

The Relevance of 7-Day Patch Test Reading

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Background: Patch test readings are usually performed on day 2 (48 hours) and day 4 (96 hours). However, reports in the literature identify delayed allergy to metals, corticosteroids, antibiotics, some preservatives, acrylic and methacrylic monomers and *p*-phenylenediamine.

Objectives: The aim of our study was to identify the benefit of performing a day 7 (168 hours) reading to identify relevant late positive reactions.

Patients/Methods/Materials: Two hundred three consecutive patients were patch tested to the British Society for Cutaneous Allergy standard series with additional test series selected according to clinical history and applied at the same time.

Results: Twenty-six patients (12.8%) had new positive reactions on day 7 (168 hours), with 28 relevant positive reactions to 21 allergens. These included mercury 0.5% (2/26); cobalt chloride 1% (2/26); colophony 20% (2/26); disperse blue mix 106/124 1% (2/26); preservatives (4/26) that included Methylchloroisothiazolinone/methylisothiazolinone, sodium metabisulfite, and diazolidinyl urea; fragrances (7/26); and gentamycin sulfate 20% (1/26).

Conclusions: These results confirm findings in the literature and support the argument for performing a day 7 reading (168 hours) to identify relevant late positive reactions.

THE TECHNIQUES of patch testing have evolved to a standardized methodology worldwide, but variations in protocols remain with regard to reading times. Most patch test reactions occur at day 3 (72 hours) or 4 (96 hours). Patch test reactions that become positive *de novo* at day 7 (168 hours) or later are regarded as late or delayed reactions. Most centers interpret patch test results at 48 hours (day 2) and at 96 hours (day 4) after placement. A third reading at day 5 to 7 identifies a proportion of relevant positive reactions where sensitivity is weak or where there is poor absorption of the allergen.¹ In contrast to a late reaction, patch test sensitization develops at least 10 days after patch-test application and is regarded as an extremely infrequent adverse event of patch testing.²

The British Society for Cutaneous Allergy (BSCA) recommends that patch test readings are performed on day 2 (48 hours) and day 4 (96 hours).³ Most dermatologists apply a standard series and additional series based on history but may add extra series after 48 hours depending on the readings at day 2, and therefore, these tests will only have a 48-hour reading. Reports in the literature identify delayed allergy, in 7% to 14% of patients, to metals, corticosteroids, antibiotics, some preservatives, acrylic and methacrylic

monomers, and *p*-phenylenediamine (PPD). However, this requires an extra reading for patients and staff, and so, a day 7 (168 hours) reading is not standard practice.

The aims of our study were to identify allergens with delayed positive reactions and to determine the role of performing an additional day 7 (168 hours) reading in identifying new relevant positive reactions.

METHODS

Two hundred three consecutive patients, older than 14 years, were tested to the BSCA standard series. Additional test series were applied at the same time, selected according to clinical history, and included cosmetic/facial, fragrances, medicaments, hairdressing, corticosteroids, textiles and dyes, dental screening battery, dental (meth)acrylate, (meth)acrylate printing series, rubber additives, plastics and glues, amalgam, epoxy, and photographic chemicals series (Chemotechnique Diagnostics, Vellinge, Sweden) and the patients' own products. Allergens were applied to the upper back using IQ ultra chambers (Chemotechnique Diagnostics). The allergens were removed after 48 hours. All patients had patch tests read and interpreted by the same consultant dermatologist at day 2 (48 hours), 4 (96 hours), and 7 (168 hours). The strength of reactions was graded on the International Contact Dermatitis Research Group grading scale.

RESULTS

Two hundred three patients had patch testing. Twenty six patients (12.8%) had new relevant positive reactions on day 7, with

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28 positive reactions to 21 allergens. These were seen to mercury 0.5% (2 patients), cobalt chloride 1% (2 patients), colophony 20% (2 patients), disperse blue mix 106/124 1% (2 patients), preservatives, fragrances, and gentamycin sulfate 20% (Table 1). Two patients had multiple relevant late reactions. A patient with suspected fragrance allergy had positive reactions to geraniol 2% and hydroxycitronellal 2%. One patient, with oral lichenoid reaction of uncertain significance, had positive reactions to mercury 0.5% and palladium chloride 2%. Forty patients, referred from the oral medicine department, were tested to the BSCA series and dental screening battery. Four patients had new positive late readings, which included mercury 0.5% (2/4), palladium chloride (1/4), diazolidinyl urea (Germal II) 2% (1/4), and fragrance mix I 8% (1/4). No new positive late reactions to corticosteroids were seen. No irritant reactions were seen.

