

Urushiol Patch Test Using the T.R.U.E. TEST System

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Background: Poison ivy, poison oak, and poison sumac are the most common causes of allergic contact dermatitis in North America. Although extensive efforts have been made to develop therapies that prevent and treat allergic contact dermatitis to these plants, there lacks an entirely effective method, besides complete avoidance. Efforts to develop a more effective preventive therapy, such as a vaccine, are ongoing. To accurately evaluate the efficacy of these new therapies, an appropriate assessment tool is needed.

Objective: The aim of this study was to evaluate the safety and appropriate doses of urushiol required for a patch test based on the hydrogel delivery system of the Thin-Layer Rapid Use Epicutaneous Patch Test.

Methods: Nine subjects were patch tested with various doses of urushiol and a negative control on day 0. Patch test sites were inspected for any local reaction on days 2, 4, 7, 14, and 21 after the initial exposure and graded by standard morphology.

Conclusions: All 9 subjects did not have any significant adverse effects. The urushiol patch test using the hydrogel delivery method demonstrated urushiol sensitivity. All doses of urushiol resulted in a local reaction, and severity of reactions was correlated with dosage of urushiol used in the patch test.

Poison ivy, poison oak, and poison sumac are the most common causes of allergic contact dermatitis in North America, affecting 10 million to 50 million Americans per year.¹ Poison ivy, oak, and sumac are members of the Anacardiaceae family of plants and belong in the genus *Toxicodendron*.² Exposure to plants of the Anacardiaceae family accounts for more allergic contact dermatitis than other plant families combined.³ Prevalence of poison ivy and poison oak sensitivity in the general adult population ranges from 50% to 70%.^{2,4} Poison ivy dermatitis affects all ethnicities and skin types, and most geographical regions in the United States are at risk.⁵ Plant dermatitis is a significant occupational hazard in agriculture and forestry and afflicts many individuals engaged in outdoor or wilderness activities. Furthermore, this can result in significant monetary expense for worker disability and medical therapy.³

To accurately evaluate the efficacy of new therapies being developed to prevent contact dermatitis secondary to poison ivy, oak, or sumac exposure, an appropriate assessment tool is required. Currently, there is no urushiol sensitivity patch test available in the United States. Therefore, the main purpose of this study was to evaluate the safety and appropriate doses of urushiol required for a patch test based on the hydrogel delivery system used by T.R.U.E. TEST (SmartPractice, Phoenix, AZ). T.R.U.E. TEST is approved by the US Food and Drug Administration as a biologic/device combination product with the intended use of patch testing for the diagnosis of contact allergy. Furthermore, we determined whether a dose-response relationship exists between the doses of urushiol and the severity of response.

METHODS

After approval of the study protocol from the Penn State College of Medicine Institutional Review Board, 9 volunteer subjects with a documented history of allergic contact dermatitis to poison ivy, oak, or sumac were entered into the study after informed consent was obtained. None of the subjects was immunocompromised. Subjects did not undergo treatment with systemic or topical corticosteroids for at least 1 month before study entry or antihistamines for at least 2 weeks before study entry through the end of the study period.

The urushiol patch test investigational product used in this study was based on the delivery system of the US Food and Drug Administration–approved T.R.U.E. TEST. The allergens (raw

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materials) were homogenized in an excipient to form a hydrophilic gel. The uniformly dispersed allergen gel preparation was coated on an impermeable backing sheet of polyester and dried to a thin film. The coated sheet was then cut into 9 × 9-mm squares (test patches) and mounted onto the surgical tape. The urushiol patch test investigational product consisted of a single piece of surgical tape with 5 occlusive polyester patches each of approximately 0.81 cm². The panel tape was composed of polyester and is coated with an acrylate-based adhesive. There are no natural rubber latex proteins, rubber components, balsams, or rosins in the adhesive or tape. Acrylate adhesives used in medical-grade tapes are typically processed to remove free monomers that may be allergenic. Each patch was coated with a film containing a uniformly dispersed, but randomly positioned, sample of urushiol at 4 strengths of 0.041, 0.081, 0.16, and 0.32 µg plus 1 patch with a negative control using ethanolic polyvinylpyrrolidone (PVP; CAS# 9003-39-8).

