

A Contemporary Fischer-Maibach Investigation: Variations in Patch Test Delivery Systems and Implications for Standardization

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Background: Standardization of patch testing has been difficult to achieve.

Objectives: This study aimed to identify physical variations in patch test systems that could affect delivery of allergens.

Methods: We compared the volume, depth, and contact area of 21 patch test delivery systems. We also filled a variety of patch test systems with different volumes of liquid (ferrous chloride) and petrolatum (disperse blue) allergens to investigate coverage and extrusion.

Results: The depth of chambers varied from minimal to greater than 1 mm. Mean areas ranged from 50 to almost 350 mm². In most chambers, even the largest volume of liquid (40 µL) seemed to be completely contained by each product's absorbent material. The amount of petrolatum required to provide 100% coverage ranged from 15 to 45 µL. The dose delivered, as defined by mg per cm², varied more than 2-fold across the systems.

Conclusions: There are considerable differences across various patch tests. Different patch test systems likely do not deliver the same dose of allergen if the same volume of excipient is applied. Appreciating the differences between different patch test systems may help refine recommendations for the amount of allergens that should be applied to different patch test systems.

In 1984, Fischer and Maibach¹ filled Finn Chambers with different weights of colored petrolatum to investigate the behavior of petrolatum in testing conditions and to recommend an ideal testing volume of petrolatum to guide clinicians. It is well established that the dose of allergen per unit area is an important metric underlying both sensitization and elicitation of responses in allergic contact dermatitis.^{2–5} Yet, how often are the implications of this finding considered in daily practice? Many clinicians assume that all patch test chambers deliver the same dose of allergen. In fact, not only is there considerable variation across patch test systems but also the use of different systems also varies across both clinics and countries. Patch test systems differ in their physical dimensions (and hence surface area and volume), the material from which chambers or patches are constructed (eg, aluminum, polypropylene, polyethylene, cotton), the presence or absence of filter

paper or coatings, the material of the tape panel (eg, nonwoven adhesive, polyurethane), and adhesive and occlusive properties. Depending on the patch test system, chambers are loaded manually by clinicians at the testing site or are preloaded in exact dosages by the manufacturer. Manual loading introduces variability in dose delivery because of differences in how allergen is dispensed (eg, pipette, syringe, dropper) and in the amount dispensed.^{1,6} Patch testing is further complicated by a myriad of other variables— allergen/excipient differences (eg, molecular weight, viscosity), patient factors (eg, sensitivity, skin type), and clinician-related factors (eg, reading times, scoring, interpretation).

Although patch testing is considered an important tool for diagnosing allergic contact dermatitis, rigorous standardization is lacking. This lack of standardization is not a purely academic concern. It hinders meaningful comparison of results across clinics and across national and international clinical trials. Improvements in standardization of the controllable aspects of the patch testing process would minimize methodological errors and promote reproducibility of results, a clear benefit for patients seeking relief from their often debilitating but preventable dermatitis.

To evaluate the effects of different patch test products on dose delivery, we compared the physical dimensions (specifically, chamber area and volume) of 21 commercially available chambers and patches and investigated the effects of different volumes of common allergens on the extent of chamber coverage and extrusion. If these parameters were better understood, it may be possible to refine

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Disclosures: Curt Hamann, MD, is the owner and Chief Executive Officer of the umbrella entity SmartHealth (Phoenix, Ariz), which manufactures and sells Finn Chambers and allergEAZE chambers and related products for patch testing, and with its subsidiary company, SmartPractice Canada, sells allergEAZE allergens. Dathan Hamann and Carsten Hamann are his sons.

DOI: 10.1097/DER.0b013e31829f28ac

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recommendations about how much allergen should be applied to different types of patch test chambers.

METHODS

Twenty-one commercially available patch test delivery systems were selected for analysis based on worldwide clinical patch testing (Fig. 1). The physical dimensions of these systems, which include both patches (no measurable depth) and chambers (measurable depth), were compared. The patch systems were Curatest (Curatest and Curatest F; Lohmann & Rauscher GmbH & Co, Neuwied, Germany) and Torii (9 and 16 mm; Torii Pharmaceutical Co, Ltd, Tokyo, Japan). The chamber systems were Finn Chambers (8-mm aluminum without and with filter paper, polypropylene-coated aluminum, and Finn Chambers AQUA; 12 mm without and with filter paper; and 18 mm without and with filter paper; SmartPractice, Phoenix, Ariz), allergEAZE (on polyurethane and on nonwoven polyester tape; formerly Hayes, Service B.V., Haarlem, the Netherlands; as of April 1, 2013, SmartPractice), Hill Top Chambers (19 and 25 mm with Webril; Hill Top Research, St Petersburg, Fla), IQ Ultra (0.8-mm depth and 0.5-mm depth [current]; Chemotechnique Diagnostics, Vellinge, Sweden), Trolab (Almirall Hermal GmbH, Reinbek, Germany), and Van der Bend Chambers (10 mm [discontinued] and 8 mm [current]; Van der Bend B.V., Brielle, the Netherlands).

Measurement of Chamber Dimensions

For each delivery system, the depth of all 10 patches or chambers on 1 patch test panel was measured with 2 exceptions. Only 2 samples each of the 19- and 25-mm Hill Top Chambers were available for measurement. If chambers were packaged individually, 10 separate chambers were measured. All patches were placed on a certified flat granite block and measured with a calibrated depth micrometer (Fred V. Fowler Company Inc, Newton, Mass). From the same panels, the diameters (d) of the base of the circular chambers or the length (l) of the sides of the square chambers were measured with a caliper (Sylvac SA, Crissier, Switzerland). Interchamber distance and the dimensions of the entire patch system were measured with a ruler.

For each type of patch or chamber, all measurements were averaged. Based on these measurements, both mean contact area and mean volume were calculated for all chamber systems. The mean contact area of square chambers was calculated as l^2 , where l equals the length of the sides and that of round chambers as πr^2 , where r equals the radius ($d/2$) of the circles. The mean volume (v) of square chambers was calculated as $v = l^3$. Finally, the mean volume of round chambers was calculated as the product of the mean contact area and mean depth. When filter paper was present in a chamber, the mean volume was the sum of the portion attributable to the material and to the unfilled portion (air gap) of the chamber.

