

Rapid Immunochromatographic Measurement of Specific Tear Immunoglobulin E in Moderate to Severe Cases of Allergic Conjunctivitis With Immfast Check J1 in the Spring

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Purpose: To evaluate whether Immfast Check J1, a new commercial immunochromatographic test for the measurement of specific immunoglobulin (Ig) E, could be used as a screening test for the diagnosis of allergic conjunctivitis.

Methods: A prospective, nonrandomized, cross-sectional study was conducted in 64 moderate to severe cases of allergic conjunctivitis (allergic group) and 44 age- and sex-matched healthy control subjects (control group). Specific IgE levels for cedar pollen, cat epithelium, and *Dermatophagoides pteronyssinus* were measured in tear fluid with the Immfast Check J1.

Results: Specific IgE levels in tear fluid could be assayed within 20 minutes in all subjects. The positive rate of specific IgE was significantly higher in the allergic group than in the control group (cedar pollen, 96.9% vs. 2.3%, $P < 0.00001$; cat epithelium, 23.4% vs. 0.0%, $P = 0.00018$; and *D. pteronyssinus*, 53.1% vs. 2.3%, $P < 0.00001$). Specific IgE scores were also significantly higher in the allergic group than in the control group (cedar pollen, $P < 0.00001$; cat epithelium, $P = 0.03907$; and *D. pteronyssinus*, $P < 0.00001$).

Conclusions: These results suggest that Immfast Check J1 is a rapid and easy test for detection of allergen-specific IgE in tear fluid samples from patients with allergic conjunctivitis. The test is reliable and easy to perform on an outpatient basis.

Key Words: allergic conjunctivitis, Immfast Check, immunochromatography, tear, specific IgE

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Seasonal allergic conjunctivitis arises from a hypersensitivity reaction to specific airborne allergens, mainly pollens, resulting in mild to severe symptoms of ocular itching,

redness, epiphora, and secretion. Detection and quantification of specific immunoglobulin (Ig) E antibodies in serum is currently the standard method^{1–2} for diagnosis of seasonal allergy. Measurement of total IgE in tear fluid is also useful for the diagnosis of allergic conjunctivitis.^{3–8}

Recently, a new commercial kit (Immfast Check J1; Mitsubishi Kagaku Iatron, Inc, Tokyo, Japan) was introduced for the rapid immunoassay of specific IgE for cedar pollen, cat dander, and house dust (*Dermatophagoides pteronyssinus*) in serum. Ikeda et al⁹ reported that the positive rate was 11 of 32 eyes in *D. pteronyssinus*, 15 of 32 eyes in cedar, and 1 of 32 eyes in cat in the Japanese Journal; however, there has been no reports in the English literature. The aim of this study was to ascertain whether the Immfast Check J1 could be used to detect allergen-specific IgE in tear fluid of patients with allergic conjunctivitis during the spring. We compared the results obtained in patients with seasonal allergic conjunctivitis and healthy control subjects in a cross-sectional study.

METHODS

Subjects

Institutional review board approval was obtained for this study, which was performed in accordance with the Helsinki Declaration of 1975 and its 1983 revision. Informed consent was obtained from each subject. Allergic conjunctivitis was diagnosed according to the guidelines of diagnosis and treatment of conjunctivitis.¹⁰ Patients with moderate or severe allergic conjunctivitis according to the criteria proposed by Shoji et al¹¹ were enrolled. A total of 64 outpatients with allergic conjunctivitis (allergic group) and 44 healthy control subjects with no history of allergic diseases and with negative skin prick test results (Phazets; Pharmacia) (control group) were selected for this trial (Table 1). None of them wore contact lenses or had been treated with topical or systemic drugs during the preceding 6 weeks.

Determination of Specific IgE in Tears

Measurement of specific IgE was performed with the Immfast Check J1 immunochromatographic assay. This kit is specifically designed for direct measurement of IgE antibodies directed against cedar pollen, cat dander, and house dust (*D. pteronyssinus*). Assay was performed according to the modified manufacturer's instructions, as described previously.^{12,13} In brief, tear fluid samples were obtained with

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TABLE 1. Results of the Immfast Check J1 Test in Healthy Control Subjects (Control Group) and Patients With Allergic Conjunctivitis (Allergic Group)

	Control Group		Allergic Group		<i>P</i>	
No. patients	44		64		—	
Age (yrs)	24.9 ± 8.1		26.9 ± 12.2		NS*	
Male/female	21/23		29/35		NS†	
Immfast Check test					—	
Test positive	1		63		—	
Test negative	43		1		<0.00001‡	
Positive rate	2.3%		98.4%		—	
Sensitivity			98.4%			
Specificity			97.7%			
Positive predictive value			98.4%			
Negative predictive value			97.7%			
Positive likelihood ratio			43.313			
	Outdoor Allergen, Cedar Pollen		Animal Allergen, Cat		Indoor Allergen, <i>D. Pteronyssinus</i>	
Specific IgE	Control Group	Allergic Group	Control Group	Allergic Group	Control Group	Allergic Group
Positive rate (%)	2.3	96.9	0.0	23.4	2.3	53.1
‡ <i>P</i>	<0.00001		0.00018		<0.00001	
Test negative	Score 0/1	43	2	44	49	30
Test positive	Score 2	1	32	0	8	22
Positive	Score 3	0	18	0	4	8
Positive	Score 4	0	12	0	3	4
§ <i>P</i>	<0.00001		0.03907		<0.00001	

Number of patients, mean ± SD, or outcome including percentages.

