

ImmunoCAP and HY•TEC enzyme immunoassays in the detection of allergen-specific IgE compared with serial skin end-point titration by receiver operating characteristic analysis

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OBJECTIVES: In this study, we compared 2 different immunoassays, HY•TEC enzyme immunoassay (Hycor Biomedical Inc, Garden Grove, CA) and Pharmacia Upjohn ImmunoCAP (Pharmacia and Upjohn, Kalamazoo, MI) using skin end-point titration as a gold standard by receiver operating characteristic analysis.

MATERIALS: One hundred patients were enrolled in this study and were tested with the above 3 modalities. The results were analyzed by receiver operating characteristic analysis, in which the curves give a decision criterion for any diagnostic test relative to its sensitivity and specificity against an accepted gold standard test. Only inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, cat dander, giant ragweed, English plantain weed, timothy grass, *Alternaria tenuis*, *Aspergillus fumigatus*, meadow fescue, oak tree, white ash tree, and lamb's-quarter weed) were studied.

RESULTS: The results obtained with both ImmunoCAP and HY•TEC showed a good correlation with skin end-point titration for *D pteronyssinus*, *D farina*, cat dander, giant ragweed, timothy grass, *A tenuis*, and meadow fescue, with sensitivities ranging from 71% to 88%. However, the sensitivity of both tests to English plantain weed, lamb's-quarter weed, oak

tree, and white ash tree was lower (66% down to 39%). Additionally, HY•TEC results were not satisfactory for detecting specific IgE to *A fumigatus* (30% sensitivity). The overall sensitivity for each test was 65.67% for the HY•TEC and 71.25% for the ImmunoCAP.

CONCLUSION: The 2 in vitro testing systems are comparable for most of the allergens, except for *A fumigatus*, English plantain, lamb's-quarter, and white ash tree; ImmunoCAP and HY•TEC would benefit from improved technology for detecting these specific IgE antibodies. The HY•TEC assay did not give acceptable results for *A fumigatus*. The overall assay characteristics were good to excellent for each system tested. (Otolaryngol Head Neck Surg 2000;122:64-70.)

In vitro testing for allergen-specific IgE has become an important part of allergy testing. Advances in technology now allow us to detect allergen-specific IgE levels more rapidly, more reliably, and more efficiently compared with a decade ago. Many tests have been demonstrated to be reliable, reproducible, and cost-effective. The Pharmacia ImmunoCAP system is an in vitro assay that uses a unique 3-dimensional solid-phase surface. This in vitro testing technique offers a semiautomated system, and the results are obtained in 6 hours. The HY•TEC enzyme immunoassay (EIA) is another new, 1-day test that also uses a solid phase with a classic solid, paper disk.

The efficiency and reliability of the Pharmacia ImmunoCAP system has been previously demonstrated by our laboratory and by the laboratories of many other institutions.¹⁻³ Similarly, HY•TEC EIA has been shown to be efficient and reliable for in vitro allergy testing.^{4,5} The Pharmacia ImmunoCAP and HY•TEC differ in the amount of manual work that requires a technician's time. After manual placement of tubes during the initial setup, the HY•TEC system runs the rest of the assay in a fully automated mode. On the other hand, the ImmunoCAP system requires initial placement, manual transfer

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of the microplate, and sample, calibrator, and control pipetting. Furthermore, the HY•TEC EIA offers easier data entry and a larger allergen-testing capacity than does the ImmunoCAP system.

There is no perfect test for allergies. However, skin end-point titration (SET) intradermal skin testing remains a clinical gold standard as a method of in vivo allergy testing. This test gives the physician semiquantitative results, as do in vitro tests, on the patients' allergies by using several different dilutions that contain standard amounts of allergen in each dilution.

