

In the beginning there was

Skin Prick Testing

Advantages

Direct contact with the patient
Cheap, Quick, Same Day Results
+/- 60 Single & Mixed Allergens available
Inhalants generally of good quality

Disadvantages

Quality of Extracts- The Good, The Bad & The Ugly
Highly Operator Dependent
Results Affected by: Treatment
Dermographism
Non-Reactive Skins: Colour, Age
Possibility of Anaphylaxis
Compare 60 Allergens to 700 in ImmunoCAP
1 Bottle (2.0ml) = +/- 70 Tests – Too much

In general, Where desensitising extracts are available quality is good. Where it is not, e.g; Foods best described as doubtful and the Prick / Prick test should be used.

SKIN PATCH TESTING

For Contact Dermatitis & Contact Intolerances. Thus almost entirely in the realm of dermatology clinics and **Specialists** only.

-700 "Allergens" available (**But** you can also make your own)

-"Fill Your Own" Chambers available

-Packing in 5ml syringes not user friendly for small units- +/- 125 patient doses / syringe.

-Wide selections of standardised panels available

For: Cosmetics

Plants

European Baseline

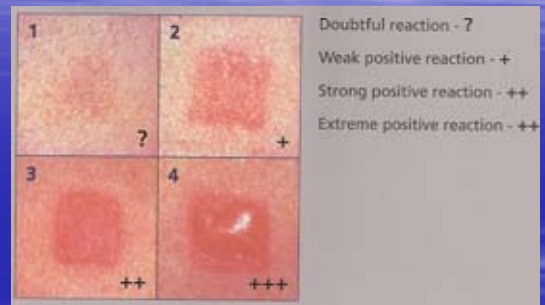
Textiles Colours & Finishes

Adverse Drug Reactions

Fragrances, etc.

Dental

Plastics & Glues



-The TRUE TEST- Ready to use European Standard Services- 2 x Panels of 12

THE CAST TEST

Cellular Antigen Stimulation Test

Initially an overnight ELISA test, then improved to 5 hours from start to finish; CAST 2000 ELISA.

FLOWCAST, FLOW2CAST, 2 hours and then 1 hour tests.

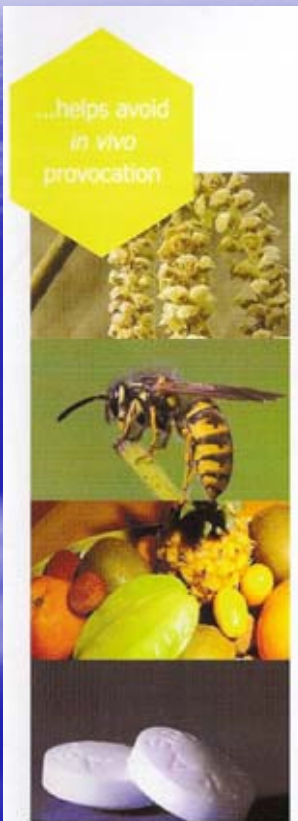
Basophils are activated by allergens and the activity is detected by flow cytometry measuring the increase of CD63 at the cellular surface. Both IgE and Sour IgE mediated reactions can be detected.

Advantages

- Especially useful for: Antibiotics, Analgesics, Anaesthetics, Food Addictives & Colorants.

Disadvantages

- Cost
- Fresh whole blood sample (Max 5 Hours)
- Flow Cytometer required



Quantitative Specific IgE

Eva Sjö Dahl, November 28, 2001

Reasons to know the quantitative specific IgE levels

- Common allergic disorders develop into severe conditions later in life
- Many conditions mistakenly believed to be IgE-related are incorrectly treated
 - Identifying the offending allergen is essential for avoidance
 - Quantification is the tool to pick out the most probable

