

ORIGINAL RESEARCH

Comparison of Intradermal Dilutional Testing With the Multi-Test II Applicator in Testing for Mold Allergy

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OBJECTIVES: To compare and correlate wheal size using the Multi-Test II applicator with the endpoint obtained by intradermal dilutional testing (IDT) for common mold allergens. To validate the safety and efficacy of modified quantitative testing (MQT) for determining immunotherapy starting doses.

STUDY DESIGN AND SETTING: Prospective study of 86 subjects with Multi-Test II and IDT for 6 common mold antigens.

RESULTS: There was 84% concordance between IDT results and the results expected from the MQT method. When IDT and MQT results differed, the MQT algorithm predicted a safer endpoint for starting immunotherapy in all but 2 cases.

CONCLUSION: The correlation between Multi-Test II and IDT is not strong enough to infer IDT endpoint from Multi-Test II results for molds. MQT is nearly as effective as formal IDT in determining endpoint.

SIGNIFICANCE: MQT appears to be a safe method for determining starting doses for immunotherapy with fungal allergens.

EBM rating: B-3b

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Accurate diagnosis of mold allergy is a problem for the otolaryngologic allergist. Mold allergy testing is complicated by a lack of standardized antigenic extracts, the large number of possible antigenic epitopes, and concern about the poor sensitivity of the cutaneous immediate hypersensitivity response. Mold allergy is appreciated as a cause of upper and lower respiratory symptoms, but developing a system for sensitive and specific testing that will lead to productive immunotherapy has proven difficult. Studies comparing skin prick testing (SPT) and intradermal

dilutional testing (IDT) specifically for mold are lacking in the current literature.

Recent work by Krouse to develop a blend of the IDT and SPT methods has led to modified quantitative testing (MQT). In this method, SPT is used initially to determine an approximate range of sensitivity, and a single weaker or more concentrated intradermal test is used to define the level of sensitivity and quantify the allergic response.¹ This method of testing has recently been adopted by the American Academy of Otolaryngic Allergy as a valid form of testing. However, no studies to date have used experimental data to validate this method of skin testing.

The purpose of this study was to compare the Multi-Test II applicator to IDT in demonstrating skin sensitivity to 6 mold extracts. We analyzed the correlation between wheal size of SPT and endpoint as determined by IDT. In addition, analysis of MQT and its comparison with traditional IDT was performed.

The hypotheses of this study were 1) wheal size using the Multi-Test II applicator correlates with endpoint dilution when testing for mold allergy, and 2) MQT is a safe alternative to IDT for obtaining quantitative data when testing for mold antigens.

METHODS

The sample consisted of 86 volunteers between the ages of 18 and 65 who were recruited in November and December 2004 from the University of Texas Medical Branch

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(UTMB) otolaryngology clinic and hospital staff. Institutional Review Board approval was obtained before initiating the study. Subjects were a combination of random volunteers comprised of patients from the otolaryngology clinic with and without allergy symptoms as well as volunteer hospital and clinic staff. Subjects were excluded if they were pregnant, had used an oral antihistamine or anti-leukotriene agent in the previous week, had a history of anaphylactic reaction to skin testing, or were currently taking a beta antagonist. One subject had a vasovagal response to intradermal skin testing and was thus excluded from the study. Another subject was found to have dermatographia and was likewise excluded from the study. For testing, the left upper arm was prepared using an alcohol swab. Subjects were simultaneously tested with *Aspergillus fumigatus*, *Alternaria alternata*, *Epicoccum nigrum*, *Stemphylium*, *Curvularia Spicifera*, and *Helminthosporium* extracts (Antigen Laboratories, Liberty, MO) using the Multi-Test II applicator (Lincoln Diagnostics, Decatur, IL) for skin prick testing and the intradermal dilutional technique (serial fivefold dilutions from antigen concentrate). Positive histamine controls were used with both the intradermal testing and SPT. Negative glycerine controls were applied in the same manner. A positive histamine and negative glycerine test by both intradermal and skin prick testing were required for entrance into the study. Stock extract (1:20 w:v dilution) was used in the Multi-Test II dip wells. All skin test results were read 15 minutes after application.

The method of application for IDT was performed as described by John Fornadley in his description of skin endpoint titration.² Endpoints were recorded as the first dilution that led to a progressive increase in wheal size. Positive intradermal tests were considered endpoints of a #2 dilution (1:500 w:v) or higher. A positive test by the Multi-Test II applicator was considered a wheal 4 mm larger than the negative (glycerine) control. Endpoint and wheal size were recorded along with the subject's age, sex, and presence or absence of subjective allergy symptoms as assessed by the question, "Do you have allergy symptoms, either occasionally or year-round?" Application and interpretation of test results were all performed by the principal investigator.