DISCUSSION

We found that interpreting patch test reactions at 48 and 96 hours is sufficient to identify most positive patch test reactions. However, 12.8% of patients had new relevant reactions at 168 hours to cobalt chloride, mercury, colophony, disperse blue mix, preservatives, fragrances, and gentamycin sulfate, which confirms previous

reports. Performing an additional 168-hour reading has implications in terms of cost, staffing, and inconvenience for patients. Therefore, it is important to appropriately select which patients may benefit from this additional reading.

Comparison with previous studies in the literature is complicated by variations in terminology of what is considered a late or delayed reaction. Various studies have defined late readings as occurring from 144 to 672 hours. Previous studies are summarized in Table 2. The terminology used internationally varies, with the North American Contact Group referring to standard 48- and 96-hour readings as day 3 and 5, while various European Groups studies refer to these readings as day 2 and 4.^{4,5} In addition, different concentrations of allergens are used, such as methylchloroisothiazolinone/methylisothiazolinone 0.02% used by the BSCA and Swedish Contact Groups and 0.01% by the North American Contact Group. Similarly, many American groups test with nickel sulfate 2.5%, whereas European centers use 5%.

Mitchell⁶ compared patch test readings at 48 and 168 hours in 499 patients. There were 107 positive reactions at 168 hours, which had been negative at 48 hours, a total of 21%. However, this study omitted the standard 96-hour reading. Twenty-four patients (4.8%) showed late reactions to neomycin. Other allergens that produced late reactions included PPD, caine mix, potassium dichromate, and Amerchol. Macfarlane et al⁵ noted a 7.2% incidence of newly positive reactions after day 4. In this study, 403 patients were examined at 48, 96, 168, and 216 hours. Late positive reactions after 96 hours were noted in 29 (7.2%) patients. The allergens that most commonly produced late positive reactions were neomycin, potassium dichromate, and cobalt.

Davis et al⁴ reported the patch tests of 372 patients at 96 hours and at least once during days 7 to 21. The new positive allergens, after 120 hours, were gold sodium thiosulfate 0.5% (22/253 patients, 6.2%), dodecyl gallate 0.25% (6/105, 5.7%), palladium chloride 2% (8/194, 4.1%), and neomycin sulfate 20% (10/253, 4.0%). They suggested that certain allergens should prompt physicians to perform a later reading including metals (gold, palladium, beryllium, nickel, cobalt), topical antibiotics (neomycin sulfate, bacitracin), and some preservatives (dodecyl gallate, thiomersal, methenamine). They reported a lower number of delayed positive reactions to PPD (0.4%), which was at variance with Hillen et al,⁷ who found delayed reactions in 1.5%. Our study also found a low incidence of delayed positive reactions to PPD, with no new positive reactions in patients tested to PPD 1.0% at 168 hours.

Jonker and Bruynzeel⁸ from Denmark compared readings at 48 and 72 hours with later readings at 144 and 168 hours. Late positive reactions after 72 hours were seen in 62 (8.2%) of 760 patients. The allergens included nickel sulfate 5% (2.6%), neomycin sulfate 20% (0.9%), tixocortol-21-pivalate 1% (0.7%), methylchloroisothiazolinone 0.02% (0.7%), and 4-*tert*-butylphenol formaldehyde resin 1% (0.7%). When considering that 77 positive reactions in 62 (8.2%) of 760 patients would have been missed, these authors concluded that it was worthwhile having an additional late reading at either 144 or 168 hours.