The clinical utility of quantitative specific IgE values

Developing allergy

- *The presence of even low levels of IgE antibodies suggest an ongoing sensitization and formation of antibodies of other specificities.*
- *In the small child, elevated egg-IgE antibody levels are associated with significantly elevated risk of developing inhalant allergies later in childhood.*

The clinical utility of quantitative specific IgE values

Suspected food allergy

- *In food allergy, IgE antibody levels have been derived indicating 95% positive and 90% negative predictive value for a clinical reaction at certain clinical conditions.*
- *Food IgE antibodies can also be used to predict which food allergies are resolving spontaneously.*
- *Low levels indicate a small, but still a risk, to develop symptoms to food exposure.*

The clinical utility of quantitative specific IgE values

Suspected inhalant allergy

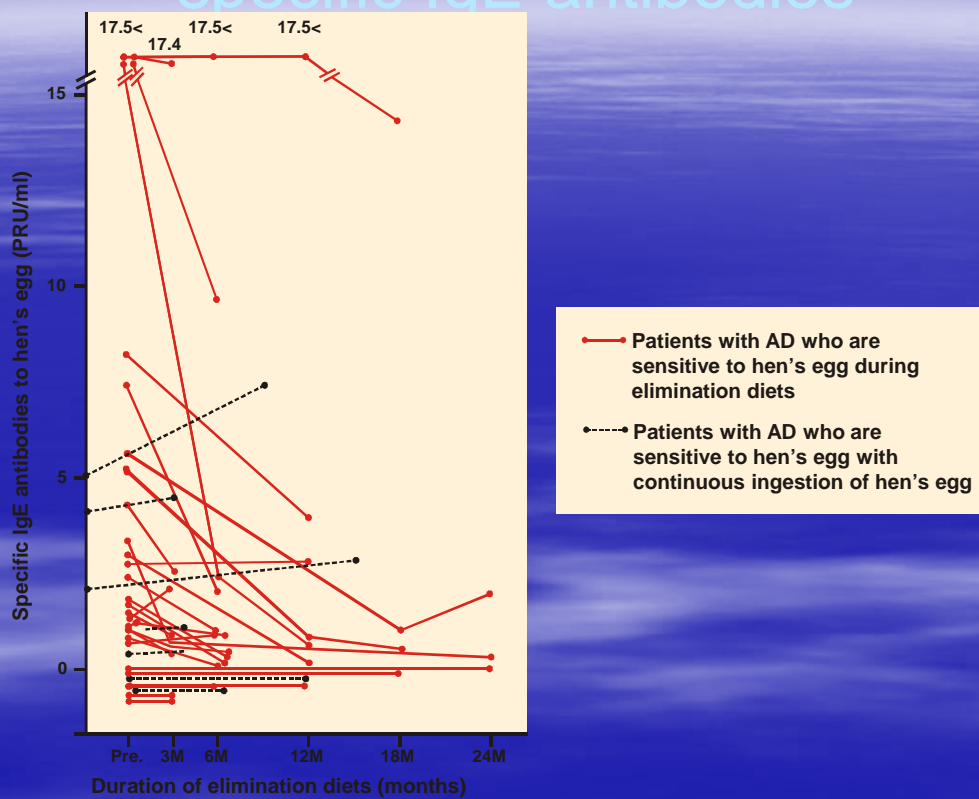
- *In cases of inhalant allergy, specific IgE antibody levels correlate closely with results of inhalation challenge studies in cat-sensitive persons.*
- *High levels of IgE antibodies to inhalant allergens indicate a high probability that clinical symptoms are associated with exposure to that particular allergen and allergic disease.*
- *Low levels indicate a low probability to develop symptoms and allergic disease associated with that particular allergen.*

*Yunginger JW et al., J Allergy Clin Immunol 2000; 105: 1077-84
Ahlstedt S, Clin Exp Allergy (in press)*

Probability × Consequence = Risk

- As some allergens can have severe consequences, even low values associated with low likelihood of symptoms need to be considered.
- In the case of sensitization to allergens such as peanut, latex or drugs that can cause anaphylaxis, any IgE antibody level represents a risk and should be regarded seriously.