For each patch test system, the manufacturer's product literature was reviewed to determine the materials used in the system (Table 1).

Visualization of Coverage and Extrusion

At room temperature, 6 chambers on each of the small patch test systems [Curatest and Curatest F, 8-mm Finn Chamber without filter paper and Finn Chambers AQUA, allergEAZE on nonwoven polyester and allergEAZE clear on polyurethane, Trolab, IQ Ultra (0.8-mm depth [discontinued] and 0.5-mm depth [current]), Van der Bend Chambers, (10 mm [discontinued] and 8 mm [current], and 9-mm Torii patch)] were filled with different volumes (15, 20, 25, 30, 35, or 40 μ L) of 1% disperse blue 124/106 in petrolatum (allergEAZE; SmartPractice Canada, Calgary, Canada). If 40 μ L did not provide 100% coverage, as defined later, testing continued with 5- μ L increments of disperse blue until 100% coverage was obtained.

The disperse blue, which was selected to visualize the results of coverage and extrusion, was dispensed with a Gilson Microman precision microliter pipette (Gilson S.A.S., Villiers-le-Bel, France) according to the manufacturer's specification. On round chambers, the disperse blue was dispensed in a ribbon across the diameter of the base; in square chambers, it was dispensed across the diagonal of the base (Fig. 2).⁷ The same pipette was used to fill the same types of chamber with liquid ferrous chloride at the same volumes as used with the disperse blue. Before the liquid was added to the aluminum Trolab and Finn Chambers, the filter paper that accompanies each type of chamber was added as would be the case in a clinical situation.

The chambers filled with disperse blue were covered with glass and tamped lightly with a finger to mimic the application of a patch test system to a patient's back. The results were photographed to provide a nonquantitative visual comparison of the coverage of the chamber and of the extent of extrusion (Fig. 2). The coverage of a patch or chamber was considered 100% when a given volume of disperse blue covered the entire base of the chamber as determined by visual inspection through the glass. This method of quantification was based on the assumption that 100% coverage of the base of the chamber would correspond to homogeneous coverage of the test area had the chamber been applied to an individual's back. Extrusion was measured at its greatest point from the rim or edge of a chamber. Extrusion was considered mild if 1 mm or less of the disperse blue spread beyond the chamber, moderate if the spread was less than or equal to 2.5 mm, and severe if the spread was beyond 2.5 mm.⁷ The liquid ferrous chloride was observed to moisten, saturate, or be unabsorbed. No glass was placed over the chambers filled with liquid (Fig. 3).

As available, manufacturers' product literature was reviewed to identify their recommendations for the optimum amount of a semisolid or liquid that should be added to their patch or chamber system.

RESULTS

Chambers were composed of aluminum, Kraton, polyethylene, or polypropylene, either as singles or embedded in panels whose



Figure 1. Panels and individual chambers of the different patch test systems used in different countries and evaluated in this study. All panels are identified left to right and are shown looking into the chamber (actual size not shown). A, AllergEAZE chambers on nonwoven polyester, allergEAZE chambers on polyurethane, and Curatest patches (Row 1). Curatest F patches, 8-mm aluminum Finn Chambers, and Finn Chambers AQUA (Row 2). B, IQ Ultra chambers (0.8-mm depth), IQ Ultra chambers (0.5-mm depth), and 9-mm Trolab chambers (Row 1). Nine-millimeter Torii patches, current 8-mm Van der Bend Chambers, and discontinued 10-mm Van der Bend Chambers (Row 2). C, Twelve-millimeter aluminum Finn Chamber without filter paper, 18-mm aluminum Finn Chamber without filter paper, 12-mm aluminum Finn Chamber with filter paper, and 18-mm aluminum Finn Chamber with filter paper (Row 1). Nineteen-millimeter Hill Top Chamber with Webril, 25-mm Hill Top Chamber with Webril, and 16-mm Torii patch (Row 2).

TABLE 1. Description of Patch Test Systems

System	Shape	Manufacturer	Chamber/Patch Material	Panel Material	Panel Width, mm	Panel Length, mm	Space Between Chambers, mm	
							Horizontal	Vertical
Patch Curatest	Round	Lohmann & Rauscher	Disk framing: polyester film/Nonwoven test disks: 60% cotton, 100% viscose, 1 side coated with polyethylene; skin-friendly polyacrylate adhesive	Nonwoven base material: white, 100% polypropylene	76	126	25	17
Curatest F	Round	Lohmann & Rauscher	Disk framing: polyester film/Nonwoven test disks: 60% cotton, 100% viscose, 1 side coated with polyethylene; skin-friendly polyacrylate adhesive	Film base: 100% polyurethane	65	125	25	17
Torii 9 mm	Round	Torii Pharmaceutical	100% Cotton nonwoven fabric with polyester film frame; acrylic-based adhesive	Polyurethane	32	114	NA	12
16 mm	Round	Torii Pharmaceutical	100% Cotton nonwoven fabric with polyester film frame; acrylic-based adhesive	Polyurethane	39	226	NA	20
Chamber AllergEAZE	Square	SmartPractice	Polyethylene terephthalate	Nonwoven	60	120	11	9
On nonwoven polyester	Square	SmartPractice	Polyethylene terephthalate	Polyurethane	60	120	11	9
Finn Chamber 8 mm	Round	SmartPractice	Aluminum	Scanpor	50	120	9	9
W/o filter paper	Round	SmartPractice	Aluminum	Scanpor	50	120	9	9
W/ filter paper	Round	SmartPractice	Polypropylene-coated aluminum	Scanpor	50	120	9	9
Polypropylene coated Finn Chambers	Round	SmartPractice	Paper-coated aluminum	Polyurethane	50	120	9	9
AQUA 12 mm	Round	SmartPractice	Aluminum	Scanpor	50	40	NA	NA
W/o filter paper	Round	SmartPractice	Aluminum	Scanpor	50	40	NA	NA
W/ filter paper	Round	SmartPractice	Aluminum	Scanpor	50	50	NA	NA
W/o filter paper	Round	SmartPractice	Aluminum	Scanpor	50	50	NA	NA
W/ filter paper	Round	SmartPractice	Kraton	Durapore	34	34	NA	NA
Hill Top Chamber	Square	Hill Top Research	Kraton	Durapore	45	45	NA	NA
19 mm w/ Webril	Square	Hill Top Research	Polyethylene	Nonwoven adhesive tape	68	143	19	12
25 mm w/ Webril	Square	Chemotechnique	Polyethylene	Nonwoven adhesive tape	52.5	117	15	12
IQ Ultra	Square	Chemotechnique	Aluminum	Not found	50	120	9	9
0.8-mm depth	Square	Almirall	Polypropylene	Not found	73	175	11	14
0.5-mm depth	Square	Van der Bend	Polypropylene	Not found	72	136	24	12
Trolab	Square	Medical Supplies	Polypropylene	Not found				
Van der Bend Chamber 10 mm (discontinued)	Square	Medical Supplies	Polypropylene	Not found				
8 mm (current)	Square	Medical Supplies	Polypropylene	Not found				