Sensitivity and specificity are 2 important concepts when the limitation of the "accuracy" of a diagnostic test is measured. The unique description of diagnostic performance depends on the arbitrary selection of a decision cut-off point. The receiver operating characteristic (ROC) is a simple and empirical description of this decision cut-off point effect, at possible combinations of the relative sensitivity and specificity for various cut-off points.^{6,7} The ROC curve allows us to predict the sensitivity and relative specificity levels at these different cut-off points.

In this study, 2 different in vitro testing systems were compared, with SET as the gold standard. The comparison of ImmunoCAP and HY•TEC with SET has not been made before. SET was chosen as a gold standard because it is the method most commonly used by otolaryngologists for the diagnosis of inhalant allergies. Although any test (including skin testing) has its limitations, clinically SET is the standard to which most otolaryngology clinicians compare a new method. Twelve common inhalant allergens were used for this study; food allergens were excluded.

METHODS AND MATERIAL

The subjects were either paid volunteers recruited by local advertisement or patients seen at the University of Chicago Allergy Clinic. Informed consent was obtained from each subject before entry into the study. The study protocol was approved by the institutional review board at the University of Chicago.

Skin tests were performed, and blood was collected from both atopic and healthy subjects. We studied 100 patients; these included atopic and healthy subjects with ages ranging from 17 to 72 years. None had had prior immunotherapy or any major medical problems. All discontinued antihistamine therapy at least 3 days before skin testing. *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, cat dander, oak tree, white ash tree, English plantain weed, timothy grass, *Alternaria tenuis*, *Aspergillus fumigatus*, meadow fescue, lamb's-quarter weed, and giant ragweed antigens were studied. Each patient was tested by SET, ImmunoCAP, and HY•TEC EIA.

Skin Test

An experienced allergy nurse performed skin testing in each of the patients. SET (5-fold dilution) as described previously was used.⁸ Histamine was used as a positive control, and glycerin was used as a negative control. The allergen concentrate was 1:20 weight/volume, dilution 1 was 1:100, dilution 2 was 1:500, dilution 3 was 1:2500, dilution 4 was 1:12,500, dilution 5 was 1:62,500, and dilution 6 was 1:312,000. Intradermal skin testing was performed at these dilutions. The end point was defined as the most concentrated dilution producing a 7-mm wheal followed by a confirmatory wheal of at least 9 mm at the next higher concentration. The end point thus obtained was used for comparison with the in vitro tests. Skin test antigens were obtained from the Bayer Pharmaceutical Division (Spokane, WA).

In Vitro Tests

The reagents and instrumentation for Pharmacia ImmunoCAP were obtained from Pharmacia and Upjohn (Kalamazoo, MI), and the reagents and instrumentation for HY•TEC EIA were obtained from Hycor Biomedical Inc (Garden Grove, CA). As noted previously, the 2 systems differ in their degree of automation; the ImmunoCAP demands more of the technician. The HY•TEC system measures the optical density without the need for replacement of the test tubes. The fluorescence of the eluate for ImmunoCAP was measured after transfer to a FluoroCount 96. The alternative scoring method (ASM) for percentage of reference fluorescence was used for ImmunoCAP. ASM percentage of reference results above 70% were accepted as positive. An experienced technician performed all of the tests.

HY•TEC detected the allergen-specific IgE concentrations by using a spectrophotometric method on a Spectra reader. Results above 0.08 IU/mL were scored as positive. The first 68 samples for the HY•TEC EIA test were duplicated for assessment of the reproducibility of this test.

ROC Analysis and Calculations

The sensitivity and specificity were calculated for each of the individual allergens for ImmunoCAP and HY•TEC, with the SET results taken as reference. ROC analysis was used for comparison of the specificity and sensitivity of ImmunoCAP with those of HY•TEC. The presence of specific IgE was defined as an end point on SET of 2 or greater for the particular antigen. For *D pteronyssinus* and *D farina*, no dilution of 1 (weight/volume 1:20) was available. Skin test results of 3 or greater were accepted as positive. ROC curves were obtained with software developed by the Department of Radiology, University of Chicago (courtesy of Charles Metz, PhD).