Effect of elimination diets on food-specific IgE antibodies



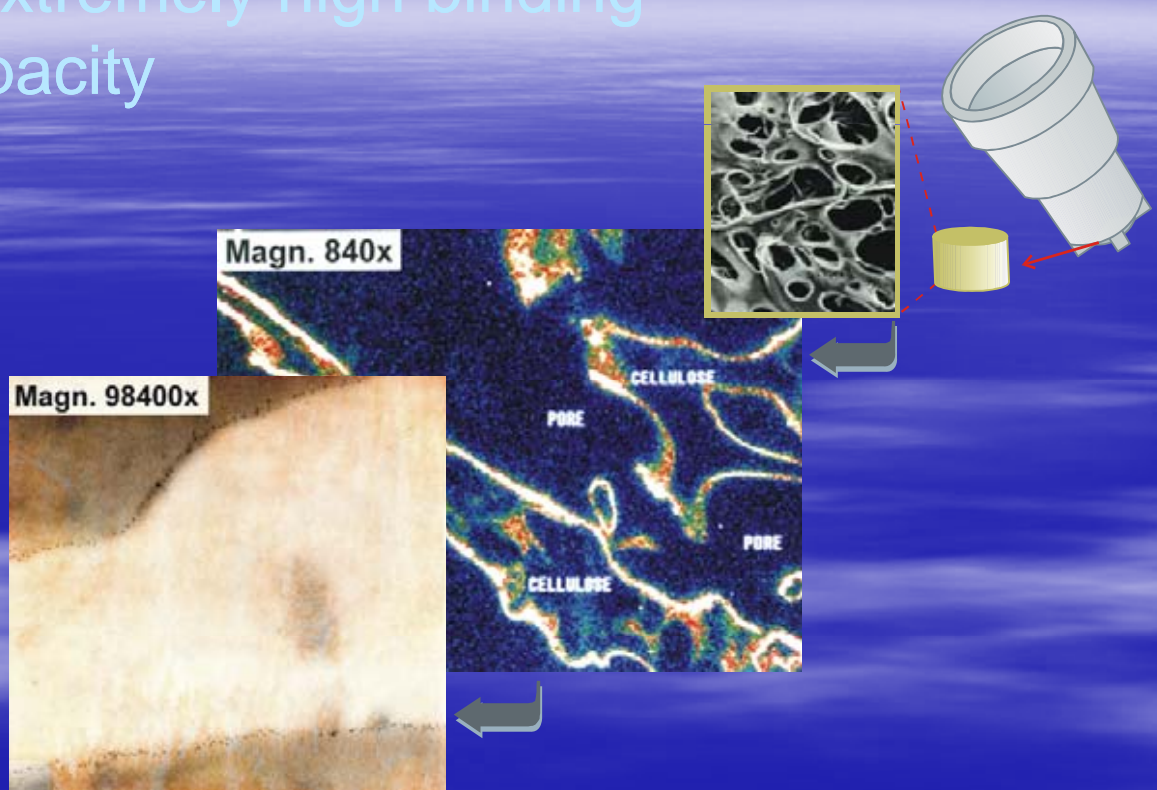
Agata H et al., *J Allergy Clin Immunol* 1993; Vol 91, No 2: 675

Prerequisites for a quantitative specific IgE test

- Excess of allergen
- Precision
 - Reproducibility
 - Repeatability
- Linearity
- Calibration traceable to WHO