Statistics

The incidence of positive skin tests for each individual antigen was computed using simple percentages. Correlation between endpoint and wheal size was calculated using a 2-tailed Spearman's rho with statistical significance determined using the *t* test. Statistical significance was considered a *P* value less than 0.05. For correlation of the two tests, 3 large groups were analyzed separately. All subjects tested were analyzed in Group 1. All subjects reporting symptoms were analyzed in Group 2. And finally, all subjects with positive skin tests were grouped together and analyzed in Group 3. Results were analyzed for all molds tested, as well as individual molds. An additional analysis was performed to compare the data obtained from formal

Table 1
Number of positive tests if an IDT of 2 is considered positive

	Positive IDT	Negative IDT
Positive SPT	51 (10.1%)	3 (0.6%)
Negative SPT	50 (9.9%)	400 (79.4%)

IDT with the data that could be expected from using the MQT algorithm developed by Krouse.¹ The final endpoint expected from MQT and the actual endpoint obtained by IDT were compared.

RESULTS

A total of 84 subjects were tested for 6 antigens for a total of 504 skin tests. There were 62 female patients and 24 male patients with an average age of 38 (range 21-58). Of the 84 subjects analyzed, 30 had positive skin tests by either SPT or IDT. Twenty-nine were positive by IDT, 17 were positive by SPT, and 16 were positive for both tests. Of those subjects that were positive by either test, 12 were positive to 3 or more antigens. Tables 1 and 2 show a summary of the allergy testing results (for all subjects) in 2 × 2 box-plot format. Table 1 shows results if an IDT endpoint of 2 is considered a positive result, and Table 2 shows the results if an IDT endpoint of 3 is considered positive. There were 13 subjects that tested positive with IDT and negative with SPT. These 13 subjects had 29 positive tests by IDT. For these subjects there were 28 endpoints at dilution #2 and 1 endpoint at dilution #3.

The incidence of positive tests as determined by IDT was *Aspergillus* 27%, *Epicoccum* 21%, *Stemphylium* 19%, *Helminthosporium* 19%, *Curvularia* 18%, and *Alternaria* 16%. The incidence of positive tests as determined by SPT was *Aspergillus* 13%, *Epicoccum* 11%, *Alternaria* 11%, *Helminthosporium* 11%, *Curvularia* 10%, and *Stemphylium* 10%.

Of the subjects that were tested, 51 (61%) reported either seasonal or perennial allergy symptoms, and 33 reported never experiencing allergy symptoms. Of the 51 subjects who reported symptoms, 21 (41%) had positive tests by IDT and 12 (24%) had positive tests by SPT. Of the 33 subjects who reported never experiencing allergy symptoms, 7

Table 2
Number of positive tests if an IDT endpoint of 3 is considered positive

	Positive IDT	Negative IDT
Positive SPT	22 (4.4%)	32 (6.3%)
Negative SPT	3 (0.6%)	447 (88.7%)

Table 3
Correlation of IDT endpoint and SPT wheal size for all patients tested

Antigen	Spearman correlation coefficient	P value
<i>Alternaria</i>	0.796	<0.01
<i>Aspergillus</i>	0.624	<0.01
<i>Curvularia</i>	0.729	<0.01
<i>Epicoccum</i>	0.646	<0.01
<i>Helminthosporium</i>	0.726	<0.01
<i>Stemphylium</i>	0.607	<0.01
All antigens tested	0.684	<0.01

(21%) had positive tests by IDT and 4 (12%) tested positive by SPT. These differences were not statistically significant.

Of the subjects reporting symptoms, 78 skin tests were positive by IDT and 41 were positive by SPT. Of those subjects without symptoms, 24 skin tests were positive by IDT and 13 were positive by SPT.

Correlation coefficients for SPT and IDT results were calculated for groups 1, 2, and 3. Tables 3, 4, and 5 show correlation coefficients for these groups as well as their respective *P* values.

Further analysis of the 504 data points was performed to compare IDT results with results expected from using the MQT algorithm proposed by Krouse. Calculation of the MQT was performed using the schematic shown in Figure 1.