TABLE 1. Positive Patch Test Reactions

Patient no.	Patch Test Reactions	48 h Day 2	96 h Day 4	168 h Day 7
1	Cobalt chloride 1.0%	—	—	+
2	Cobalt chloride 1.0%	—	—	+
3	Mercury 0.5%	—	—	+
	Palladium chloride 2%			+
4	Mercury 0.5%	—	—	+
5	Disperse blue mix 106/124 1%	—	—	+
6	Disperse blue mix 106/124 1%	—	—	+
7	Colophony 20%	—	—	+
8	Colophony 20%	—	—	+
9	Geraniol 2%	—	—	+
	Hydroxycitronellal 2%	—		+
10	Diphenylguanidine 1%	—	—	+
11	Tea tree oil 5%	—	—	+
12	Sorbic acid 2%	—	—	+
13	Cinnamyl alcohol 2%	—	—	+
14	Sodium metabisulfite 1%	—	—	+
15	Ammonium persulfate 2.5%	—	—	+
16	Ethyleneurea, melamine formaldehyde (fix AC)5%	—	—	+
17	Fragrance mix I 8%	—	—	+
18	Methylchloroisothiazolinone/ Methylisothiazolinone 0.01%	—	—	+
19	Propyl gallate 1%	—	—	+
20	Diazolidinyl urea Germal II 2%	—	—	+
21	Gentamycin sulfate	—	—	+
22	Amerchol L 101 50%	—	—	+/-
23	2 Hydroxy-5-methylphenyl benzotriazol 1%	—	—	+

TABLE 2. Review of Literature of Late and Delayed Patch Test Reading

Study	Patients	Allergens	Patch Tests Read	Conclusions
Mitchell ⁶	499	Wide range of allergens.	48 h 168 h	21% had positive reactions at 168 h, which were negative at 48 h. Late reactions noted to neomycin.
Macfarlane et al ⁵	403	Wide range of allergens including neomycin, potassium dichromate, and cobalt.	48 h, 96 h, 168 h, 216 h Paired readings 48 h and 96 h, 96 h and 168 h	Late positive reactions after 96 h were noted in 7.2% of patients. Commonest late reaction allergens included neomycin, potassium dichromate, and cobalt. Paired readings at 96 h, 168 h of greater use than paired readings at 48 h, 96 h.
Davis ⁴	372	Patients with suspected allergy to metals and corticosteroids. Wide range of allergens.	Tested at 96 h and at least once during days 7–21	The new positive allergens, after 120h included gold sodium thiosulfate, dodecyl gallate, palladium chloride, neomycin sulfate. ⁷ Certain allergens should prompt physicians to perform a later reading including metals (gold, palladium, beryllium, nickel, cobalt), topical antibiotics (neomycin sulfate, bacitracin), and some preservatives (dodecyl gallate, thiomersal, methenamine).
Hillen et al ⁷	1748, 1748, 812	PPD, epoxy resin, nickel sulfate	168 h, 336 h, 504 h (day 7, 14, 21)	1.5% of patients had delayed reaction to PPD and 0.3% to epoxy resin. Positive reactions at later readings after day 7 due to patch test sensitization. Late readings not done for nickel sulfate allergies.
Jonker and Bruynzeel ⁸	760	European baseline series Additional series	Compared paired readings at 48 h, 72 h, with readings at 144 h, 168 h	8.2% of patients had late reactions after 72 h. Commonest allergens involved nickel sulfate, neomycin sulfate, tixocortol-21-pivalate, methylchloroisothiazolinone, 4- <i>tert</i> -butylphenol formaldehyde resin. Authors recommended an additional late reading at either 144 or 168 h.
Geier et al ⁹	1096, 1243, 1136	Standard series	Paired readings in 3 groups: 72 h, 96 h, 72 h, 120 h, 72 h, 144 h	Readings at 144 h identified more positive results with late reactions seen in neomycin sulfate, cobalt chloride, and PPD in the 72 h/144 h group.
Isaksson et al ¹⁰	12	2-Hydroxyethyl methacrylate, ethyleneglycol dimethacrylate tested to serial dilutions	72 h, 168 h Further readings at day 10, 14, 21, 24, 28	Reactions to 2-hydroxyethyl methacrylate and ethyleneglycol dimethacrylate may occur after 1 week. Reading at 72 h and 168 h failed to identify 16% of reactions to 2-hydroxyethyl methacrylate and 5.3% of reactions to ethyleneglycol dimethacrylate.
Isaksson et al ¹³	5432, 3379	Corticosteroid mixes	48 h, 72 h, 48 h, 72 h, 168 h	30% of reactions to corticosteroid markers are missed if a 168 h reading is omitted.
Davis et al ¹⁴	135	Corticosteroid series	96 h and 1 further reading from day 7–21	Extending reading after 168 h was of little value. Only 2 reactions were seen after 96 h.
Matiz et al ¹⁵	38 pediatric patients	Standard series	3 readings: 48 h 72–96 h 168–216 h	50% of children had a positive reaction at 168–216 h (day 7–9), and of these, 5 (13%) had newly positive reactions. Late positive allergens included quaternium 15, formaldehyde, diazolidinyl urea, epoxy resin, neomycin sulfate and <i>p</i> - <i>tert</i> -butylphenol formaldehyde resin. Delayed reactions should be considered in pediatric patients.

