

Laboratory evaluation of a commercial immunoassay for fire ant allergen-specific IgE antibodies

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Background: *In vitro* testing for fire ant sensitization would be useful for research purposes and in special clinical situations.

Methods: Laboratory performance of a commercial assay (Pharmacia CAP System, [PCS]), for specific IgE to *Solenopsis invicta* whole body extract was studied in 46 persons. Assay results were compared with those of venom skin testing, RAST, and ELISA. The manufacturer's global cutoffs were compared with cutoffs set by using methods derived from analytical detection limit theory.

Results: Thirty-two study subjects had positive skin test results, and 14 had negative results. Raw PCS data demonstrated a high level of correlation with RAST ($\rho = 0.941$) and ELISA ($\rho = 0.931$), and showed good correlation with skin testing ($\rho = -0.769$). Analysis of binormal receiver operating characteristic curves, using skin test results as the reference standard, demonstrated no difference in performance among the three assays. The fixed global quantitative cutoff of 0.35 kUa/L was relatively insensitive. Use of the manufacturer's qualitative alternate scoring method cutoff substantially increased sensitivity without loss of specificity, as did lower limit of detection set by use of diluent.

Conclusions: In situations in which skin testing for fire ant sensitization is not feasible, PCS appears to be an acceptable *in vitro* alternative method for determination of fire ant allergen-specific IgE. (*J ALLERGY CLIN IMMUNOL* 1995;96:182-7.)

Key words: Allergy diagnosis, imported fire ant, skin testing, *in vitro* testing, ROC curve

As the prevalence of adverse reactions to imported fire ants (*Solenopsis invicta*) increases in the southeastern United States,^{1,2} the reliable diagnosis of hypersensitivity to fire ant venom is crucial. Clinical manifestations have been well defined and are the cornerstone of diagnosis,³⁻⁶ but demonstration of allergic sensitization is necessary for confirmation of the diagnosis and is necessary for the

Abbreviations used

IFA-V:	Imported fire ant venom
IFA-WBE:	Imported fire ant whole body extract
LLD:	Lower limit of detection
PCS:	Pharmacia CAP System
ROC:	Receiver operating characteristic

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initiation of immunotherapy. Whether it is performed *in vivo* or *in vitro*, by using whole body extract or venom, the detection of allergen-specific IgE is relevant diagnostically only when interpreted in the context of the clinical history and physical examination.

For the winged members of the order Hymenoptera, venom (rather than whole body extract) is the accepted standard for diagnosis and treatment.^{1,7,8} Venom is also the most appropriate reagent for

clinical and laboratory diagnosis of fire ant allergy,⁹⁻¹³ but imported fire ant venom is not commercially available and is difficult to obtain. Each ant contains only 10 to 100 ng of venom protein—1000 times less protein per insect than for other hymenoptera.^{3-5, 14, 15} Thus imported fire ant whole body extract (IFA-WBE) is the only reagent currently available and licensed for diagnostic testing and immunotherapy. IFA-WBE does contain relevant *Solenopsis invicta* allergens, but their concentration is reduced relative to venom and may vary from batch to batch.^{6, 12, 16-22} Still, the venom content in IFA-WBE appears to be adequate for accurate skin testing and provides effective desensitization.^{8, 13, 15, 21, 23}

Skin testing remains the most sensitive and clinically efficient means of detecting allergen-specific IgE,^{8, 24, 25} but laboratory assays for allergen-specific IgE are useful in research and for occasional patients in whom skin testing is not feasible. ELISA and RAST assays using IFA-WBE and venom have been described.^{11-13, 21, 26, 27} The Pharmacia CAP System (PCS) has been found to be a useful assay in the evaluation of bee and wasp venom allergy, and it may offer advantages over conventional RAST.^{28, 29} This study was designed to compare performance of this assay, using IFA-WBE solid phase, with that of venom RAST and venom ELISA. Skin test results were used as the reference standard.