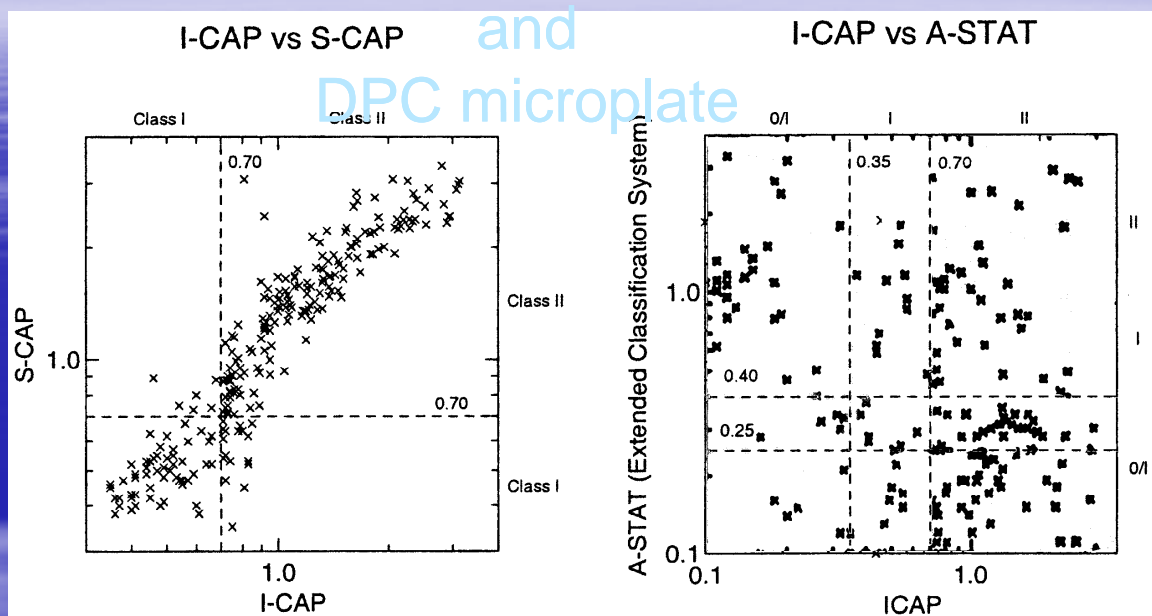
Solid phase: ImmunoCAP™

– extremely high binding capacity



L. Sevés & A. Sandell, 1992

Comparison of results from different laboratories running Pharmacia CAP System and DPC microplate



I-CAP: Pharmacia CAP System (Laboratory I)
S-CAP: Pharmacia CAP System (Laboratory II)
A-STAT: AlaSTAT, DPC (Laboratory III)

Scatter plots of values from methods related to their own cut-off values between class 0 / 1 and class 1 / 2.

The meaning of a class result is totally different for different specific IgE measuring methods. Class X for A-STAT is not the same as class X for S-CAP. Kendall's W test showed significant different results from different assays. Kendall's W across all assays was 0.120; $\chi^2 = 49.41$; $p < 0.01$

Allergy care

Diagnosis

An early identification of offending allergens



Limitation of allergen exposure with appropriate treatment



Prognosis

Decrease of the severity of reactions and a delay of the disease progression



Better outcome

Phadia ImmunoCAP System™



ImmunoCAP technology

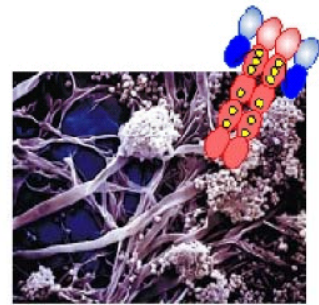
Unequaled performance!

Why and how?

