experimental Therapeutics*, Vol 83, 1944, pp. 377-390.

[3] Shelanski, H. A., and Shelanski, M. V., "A New Technique of Human Patch Test," *Proc. Sci. Sect. Toilet Goods Assoc.* , Vol 19, 1953, pp. 46-49.

[4] Henderson, C. R., and Riley, E. C., "Certain Statistical Considerations in Patch Testing," *Journal of Investigative Dermatology* , Vol 6, 1945, pp. 227-232.

Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Terminology

2.1 *Definitions:*

2.1.1 *allergen, n*—a substance capable of causing an allergic reaction.

2.1.2 *allergic contact dermatitis (ACD), n*— a Type IV delayed-in-time dermatitis that is caused by skin contact with a hapten that evokes a cell-mediated (delayed-type hyersensitivity) immune response.

2.1.3 *allergic contact dermatitis reaction, n*—an adverse immune response following exposure to chemical (non-protein) allergens.

2.1.4 *antigen, n*—any substance that provokes an immune response when introduced into the body.

2.1.5 *atopic dermatitis, n*—the most common form of chronic inflammatory dermatitis.

2.1.5.1 *Discussion*—Although immunologic mechanisms may play a role in producing this dermatitis, the role of any allergen in producing and sustaining this morphologically similar dermatitis is not proven or as clearly understood as classical allergic contact dermatitis.

2.1.6 *blister, n*—a vesicle containing serum.

2.1.7 *bullae, n*—synonymous with blister.

2.1.8 *cell-mediated immunity, n*—that portion of the immune system mediated by white blood cells called T-cells or T-lymphocytes.

2.1.9 *challenge test, n*—a medical procedure used to identify a substance to which a person is sensitive by deliberately re-exposing them to that substance in an attempt to reproduce the reaction.

2.1.10 *dermatitis, n*—inflammation of the skin evidenced by itching, redness, and various skin lesions.

2.1.11 *diagnostic patch tests, n*—a form of skin testing in which suspected allergens are applied to the skin, covered, and observed 48 to 96 h or more later to see if a reaction occurs.

2.1.11.1 *Discussion*—This test is often used to identify possible causes of allergic contact dermatitis.

2.1.12 *eczema, n*—synonymous with dermatitis.

2.1.13 *edema, n*—swelling caused by excessive infiltration of fluid into the skin.

2.1.14 *erythema, n*—synonymous with redness of the skin.

2.1.15 *immune response, n*—the activity of specialized cells or their products against antigens and allergens introduced to the body.

2.1.16 *immunize, v*—to render a patient immune from foreign substances.

2.1.17 *induration, n*—hardening of a tissue due to edema and cellular infiltration.

2.1.18 *inflammation, n*—a basic response of the body to injury, usually characterized by redness of the skin, warmth, swelling, and pain.

2.1.19 *irritation, n*—a chemically induced dermatosis without immunological involvement.

2.1.20 *mast cells, n*—tissue cells that contain packets of biochemicals responsible for the symptoms of allergy.

2.1.20.1 *Discussion*—When allergens attach to I_gE antibodies sitting on the surface of these cells, a signal is sent, causing them to release these biochemical mediators of allergy.

2.1.21 *mediators, n*—soluble products of immune cells that interact and/or activate other parts of the immune system.

2.1.22 *mild irritant control, n*—a substance that will produce a minimally perceptible dermatitis.

2.1.23 *neutral control, n*—a substance, such as water, that through clinical usage, has not been found to be an allergen.

2.1.24 *papules, n*—small, solid red elevations of the skin.

2.1.25 *predictive patch test, n*—a repeat insult patch test (RIPT) used as a toxicology test to determine the potential for ACD.

2.1.26 *sensitive, v*—to expose to an antigen, provoking an immune response so that on re-exposure to that antigen, a more advanced secondary response occurs. Synonymous with immunize.

2.1.27 *study material, n*—a synthetic or natural polymer material used as a medical glove or as a part of a medical glove.

2.1.28 *vesicles, n*—small circumscribed fluid-filled elevations of the skin smaller than a blister.

3. Summary of Test Method

3.1 A general medical history of the study subjects should be taken and include information on dermatologic conditions and sensitivities to specific compounds. Studies conducted in accordance with this human RIPT protocol will employ a minimum of 200 study subjects. Prior to evaluating the material in a human RIPT, acceptable toxicology data should be obtained. The sensitization potential of the study material is evaluated in a test panel of a minimum group size of 200 subjects. The study panel should include men and women. The induction phase of the human RIPT includes 10 multiple 48-h (72-h on weekends) patches at the same site typically on the upper back with no rest between repatching except for scoring. The patch site is graded for skin responses prior to each subsequent patch application. In the event of any significant erythema, the site of patch application should be moved to another location to confirm the reaction. Following the completion of the induction phase, there is approximately a 21 day rest period to allow the development of latent sensitization. This is followed by two consecutive 48-h challenge patches applied to naive sites. Responses are evaluated after the removal of each consecutive 48-h patch application. A minimum of two delayed skin site gradings is required to differentiate irritation from sensitization reactions. If the results are equivocal, a second challenge, after the original challenge dermatitis has cleared, may be conducted to ensure that sensitization was not overlooked.