METHODS

Subjects

Forty-six persons from a fire ant-endemic area underwent quantitative intradermal skin testing with *Solenopsis invicta* fire ant venom (Vespa Laboratories, Inc., Spring Mills, Pa.) in a previous study of the safety and efficacy of fire ant venom in the diagnosis of fire ant allergy.⁹ Thirty-two subjects were reactive and 14 were nonreactive at a concentration of 0.1 µg/ml or less. Of the 32 subjects reactive to the skin test, 25 had a history of a systemic reaction, six had only local reactions, and one did not recall a fire ant sting. Of the 14 subjects not reactive to the skin test, seven were stung but had only a local reaction and seven did not recall a previous fire ant sting. Sera obtained at the time of skin testing were frozen at -70°C and used to evaluate the utility of RAST and ELISA methods for detection of fire ant venom specific IgE²⁷; these sera were also used in this study. Skin testing and phlebotomy were performed after each patient reviewed and signed an informed consent document approved by the human assurance committee of the Medical College of Georgia.

Immunoassay for IFA-WBE-specific IgE

Sera were assayed for IFA-WBE-specific IgE with the Pharmacia CAP System fluorescent enzyme immunoas-

say method (Pharmacia Diagnostics, Uppsala, Sweden),³⁰ as well as the commercially available IFA-WBE solid phase. All reagents were purchased from the manufacturer. The assay was performed as described in the manufacturer's instructions. Solid-phase background (assessment of nonspecific binding) was measured in six replicates by using sample diluent (Pharmacia) as well as serum from a single atopic patient who had never been stung by a fire ant.

Statistical analysis

Assay results were compared with skin test, RAST, and ELISA results by means of Spearman's rank correlation coefficient. Results of the three in vitro assays were compared with each other by means of binormal receiver operating characteristic (ROC) curves, fitted by using the program CLABROC (Charles Metz, PhD; University of Chicago) as previously described,²⁵ and using skin test results as the reference standard. Additionally, conventional calculations of sensitivity, specificity, and efficiency were performed at four possible assay cutoffs³¹: (1) the manufacturer's recommended fixed global quantitative cutoff (0.35 kUa/L), (2) the manufacturer's alternate scoring method qualitative cutoff (60% of the fluorescence response of the 0.35 kU/L calibration standard), using background measurements of (3) sample diluent or (4) serum from a single atopic patient not previously stung by a fire ant to set lower limit of detection (LLD), defined, as is conventional, as mean background response plus 3 standard deviations; because both α and β were set at 0.05, there is, in theory, about a 5% chance of misclassifying a result when samples are assayed at the LLD.^{32, 33}

RESULTS

IFA-WBE-specific IgE levels ranged from <0.35 to 659 kUa/L. Raw PCS data (fluorescence) correlated well with RAST ($\rho = 0.94$), ELISA ($\rho = 0.93$) and skin tests ($\rho = -0.79$) (Fig. 1). Analysis of ROC curves demonstrated no statistical difference in performance of RAST ($A_z = 0.92$), ELISA ($A_z = 0.91$) and PCS ($A_z = 0.90$) (Fig. 2).

Conventional calculations of sensitivity and specificity for RAST, ELISA, and PCS relative to skin testing are presented in Table I. At the defined cutoffs,²⁷ sensitivities for both RAST and ELISA were 0.81 and 0.75, respectively, with specificities of 0.93 and 1.00. For PCS, 27 of the 32 patients with positive skin test results had values greater than the manufacturer's fixed global quantitative cutoff (0.35 kUa/L), yielding a sensitivity of 0.84. Ten of the 14 patients with negative skin test results had values less than the 0.35 kUa/L cutoff, for a specificity of 0.71. Use of the manufacturer's alternate scoring method cutoff increased sensitivity (1.00) without a corresponding

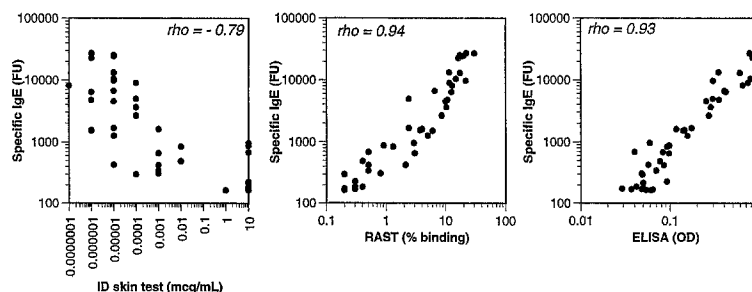


FIG. 1. Correlation of test results from the Pharmacia CAP System with (left to right) skin test reactivity, RAST, and ELISA.