We used the DataDesk program (DataDesk 3.0, 1989; Data Description, Ithaca, NY) for the Macintosh to calculate Pearson product-moment correlation coefficients and to assess

Table 1. Sensitivity and specificity for individual antigens

Antigens	Sensitivity		Specificity	
	ImmunoCAP	HY•TEC	ImmunoCAP	HY•TEC
<i>D pteronyssinus</i>	70.83% (34/48)	64.58% (31/48)	94.23% (49/52)	94.23% (49/52)
<i>D farina</i>	70.21% (33/47)	74.46% (35/47)	92.45% (49/53)	81.11% (43/53)
Cat dander	84.44% (38/45)	84.44% (38/45)	94.54% (52/55)	81.81% (45/55)
Meadow fescue	80.00% (36/45)	77.77% (35/45)	92.72% (51/55)	96.36% (53/55)
Timothy grass	84.21% (32/38)	86.84% (33/38)	90.32% (56/62)	93.54% (58/62)
<i>A fumigatus</i>	70.00% (7/10)	30% (3/10)	88.88% (80/90)	97.77% (88/90)
<i>A tenuis</i>	88.46% (23/26)	80.76% (21/26)	97.29% (72/74)	95.94% (71/74)
Oak tree	60.00% (21/35)	65.71% (23/35)	93.84% (61/65)	89.23% (58/65)
White ash tree	58.33% (21/36)	55.55% (20/36)	93.75% (60/64)	95.38% (62/64)
Giant ragweed	78.43% (40/51)	84.31% (43/51)	97.95% (48/49)	95.91% (47/49)
English plantain	58.33% (21/36)	44.44% (16/36)	95.31% (61/64)	93.75% (60/64)
Lamb's-quarter	53.65% (22/41)	39.02% (16/41)	96.61% (57/59)	94.91% (56/59)

Parentheses contain the (test+/true+) for sensitivity and (test-/true-) for specificity.

the reproducibility of the duplicate runs of HY•TEC in the first 68 patients.

The true-positive results detected by either ImmunoCAP or HY•TEC were also compared with skin test results for correlation of class scores. For ImmunoCAP ASM percentage of reference, results were converted to class scores ranging from 0 to 6. Scores of 1 or greater were accepted as positive. Similarly, HY•TEC results (in international units per milliliter) were converted to class scores of 0 to 6; again, scores equal to or greater than 1 were accepted as positive. For assessment of this correlation, the true-positive class scores obtained by either ImmunoCAP or HY•TEC were simply subtracted from SET results. A result of 0 was labeled as no class difference. A value of -1 indicated SET results 1 class higher than ImmunoCAP or HY•TEC results. Values of -2 or less were labeled as underclassifications as well and indicated that SET results were 2 classes or more higher than ImmunoCAP or HY•TEC results. A value of 1 indicated that in vitro test class scores were higher than SET scores, and results that were higher than 1 class difference were labeled as overclassifications.

RESULTS

Of the 100 subjects, 91 were found to be allergic to at least 1 of the tested allergens according to the SET test results. The most common allergens were giant ragweed (51/100), *D pteronyssinus* (48/100), *D farina* (47/100), cat dander (45/100), and meadow fescue (45/100). For mold allergies, only 10 of the 100 subjects were allergic to *A fumigatus*, and 26 of the 100 were allergic to *A tenuis*.

Duplicate HY•TEC samples were run for the first 68 patients. The overall Pearson product-moment correlation coefficient for the HY•TEC result and its duplicate was 0.97, which was nearly perfect; it ranged from 0.89 to 1.0 for individual antigens.

