



ImmunoCAP[®]

Specific IgE blood test

The ImmunoCAP Specific IgE blood test has been cleared by the FDA for quantitative measurement of specific IgE.



TECHNOLOGY YOU CAN COUNT ON

Allergic disease and allergens are complex in nature. Therefore, the design and production of high quality assays is an intricate task. Pharmacia has both pioneered and mastered the technology required to produce superior assays.

The ImmunoCAP® Specific IgE blood test is cleared by the U.S. Food and Drug Administration (FDA) to quantitatively measure levels of specific IgE. A comparative study published in the *Journal of Allergy and Clinical Immunology* demonstrated that ImmunoCAP is the most accurate specific IgE test available and should be considered the standard for serologic testing!¹ This means ImmunoCAP can accurately determine *if* patients are allergic and exactly *what* they are allergic to.

The ImmunoCAP contains the solid phase component of the Pharmacia *in vitro* immunodiagnostic system. It measures specific IgE unique to the respective allergen bound to the ImmunoCAP solid phase. The ImmunoCAP consists of a reaction chamber containing a cellulose sponge matrix, which is a flexible hydrophilic polymer to which allergenic components are covalently coupled. The ImmunoCAP is used in both the Pharmacia CAP System™ and the UniCAP® System. This three-dimensional solid phase has a high binding capacity and is an excellent carrier of allergens. Other features include



- **a porous structure that absorbs the entire serum sample into the cellulose sponge, resulting in good reaction kinetics and providing test results in hours, not days**
- **high elasticity for efficient washes and, therefore, low background levels**
- **a design that allows for automation, reducing labor costs and hands-on time**

The design of the solid phase is crucial to the performance characteristics of an assay, because of the complexity of allergen source materials and the diversity of individual IgE antibody specificities. To provide quantitative results and a high sensitivity, it is critical that the capacity of the solid phase is high enough to bind all clinically relevant proteins of the allergen.

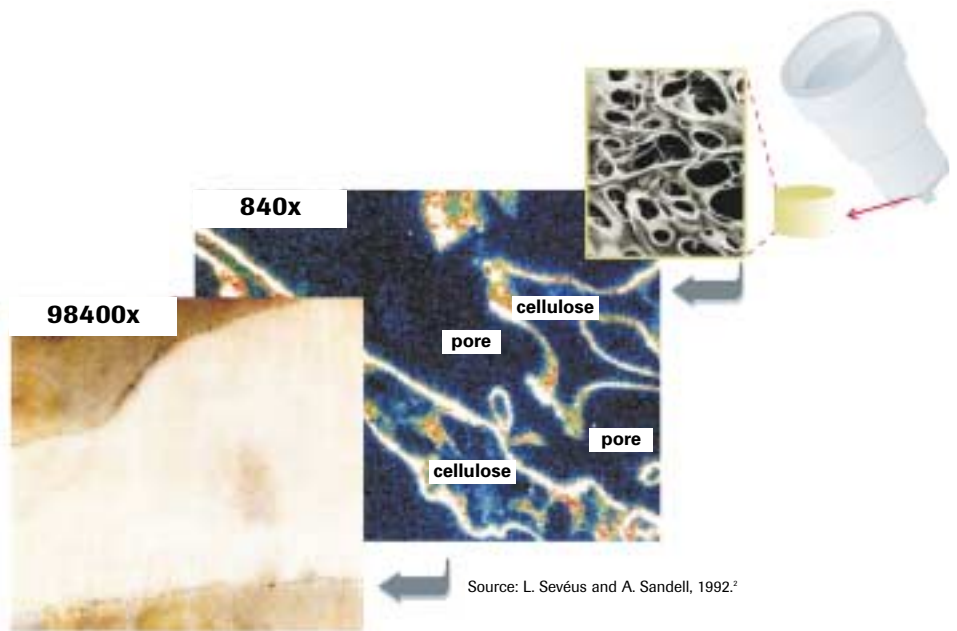
SOLID PHASE: ImmunoCAP®

These pictures show the cellulose polymer under different degrees of magnification. Efficient utilization of the large surface and the preserved condition of proteins covalently coupled to the surface are demonstrated with different techniques and magnifications.

White areas indicate the binding of fluorescence-labeled protein to the activated cellulose polymer. The high density of white areas seen in the picture shows that the surface of the solid phase is loaded with proteins, and demonstrates that the large surface of the solid phase is efficiently utilized.