Urushiols were supplied by ElSohly Laboratories, Inc (Oxford, MS). Urushiols are purified after the general procedure previously described by ElSohly et al.⁶ Briefly, organic solvent extraction (ethanol) of poison ivy leaves and small stems was followed by partitioning of the extract between water and chloroform and silica gel chromatography of the chloroform fraction using chloroform as the elution solvent. Select fractions of the column eluate are dried and further extracted by partition in organic solvents (acetonitrile and hexane), with the acetonitrile portion collected and dried. The final extract contains 61.5% wt/wt urushiols, predominately comprising the triunsaturated congener of pentadecylcatechol (PDC), together with some monounsaturated and diunsaturated congeners of PDC, as well as a small amount of PDC. For the preparation of the skin testing patches, the urushiol extract is dissolved in ethanol to create a solution of 5-mg/mL urushiols (based on 61.5% urushiol content). The urushiol solution is stored in amber glass vials at 2°C to 8°C and was evaluated for stability through 12 months. The refrigerated and frozen samples of urushiol solution, analyzed by gas chromatography–flame ionization detector for urushiol concentration on days 0, 7, 14, 29, 57, 97, 185, 275, and 365, were shown to be stable without undergoing degradation for at least 12 months.

The urushiol patch panel was prepared by mixing urushiols with PVP and coating a sheet of occlusive polyester. The desired concentration of urushiol ethanolic solution was mixed with the PVP excipient until a uniform blend was achieved. The gel was then allowed to swell appropriately and centrifuged as necessary to remove air bubbles. The occlusive polyester sheet was pretreated to apply an electrostatic charge, and then the urushiol-gel mixture was applied to the polyester sheet. The urushiol-gel-coated sheets were dried, trimmed, and packed into a labeled laminate foil pouch and stored at 2°C to 8°C. Stability was demonstrated by the urushiol-gel-coated sheets for 12 months at 2°C to 8°C. To prepare the patch test, the urushiol-gel sheets were cut into small square patches (approximately 0.81 cm²) and placed onto the appropriate location on the adhesive surgical tape panel. The finished investigational panels were covered with a protective sheet and sealed in a

laminated foil pouch. They were then labeled and dispensed to the study investigators for use on the study subjects.^{7–9}

A patch of the randomly positioned samples of urushiol at 4 strengths and negative control was applied on the back of each subject. Once administered (on day 0), the patch test remained on the subject for 48 hours and then was removed (day 2). Upon removal on day 2, the first assessment and interpretation of the results were performed. Additional assessments and interpretations were performed on days 4, 7, 14, and 21. Study subjects and the investigator were blind to the arrangement of the test articles and placebo on each patch test. Severity of the reactions was graded according to standard morphology: 0 (no reaction), 1+ (nonvesicular with erythema, infiltration, and possibly papules), 2+ (edematous or vesicular reaction), 3+ (spreading, bullous, ulcerative reaction), and +/- (macular erythema). Symptoms such as pruritus and burning and any adverse events were assessed and documented. Irritancy was differentiated from a true allergic contact dermatitis response by the duration of the reaction. Response to treatment, which was initially coded as 0, +/- , 1, 2, or 3, was recoded in a binary fashion as follows: 0, no response; all other values, response. A repeated-measures binary logistic regression model was fit with SAS PROC GENMOD (SAS 9.4; SAS Institute, Cary, NC) using an autoregressive correlation structure. Both dosage and time were included as categorical explanatory variables. However, the model did not include an interaction term because of the limited number of subjects. Odds ratio estimates for each nonzero dosage versus control were obtained by exponentiating the appropriate parameter estimates.

RESULTS

Nine subjects were enrolled in the study, and they included 3 male and 6 female individuals aged 22 to 59 years (mean age, 46 years). All subjects completed the study. One subject had a mild flare of dyshidrotic hand eczema, which was treated with clobetasol cream for 1 day. Each of the doses of urushiol compared with placebo on the patch test yielded a reaction. Despite the limitation of a small sample size, the odds ratios for each of the doses were much greater than and did not include 1 (the null value), which confirms that each dose of urushiol used for patch testing yielded a reaction (Table 1). Furthermore, the odds ratio was greater as the dose of urushiol increased, which indicates that higher doses yield more severe reactions compared with placebo in all of the days.