The overall sensitivity of ImmunoCAP was 72.92% (334/458), and that of HY•TEC was 67.24% (308/458). The specificity was calculated as 93.80% (696/742) for ImmunoCAP and 92.99% (690/742) for HY•TEC. The sensitivity of ImmunoCAP ranged from 53.65% to 88.46%, and the sensitivity of HY•TEC ranged from 30.00% to 86.84%. High sensitivity was obtained for ImmunoCAP results for cat dander (84.44%), timothy grass (84.21%), *A tenuis* (88.46%), meadow fescue (80.00%), and giant ragweed (78.43%). High sensitivity was obtained for HY•TEC results for cat dander (84.44%), *A tenuis* (80.76%), timothy grass (86.84%), meadow fescue (77.77%), and giant ragweed (84.31%). The specificity of ImmunoCAP ranged from 89% to 98%, and the specificity of HY•TEC ranged from 81% to 96% (Table 1).

The sensitivity of HY•TEC was slightly higher than that of ImmunoCAP for timothy grass (86.84%), giant ragweed (84.31%), and *D farina* (74.46%). The sensitivity of HY•TEC to white ash tree (55.55%), English plantain (44.44%), lamb's-quarter (39.02%), and *Aspergillus* (30.00%) was low. Although ImmunoCAP results for white ash tree (58.33%), English plantain (58.33%), and lamb's-quarter (53.65%) were slightly higher than those for HY•TEC, the sensitivity to these allergens was also low for ImmunoCAP. The sensitivity to oak tree was low in both in vitro tests; however, the HY•TEC results for oak tree were slightly better than those of ImmunoCAP (65.71% vs 60.00%).

ROC Analysis

ROC analysis compares the sensitivity of 2 diagnostic procedures at a particular specificity. In an ROC graph, the y-axis represents the true-positive results, known as the sensitivity, and the x-axis represents the

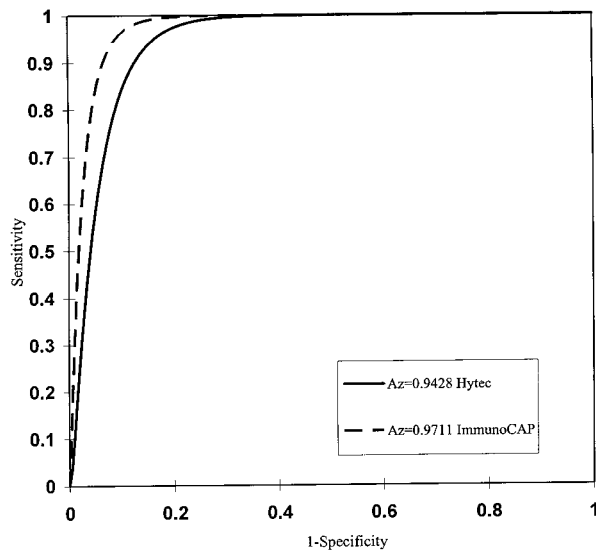


Fig 1. *D pteronyssinus* ROC curve.

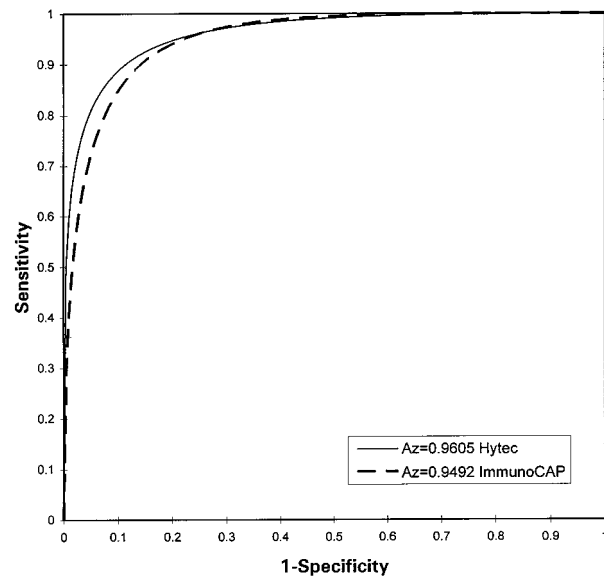


Fig 3. Meadow fescue ROC curve.