NA, not applicable; W/, with; W/o, without.

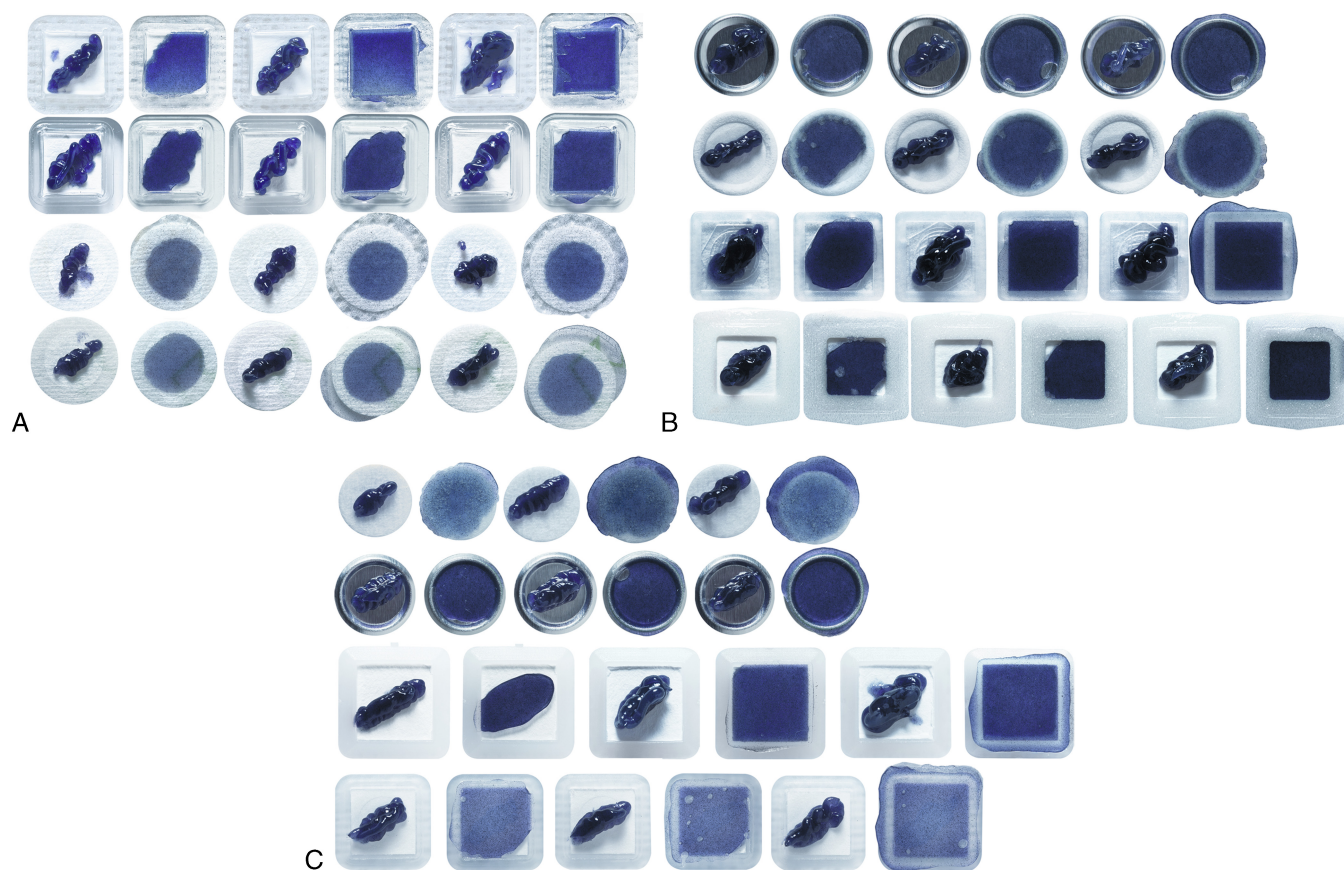


Figure 2. Sample of patch test chambers and patches with disperse blue before and after glass was applied to visualize the spread and extrusion of the allergen (original magnification approximately $\times 185$). Volumes (left to right) shown reflect volumes immediately lower than, at, or more than 100% coverage. As would be expected, the coverage decreased progressively with even lower volumes that provided less than 100% coverage and hence were not shown. Volumes greater than those providing 100% coverage were associated with progressively more extrusion (also not shown). A, AllergEAZE nonwoven polyester: 30, 35 (100%), and 40 μL (Row 1). AllergEAZE on polyurethane: 30, 35, and 40 μL (100%; Row 2). Curatest: 15, 20, and 25 μL (Row 3). Based on the amount of extrusion that is visible because placement of the ribbon of petrolatum was not precisely on the diameter of the patch, 15 μL likely would provide 100% coverage as it did for Curatest F. Curatest F: 15 (100%), 20, and 25 μL (Row 4). B, Finn Chamber without filter paper: 25, 30 (most likely 100% based on presence of air bubble and extrusion), and 35 μL (Row 1). Finn Chambers AQUA: 25, 30, and 35 μL (Row 2). Based on the extrusion, 25 to 30 μL would provide 100% coverage. IQ Ultra chamber (0.8-mm depth): 30, 35 (100%), and 40 μL (Row 3). Although 35 μL was required to provide complete coverage, this volume was also associated with 1 mm of extrusion. IQ Ultra chamber (0.5-mm depth): 20, 25, and 30 μL (100%; Row 4). Although 30 μL was needed to obtain 100% coverage, this volume was associated with 3 mm of extrusion. C, Nine-millimeter Torii: 15, 20, and 25 μL (Row 1). Given the amount of extrusion associated with 20 μL , 15 μL would likely have provided 100% coverage. Trolab: 30 (almost 100%), 35 (100% but with moderate extrusion), and 40 μL (Row 2). Ten-millimeter Van der Bend Chamber (discontinued): 40, 45 (100%), and 50 μL ; less than 1 mm of extrusion was associated with 100% coverage (Row 3). Eight-millimeter Van der Bend Chamber (current): 30, 35 (100%), and 40 μL (Row 4). Bubbles reflect the nonuniform adherence of the disperse blue to the glass.

