

Comparison of reactivity to allergens using the TRUE Test and IQ chamber system

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The reproducibility of the patch test is of great importance to its diagnostic utility. The objective of this study was to quantitatively and qualitatively compare the reproducibility of patch test reactions between the manufacturer-loaded TRUE Test[®] (TT) panel and the investigator-loaded IQ[®] Chamber (IQC) system using the European Standard Series (ESS) (Chemotechnique Diagnostics, Malmö, Sweden). Consecutive patients were simultaneously patch tested with 21 allergens using the TT and IQC system. The concordance and discordance of the 2 methods were analysed. Testing was performed in 207 patients (85 men and 122 women) who yielded 317 positive reactions, 188 with the IQ system and 129 with the TT. A total of 204 tests were positive with both methods, showing a positive concordance rate of 64.4%, with IQC producing more positive results. A high concordance rate of positive results was achieved for Cl+ Me– isothiazolinone (81.5%), nickel sulfate (79%), formaldehyde (76.9%), and paraphenylenediamine (PPD) (72.7%); a moderate concordance was noted for quaternium 15 (66.7%), potassium dichromate (64.3%), and fragrance mix (58.1%); and a low concordance was noted for cobalt chloride (27.6%) and Balsam of Peru (18.2%). The IQC and the TT are similar in finding allergy to allergens with high concordance. The IQC system has an advantage in detecting clinically relevant reactions to allergens that have a moderate and a low concordance.

Key words: European standard series; IQ[®] chambers; patch testing; reproducibility; TRUE Test.
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The reproducibility of patch tests is of great importance for their diagnostic utility. Several studies have addressed the question of reproducibility using different approaches including synchronous left- versus right-sided patch testing with allergens applied in the investigator-loaded Finn Chambers (1, 2), or manufacturer-loaded test systems such as Epiguick (3) and TRUE Tests (TT) (4) and identical test allergens from different commercial sources (5).

The standardization of patch testing remains controversial. Some authors suggest that patch testing using Finn Chambers[®] (Epitest Ltd. OY, Tuusula, Finland) is not as standardized and accurate as the thin layer rapid use epicutaneous tests TT (Pharmacia, Uppsala, Sweden), which are preloaded with allergen. Reproducibility with positive patch tests using the Finn Chambers is around 70% (6) to 95% (5) and using TTs, it is about 95% (4).

Gollhausen et al. (7) reported a 37.9% nonreproducibility rate for the Finn Chamber compared

with 17.9% for the TT. Concordance between the 2 methods varied from 63% to 78% in different studies (7–14). 1 group concluded that the Finn Chamber is superior to the TT in detecting relevant contact allergens, such as fragrance mix, Balsam of Peru, and thiuram mix (14).

The aim of this study was to assess the reproducibility of the TT against the IQ[®] Chamber (IQC) method using the European Standard Series (ESS) (Chemotechnique Diagnostics, Malmö, Sweden). The concordance and discordance of the paired results were calculated taking clinical relevance into account. To refine our results, we qualitatively evaluated the intensity of the positive reactions in the 2 tests.

Material and Methods

The cohort of this study included consecutive patients with suspected contact allergy who were referred to 2 dermatology patch testing clinics of 2 medical centres from November 2003 to May

2004. 2 investigators (1 in each centre) read the reactions. This trial was not randomized or blinded.

After informed consent was provided, the patients were patch tested with the TT panel and the IQC method using the ESS. Additional series were applied as required for the individual patients. For purposes of the study, we selected for comparison 21 allergens from the 23 allergens of the ESS and 21 corresponding allergens from

the 24 allergens of the TT panel, as shown in Table 1. The allergens applied on the IQC square chambers were placed on unaffected skin of the right upper back and the TT allergens were placed on the left side. The patches were removed after 2 days, and readings were performed after 48 and 72 hr, according to the standard patch test recommendations (15). All positive responses were recorded and clinically scored for intensity as follows: +1 – erythema and infiltration; +2 – erythema,