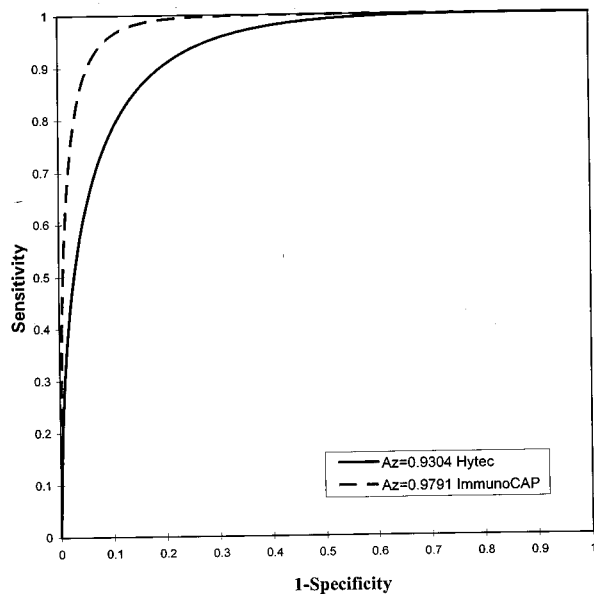


Fig 2. Cat dander ROC curve.

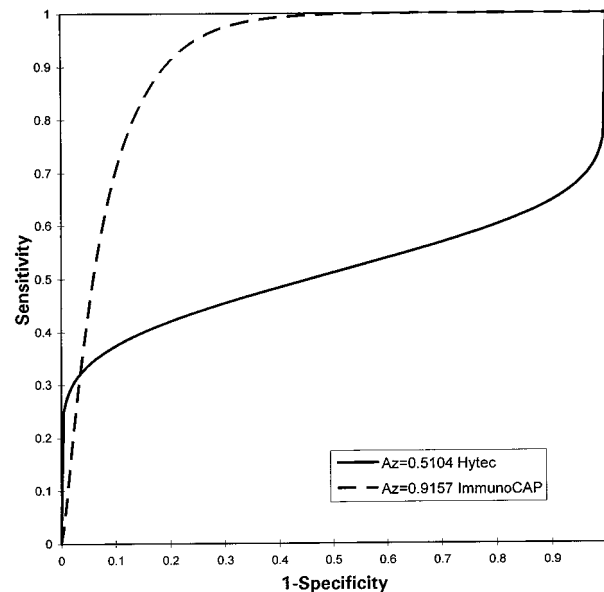


Fig 4. *A fumigatus* ROC curve.

false-positive results, defined as $1 - \text{True-negative results}$, or $1 - \text{Specificity}$. Depending on the decision criteria for the positive and negative results, ROC analysis provides a particular curve. The slope of the ROC curve usually decreases as the number of false-positive results increases. If there are no false-positive results, ROC analysis will not result in a graph. Generally, better decision or detection performance is indicated by an ROC curve that is higher or to the left in the ROC space.

Dust mites. For *D pteronyssinus* and *D farina*, the area indexes as denoted by the area (Az) under the ImmunoCAP ROC curves were 0.9711 and 0.9449. The HY•TEC Az values were 0.9428 for *D pteronyssinus* and 0.8446 for *D farina* (Fig 1). The performance of ImmunoCAP was better than that of HY•TEC on the ROC curve for these allergens.