On day 2, reactions varied from +/- to 2+ (Fig. 1). On day 4 (Fig. 2), reactions were stronger than those on day 2 (Fig. 1). On

TABLE 1. Odds Ratio of the Reactions Elicited by the Various Doses of Urushiol Compared With Placebo

Dose, µg	Odds Ratio	95% Confidence Interval
0.041	475.5	30.7–7376.8
0.081	1927	132.7–27,981.5
0.16	3204.1	185.7–55,270.8
0.32	7188.9	378.0–136,721.3

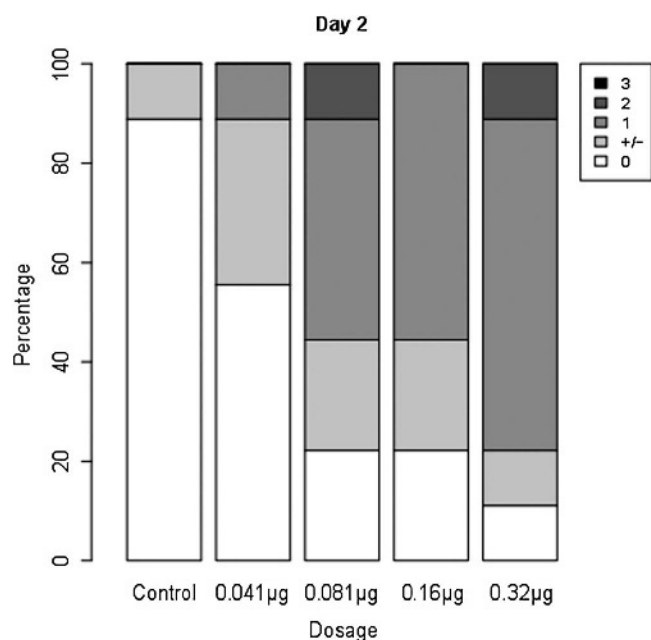


Figure 1. Day 2 reactions to various doses of urushiol.

day 7 (Fig. 3), only 1 patient had a 3+ reaction. On day 14 (Fig. 4), reactions were less intense than those on days 4 (Fig. 2) and 7 (Fig. 3), and on day 21 (Fig. 5), all subjects had the weakest reactions.

Figure 6 illustrates the intensity of the reaction for the various doses. There appears to be a dose-response relationship between the dose of urushiol and the reaction that it yields. For instance, the strongest dose, 0.32 µg, appears to yield the strongest reaction, reflected by the highest number of patients with 1+, 2+, or 3+ reaction compared with the weaker doses. Furthermore, the only time there was a 3+ reaction was in the 2 higher doses, 0.16

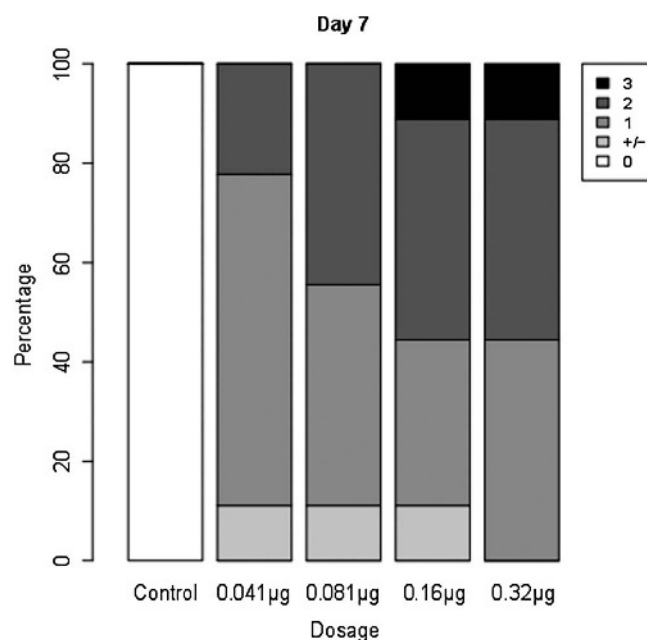


Figure 3. Day 7 reactions to various doses of urushiol.

and 0.32 µg. One patient on day 4 had a 3+ reaction to the 2 higher doses and a 2+ reaction to the 2 lower doses (Fig. 7). Moreover, Figure 8 demonstrates that the peak of severity of response was on days 4 or 7 for all patients.