composition also varied. Twelve chambers and patches were round, and 9 chambers were square (Table 1).

Measurements

The physical dimensions of the chambers varied considerably (Tables 1 and 2). As would be expected, the mean depth of the patches (Curatest and Torii) was minimal (<0.25 mm), as was that of the 8-, 12-, and 18-mm Finn Chambers with filter paper and that of the 8-mm Van der Bend Chamber. Six chambers had an intermediate depth, varying from 0.34 mm to 0.58 mm. The depth of 7 chambers exceeded 0.6 mm (range, 0.61 to >1 mm). The area of 14 systems ranged from approximately 50 to 81 mm^2 . The mean

calculated areas ranged from as small as approximately 50 mm^2 for the Curatest patches to more than 165 mm^2 for the 4 largest systems. The 18-mm Finn Chamber had the largest mean area (346.36 mm^2). Mean calculated total volume ranged from as little as approximately 23 μL in the 8-mm Finn Chamber with filter paper to almost 700 μL in the large Hill Top Chamber.

Coverage and Extrusion With Petrolatum

Across the systems evaluated, the minimum volume of disperse blue needed for 100% coverage ranged from 15 to 45 μL (Table 3, Fig. 2). The area of the Curatest and 9-mm Torii patches was covered completely and associated with minor extrusion at the

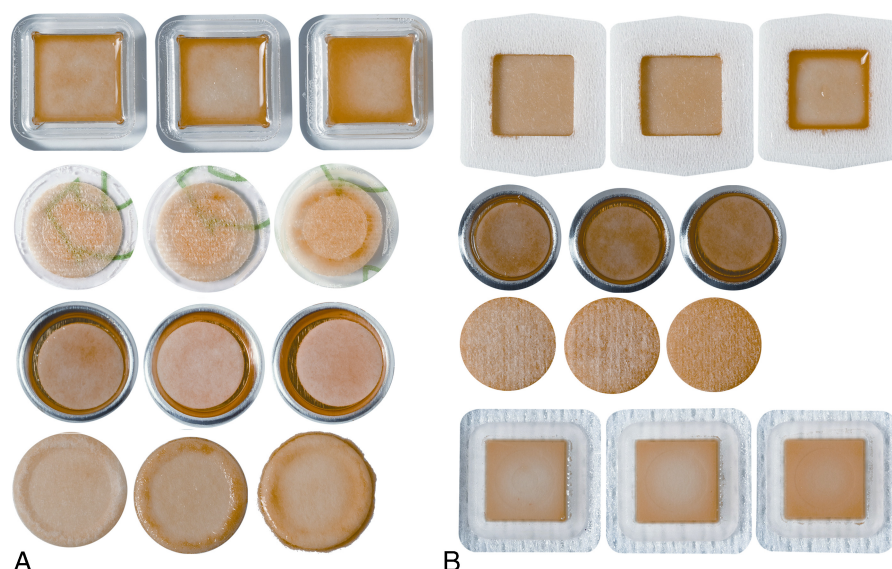


Figure 3. Samples of patch test chambers and patches show the volumes of liquid at or beyond the absorbency threshold up to the maximum volume used (40 μ L). In all cases from left to right, the volumes are 30, 35, and 40 μ L (original magnification approximately $\times 185$). A, AllergEAZE on polyurethane: the same result was obtained for allergEAZE woven (not shown; Row 1). Curatest: only a slight amount of liquid remained unabsorbed at 40 μ L (Row 2). Eight-millimeter Finn Chamber with filter paper: the amount of unabsorbed liquid increased as its volume increased; 25 μ L (not shown) provided 100% coverage (Row 3). Eight-millimeter Finn Chambers AQUA: a slight amount of liquid remained unabsorbed when the volume reached 40 μ L (Row 4). B, Half-millimeter depth IQ Ultra: a slight excess of liquid was unabsorbed at 30 μ L and increased with the increase in volume thereafter (Row 1). Trolab with filter paper: such as the Finn Chamber, 100% coverage would have been 25 μ L, with unabsorbed excess liquid increasing with volume (Row 2). Nine-millimeter Torii patch: all liquid was absorbed at all volumes (Row 3). Discontinued 10-mm Van der Bend patch: all liquid was retained in the chamber; there was no unabsorbed liquid (Row 4).

smallest volume (15 μ L) of disperse blue. Not surprisingly, the extent of extrusion increased as the volume of petrolatum increased. The 2 types of allergEAZE chambers required 35 to 40 μ L to cover the entire area and were associated with minimal extrusion. The entire surface area of the base of the 8-mm Finn Chambers was covered when the volume of disperse blue was between 25 and 30 μ L, and slight extrusion was visible with both volumes. The 8-mm Trolab required 30 to 35 μ L to obtain complete coverage. A volume of 25 μ L almost covered the 0.5-mm depth IQ Ultra. A volume of 30 μ L provided 100% coverage, but a 3 mm of extrusion was also visible. The coverage of the 0.8-mm depth IQ Ultra chamber was almost 100% at 30 μ L, and mild extrusion was present. The current 8-mm Van der Bend Chamber was completely covered when the volume of disperse blue reached 30 to 35 μ L; mild extrusion was associated with both amounts. It took 45 μ L of disperse blue to achieve 100% coverage of the discontinued 10-mm Van der Bend Chamber. Extrusion was less than 1 mm. As would be expected for the large chamber systems, the maximum volume of 40 μ L failed to cover the entire base. As an example, the 16-mm Torii patch is shown with 40 μ L of disperse blue (Fig. 4).