The Facts

...involved in specific IgE testing

- ① IgE - one of five immunoglobulin classes
- ② Extremely low serum concentration of specific IgE
- ③ Allergen characteristics
- ④ Multitude of allergen sources
- ⑤ Complexity of an allergen source
- ⑥ Variation of allergen sources
- ⑦ Diversity of patient IgE specificities



The Facts

IgE - one of five immunoglobulin classes

ImmunoCAP Technology



IgE



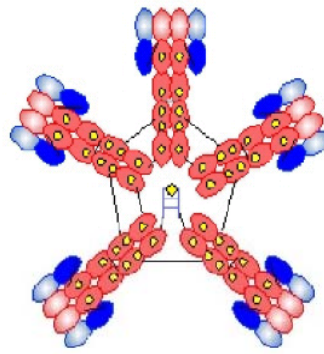
IgG1



IgA1



IgD



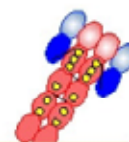
IgM

- Variable domain light chain
- Constant domain light chain
- Variable domain heavy chain
- Constant domain heavy chain
- Carbohydrate chain

The Facts

Extremely low serum concentration of specific IgE

Physicochemical properties of human immunoglobulin classes



Property	Immunoglobulin type									
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	sIgA	IgD	IgE
heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	α_2/α	δ	ϵ
mean serum conc. (mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.05	0.03	0.00005
sedimentation constant	7s	7s	7s	7s	19s	7s	7s	11s	7s	8s
mol. wt ($\times 10^3$)	146	146	170	146	970	160	160	385	184	188
half-life (days)	21	20	7	21	10	6	6	?	3	2
% intravascular distribution	45	45	45	45	80	42	42	trace	75	50
carbohydrate (%)	2-3	2-3	2-3	2-3	12	7-11	7-11	7-11	9-14	12

IC-11

Reference: Immunology, 5th ed., Roitt, Brostoff, Male, 1998

The Facts

Allergen characteristics

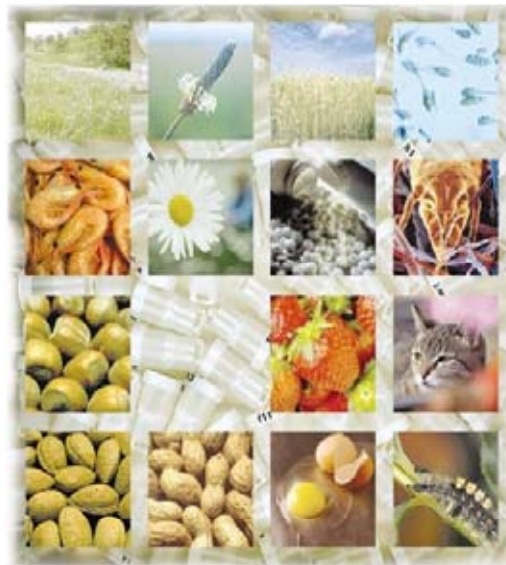


- No common structure
- Proteins / glycoproteins, haptens
- Molecular weight 5 000 - 150 000 Da
- Isoelectric point 4 - 7
- Allergen determinants:
 - Sequential and conformational