DISCUSSION

Typically, a sensitized individual develops an intensely pruritic, erythematous eruption within 24 to 48 hours but can range from 5 hours to 15 days.¹⁰ This reaction is described as streaks or areas of erythema and edematous papules, which precede vesicles and

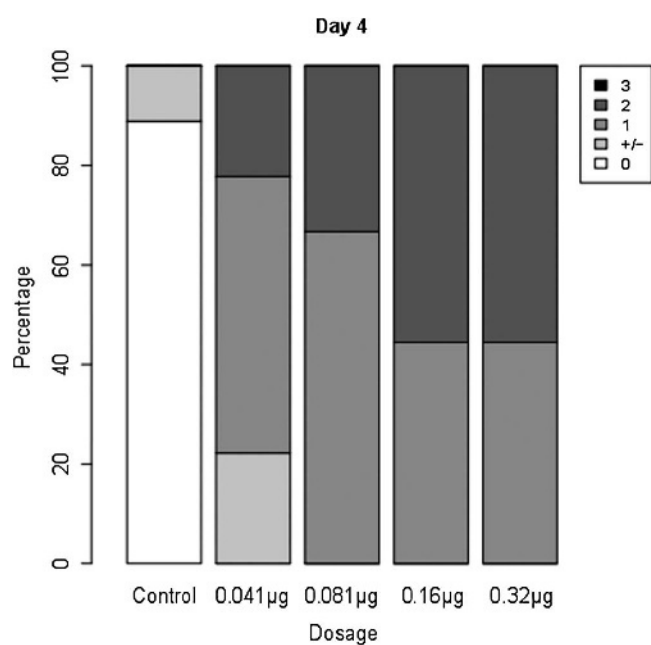


Figure 2. Day 4 reactions to various doses of urushiol.

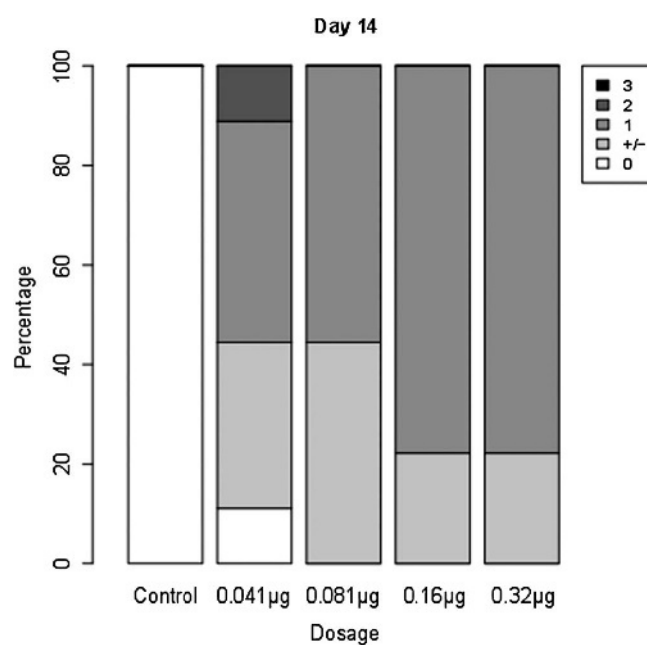


Figure 4. Day 14 reactions to various doses of urushiol.