Coverage and Extrusion With Liquid

All of the brands of smaller (≤ 10 mm) patch test systems were completely covered by all 6 volumes of liquid. Unabsorbed liquid was visible in the 2 types of allergEAZE chambers, the 8-mm Finn

Chamber with filter paper, and the Trolab chamber with filter paper when liquid volumes were 30 μ L or greater (Fig. 3). In the Curatest F, Finn Chambers AQUA, 0.5-mm depth IQ Ultra, 9-mm Torii, and discontinued 10-mm Van der Bend systems, unabsorbed liquid was minimal even with the largest volume of 40 μ L.

DISCUSSION

More than 40 years ago, the International Contact Dermatitis Research Group (ICDRG) devised guidelines concerning (1) the optimal concentrations of allergens for testing and (2) a standardized system for reading and interpreting the results.^{8,9} Overall, the international community widely accepted these recommendations, introducing standardization to patch testing. The simplicity of patch testing techniques belies complex variables that may affect patch test results. Persistent discussions deliberate the need for standardization, especially for reading and interpretation.^{10,11} However, before reading standardization can be realized, clinicians must appreciate the physical variables that can affect the delivered dose of allergen. After all, the delivered dose is ultimately responsible for the reactions that clinicians must read and interpret.

Volume and Coverage of Chambers

In 1984, Fischer and Maibach¹² filled Finn Chambers with different masses of petrolatum incorporating methylene blue (7.50–34 mg in 2.5 mg increments) and examined the distribution of the material on the skin. Based on a specific gravity of 0.83, they

TABLE 2. Measured and Calculated Dimensions of Patch Test Systems

System	Chamber Length or Diameter, mm	Internal Diameter, mm	Mean Area, mm ²	Depth mean (range), mm	Calculated Volume, μ L		
					Air Gap	Absorbent Media	Total
AllergEAZE							
Nonwoven polyester	8	8	64	0.70 (0.65–0.76)	44.99	21.76	66.75
On polyurethane	8	8	64	0.77 (0.68–0.83)	49.54	21.76	71.30
Curatest							
Curatest	12	8	50.27	0.17 (0.12–0.23)	8.60	24.88	33.48
Curatest F	12	8	50.27	0.13 (0.10–0.16)	6.74	22.62	29.36
Finn Chamber							
8 mm							
W/o filter paper	9.5	8	70.88	0.44 (0.41–0.48)	26.58	0	26.58
W/ filter paper	9.5	8	70.88	0.14 (0.11–0.18)	8.60	14.58	23.18
Polypropylene coated	9.5	8	70.88	0.41 (0.40–0.41)	24.47	0	24.47
Finn Chambers AQUA	9.5	8	70.88	0.42 (0.41–0.45)	25.32	17.11	42.42
12 mm							
W/o filter paper	14.5	12	165.13	0.61 (0.61–0.62)	84.52	0	84.52
W/ filter paper	14.5	12	165.13	0.22 (0.20–0.23)	30.61	43.72	74.33
18 mm							
W/o filter paper	21	18	346.36	1.02 (1.01–1.03)	303.13	0	303.13
W/ filter paper	21	18	346.36	0.11 (0.09–0.12)	32.55	241.75	274.30
Hill Top Chamber*							
19 mm w/ Webril	15	10.84	92.29	0.62 (0.61–0.63)	57.22	245.44	302.66
25 mm w/ Webril	20	17.5	240.53	0.54 (0.49–0.58)	128.68	567.06	695.74
IQ Ultra							
0.8-mm depth	9	9	81	0.81 (0.77–0.85)	65.37	0	65.37
0.5-mm depth	11	8	64	0.39 (0.34–0.45)	25.22	45.98	71.20
Torii							
9 mm	9	9	63.62	0 (0)	0	42.62	42.62
16 mm	16	16	201.06	0 (0)	0	126.67	126.67
Trolab	9	7.65	63.62	0.51 (0.47–0.54)	27.71	0	27.71
Van der Bend Chamber							
10 mm (discontinued)	10	10	100	0.78 (0.75–0.84)	78.10	33.00	111.10
8 mm (current)	8	8	64	0.24 (0.20–0.29)	15.36	46.08	64.44

*Based on the average of only 2 chambers.

WI, with; W/o, without.

concluded that the optimum volume of petrolatum that should be dispensed into a Finn Chamber is 12 to 15 μ L, which converts to 10 to 12.5 mg. In our analysis, only the 2 Curatest patches and the 9-mm Torii patch provided 100% coverage with 15 μ L of petrolatum as recommended by Fischer and Maibach. The depth of these products was minimal.

The 12 to 15 μ L volume recommended by Fischer and Maibach¹² corresponds to the manufacturer's recommendation of filling Finn Chambers slightly more than half of their volume (mean of 8-mm aluminum without filter paper and polypropylene-coated chambers, 26.58 and 24.47 μ L, respectively). In our experiment, however, 30 μ L (25 mg) of petrolatum was required to fill the 8-mm aluminum Finn Chamber to obtain complete coverage.

More recently, Bruze and coworkers⁷ patch tested 12 volunteers with Finn and Van der Bend Chambers filled with petrolatum with green dye (10–30 mg in 5 mg increments). Bruze et al⁷ used the discontinued 10-mm Van der Bend Chamber (Isaksson M, personal communication, 2013). Their criterion was 100% coverage

in green corresponding to the area of the chamber as visualized on the skin in at least 95% of the tests. They concluded that the optimum amount of petrolatum that should be dispensed in an 8-mm Finn Chamber was 20 mg. Based on Fischer and Maibach's¹ specific gravity of 0.83, 20 mg is equivalent to 24 μ L. As visualized under glass in our study, 30 μ L of disperse blue was needed to provide 100% coverage of the 8-mm aluminum Finn Chamber. Because the patch test systems were occluded with glass instead of skin, the generalizability of these findings to in vivo use is unclear.