The Facts

ImmunoCAP Technology

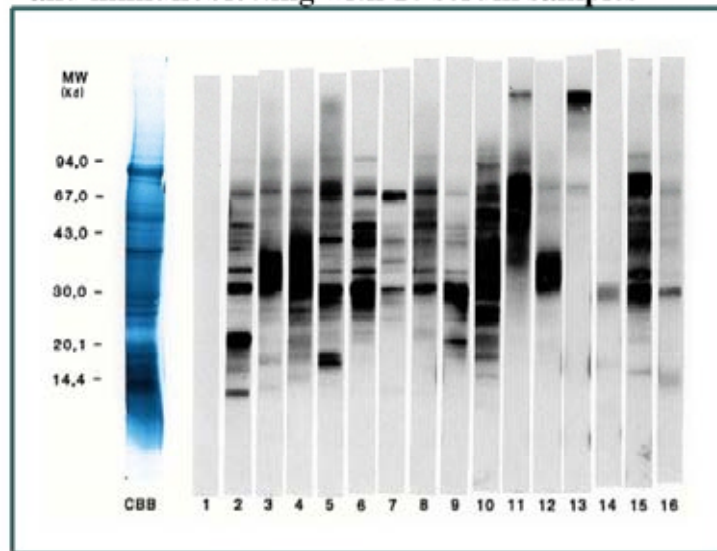
Multitude of allergen sources



The Facts

Complexity of an allergen source

SDS-gPAGE of an allergen
and immunoblotting with 16 serum samples



W. Rolfsen et al, 1987

The Facts

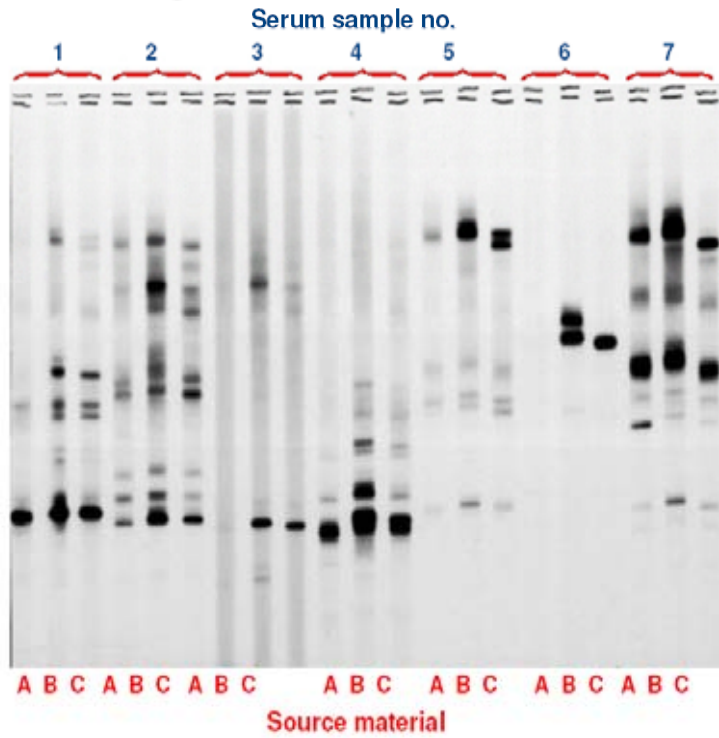
ImmunoCAP Technology



IC-15

Variation of allergen sources

Immunoblotting: Source material comparison, *Aspergillus fumigatus*

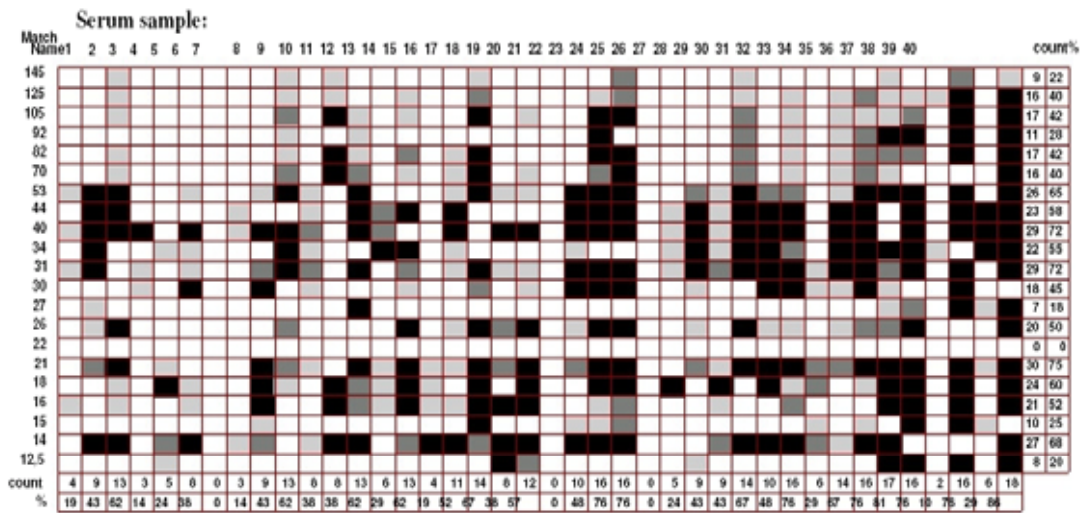


The Facts

ImmunoCAP Technology

Diversity of IgE specificities

Allergogram, Peanut



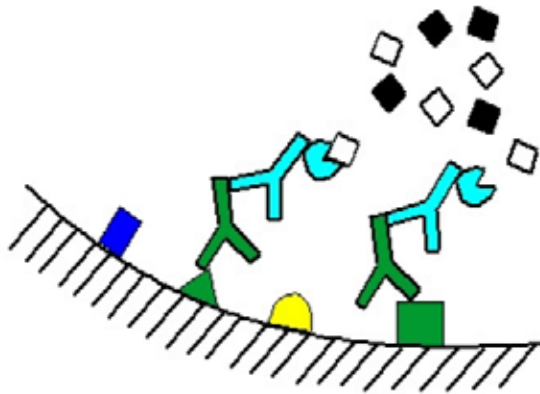