*Unpaired *t* test.† χ^2 test.

‡Fisher exact test.

§Mann–Whitney *U* test.

NS, not significant.

Schirmer test strips (Schirmer Tear Production Measuring Strips; Showa Yakuhin Kako, Tokyo, Japan), and 5-mm lengths of filter paper soaked with tear samples were placed into test tubes containing 100 μ L of reaction buffer solution. After mixing of the tears with the reaction buffer by gentle pipetting for 10 seconds at room temperature, the mixture was transferred to an Immfast Check J1 cassette. Then 200 μ L of enzyme substrate, a mixture of 5-bromo-4-chloro-indolylphosphate and nitroblue tetrazolium (BCIP/NBT), was added to the substrate reservoir pad immediately after sample application. Specific IgE antibodies for cedar pollen, cat epithelium, and *D. pteronyssinus* in the samples first react with the alkaline phosphatase-labeled mouse antihuman IgE antibody (Ab) (ALP-labeled anti-IgE) in the reaction buffer. ALP-labeled anti-IgE is bound to specific IgE for cedar pollen, cat epithelium, or *D. pteronyssinus* at different capture sites. *D. pteronyssinus* antigen (Ag) is immobilized at the first line on the membrane, cat epithelium Ag is at the second line, and cedar pollen Ag is at the third line. When the substrate solution (BCIP/NBT) reaches the control line on the membrane, it reacts with ALP in the ALP-labeled allergen-IgE-antispecific-IgE-Ab complex. Reaction of ALP with BCIP/NBT results in the formation of a blue–purple complex at each test line.

Twenty minutes after sample application, the color of the test line was assessed according to the manufacturer's

instructions. The intensity of the blue color of the test line depends on the level of each specific IgE (Fig. 1). The assay was calibrated against a World Health Organization standard for IgE in the range 0.35–100 kU/L. The results were classified into 4 categories according to a semi-quantitative scale: class 0/1 (no signal, 0.35–0.69 kU/L); class 2 (weaker than line A, 0.70–3.49 kU/L); class 3 (similar to or stronger than line A and weaker than line B, 3.50–17.49 kU/L); and class 4 (similar to or stronger than line B, ≥ 17.50 kU/L) as described in the manufacturer's instructions (Fig. 1). Positive results were determined by visual observation and confirmed using Scion Image software (Scion Corporation, Frederick, MD).

Statistical Analyses

Mean values were compared with the unpaired *t* test. Frequency analysis was performed by using the χ^2 or Fisher exact test. The Mann–Whitney *U* test was used for the comparison of semiquantitative measurements between the 2 groups. Sensitivity, specificity, and positive and negative predictive values were calculated with standard formulae. The level of significance was set at *P* < 0.05. Calculations were done using the Stat View statistical software package (Abacus Concepts, Berkeley, CA).

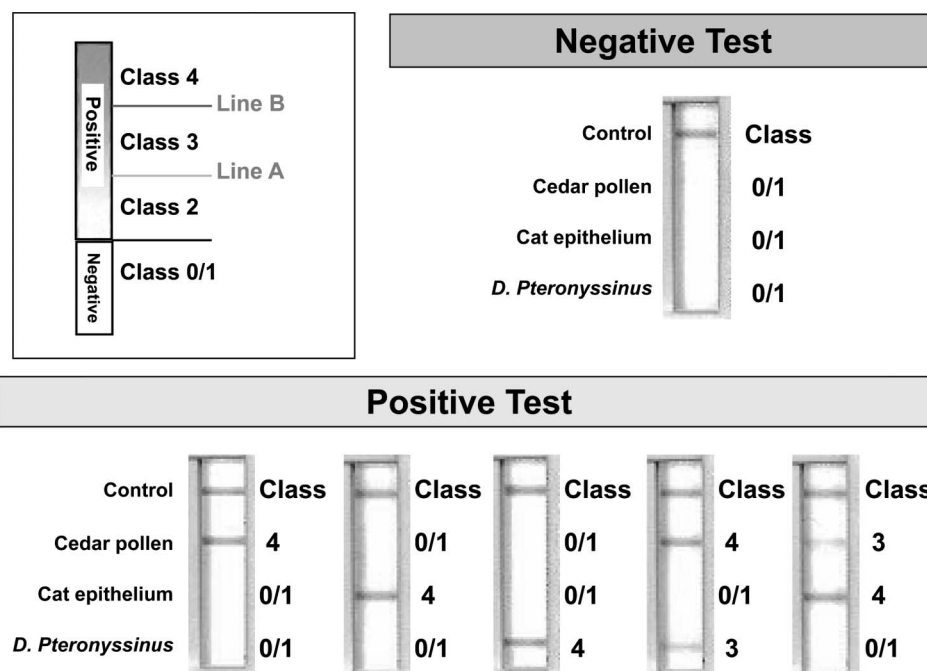


FIGURE 1. Photographs showing results of the Immfast Check J1 test. The results are graded according to the manufacturer's instructions on a scale of 0–4 as follows: class 0/1, no line; class 2, weaker than line A; class 3, similar to or stronger than line A and weaker than line B; and class 4, similar to or stronger than line B. Positive results were determined by visual observation and confirmed using Scion Image software.