Black dots indicate binding of gold-labeled antibodies to the allergen-coupled cellulose polymer, demonstrating the allergens still have intact epitopes recognizable by antibodies after coupling of the allergen to the solid phase polymer.

ImmunoCAP binds almost 150 times more protein than a passively coated tube and at least 3 times more protein than a paper disc.³



COMPREHENSIVE SOURCE MATERIAL STANDARDIZATION

The ImmunoCAP standardization process begins with selecting the optimal allergen source material and involves strict quality assurance during the production of the assay reagents. The standardization procedure at Pharmacia includes clinically based performance specifications. It also requires extensive supplies of patient serum samples and comparisons to several production lots of allergen source material.

CLINICALLY IMPORTANT ALLERGEN COMPONENTS

Another important aspect of allergen source material standardization is The Serum Collection Bank at Pharmacia. The Serum Collection Bank has registered more than 39,000 samples since its establishment in 1974 as a worldwide serum and plasma collection program. Physicians and blood donor centers in different geographical locations throughout the world supply the company with serum or plasma documented and characterized by specific IgE, IgG, and IgA. These samples are used for allergen standardization purposes, quality control, product production, and for our external, international quality assessment program, Quality Club.

STANDARDIZED AND QUANTITATIVE

Two important requirements for a quantitative test for specific IgE antibodies are 1) the ability to report results in internationally standardized units, and 2) the demonstration of parallelism between calibrator and sample dilutions.

For total IgE protein, a mass unit (U) is defined by the World Health Organization (WHO) International Reference Preparation 75/502; 1 IU has been shown to be equal to 2.4 ng IgE.⁴

In UniCAP® Specific IgE and Pharmacia CAP System™ Specific IgE, total IgE protein is used for calibration of the assays. The total IgE concentrations are expressed as a concentration of International Units IgE (kU/L), and the calibrators are directly traceable to the WHO International Reference Preparation for human IgE 75/502 (see figure below). Measured response values for allergen specific IgE antibodies are evaluated against a total IgE calibration curve and are expressed as a concentration in allergen specific units (kU_A/L).

U = international unit for IgE as defined by WHO International Reference 75/502

U_A = allergen specific unit

An excess of bound solid-phase allergens permits quantitative binding of specific IgE over a wide concentration range. The demonstration of parallelism when diluting calibrators and patient samples (positive for different allergens) indicates the assay design provides quantitative results. Quantitative results for specific IgE are measured in kU_A/L, where A represents the amount of allergen-specific antibodies. Quantitative reproducible results are important to clinicians and can only be obtained using ImmunoCAP® technology.

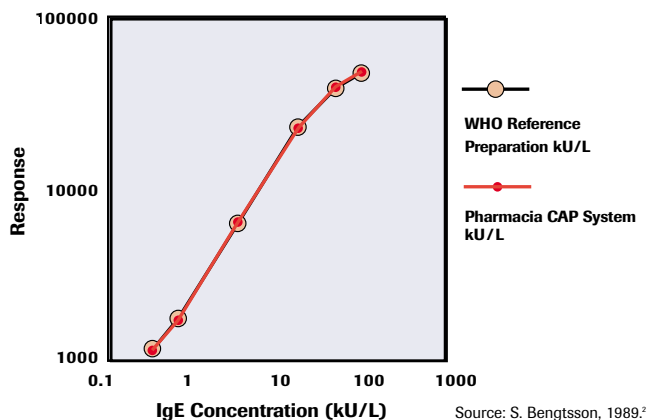
IMMUNOCAP ASSAYS ARE HIGHLY SPECIFIC AND SENSITIVE

The high binding capacity of ImmunoCAP essentially removes the risk of competition or interference by antibodies of immunoglobulin classes that are not being measured. Together with the use of well characterized allergens and a solid phase that is easy to wash efficiently, this ensures excellent specificity.

High sensitivity, allowing more positive patient results to be detected compared to non-ImmunoCAP systems, is the result of the efficient binding of allergens to ImmunoCAP and of improving detection of low concentrations of antibodies.