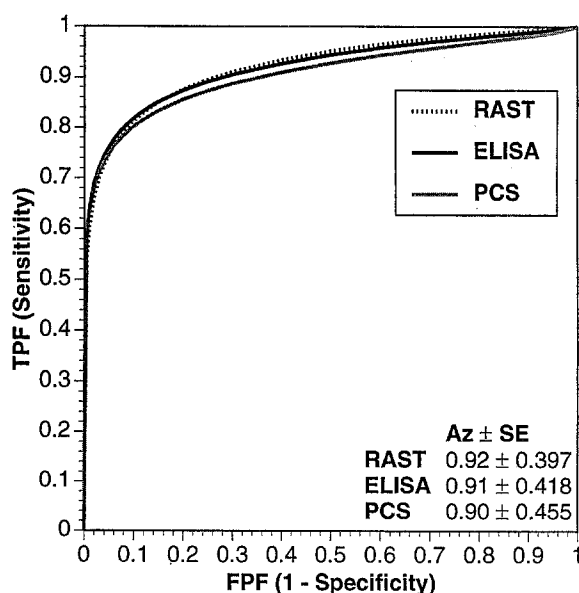


FIG. 2. Binormal ROC curves illustrating sensitivity and specificity of the Pharmacia CAP System, RAST, and ELISA, using skin test reactivity as reference. Areas under the curve (Az) do not differ statistically.

loss of specificity, as did the cutoff set by use of LLD determined with the diluent. Use of the assay cutoff set with LLD determined using atopic serum resulted in a substantial loss in specificity (Table II).

Examination of individual data revealed four persons whose results were positive by PCS and negative by skin testing and ELISA; three of the four had negative results by RAST (Table III). Of these four subjects, two did not recall a fire ant sting and two had only local reactions; one is a scientist who, in the course of extensive research with fire ants, has had substantial exposure to IFA whole body antigens. Two of the five patients with a positive skin test and negative PCS assay results

experienced systemic reactions; the other three had been stung but experienced only a local reaction.

DISCUSSION

The efficacy of venom RAST and venom ELISA in the diagnosis of fire ant allergy has been well documented.^{9-11, 27} By using a whole body extract for the solid phase, PCS produced results similar to that of the venom immunoassays. As is the case for other allergenic substances,³² the quantitative cutoff used in PCS may be too conservative; use of the manufacturer's alternate scoring method or a lower limit of detection set using sample diluent substantially increased sensitivity without loss of specificity. These findings are reflected in the ROC curves (Fig. 2), which show the normalized relationship between sensitivity and specificity over the entire assay ranges. Wide confidence intervals (not plotted) were observed because of the relatively small number of patients studied. (See the confidence intervals in Tables I and II.) Nonetheless, the areas under the three ROC curves are sufficiently similar that it is unlikely that having studied a larger number of patients would have affected study outcome. Certainly, however, analysis of several hundred samples would have shown a clear loss of specificity as sensitivity was increased by lowering cutoff. Results of this study illustrate the mathematical relationship between the false-positive rate and false-negative rate as the assay cutoff is varied.³⁴ For example, examination of ELISA data suggests that this assay cutoff was set to minimize the false-positive rate, at the cost of a relatively high false-negative rate.

As previously shown, when low levels of specific IgE are assayed (as would seem indicated in the evaluation of suspected Hymenoptera allergy), measurement of nonspecific background is critically important as cutoffs are lowered.³² It is not yet clear whether the relationship between back-

TABLE I. Sensitivity and specificity for detection of fire ant specific IgE, using skin testing as the reference standard

In vitro test	Sensitivity	95% CI	Specificity	95% CI
RAST	0.81	0.67-0.95	0.93	0.79-1.00
ELISA	0.75	0.64-0.94	1.00	—
PCS (<0.35 kUa/L)	0.84	0.74-0.96	0.71	0.46-0.96
PCS (ASM)	1.00	—	0.71	0.46-0.96

CI, Confidence interval; PCS, Pharmacia CAP System; ASM, alternate scoring method.