RESULTS

The Immfast Check J1 gave positive results for each of the 3 antibodies in 63 patients with allergic conjunctivitis (98.4%) and 1 control subject (2.3%, Table 1). The difference in outcomes was significant ($P < 0.00001$, Fisher exact test, Table 1). The sensitivity, specificity, positive and negative predictive values, and positive likelihood ratio are shown in Table 1.

For each specific Ab, significant differences were found between the patients with allergic conjunctivitis and the controls in the positive results (cedar pollen, 96.9% vs. 2.3%, $P < 0.00001$; cat epithelium, 23.4% vs. 0.0%, $P = 0.00018$; and *D. pteronyssinus*, 53.1% vs. 2.3%, $P < 0.00001$, Fisher exact test) and in the grade of positivity (cedar pollen, $P < 0.00001$; cat epithelium, $P = 0.03907$; and *D. pteronyssinus*, $P < 0.00001$, Mann–Whitney U test) (Table 1).

DISCUSSION

In this study, the Immfast Check J1 showed a very high sensitivity (98.4%) and specificity (97.7%). The level of each specific Ab in tear fluid was significantly higher in the allergic group than in the control group. Therefore, the Immfast Check J1 is useful for detection of allergen-specific IgE in tear samples from patients with allergic conjunctivitis. The present rates were similar to or higher than those reported by Ikeda et al.⁹ The reason is that the allergic group consisted of patients with moderate to severe allergic conjunctivitis.

Our previous study showed that a capsulated hydrophilic carrier polymer radioallergosorbent test for cedar pollen Ab was positive in 52.5% (21 of 40) of patients with allergic conjunctivitis during the spring.¹ The positive rate for serum in that study was lower than with tear samples in the present study. This difference between tears and serum suggests that

local production of IgE Ab may be the main factor influencing the severity of allergic conjunctivitis. Determination of locally produced specific IgE in tear fluid with the Immfast Check J1 is specific, sensitive, and reproducible. Therefore, it is a practical alternative method for the clinical evaluation of local allergic reactions in the conjunctiva, rather than measurement of specific IgE in serum.

Detection of allergen-specific IgE in serum is normally performed with an enzyme-linked immunosorbent assay and CAP radioallergosorbent test as standard methods.^{14–18} However, these assays require more time for analysis. With the Immfast Check J1, we could assay the specific IgE levels in fluid within 20 minutes. This would be of considerable benefit to patients because allergic conjunctivitis could be diagnosed during the first visit. All that is needed is to touch a piece of filter paper to the lower fornix region of the conjunctiva. Therefore, we conclude that the Immfast Check J1 is a rapid, sterile, and noninvasive method for confirmation of allergic conjunctivitis.

In this study, the positive rate for specific IgE directed against cedar pollen was high (96.9%), suggesting that outdoor allergens like cedar pollen are an important cause of springtime allergy in Japan. Allergens can be divided into 2 categories according to their source (indoor or outdoor). Cedar pollen is a typical outdoor allergen in Japan. On the other hand, house dust mites and animal dander (usually from cats) are the major indoor allergens. Outdoor allergens may be more closely related to seasonal allergic conjunctivitis, whereas indoor ones are more frequently associated with perennial allergic conjunctivitis. The Immfast Check J1 tests these 3 major allergens associated with allergic diseases. Our previous studies showed a high correlation between allergen-specific IgE levels for indoor and outdoor allergens.^{1,2} Positivity for specific IgE antibodies against *D. pteronyssinus* Ag was associated with

sensitivity to animals, other mites, insects, and molds such as animal skin (cat epithelium/dander, dog, guinea pig, rat, and mouse), house dust, *Acarus*, moth, *Candida*, and *Alternaria tenuis*. In contrast, cedar pollen seemed to be associated with sensitivity to other pollens such as cypress, ragweed, and mugwort. These results suggest that both indoor and outdoor allergens can cross-react and have common epitopes.^{1,2} Therefore, examination of these 3 specific IgE Abs can be predictive for other possible allergen-specific Abs.

One limitation of the present study is that we selected patients with moderate to severe allergic conjunctivitis, so we need to reevaluate the sensitivity of the kit in patients with mild allergic conjunctivitis. In conclusion, our study demonstrated that analysis of tear fluid samples with the Immfast Check J1 is a rapid and easy method for diagnosing allergic conjunctivitis and has advantages for detecting the specific IgE Abs responsible for allergic conjunctivitis. Patients with high Ag levels can be advised about which allergens to avoid and treat. In the future, we plan to investigate the relationship between the severity of allergic conjunctivitis and specific IgE levels in tears.

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