Table 1. Matched allergens from the ESS and TT

Allergen from the IQC	Concentration (% w/w)	Allergen from the TT panel	Concentration ($\mu\text{g}/\text{cm}^2$)
Potassium dichromate	0.5 pet.	Potassium dichromate	200
4-Phenylenediamine base	1.0 pet.	<i>p</i> -Phenylenediamine	90
Thiuram mix	1.0	Thiuram mix	25
Tetramethylthiuram monosulfide	0.25	Tetramethylthiuram monosulfide	EP
Tetramethylthiuram disulfide	0.25	Tetramethylthiuram disulfide	EP
Tetraethylthiuram disulfide	0.25	Disulfiram	EP
Dipenthmethylenethiuram disulfide	0.25	Dipenthmethylenethiuram disulfide	EP
Neomycin sulfate	20.0 pet.	Neomycin sulfate EP	230
Cobalt chloride	1.0 pet.	Cobalt chloride	20
Benzocaine	5.0 pet.	Caine Mix	630
		Benzocaine EP	5 parts
		Cinchocaine hydrochloride BP	1 part
		Amethocaine hydrochloride EP	1 part
Nickel sulfate	5.0 pet.	Nickel sulfate	200
Clioquinol (Chinoform, Vioform)	5.0 pet.	Quinoline mix	190
		Clioquinol	EP
		Chlorquinaldol	EP
Colophony	20 pet.	Colophony	850
Paraben mix	16.0 pet.	Paraben mix	1000
Methyl-4-hydroxybenzoate	4.0	Methyl-4-hydroxybenzoate	EP
Ethyl-4-hydroxybenzoate	4.0	Ethyl-4-hydroxybenzoate	EP
Propyl-4-hydroxybenzoate	4.0	Propyl-4-hydroxybenzoate	EP
Butyl-4-hydroxybenzoate	4.0	Butyl-4-hydroxybenzoate	EP
		Benzylparahydroxybenzoate	EP
<i>N</i> -isopropyl- <i>n</i> -phenyl-4-phenylenediamine	0.1 pet.	Black Rubber mix	75
		<i>N</i> -isopropyl- <i>n</i> -phenyl paraphenylenediamine	2 part
		<i>N</i> -cyclohexyl- <i>N</i> phenyl paraphenylenediamine	5 parts
		<i>N,N</i> -diphenyl paraphenylenediamine	5 parts
Wool alcohols	30.0 pet.	Wool alcohols	1000
Mercapto mix	2.0 pet.	Mercapto mix	75
<i>N</i> -cyclohexylbenothiazyl sulfonamide	0.5	<i>N</i> -cyclohexylbenothiazyl sulfonamide	EW
Mercaptobenzothiazole	0.5		
Dibenzothiazyl disulfide	0.5	Dibenzothiazyl disulfide	EW
Morpholinylmercapto benzothiazole	0.5	Morpholinylmercapto-benzothiazole	EW
Epoxy resin	1.0 pet.	Epoxy resin	50
Balsam of Peru	25.0 pet.	Balsam of Peru	800
4- <i>tert</i> -Butylphenol formaldehyde resin	1.0 pet.	<i>p</i> - <i>tert</i> -Butylphenol formaldehyde resin	40
Mercaptobenzothiazole	2.0 pet.	Mercaptobenzothiazole	75
Formaldehyde	1.0 aq.	Formaldehyde	180
Fragrance mix	8.0 pet.	Fragrance mix	430
Cinnamic alcohol	1.0	Cinnamyl alcohol	4 parts
Cinnamic aldehyde	1.0	Cinnamaldehyde	2 parts
Hydroxycitronellal	1.0	Hydroxycitronellal	4 parts
Amylcinnamaldehyde	1.0	α -Amylcinnamaldehyde	1 part
Geraniol	1.0	Geraniol	5 parts
Eugenol	1.0	Eugenol	2 parts
Isoeugenol	1.0	Isoeugenol	1 part
Oak moss absolute	1.0	Oak moss absolute	5 parts
Quaternium 15	1.0 pet.	Quaternium 15	100
Cl+ Me– isothiazolinone	0.01 aq.	Cl+ Me– isothiazolinone	4

Aq., aqua; IQC, IQ Chamber using the ESS; EP, equal parts; ESS, European Standard Series; EW, equal weight; pet., petrolatum; TT, TRUE Test.

infiltration, and papules or papulo-vesicles; and +3 – severe erythema, infiltration, and coalescing vesicles. Irritant and doubtful reactions were included in the negative-results group. Demonstrated exposure was also assessed. A positive reaction was considered to have shown exposure if the patient was known to have cutaneous exposure to a product known to contain the allergen to which the patient reacted. The exposure assessment was based on information from packages and safety data sheet when available. Negative concordance was defined as a negative reaction to the same allergen in both systems, positive concordance – positive reaction to the same allergen in both systems, or discordance – positive reaction of an allergen in 1 system and negative reaction to the same allergen in the other system. The formulas used to calculate positive and negative concordance are shown in Chart 1.