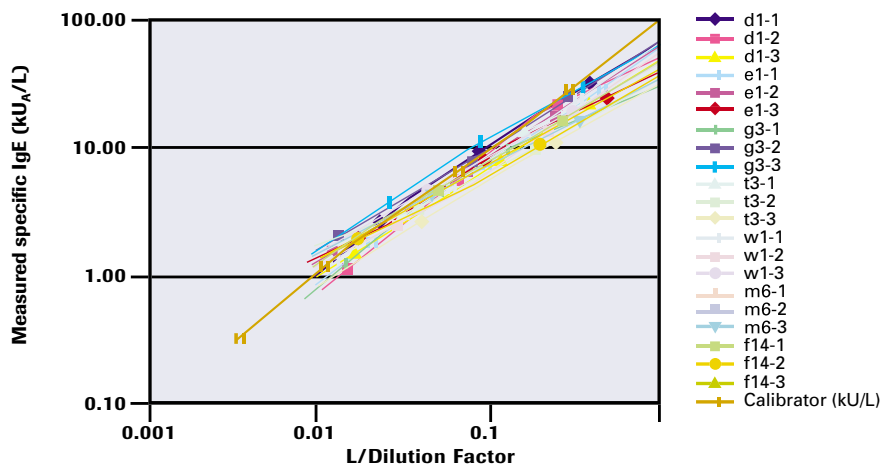
CALIBRATION

WHO IgE Reference Preparation 75/502 and Pharmacia CAP System calibrator



This calibration design permits a wide measuring range and ensures stability over time.

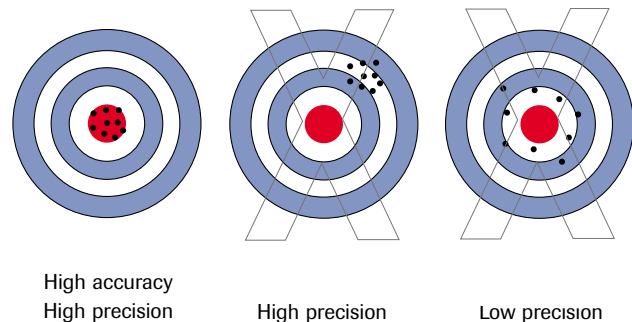
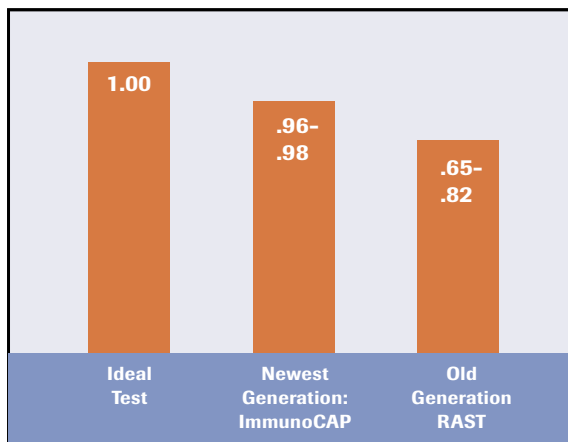
PARALLELISM (PHARMACIA CAP SYSTEM)



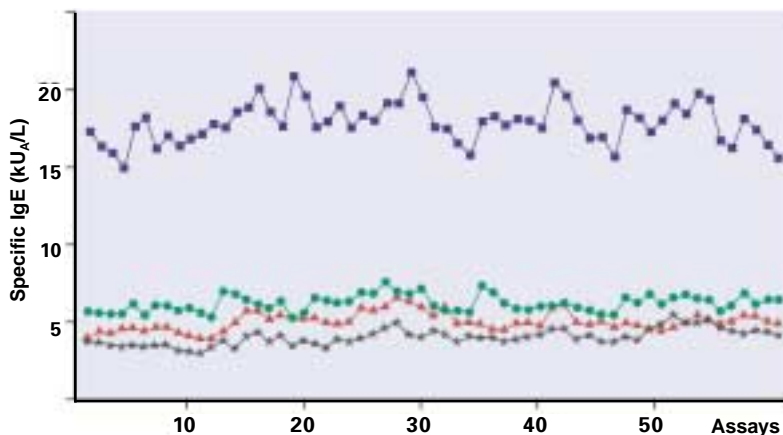
ACCURACY AND PRECISION OF IMMUNOASSAYS FOR SPECIFIC IgE

Based on revolutionary ImmunoCAP® technology, the ImmunoCAP assays are a safe, effective, and reliable alternative to traditional allergy testing methods.