Geier et al⁹ compared paired patch test readings in 3 groups of patients, with 2 readings in each group. One group had readings at 72 and 96 hours, 1 group had readings at 72 and 120 hours, and the final group had readings at 72 and 144 hours. Performing later readings at 144 hours identified more positive results, with more late reactions seen with neomycin sulfate, cobalt chloride, and PPD in the 72 hours/144 hours group. Of all positive reactions, newly positive reactions after 72 hours were seen in 14.8% of the 72 hours/96 hours group (255/1725 patients), 22.7% in the 72 hours/120 hours group (355/1565 patients), and 17.9% in the 72 hours/144 hours group (279/1561 patients).

Acrylic and methacrylic monomers, used in dental restorative work, are also known to cause delayed reactions, which sometimes

appear after 168 hours. In a study to determine the development and course of patch test reactions to 2-hydroxyethyl methacrylate in allergic patients, 12 patients were retested by serial dilutions.¹⁰ Readings at 72 and 168 hours failed to identify 16% of reactions to 2-hydroxyethyl methacrylate and 5.3% of reactions to ethyleneglycol dimethacrylate because these reactions appeared more than 1 week later.

Corticosteroids are also considered to cause delayed reactions.^{11,12} It has been reported that up to 30% of corticosteroid allergy can be missed if a late reading is not done.¹³ This study by Isaksson et al¹³ compared readings to 2 concentrations of corticosteroid mixes at 48 hours/72 hours with readings at 168 hours and concluded that a 168-hour reading was necessary. However,

a 96-hour reading was omitted in this study. Davis et al¹⁴ examined the role of delayed readings in corticosteroid allergy after day 5 (96 hours) in 135 patients. Patients were tested at 96 hours, and these results were compared with readings from day 7 to 10. Newly positive readings were only seen in 2 patients after 96 hours, both to budesonide 0.1%: 1 at 168 hours and 1 at 192 hours. Our results did not show any new positive results to budesonide 0.1% at 168 hours. All patients were tested to tixocortol pivalate 1.0% and budesonide 0.1% as part of the BSCA standard series, but we did not specifically test patients to the corticosteroid batteries unless clinically suspected.

Delayed reactions should be considered in pediatric patients as well. A prospective study was performed in 38 children with allergic contact dermatitis with readings at 48 hours, at 72 to 96 hours, and again at 168 to 216 hours for a final late delayed reading.¹⁵ Nineteen children (50%) had a positive reaction at 168 to 216 hours (day 7–9), and of these, 5 (13%) had newly positive reactions. Late positive allergens included quaternium 15 2%, formaldehyde 1%, diazolidinyl urea 1%, epoxy resin 1%, neomycin sulfate 20%, and *p*-tert-butylphenol formaldehyde resin 1%. Formaldehyde-releasing preservatives are widely used in skin care products for both children and adults and are an important cause of contact allergy. Dermatologists should be aware of the potential for the development of late reactions to formaldehyde-related allergens in these groups.¹⁶

A limitation of our study was the relatively small number of patients included. However, all patients in this study had 168-hour reading performed, as was routine practice in our department at that time. Patients were not selected to have a 168-hour reading based on clinical suspicion of having allergy to allergens causing potential late reactions such as metals, preservatives, or antibiotics, which would have introduced a positive bias into our results. Therefore, the rate of 12.8% reflects the number of relevant patch test reactions that may be identified when unselected patients have a third reading at 168 hours performed.

Performing a 168-hour reading is important to determine relevant late reactions. In our opinion, the evidence outweighs the drawback of an extra visit in terms of the health care cost of an additional hospital/clinic visit and the time required for physicians to review the patch test sites.

CONCLUSIONS

This study confirms that late reactions occur to metals, fragrances, preservatives, colophony, and dyes. Reports in the literature add acrylates and corticosteroids to the list. The incidence varies

between 7% and 14%, thus supporting the need for 168-hour readings when patch testing. This applies in particular to patients with facial, hand, dental, and occupational contact allergy.

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