Eighty-three tests yielded results that were different from those predicted by the MQT method. Conversely, there was an 84% concordance between our results and the results expected by the MQT method. Of the 83 IDT results that were different from those expected from MQT, 51 yielded results one dilution more concentrated than what would be expected with MQT (ie, MQT predicts a starting immunotherapy concentration one dilution weaker than IDT). Thirty tests yielded results two dilutions more concentrated than would be expected with MQT. Two yielded results one dilution less concentrated than would be expected with MQT (Dilution #4 by our testing, Dilution #3 expected by MQT). Fifty results would have been classified as negative

Table 4
Correlation of IDT endpoint and SPT wheal size for patients with allergy symptoms

Antigen	Spearman correlation coefficient	P value
<i>Alternaria</i>	0.766	<0.01
<i>Aspergillus</i>	0.671	<0.01
<i>Curvularia</i>	0.811	<0.01
<i>Epicoccum</i>	0.669	<0.01
<i>Helminthosporium</i>	0.764	<0.01
<i>Stemphylium</i>	0.714	<0.01
All antigens tested	0.731	<0.01

Table 5
Correlation of IDT endpoint and SPT wheal size for responders to either test

Antigen	Spearman correlation coefficient	P value
<i>Alternaria</i>	0.509	NS
<i>Aspergillus</i>	0.220	NS
<i>Curvularia</i>	0.628	<0.05
<i>Epicoccum</i>	0.329	NS
<i>Helminthosporium</i>	0.465	NS
<i>Stemphylium</i>	0.283	NS
All antigens tested	0.396	<0.01

NS, not significant.

tests by SPT that would have resulted in positive tests by MQT (45 endpoints of 2, 3 endpoints of 3, and 2 endpoints of 4). Thus, the addition of intradermal testing with the MQT protocol yields a 10% higher number of positive tests when compared to SPT alone.

DISCUSSION

Mold allergy is difficult to diagnose for several reasons. Molds in general may present no seasonal variation, making the patient's history less reliable. Also, there are over 200,000 species of mold that have been identified, and a single fungal species can produce more than 40 different proteins that stimulate IgE production; thus, distinguishing clinically significant mold antigens is problematic. Finally, standardization of mold extract has proven most difficult. In contrast to other inhalant allergens, mold extracts tend to have low allergenic activity and vary considerably from batch to batch.³

Mold allergy has been previously studied at UTMB.⁴ In our study, 35% of subjects tested positive to at least one mold allergen. This result is similar to the 32% of patients found by Calhoun.⁵ However, this result is higher than other studies and is likely explained by higher mold exposure in our population. The subtropical climate, high humidity, and mild winters of Galveston and south Texas may explain our increased prevalence of mold allergy.

Intradermal dilutional testing and skin prick testing have been previously compared. Studies have suggested that SPT is more accurate than IDT in predicting the response to nasal challenge for grass, cat, ragweed, and dust antigens.⁶⁻⁹ The advantages of SPT, as stated by Nelson et al, include improved safety, less technical demand, increased speed, decreased cost, decreased pain, easier interpretation, and better correlation with clinical symptoms.⁶ However, intradermal testing also has certain advantages including more sensitive and reproducible results, and IDT yields quantitative information that allows estimation of safe starting doses for immunotherapy. Proponents of IDT also argue that IDT-