Sensitivity, specificity, efficiency and nonreproducibility were calculated according to the formulas described by Brasch et al. (1).

Results

Simultaneous testing was performed in 207 patients, 85 men and 122 women, with mean age 44.5 ± 17.7 years. Contact dermatitis had been present for a median of 12 months before patch testing. 15% of the patients had a history of atopic disease (atopic dermatitis, asthma and hay fever).

A total of 9725 individual patch tests were obtained. The findings were compared only for the 21 preselected allergens that appear in both series, for a total of 8694 tests.

317 positive reactions were observed (3.6% of 8694 tests), 188 with the IQC system and 129 with TT. 204 tests (102 pairs) were positive in both groups, showing a positive concordance rate of 64.4% [calculated according to the formula, $102 \times 2 / (188 + 129) \times 100$].

A total of 8377 negative reactions were observed (96.4% of 8694 tests). There were 2352 negative reactions observed in the IQC system (112 patients) and 2583 observed in the TT system

(123 patients). Concordant negative results were noted in 4326 tests (103 pairs = 206×21 allergens), showing a negative concordance rate of 87.7%, which was calculated according to the formula $[(103 \times 2 \times 21) / (2352 + 2583)] \times 100$. The residual 3442 patch tests constituted the negative patches of patients who had at least 1 positive result either in the IQC or in the TT series.

The comparative results of testing with each individual allergen are shown in Table 2. Good concordance of positive results was achieved for Cl+ Me– isothiazolinone (81.5%), nickel sulfate (79%), formaldehyde (76.9%), and paraphenylenediamine (PPD) (72.7%). Moderate concordance of the results was obtained for quaternium 15 (66.7%), potassium dichromate (64.3%), and fragrance mix (58.1%), and low concordance was obtained for cobalt chloride (27.6%) and Balsam of Peru (18.2%). The number of positive results obtained for colophony, paraben mix, clioquinol, mercapto mix, and mercaptobenzothiazole were too small for statistical comparison. The intensity levels of responses in the 2 tests are shown in Table 3. Concordance of reaction strength was observed in 72.5% of the cases and discordance in 27.5%. The allergens with the greatest discordance in intensity levels of response were Cl+ Me– isothiazolinone, which yielded stronger positive reactions in the TT, followed by fragrance mix. In most of the paired reactions with uneven strength, the difference in clinical scoring did not surpass 1+.

Efficiency, specificity, sensitivity, and nonreproducibility were calculated for all the allergens. The most significant results for the 9 allergens that triggered the greatest number of positive responses are shown in Table 3. Efficiency and specificity were high for all 9 allergens (between 0.90 and 0.99). However, the sensitivity results (indicating also reproducibility) varied. The highest sensitivities were observed for Cl+ Me– isothiazolinone (0.69) and nickel sulfate (0.65) and the lowest for Balsam of Peru (0.10) and cobalt chloride (0.16). Accordingly, the nonreproducibility was lowest for Cl+ Me– isothiazolinone (0.31)

$$\% \text{positive concordance} = \frac{\text{concordant positive allergens (IQC, TT)} \times 2}{(\text{total positive allergens, IQC}) + (\text{total positive allergens, TT})} \times 100$$

$$\% \text{negative concordance} = \frac{\text{concordant negative allergens (IQC, TT)} \times 2 \times 21a}{(\text{total negative allergens, ESS} \times 21a) + (\text{total negative allergens, TT} \times 21a)} \times 100$$

where 21a represents the number of allergens that appeared in both tests; IQC, IQ Chamber using the European Standard Series allergens and TT, TRUE tests.

Chart 1. Formulas used to calculate positive and negative concordance.