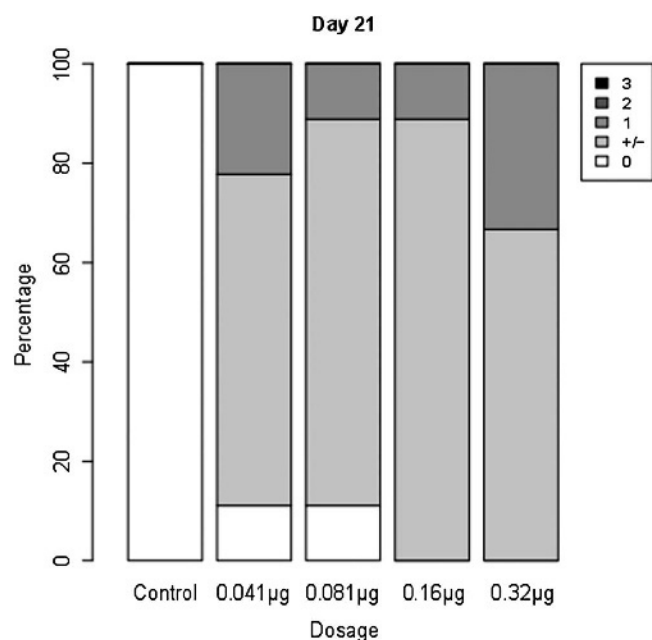


Figure 5. Day 21 reactions to various doses of urushiol.

bullae. The mildest reaction (+/- or 1+) generally lacks vesicles. On the other hand, severe reactions (2+ or 3+) can be characterized by vesicles to confluent bullae. Individual variations in the course of experimentally induced allergic contact dermatitis from urushiol in previously sensitized adults do exist. One study documented the range of individual variations in both reaction time and severity, which most likely represent differences in underlying immune responses between individuals.¹⁰ For instance, the onset of reactions ranged from 4 hours to 4 days, peak reactions occurred from 1 to 14 days, and maximum severity of reactions ranged from

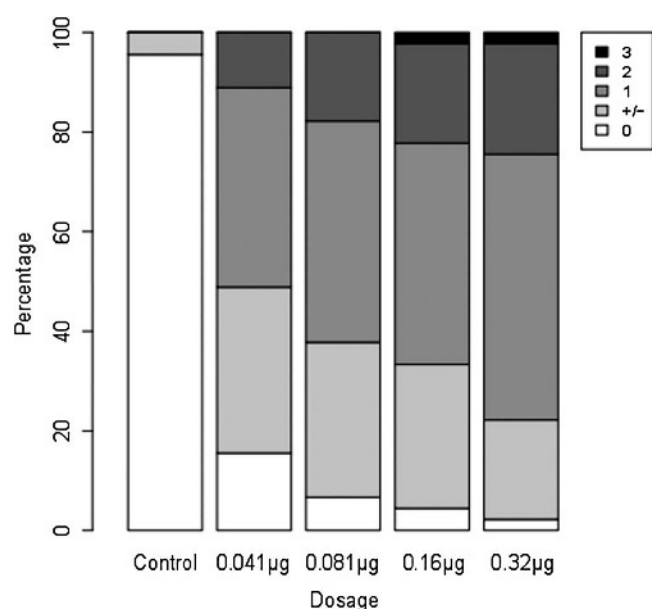


Figure 6. Composite reactions to various doses of urushiol.