- In clinical tests, ImmunoCAP technology has been demonstrated to be close to an ideal lab determination.
- In a recent study,¹ laboratories evaluated more than 12,000 blinded serum samples containing various levels of specific IgE, using a number of lab tests in order to compare accuracy and precision. The summary bar graph below left reflects the average standardized slope coefficients; the ideal would be 1.0. The results in the study ranged from 0.65 to 0.98.
- The ImmunoCAP technology produced the best assessments (0.96 to 0.98), performing almost as well as the ideal standard. RAST™ tests ranged from 0.65 to 0.82. As researchers concluded, “The Pharmacia CAP System™ performed well when compared with the standard of an ideal assay, although other assays often did not perform up to this standard.”¹



REPRODUCIBLE



Analysis of results for UniCAP® and Pharmacia CAP System instrumentation from both internal and external quality assessment programs demonstrates a highly accurate and precise assay with an average coefficient of variation below 15% for specific IgE, including lot-to-lot and person-to-person variation.

According to studies published in the *Journal of Allergy and Clinical Immunology* and *Annals of Allergy*, the ImmunoCAP® test from Pharmacia should be considered the standard specific IgE serologic test method.¹⁶ The results demonstrated superior reliability and accuracy over other specific IgE *in vitro* methodologies, including RAST.™

Lab precision summary for positive readings (CV)* Nondilution samples						
Allergen	A-RAST	A-STAT	C-RAST	I-CAP	L-RAST	S-CAP
d2 <i>Dermatophagoides farinae</i>	17.7%	17.7%	23.7%	8.8%	9.9%	11.2%
e1 Cat Dander	16.8%	18.8%	5.3%	11.3%	9.7%	14.1%
e5 Dog Dander	17.6%	19.7%	11.0%	10.3%	15.4%	7.4%
g2 Bermuda Grass (<i>Cynodon dactylon</i>)	19.7%	28.7%	8.2%	11.6%	14.2%	11.1%
g6 Timothy Grass(<i>Phleum pratense</i>)	16.9%	22.8%	5.4%	9.4%	14.0%	13.9%
t7 White Oak (<i>Quercus alba</i>)	17.4%	16.5%	10.5%	7.0%	12.9%	11.6%
w1 Short Ragweed (<i>Ambrosia elatior</i>)	18.2%	24.0%	7.5%	8.7%	14.1%	6.5%
w3 Giant Ragweed (<i>Ambrosia trifida</i>)	24.6%	28.2%	10.0%	11.8%	9.9%	12.0%
w6 Mugwort Sage (<i>Artemisia vulgaris</i>)	22.0%	49.1%	7.8%	12.6%	12.9%	10.5%
w11 Russian Thistle (<i>Salsola kali</i>)	16.3%	27.0%	16.0%	8.1%	14.8%	8.5%
w14 Rough Pigweed (<i>Amaranthus retroflexus</i>)	16.3%	17.8%	23.5%	8.1%	15.0%	13.1%
m1 <i>Penicillium notatum</i>	20.8%	41.4%	15.1%	8.5%	17.2%	11.0%
m2 <i>Cladosporium herbarum</i>	26.1%	32.1%	15.3%	10.4%	11.9%	10.3%
m3 <i>Aspergillus fumigatus</i>	20.7%	26.8%	14.9%	8.2%	11.5%	10.7%
m4 <i>Mucor racemosus</i>	21.7%	27.2%	28.4%	11.2%	31.3%	9.4%
m5 <i>Candida albicans</i>	20.4%	25.7%	15.7%	11.2%	17.0%	12.5%
m6 <i>Alternaria tenuis/alternata</i>	23.4%	18.3%	9.6%	10.8%	8.5%	10.6%
Average for lab	20.3%	27.5%	13.4%	9.8%	14.7%	10.8%

A-STAT: AlaSTAT® by Allercare (Diagnostic Products Corporation)

I-CAP: Pharmacia CAP System™ by IBT Reference Laboratory

S-CAP: Pharmacia CAP System by SmithKline Beecham Laboratories (now Quest)

A-RAST: Modified RAST by Allergy Testing Laboratories (license from Hyco Biomedical)

C-RAST: Modified RAST by Commonwealth Medical Laboratory (Hycor Biomedical)

L-RAST: Modified RAST by Laboratory Corp of America (license from Hyco Biomedical)

*ideally zero

SUMMARY

The new-generation ImmunoCAP technology design provides an extremely high binding capacity using the cellulose polymer sponge. This capacity, along with extensive quality control measures, excellent allergen and reagent source materials, and calibration to WHO reference preparations, results in both high sensitivity and high specificity for assays based on this technology. In addition, the structural design of ImmunoCAP allows for automation and thorough elimination of unbound nonspecific material in the reaction complex, resulting in highly reproducible, accurate, and quantitative results.

The ImmunoCAP Specific IgE blood test has been cleared by the FDA for quantitative measurement of specific IgE.

REFERENCES

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