Bruze et al⁷ also found that the green-dyed petrolatum adhered to the bottom of the discontinued Van der Bend Chambers (10 mm), regardless of the amount dispensed, even after the patches were worn for 2 days. Petrolatum tends to liquefy at body temperature, but it is possible that the greater overall volume of the chamber, its material, or both served as an impediment to liquefaction of the petrolatum. The conductivity of heat by aluminum is relatively high. Consequently, petrolatum in aluminum chambers may liquefy more readily than in plastic chambers. A volume of 45 μ L (37 mg)

TABLE 3. Coverage of Patch Test Chambers by Volume of 1% Disperse Blue in Petrolatum, Extrusion, and Equivalent Dose in mg/cm²* at 100% Coverage

Patch System	Coverage, %		Extrusion†			Dose at 100% Coverage, mg/cm ²
	<100 μ L (mg)	100 μ L (mg)	Mild, μ L	Moderate, μ L	Excessive, μ L	
AllergEAZE						
Nonwoven polyester	15–30 (12.5–25)	35 (29)	35–40	N/A	N/A	0.45
On polyurethane	15–35 (12.5–29)	40 (33)	35–40	N/A	N/A	0.52
Curatest						
Curatest	N/A	15 (12.5)	N/A	20, 25, 35	30, 40	0.20
Curatest F	N/A	15 (12.5)	N/A	20–40	N/A	0.20
Finn Chamber						
8 mm w/o filter paper	15–25 (12.5–21)	30 (25)	30	35–40	N/A	0.39
8-mm Finn Chambers AQUA	15–20 (12.5–16.6)	25–30 (21–25)	25–30	35–40	N/A	0.33–0.39
IQ Ultra						
0.8-mm depth (old)	15–25 (12.5–21)	30–35 (25–29)	35	N/A	40	0.32–0.36
0.5-mm depth (new)	15–20 (12.5–16.6)	25–30 (21–25)	N/A	N/A	30–40	0.33–0.39
Torii						
9 mm	N/A	15 (12.5)	15	20–35	40	0.15
16 mm	15–40 (12.5–33)	N/A	40	N/A	N/A	N/A
Trolab	15–25 (12.5–21)	30 (25)	35	40	N/A	0.39
Van der Bend Chamber						
10 mm (discontinued)	15–40 (12.5–33)	45 (37)	45–50	N/A	N/A	0.37
8 mm (current)	15–25 (12.5–21)	30–35 (25–29)	30–35	40	N/A	0.39–0.45

*Based on conversion factor of 0.83 as used by Fischer and Maibach (11,776).

†Mild extrusion: less than 1 mm beyond rim at point of greatest extrusion. Moderate extrusion: less than 2.5 mm beyond rim at point of greatest extrusion. Excessive extrusion: greater than 2.5 mm beyond rim at point of greatest extrusion.

N/A, not applicable; W/o, without.

of petrolatum was needed to cover 100% of the base of the discontinued 10-mm Van der Bend Chamber as visualized under the glass. In contrast, 30 to 35 μ L (25–29 mg) of the disperse blue provided 100% coverage of the more recently introduced 8-mm Van der Bend Chamber with minimal extrusion.

All systems except the 2 Curatest patches and the 9-mm Torii patch required greater volumes of disperse blue than Fisher and Maibach's recommendation of 12 to 15 μ L (10–12.5 mg) to provide complete or almost complete coverage (Table 3, Fig. 2). Although Bruze et al⁷ concluded that 20 mg was the optimal amount of petrolatum for the 8-mm Finn Chambers, 30 mg was needed to provide 100% coverage as visualized under glass. For the remainder of the patch test systems, 25 to 45 μ L (21–33 mg) of the disperse blue was required to provide 100% coverage (Table 3). Not all systems can be assumed to deliver the same amount even if the same volume of allergen is carefully measured and dispensed. Given that the ICDRG defines a positive patch test reading as corresponding to a reaction that fills the entire area of a chamber, the use of larger or deeper chambers that fail to provide complete coverage could bias readings toward false negatives, allowing clinically relevant allergens to escape detection.

Our method of applying glass over the chambers to visualize the coverage may limit generalization to the clinical application of chambers for several reasons. First, the physical properties of the skin differ from those of smooth glass; textural differences and the presence of hair may affect the way petrolatum contacts the

skin. Second, the volume of the chamber may change, depending on the size and shape of a patient's back. Third, the duration of occlusion during an actual patch test is considerably longer than in our evaluation. Fourth, 100% coverage of the entire base of a chamber by disperse blue was intended to mimic the complete footprint of a chamber as required by the ICDRG for a positive patch test reading. However, the volume of disperse blue that met this criterion may overestimate the amount needed in clinical situations because petrolatum liquefies at body temperature.

Regardless of how carefully the disperse blue was applied, it was challenging to consistently place it uniformly across each chamber and patch. The placement of a ribbon of the allergen slightly off the diameter of a chamber was sufficient to cause



Figure 4. As was the case for all the large patch test systems, 100% coverage was not obtained with the 16-mm Torii patch even with the largest volume of disperse blue used in this study (40 μ L). These large patches and chambers should be reserved for special uses (approximately actual size).

asymmetric extrusion while not quite covering the patch (eg, Curatest with 15 μ L; Fig. 2). Nonetheless, our visual demonstration does provide an indication of how coverage and extrusion of carefully measured amounts of an allergen in petrolatum can vary across different patch test systems when occluded.

In our study, even the smallest volume (15 μ L) of liquid saturated the absorbent material in the small patch test systems. The volumes dispensed moistened the entire surface area but absorbed a sufficient amount to avoid loss of liquid during placement. Excess liquid became visible only when volumes ranged from 30 to 40 μ L, depending on the chamber (Fig. 3). Nonetheless, particular care must be exerted when dispensing liquid allergens. When clinicians lift a patch test panel to place it on a patient's back, they need to be aware that unabsorbed or excess liquid can drip down the panel contaminating other allergens. Volatility can also be an issue with liquid allergens. Therefore, liquid allergens should only be dispensed immediately before application on a patient's back.