Table 2. Comparative results for allergens tested with IQC with ESS allergens and TT panel

Allergen	+ tests, IQC	+ tests, TT	+ tests, IQC + TT	% concordance	Discordance in IQC	Discordance in TT	Efficiency	Sensitivity	Specificity	Non-reproducibility
Potassium dichromate	19	9	9	64.3	10	0	0.95	0.47	0.95	0.53
4-Phenylenediamine base	5	6	4	72.7	1	2	0.99	0.57	0.99	0.43
Thiuram mix	4	3	1	28.6	3	2				
Neomycin sulfate	2	4	2	66.7	0	2				
Cobalt chloride	23	6	4	27.6	19	2	0.90	0.16	0.90	0.84
Benzocaine	4	3	2	57.1	2	1				
Nickel sulfate	41	40	32	79.0	9	8	0.92	0.65	0.90	0.35
Clioquinol	0	1	0	0.0	0	1				
Colophony	1	1	1	100.0	0	0				
Paraben mix	2	2	2	100.0	0	0				
N-Isopropyl-n-phenyl-4-phenylenediamine	3	3	2	66.7	1	1				
Wool alcohols	0	0	0	0.0	0	0				
Mercapto mix	2	2	2	100.0	0	0				
Epoxy resin	4	2	2	66.7	2	0				
Balsam of Peru	19	3	2	18.2	17	1	0.91	0.10	0.91	0.90
4-tert-Butylphenol formaldehyde resin	4	3	3	85.7	1	0				
Mercaptobenzothiazole	2	1	1	66.7	1	0				
Formaldehyde	16	10	10	76.9	6	0	0.97	0.63	0.97	0.38
Fragrance mix	19	12	9	58.1	10	3	0.94	0.41	0.93	0.59
Quaternium 15	3	6	3	66.7	0	3	0.99	0.50	0.99	0.50
Cl+ Me– isothiazolinone	15	12	11	81.5	4	1	0.98	0.69	0.97	0.31
Total	188	129	102	64.4	86	27				

ESS, European Standard Series; IQC, IQ Chamber using the ESS; TT, TRUE test.

Table 3. Comparison of intensity levels of the paired allergic reactions

Allergen	+1		+2		+3	
	IQC	TT	IQC	TT	IQC	TT
Potassium	6	6	3	3		
PPD	1	1	1	1	2	2
Thiuram mix		1	1			
Neomycin	2	1				1
Cobalt chloride	3	4	1			
Benzocaine	2	2				
Nickel sulfate	20	22	10	8	2	2
Colophony	1	1				
Paraben mix	2	2				
N-Isopropyl-N-phenyl-4 phenylenediamine (IPPD)	2	2				
Mercapto mix	1	2	1			
Epoxy resin		1	2	1		
Balsam Peru	1	1	1	1		
Butylphenol	3	2		1		
formaldehyde resin						
Mercaptobenzothiazole (MBT)		1	1			
Formaldehyde	8	8	2	2		
Fragrance mix	7	6	1	2	1	1
Quaternium 15	3	2		1		
Isothiazolinone	6	3	3	5	2	3
Total	68	68	27	25	7	9

IQC, IQ Chamber using the ESS; PPD, paraphenylenediamine; TT, TRUE test.

and nickel sulfate (0.35) and highest for cobalt chloride (0.84) and Balsam of Peru (0.90).

Positive reactions with demonstrated exposure are shown in Table 4. The TT failed to detect 78% of the clinically relevant reactions to cobalt chloride in the IQC method, whereas the IQC method failed to identify only 4% of the clinically relevant reactions to cobalt chloride in the TT. Similarly, the TT failed to detect 80% of the clinically relevant reactions to Balsam of Peru in the IQC method, whereas the IQC method detected all the positive results obtained with the TT. In the case of quaternium 15, the IQC method failed to detect 50% of the clinically relevant reaction obtained with the TT, whereas the TT did not miss any of the positive reactions obtained in the IQC method.