Cat dander. The Az value for ImmunoCAP was 0.9791, and for HY•TEC it was 0.9304 (Fig 2). Although the sensitivity of the 2 systems was the same

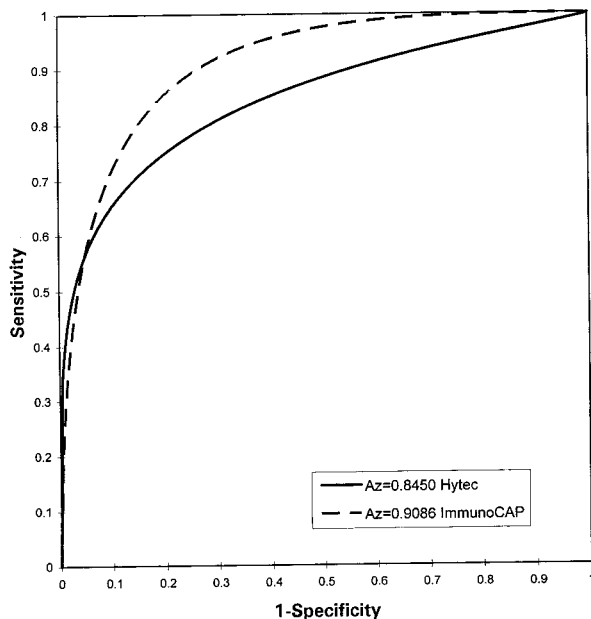


Fig 5. Oak tree ROC curve.

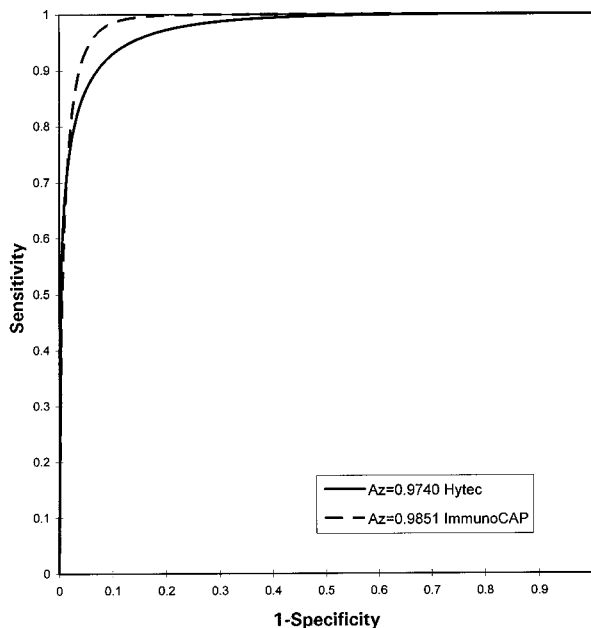


Fig 6. Giant ragweed ROC curve.

at a fixed specificity, the performance of ImmunoCAP was seen to be better than that of HY•TEC by ROC analysis.

Grass. For meadow fescue, the area under the ROC curve for HY•TEC was 0.9605, and for ImmunoCAP it was 0.9492 (Fig 3). For timothy grass, it was 0.9528 for HY•TEC and 0.9277 for ImmunoCAP.

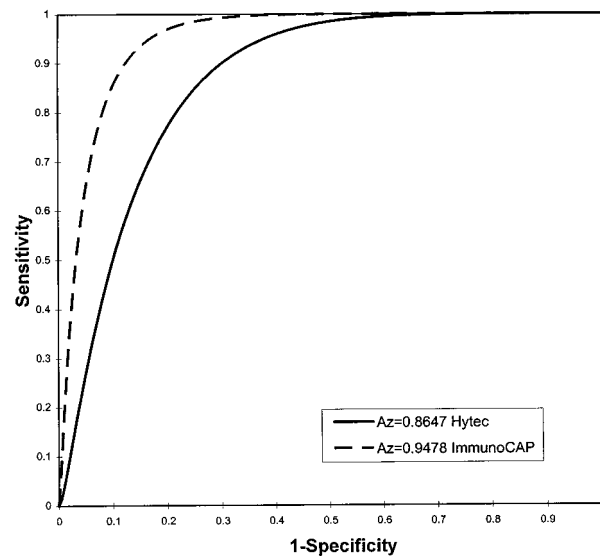


Fig 7. Overall results ROC curve.