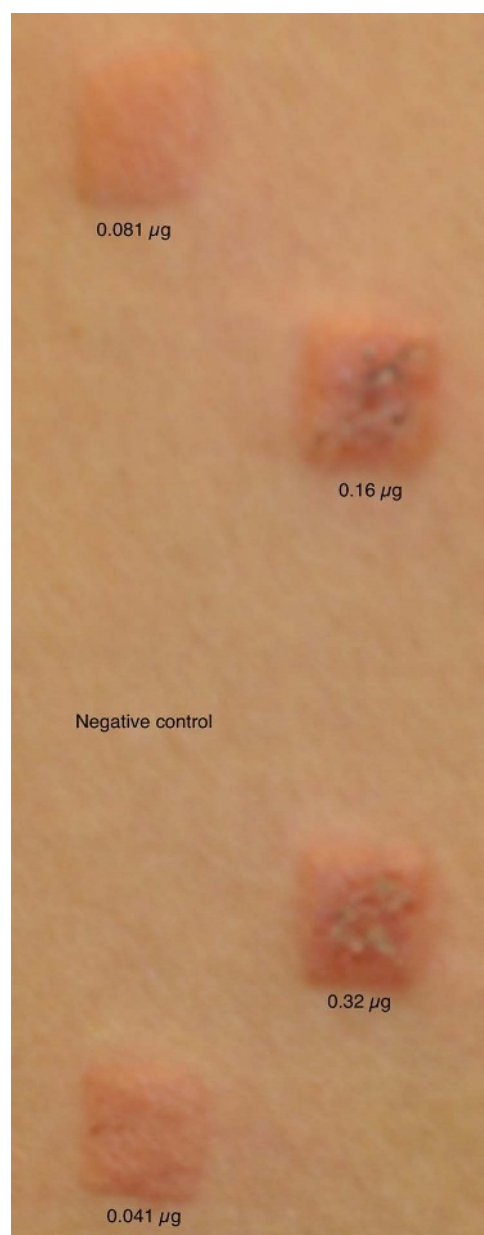


Figure 7. Patch test administration site of subject 1 on day 7. The reactions for negative control, 0.41, 0.81, 0.16, and 0.32 µg were 0, 2+, 2+, 3+, and 3+, respectively.

2+ to 3+ (erythema, edema, vesiculation, ulcerative breakdown). Our study likewise confirms that individuals vary in both severity of response and reaction time. Most of our subjects had a mild to moderate reaction (+/-, 1+, 2+). Only 1 subject had a 3+ reaction, characterized by a spreading, bullous, ulcerative reaction. The peak intensity of reaction, regardless of the doses of urushiol, was seen on day 4 or 7 for all subjects. This also verifies that the reaction elicited is a true allergic contact dermatitis reaction rather than an irritant contact dermatitis reaction because, typically at 96 hours, irritant responses may be faded, whereas allergic ones may continue to be unabated.¹¹ Furthermore, we demonstrate a dose-response

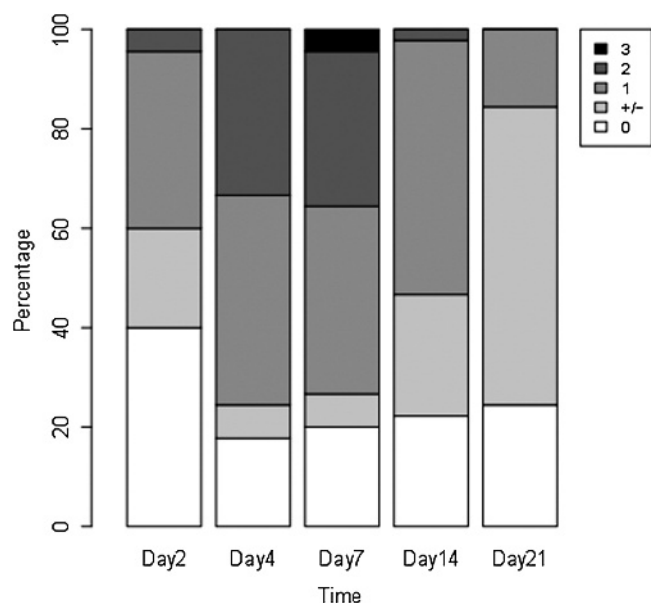


Figure 8. Composite reactions on various days of the study.

relationship for the 4 doses of urushiol in the patch test. The stronger the dose of urushiol, the stronger the reaction. Similarly, Epstein et al¹² found a dose-response relationship of dilutions of urushiol in the number of subjects with a positive reaction.

Although many methods and treatments have been developed to destroy the plant or protect the patient, none has been proven to be wholly effective.¹³ The only infallible method is proper identification and complete avoidance, which can be nearly impossible to do. Therefore, continued efforts to prevent contact dermatitis secondary to poison ivy, oak, or sumac exposure are being made, including the novel poison ivy vaccine. To accurately evaluate the efficacy of these new therapies, there is a need for an appropriate assessment tool, which currently does not exist. We have demonstrated that the urushiol sensitivity patch test using a T.R.U.E. TEST-like allergen delivery system demonstrated it as an effective

way of measuring urushiol sensitivity and had no significant adverse effects.

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