TABLE II. Sensitivity and specificity of PCS whole body fire ant solid phase relative to venom skin test results using different assay cutoffs

Cutoff	Sensitivity	95% CI	Specificity	95% CI
0.35 kUa/L quantitative	0.84	0.74-0.96	0.71	0.46-0.96
ASM (fixed)	1.00	—	0.71	0.46-0.96
Diluent (bkgd based)	1.00	—	0.71	0.46-0.96
Atopic control (bkgd based)	1.00	—	0.57	0.28-0.84

CI, Confidence interval; PCS, Pharmacia CAP System; ASM, alternate scoring method; bkgd, background.

TABLE III. Individual data for subjects with presumed false-positive and false-negative immunoassay results

Subject	Clinical history	Skin test reactivity (μg/ml)	RAST (% binding) cutoff 2.0	ELISA (OD) cutoff 0.1	PCS (FU) cutoff 478 0.35 kUa/L
1	Never stung	Negative	0.5	0.083	689
2	Never stung	Negative	0.9	0.098	880
3	Local rxn	Negative	2.9	0.059	969
4	Local rxn	Negative	0	0.04	690
5	Local rxn	0.01	1.3	0.092	843
6	Local rxn	0.001	0.5	0.085	424*
7	Never stung	0.01	0.4	0.077	491*
8	Local rxn	0.001	0.5	0.07	346*
9	Local rxn	0.0001	0.2	0.049	301*
10	Systemic rxn	0.00001	2.1	0.057	428*
11	Systemic rxn	0.001	0.8	0.048	311*
12	Local rxn	0.001	3.0	0.097	658

OD, Optical density; PCS, Pharmacia CAP System; FU, fluorescence units; ASM, alternate scoring method; rxn, reaction.

*PCS < 0.35 kUa/L, but positive by ASM; ASM cutoff was 287 FU.

ground and lower limits of detection must be determined only once, in all assay runs, or only when new lots of reagents are used.

The ROC curve is a plot of statistical sensitivity versus specificity over the entire range of data analyzed. In the comparative evaluation of specific IgE immunoassays by ROC curve analysis, the assays need not report results in the same units and a predetermined assay cutoff will not affect outcome of the analysis. ROC curves may be used to compare performance characteristics of immunoassays even when the reference standard is not perfect.³⁵

In a previous comparison of the PCS and commercial RAST methods for detection of specific IgE to inhaled allergens, PCS was found to be favorable for measuring specific IgE.^{25, 36} This new assay method is also more sensitive than RAST for detection of specific IgE to bee and wasp venoms in studies that did not evaluate results less than 0.35 kUa/L.^{28, 29}

The finding that the PCS utilizing a whole body solid phase yielded similar results to venom RAST and venom ELISA is surprising because in previous studies whole body extract has been reported as being less efficient than venom for in vitro

testing.^{9-11,27} Perhaps this finding is related to the increased binding capacity of the PCS solid phase, a three-dimensional structure with increased surface area, which can bind three times more protein than the classic cellulose disk and about 50 times more than can be absorbed on a coated tube.^{30,37}

Four patients, two without a history of a fire ant sting, had negative skin test results to the venom, but clearly had positive results by PCS. One also had positive results by RAST. The presence of fire ant body antigens could account for the relatively low specificity of the whole body PCS, because many patients are allergic to inhaled insect parts.³⁸ Alternatively, the patients could have reacted to a fire ant venom allergen present on the PCS solid phase (but not in the venom preparation used for skin testing), or the skin test result could be false negative for other reasons. Thus, despite comparable ability to detect allergen-specific IgE for fire ant using whole body extract, the solid phase performance might be substantially improved by use of a well-characterized venom source with verification of binding of all four fire ant venom allergens.

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