Molds. For *A tenuis*, the Az value for ImmunoCAP was 0.9822, and for HY•TEC it was 0.9660. However, for *Aspergillus*, the area under the ImmunoCAP curve was 0.9157, but for HY•TEC it was 0.5104 (Fig 4).

Tree pollens. The Az value under the oak tree ROC curve for ImmunoCAP was 0.9086, and under the HY•TEC curve it was 0.8450 (Fig 5). For white ash tree, the Az value was 0.8077 for HY•TEC and 0.7525 for ImmunoCAP. Thus the performance of HY•TEC was better than that of ImmunoCAP for white ash tree pollen.

Weeds. The area under the ROC curve for giant ragweed was 0.9851 for ImmunoCAP and 0.9740 for HY•TEC (Fig 6). Both of these results were very close to perfect. For lamb's-quarter, Az was 0.9618 for ImmunoCAP and 0.8783 for HY•TEC. However, for English plantain it was 0.7910 for ImmunoCAP and 0.7241 for HY•TEC.

Overall Results

The ROC curve for the SET test results used as the gold standard was compared with ImmunoCAP and HY•TEC test results. The Az value for ImmunoCAP was 0.9478 for all allergens combined, and for HY•TEC it was 0.8647 (Fig 7).

Sixty-eight percent (228/334) of true-positive ImmunoCAP results were within 1 class difference when compared with SET test results, and for HY•TEC that concordance was calculated as 62% (193/308) (Fig 8). Eighty-one percent (251/308) of HY•TEC true-positive results were detected within the same class or 1 or 2 classes below SET results, and for ImmunoCAP this concordance was 69% (181/334). Five percent (15/308)

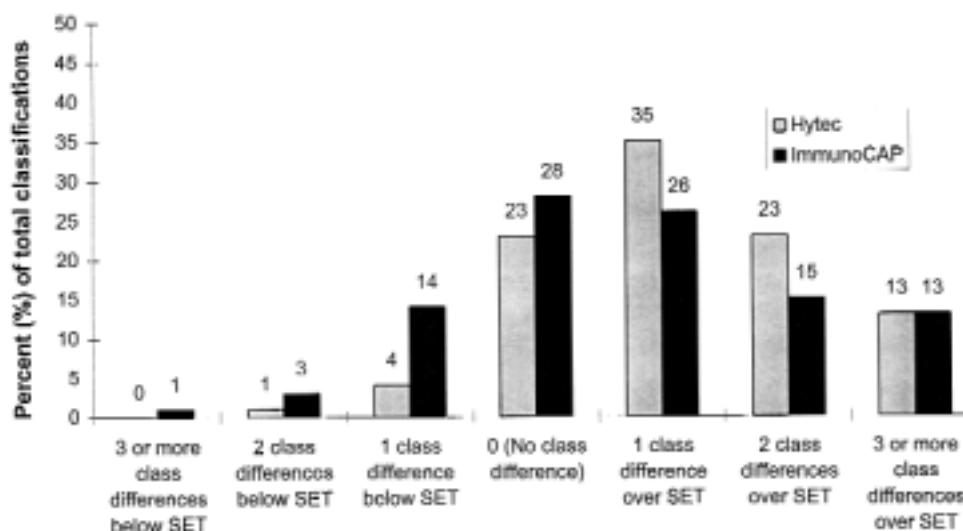


Fig 8. Class differences from SET.

of HY•TEC results and 18% (59/334) of ImmunoCAP results were overclassified according to SET test results.