4. Significance and Use

4.1 This RIPT method assesses the potential of skin sensitization with a particular medical product by repeated topical applications to the skin of selected subjects. This is a procedure that has the potential to detect many, but not all, sensitzers. This requires multiple applications to induce a cell-mediated Type IV immune response sufficient to cause an allergic reaction.

4.2 In general, the sensitization procedure requires 10 multiple 48-h (72-h on weekends) applications of patches containing the study material over a three-week induction phase. Induction is followed by approximately a 21 day rest phase to allow the development of any latent sensitization. Study subjects are then challenged by the application of two consecutive 48-h patches of the study material to naive sites. Responses are evaluated and graded after the removal of each consecutive 48-h patch application.

4.3 Although this test method is a clinical method, it may be used as part of a risk analysis to determine the potential for Type IV allergic contact dermatitis.

4.4 This test method assumes that good clinical practices will be utilized, including adequate training of practitioners.

5. Interferences and Precautions

5.1 During the course of the study, the area of the study subjects where the patch is applied should not be bathed, showered, or washed. The patch area must stay dry. Wet patches can be a source of mild irritation reactions.

5.2 Caution: Patch testing can involve a certain risk to the subject due to sensitization or raising of the level of sensitivity to the study material.

6. Experimental Plan

6.1 *Subject Inclusion/Selection Criteria:*

6.1.1 Subjects ranging from 18 to 65 years.

6.1.2 Subjects who complete a medical/personal history form.

6.1.3 Subjects who have read, understood, and signed an informed consent agreement.

6.1.4 Subjects should include both male and female.

6.2 *Subject Exclusion/Rejection Criteria:*

6.2.1 Subjects with skin disease that, in the opinion of the investigator, could interfere with the evaluation.

6.2.2 Subjects taking medications that, in the opinion of the investigator, would interfere with the study.

6.2.3 Subjects with clinically significant psoriasis, eczema, or atopic dermatitis.

6.2.4 Subjects who are pregnant or become pregnant during the study.

6.2.5 Subjects with known sensitivity to natural rubber and rubber chemicals.

6.2.6 Subjects who have acquired a recent marked skin tanning or sunburn that, in the opinion of the investigator, would interfere with the study.

6.2.7 Subjects who have undergone any type of sensitization testing within the last thirty days.

6.2.8 Subjects who are lactating women.

6.2.9 Subjects exogenously or endogenously immunosuppressed.

6.3 *Study Group:*

6.3.1 *Sample Size*—A minimum of 200 subjects will complete the study.

6.3.2 *Clinical Sites:*

6.3.2.1 One clinical location with a minimum total sample size of 200 subjects.

6.3.2.2 The testing may be done in a single clinical location but avoiding extreme climatic conditions.

7. Institutional Review and Informed Consent

7.1 *Institutional Review*—The method for this study should be reviewed by an appropriate Institutional Review Board (IRB).

7.2 *Informed Consent*—An informed consent document should be obtained from each study subject prior to initiating the study.

8. Study Materials and Patch

8.1 The patch will be an adhesive bandage with a 2 by 2-cm or larger Webril pad (or equivalent) affixed.

8.2 All study materials should be applied in an amount proportionate to the size of the 2 by 2-cm or larger patch.

8.3 A Neutral Control patch will be a adhesive bandage with a 2 by 2-cm or larger Webril pad (or equivalent) wetted with 0.2 mL of distilled or deionized water.

8.4 The study glove material should be applied so that the inside glove surface is exposed to the skin of the test subject.

9. Study Design

9.1 The human RIPT is performed to determine the potential of the product for sensitization under conditions relative to anticipated consumer exposure.

9.2 *Patch Site:*

9.2.1 Patches should be applied to the upper back area, either to the right or left of the midline. (The arm may be used as an alternative patch site to the back area.)

9.2.2 The upper back, either to the right or left of the midline, is the most common site used for patch testing. This area has been preferred because of its larger, more uniform surface; it is more accommodating to multiple tests. For many volunteer subjects, testing at this site is less obtrusive. However, there are occasions when the upper arm or forearm may be the preferred site. There is no data that supports the superiority of one of these skin sites over another for inducing experimental sensitization.

9.3 *Study Description:*

9.3.1 *Induction Phase*—The induction phase of the RIPT includes 10 multiple 48-h (72-h on weekends) patches at a single site on the upper back with no rest between re-patching. The patch site is graded for skin reactions just prior to each application. The patch site is moved slightly in the event of significant erythema or irritation to confirm the reaction.