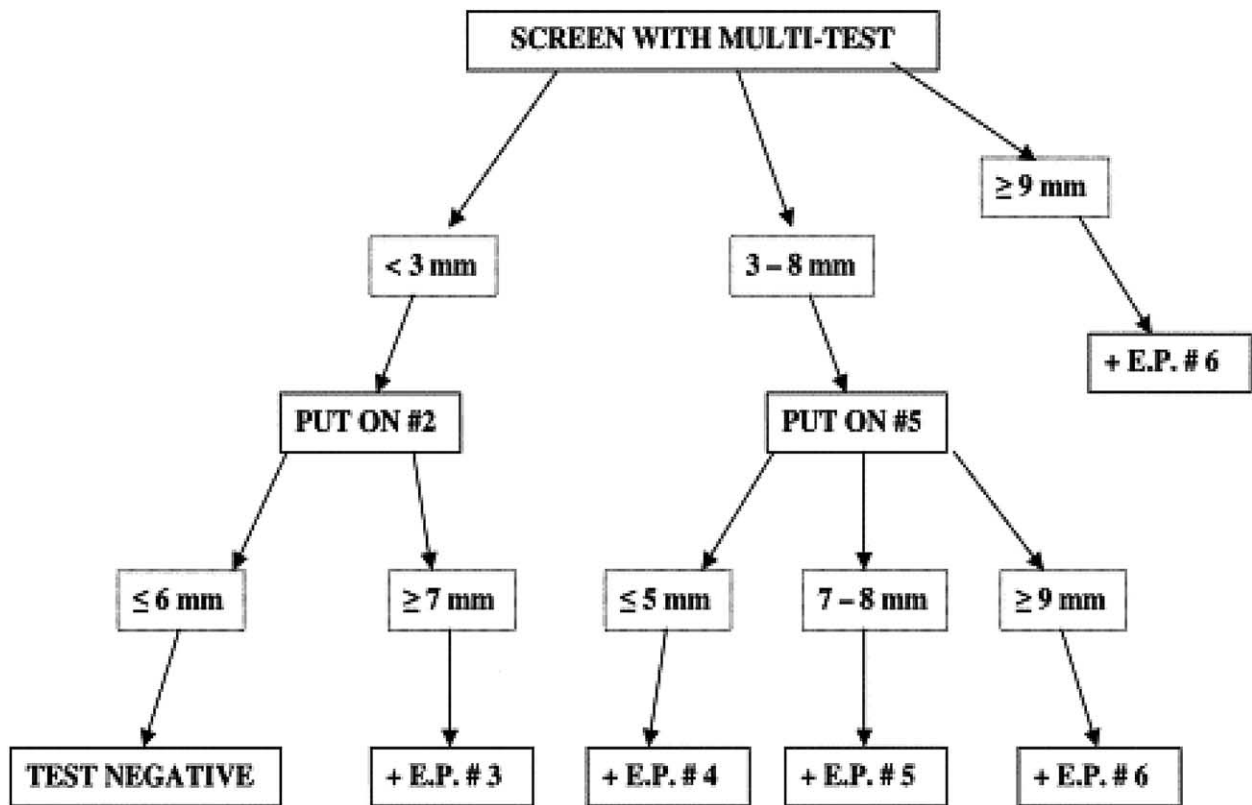


Figure 1 The MQT algorithm. Reprinted from *Otolaryngology–Head and Neck Surgery*, Volume 129, October 2003 (Krouse J, Mabry R, Skin testing for inhalant allergy 2003; current strategies), with permission from the American Academy of Otolaryngology–Head and Neck Surgery Foundation, Inc.

directed immunotherapy can be safely started at more concentrated doses, allowing rapid titration to the maximally tolerated doses, and that immunotherapy can be started safely during seasons of maximal exposure.¹⁰ Given these arguments, it is important to determine the situations where a particular method of testing is advantageous and to study combined testing methods such as MQT.

Questions have long surrounded SPT sensitivity in the diagnosis of mold allergy. This controversy has revolved around the fact that mold allergy is not as commonly diagnosed as pollen and grass allergy despite the fact that airborne mold spores are found in far higher concentrations.³ There is some concern that commercial extracts may be too dilute and therefore not suitable for SPT and that IDT should be a first-line test in the diagnosis of mold allergy. One recent study has suggested that SPT may have sensitivities as low as 44% when compared to nasal challenge. Surprisingly, the sensitivity increases to only 58% with IDT.¹¹ The authors concluded that a non-IgE-mediated reaction was responsible for those subjects who had symptoms without positive skin tests.

Simons et al recently published a paper correlating endpoint to wheal size using a grading system 1+ to 4+.¹² They found correlation coefficients that varied widely depending on the antigen tested. The only mold they tested was *Helminthosporium*, which interestingly had a correlation of -0.303. They found a higher number of patients that

had positive tests using IDT as compared to SPT and concluded that IDT was more sensitive than SPT, but the significance of these positive tests in clinically significant allergy was unknown.

When examining the correlation between SPT and IDT, the subject grouping had a large influence on the results. Our study showed good correlation between the two tests when comparing all subjects tested (Group 1). However, this number is skewed by the large number of negative tests. When looking at only positive tests, there was a poor correlation (Group 3). Analysis of subjects with symptoms (Group 2) showed a correlation of 0.73. This group of subjects represents those who may be allergy tested by the clinician, and these results are the most clinically relevant. This group of subjects had adequate correlation of results to support the use of either IDT or SPT in screening patients for mold allergy.

Our analysis also supports the use of MQT in determining a starting point for immunotherapy for mold allergy. There was an overall concordance of 84% between the expected MQT endpoint and the endpoint determined by IDT. Only twice was the MQT result more concentrated (a lower dilution) than our results from intradermal testing. Both of these instances occurred with negative SPT and endpoints of 4 by IDT. We would expect in this case an endpoint of 3 by MQT, and immunotherapy might start at one dilution more concentrated than would be the case with

standard IDT. Of the other 81 results where MQT and IDT differed, a more dilute, and likely safer, starting dose would be obtained from MQT. In the other 421 intradermal tests performed, MQT would have given identical results.

CONCLUSION

Mold allergy is a common entity in the Texas gulf coast region. Although a correlation exists between Multi-Test II and IDT, it is not strong enough to infer endpoint from Multi-Test II results in determining starting immunotherapy doses. SPT and IDT are both useful allergy tests in outpatient screening for molds. There is strong agreement between MQT and formal IDT in determining endpoint for mold allergens, and MQT is a safe way to obtain quantitative data for immunotherapy.

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