DISCUSSION

ROC analysis is based on statistical decision theory and was first developed for signal detection to solve problems that had occurred in the development of radar systems. ROC analysis has been used in experimental psychology and psychophysics studies. In the 1970s, ROC analysis began to be used in the analysis of medical imaging.⁹

In the assessment of the reliability of a diagnostic procedure, sensitivity and specificity are 2 important concepts. The true-positive fraction represents sensitivity, and the true-negative fraction represents specificity. On an ROC curve, the y-axis represents the true-positive fraction, and the x-axis represents the false-positive fraction, which is equal to $1 - \text{True-negative fraction}$ or $1 - \text{Specificity}$. From the practical perspective of ROC analysis, the combinations for the true-positive fraction and the false-positive fraction obtained from each test merely represent 1 point on an ROC curve.

In this study, results equal to or greater than 0.08 IU/mL for HY•TEC and percentage of reference results equal to or greater than 70% were taken as positive results when data were entered for ROC analysis. Depending on the decision criteria between the positive and negative results, ROC analysis is represented by a curve counting the false-positive results relative to the true-positive results. The slope of the ROC curve usually decreases as the number of false-positive results increases. This method offers the possibility of comparing 2 systems' sensitivities at any particular specificity.

Generally, better decision or detection performance is indicated by an ROC curve that is higher or to the left in the ROC space.

The area under an ROC curve when it is plotted in the unit square (denoted by A_z) also provides a useful summary index of inherent discrimination performance that is independent of possible variations in the confidence threshold. The area A_z can be interpreted as the average value of sensitivity on the corresponding ROC curve if the system's specificity is selected randomly between 0.0 and 1.0, or equivalently, as the average of specificity on the ROC curve if sensitivity is selected randomly between 0.0 and 1.0.

According to ROC analysis, ImmunoCAP results for giant ragweed, lamb's-quarter, *A tenuis*, cat dander, *D pteronyssinus*, *D farina*, *A fumigatus*, timothy grass, meadow fescue, and oak tree were close to perfect according to A_z values. Again, according to the ROC curves, the performance of HY•TEC was slightly better than the performance of ImmunoCAP for timothy grass and meadow fescue. However, the actual sensitivities for *D pteronyssinus*, *D farina*, oak tree, and lamb's-quarter were not high; the reason for the high A_z values obtained by ROC analysis is the high specificity and low number of false-positive results.

Both tests' sensitivities to timothy grass, meadow fescue, cat dander, ragweed, and *Alternaria* were high. The sensitivities of HY•TEC for giant ragweed, *D farina* (74%), and meadow fescue (87%) were better than those of ImmunoCAP at specificities differing between 81% and 94%. However, for *Aspergillus*, although the sensitivity of ImmunoCAP was 70%, the sensitivity of HY•TEC was 30%. HY•TEC's sensitivity to English

plantain (44%), lamb's-quarter (39%) and white ash tree (55%) was also low. However, ImmunoCAP results for English plantain (58% sensitivity), lamb's-quarter (54%), and white ash tree (58%) were only slightly better.

Because HY•TEC is a new testing system, we also tested its reproducibility. Concordance of the duplicates was close to perfect (97%), which was similar to our previous findings with ImmunoCAP.¹

Eighteen percent of ImmunoCAP results and 6% of HY•TEC results were overclassified according to SET results. Fifty-eight percent of HY•TEC results and 54% of ImmunoCAP results were detected within the same class or 1 class lower than SET results. These results show that there is tendency toward underclassification relative to SET. Calculations for immunotherapy using in vitro test results should take this tendency to underclassification into account.

CONCLUSION

ROC analysis is a useful method for comparing diagnostic procedures with different sensitivities and specificities. Both ImmunoCAP and HY•TEC are reliable, reproducible, and effective in the detection of inhalant allergies. The sensitivity and specificity of HY•TEC are highly comparable with those of ImmunoCAP for *D pteronyssinus*, *D farina*, cat dander, timothy grass, meadow fescue, *A tenuis*, giant ragweed, and oak tree. However, both in vitro tests revealed low sensitivity for

English plantain, lamb's-quarter, and white ash tree. The sensitivity of HY•TEC to *Aspergillus* was also low. Overall though, the assay characteristics were good for each system tested.

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