Others have also compared the coverage of test chambers by liquid allergens. Isaksson et al¹³ used the Finn Chamber and the 10-mm discontinued Van der Bend Chamber to patch test with green solutions of water, acetone, and alcohol; 15 μ L was micro-pipetted into the former and 20 μ L into the latter. Positive reactions were defined as 100% coverage in at least 95% of the tests with fewer than 5% of the chambers and patches exhibiting more than 2 mm of extrusion beyond their border. When ethanol was used, both chambers were associated with 100% positive reactions. When water and acetone were used in Finn Chambers, the rate of positive reactions was 96%. However, the rate of extrusion beyond 2 mm was also 96%. In contrast, none of the Van der Bend Chambers were associated with extrusion, regardless of the solution. The authors concluded that water solutions should be tested in Finn Chambers, whereas acetone and alcohol solutions would be better tested in Van der Bend Chambers. Whether this relationship would still hold true for the current, smaller Van der Bend Chamber is unknown. Technique, of course, can also introduce variability. When comparing results across studies, it must be kept in mind that Isaksson et al¹³ patch tested individuals, whereas we used glass to visualize spread and extrusion.

Timing is another factor that can contribute variability to patch testing. Fischer and Maibach¹² found that 0.5 mg (0.5 μ L) of water evaporated after 1 minute and then continued evaporating at a rate of 0.33 to 0.38 mg/min until only 1 to 2 mg remained. They reported that the evaporation rate for ethanol was 2 mg/min and that of acetone was 6 mg/min. Isaksson et al¹³ applied patches to the back after the solution was applied to 1 set of patches, but they did not report the actual length of time involved. We did not account for time in our study either, but we did observe that the larger volumes of liquid tended to be absorbed over time and that smaller amounts rapidly dried. Timing between dispensing and application of chambers warrants closer scrutiny.

In general, manufacturers of patch test systems offer scant guidance in the amount of allergen to be used with their product. For example, the instructions for the Curatest patch state that the

test substances should be applied to the test disk, but neither the amount of petrolatum nor the liquid that should be dispensed is mentioned. Based on a translation of the Japanese instructions for the Torii patch, the recommended amounts are 1 drop of liquid, 1 drop of metal, and a small amount of petrolatum. Droppers have been shown to be imprecise in liquid allergen administration.¹⁴ A Hill Top Chamber Fact Sheet provided only the weight and volume of their chambers at absolute capacity with no specific instructions for dispensing either liquid or petrolatum. The instruction for filling Finn Chambers with a semisolid such as petrolatum is to apply allergen to slightly more than half the volume of the chamber (a 5- to 6-mm bar with a 2-mm diameter). The instructions for dispensing liquid to Finn Chambers state that a disk of filter paper should be moistened without excess, any of which should be removed with porous paper. For allergEAZE, the most recent instructions recommend dispensing approximately 20 μ L of a semisolid diagonally across the base of the chamber. Based on our visualization technique, 35 to 40 μ L of petrolatum was needed to provide 100% coverage under glass. Instructions for both IQ Ultra chambers state that a drop of allergen (approximately 25 μ L) should be applied to filter paper; instructions for an allergen in petrolatum were not included.

The instructions for the recently introduced 8-mm Van der Bend Chamber note only that the maximum amount of substance should be 40 μ L. Based on visualization under the glass, 100% coverage was almost provided by 30 μ L of disperse blue, whereas 40 μ L moderately extruded beyond the outer edge of the chamber. Although the 8-mm Van der Bend Chamber is smaller than the discontinued 10-mm chamber, it is still manufactured from the same material (polypropylene). Consequently, further testing would be necessary to determine if petrolatum adheres to the 8-mm chamber as it did to the 10-mm chamber as reported by Bruze et al.⁷ In the discontinued 10-mm Van der Bend Chamber, 45 μ L of disperse blue was required to provide 100% coverage as visualized under glass; thus, the instructions for filling the Van der Bend Chamber likely does not cover the complete patch area. This finding is not just of historical interest. It raises the possibility that data collected using the discontinued Van der Bend Chamber could reflect insufficient contact between allergen and patient. For example, national differences in the prevalence of allergies could be underreported if data collection was based on the use of the discontinued Van der Bend Chamber. In future studies, it seems advisable to report the dimensions of a chamber along with its product name to avoid confusion in understanding the literature because products change over time.

These data raise another concern with patch testing interpretation. Consider, for example, a patient who has a reaction to an allergen on a patch test but the skin response (infiltrated erythema) does not correspond to the entire area of the chamber. Based on the ICDRG standard, such a response would be considered an irritant, doubtful, or negative. However, it also could reflect incomplete transfer of the allergen to the skin because an inappropriate amount was dispensed to the chamber selected for use or

because the allergen adhered to the chamber with little or none touching the skin. False negatives preclude a diagnosis and prevent effective management for patients with allergic contact dermatitis. Thus, clinicians may consider retesting in the case of a partial or doubtful response in large chambers.

Bruze et al⁷ noted that a test area completely covered with allergen and with less than 2.5 mm of extrusion (so as not to interfere with surrounding test sites) satisfies the ICDRG prerequisites for a positive patch test. In our study, only the small patches were associated with more than 2.5 mm of extrusion and only with the higher volumes of the disperse blue (30–40 μ L; Fig. 2, Table 3). On all the patch test systems that we evaluated, interchamber distances ranged from 9 to 25 mm (Table 1). Theoretically, 2.5 to 3 mm of extrusion may be within acceptable limits. However, no firm data are available on whether allergen in petrolatum extruded from a chamber would continue to spread after 24 to 48 hours of wear, which provides an opportunity for further liquefaction in an actual patch testing situation. Most experts, however, recommend avoiding adjacent placement of cross-reacting or co-reacting allergens such as formaldehyde and releasers, fragrances/myroxylon, metals, and topical antibiotics (eg, neomycin and bacitracin). When excessive extrusion is apparent and a skin response is present, clinicians should rule out cross- and co-reactions. When a patch test panel is removed, clinicians should observe whether the panel adhesion was poor, whether an extrusion occurred, and whether the allergen remained in the chamber. Such information may help clarify interpretation of a reaction and future diagnostic choices.