Discussion

Factors that influence the outcome of a patch test in a sensitized person include the test concentration and the dose delivered, the amount of applied material, and the use of an appropriate vehicle. Test reproducibility may be biased by these factors and by regional and intraregional variations in skin absorption and responsiveness, skin hyper-reactivity or hyporeactivity, and subclinical sensitization. Nevertheless, Ale and Maibach (4) showed a 95% reproducibility rate for positive results in the TT. They concluded that patch test-

Table 4. Demonstrated exposure of the positive patch test reactions

Allergen	Reactions with demonstrated exposure			
	IQC+ TT+ (%)	IQC+ TT- (%)	IQC- TT+ (%)	N
Potassium	9 (47)	10 (53)		19
PPD	4 (57)	1 (14)	2 (29)	7
Cobalt	4 (17)	18 (78)	1 (4)	23
Nickel	16 (76)	1 (5)	4 (19)	21
Balsam of Peru	2 (20)	8 (80)		10
Formaldehyde	10 (63)	6 (37)		16
Fragrance mix	9 (50)	8 (44)	1 (6)	18
Quaternium 15	3 (50)		3 (50)	6
Isothiazolinone	11 (69)	4 (25)	1 (6)	16

IQC, IQ Chamber using the European Standard Series; PPD, paraphenylenediamine; TT, True test.

ing is a reasonably reproducible procedure as long as methodological error is minimized. Schiessl et al. (5) reported 95% concordant allergic reactions using the Finn Chamber method and identical test allergens from different commercial sources.

In this study, the positive concordance between the IQC and the TT was 64.4%, which is close to the results reported by Wilkinson et al. (12) and Goh (13). The positive concordance differed by individual allergens tested, being high for isothiazolinone, nickel, formaldehyde, and PPD. Accordingly, the sensitivity of these allergens was high (0.57–0.67), and their nonreproducibility was low (0.31–0.4). The IQC Chamber method produced more positive results than the TT for isothiazolinone, nickel, and formaldehyde, and the TT produced slightly more positive results for PPD. These findings disagree with those of Goh (13), who showed a greater frequency of positive reactions to PPD with the Finn Chamber technique. Our results also differ from those of Suneja and Belsito (14), who reported a somewhat better performance of the TT in detecting relevant reactions to nickel and Cl+ Me– isothiazolinone.

Fragrance mix and potassium dichromate produced more positive results with the IQC method than with the TT, and the concordance of positive results for these allergens between the 2 methods was moderate (58.1%–64.3%). Their nonreproducibility was moderate as well (0.53–0.59).

Our findings for quaternium 15, for which the 2 methods showed a moderate concordance (66.7%) and which triggered more positive results in the TT, agree with those of Wilkinson et al. (12). The 2 allergens with a low concordance of positive results, namely Balsam of Peru and cobalt chloride, also produced a significantly greater number of positive results with the IQC method. These findings are supported by several studies showing the Finn Chamber methodology to be superior to the TT in detecting reactions to fragrance mix and

Balsam of Peru (4, 8, 13, 14). In addition, Fernandez Vozmediano and Hita (8) also found the Finn Chamber better for detecting allergies to potassium dichromate, nickel, and cobalt.

The nonreproducibility of allergens with moderate and low concordance may be influenced by different sources. In a manually dispensed system, such as the Finn Chambers, the optimal recommended working volume of 12–15 µl (16) varies within a wider range and can lead to inaccurate results (17, 18). This variation was reported to be higher when testing allergens in solution (19, 20). The uneven distribution of test substances within the petrolatum, producing droplets of high concentration of test substance (21), is another source of variability.

The discordance in results for certain allergens between the 2 methods may be due to false-positive reactions with the IQC. This was shown by the high discordance of positive results for this test and the high false-negative reactions with the TT (high discordance of negative results). In this respect, demonstrated exposure proved a useful, although imperfect, tool to evaluate discordant reactions, as shown also by other studies (13, 14). Last, although the allergens from the IQC and TT are corresponding, they are not exact matches in regard to concentration and composition.

This study has some limitations such as not being blinded, randomized having small number of positive results for some allergens (colophony, paraben mix, clioquinol, mercapto mix and mercaptobenzothiazole), which made statistical interpretation impossible.

Our study shows that both the IQC and the TT can detect allergy to Cl+ Me– isothiazolinone (81.5%), nickel sulfate (79%), formaldehyde (76.9%), and PPD (72.7%) with high concordance. There was also a good concordance in intensity in 72.5% of the paired positive reactions. The IQC method had an advantage in detecting clinically relevant reactions to allergens with moderate concordance, such as potassium dichromate (64.3%) and fragrance mix (58.1%), and with low concordance, such as cobalt chloride (27.6%) and Balsam of Peru (18.2%). Thus, if the TT is used, positive results for these allergens may be lost.

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