None of the volumes completely covered the base of the large chambers (>10 mm), indicating that these systems should be reserved for special testing circumstances such as with a patient's personal products. Large chambers are also appropriate for testing for food allergies. Niggemann et al¹⁵ reported that sensitivity, specificity, positive and negative predictive values, and efficiency of the 12-mm Finn Chambers were better than that of the 6-mm Finn Chambers for atopic patch testing of cow and soy milks, eggs, and wheat powder in infants and children.

Chamber Size and Dose per Unit Area

As Friedmann and others^{2–5} have shown, dose per unit area is the critical factor underlying sensitization. Allergen dose is determined by the concentration of allergen and the volume or amount applied to the patient as a function of the area of the skin exposed to the allergen (ie, dose per cm^2). Although the data from our study confirm, the volume of disperse blue that provided 100% coverage (as required by the ICDRG criterion for a positive patch test response) varied with the size of the chamber. How then do the dimensions of a chamber affect dose delivery?

Based on the volume of 1% disperse blue that provided 100% coverage with minimal extrusion in this study and using Fischer and Maibach's conversion factor of petrolatum of 0.83, the dose per area in the chambers evaluated ranged from approximately

15 mg/cm^2 with the 9-mm Torii patch to more than twice that amount for the allergEAZE chamber on polyurethane (52 mg/cm^2 , Table 3). To avoid exposing patients to an inadequate or excessive dose, clinicians who patch test should be cognizant of this variation and choose their chambers and amount of allergen accordingly.

Bruze et al¹⁶ found that intraindividual and interindividual variability affects the amount of both petrolatum and liquid allergen preparations applied at patch testing by technicians. Well-practiced technicians can dispense the amount of petrolatum-based allergens within a limited range, but they must be trained to a desired target amount. That is, with practice, clinicians can roughly calibrate themselves to dispense a known amount of petrolatum. Nonetheless, variation will always exist if the allergen is dispensed to a patch or chamber directly from prefilled syringes or other containers and actual dose delivered will likewise be affected. Furthermore, Jaimes et al¹⁷ compared 3 technicians and found that the mean amount of petrolatum also varied with the size of the syringe from which it was dispensed. There was less variability when petrolatum was expressed from small syringes (0.5 and 1.0 mL) compared with large syringes (5 mL). Many commercial allergens are prepackaged in 5-mL syringes.

Frick-Engfeldt et al¹⁸ compared 4 technicians using 3 different techniques to dispense aqueous solutions of allergens (formaldehyde and methylchloroisothiazolinone/methylisothiazolinone) to Finn Chambers. The 3 techniques were micropipetting, dripping the solution, and dripping the solution and wiping away excess solution. The micropipette technique was the most accurate and the most precise, and it was associated with the least interindividual variation. These studies, coupled with our findings, underscore that micropipetting allergens into an appropriately sized chamber is the only way to dispense a dose accurately. We recommend pipetting be the standard for research protocols. As the glass visualization experiment shows, slight variations in where the allergen is placed on a chamber can also affect the pattern of spread and extrusion, but whether this finding has clinical relevance is unknown.

If the target dose of an allergen is known based on its percent concentration, the amount dispensed can be scaled up or down, depending on the size of the chamber. For example, the German Contact Dermatitis Research Group has concluded that 1% paraphenylenediamine (PPD) represents a sensitizing concentration. To reduce sensitization, the group now recommends that a 0.35% concentration of PPD be used in patch testing.^{19–21} If we assume that 20 mg of 0.35% PPD is dispensed in a chamber with an area of 0.64 cm^2 , the target dose of PPD would be 0.11 $\text{mg PPD}/\text{cm}^2$ ($20 \times 0.0035/0.64$). The same target dose could be achieved by dispensing only 7 mg of 1% PPD. However, the corresponding volume would be less than 9 μ L, which would have been too little to provide complete coverage on glass even with a small patch test system such as Curatest or Torii. Again, however, our ability to generalize these results to how various patch test systems perform in vivo is limited. With its standardized, prefilled dose of PPD at 0.09 mg/cm^2 with only 1 mg of excipient, T.R.U.E. TEST (SmartPractice, Phoenix, AZ) is an alternative option.

Although we based all calculations in this analysis on the specific gravity used by Fischer and Maibach¹² for ease of discussion, individual formulations and grades of petrolatum also vary and are often proprietary. Different products will therefore have different melting points and viscosities, which would likely affect their performance in patch testing. We found that the specific gravity of petrolatum alone ranges from as low as Fischer and Maibach's 0.83 to as high as 0.91 (eg, see <http://www.sciencestuff.com/msds/C2213.html>, www.dormer.ca/pdf_msds/m-032.pdf, <http://www.inchem.org/documents/icsc/icsc/eics1440.htm>). Furthermore, the concentration of allergen in petrolatum varies across the many commercially available allergens and with individualized clinic preparations. Consequently, we did not attempt to account for this variable and its effect is unknown but bears consideration.

CONCLUSIONS

Patch testing is the criterion standard for diagnosing allergic contact dermatitis; however, it lacks rigorous standardization. The study performed by Fischer and Maibach¹² provides a historical precedent for the use of this study. We hope that this work may catalyze further interest in standardization and promote discussion concerning guidelines for testing volumes in different patches.

In this study, we discussed and demonstrated the following: (1) There are considerable physical differences across patch testing systems that may affect comparison and repeatability of patch test results. (2) Instructions from manufacturers on how to load patch test systems are often incomplete and are a barrier to standardization. (3) We present test volumes for each patch that provide area coverage while minimizing extrusion in vitro. (4) There is evidence that chambers used as instructed may be unable to present allergen in sufficient area to meet the ICDRG criteria for a positive patch test. (5) Patches that lack chambers, such as the Torii, can only be used with small amounts of allergens (approximately 15 μ L). (6) Different patch test systems likely do not deliver the same dose of allergen if the same volume of excipient is applied. (7) We recommend that in future studies, the dimensions of chambers used and testing volumes be reported in addition to the brand or manufacturer.

ACKNOWLEDGMENTS

The authors thank Jack Manemann and Mike Klenk for their assistance in measuring chamber dimensions, Kathryn Shannon for her assistance in preparing the patch test chambers, and Dave Bulno for photography.

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