H. Perborn, 1997

The Design

Immunoassay principle

Sequential immunometric assay

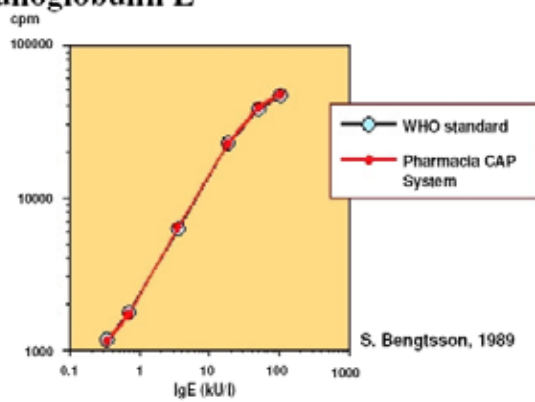
- ✓ high sensitivity and specificity
- ✓ high precision
- ✓ wide measuring range



The Design

Calibration

- ✓ Calibrators: human IgE
- ✓ Anti-IgE ImmunoCAP
- ✓ Calibration to the 2nd International Reference Preparation 75/502 of Human Serum Immunoglobulin E from WHO



WHO IgE Ref. Prep. 75/502 and Pharmacia CAP System calibrator

The Design

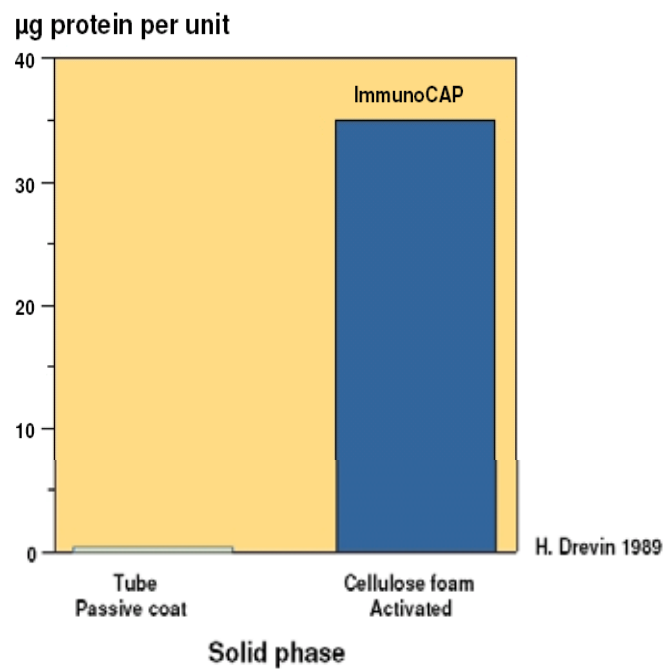
ImmunoCAP Technology



Solid phase: ImmunoCAP

Extremely high binding capacity

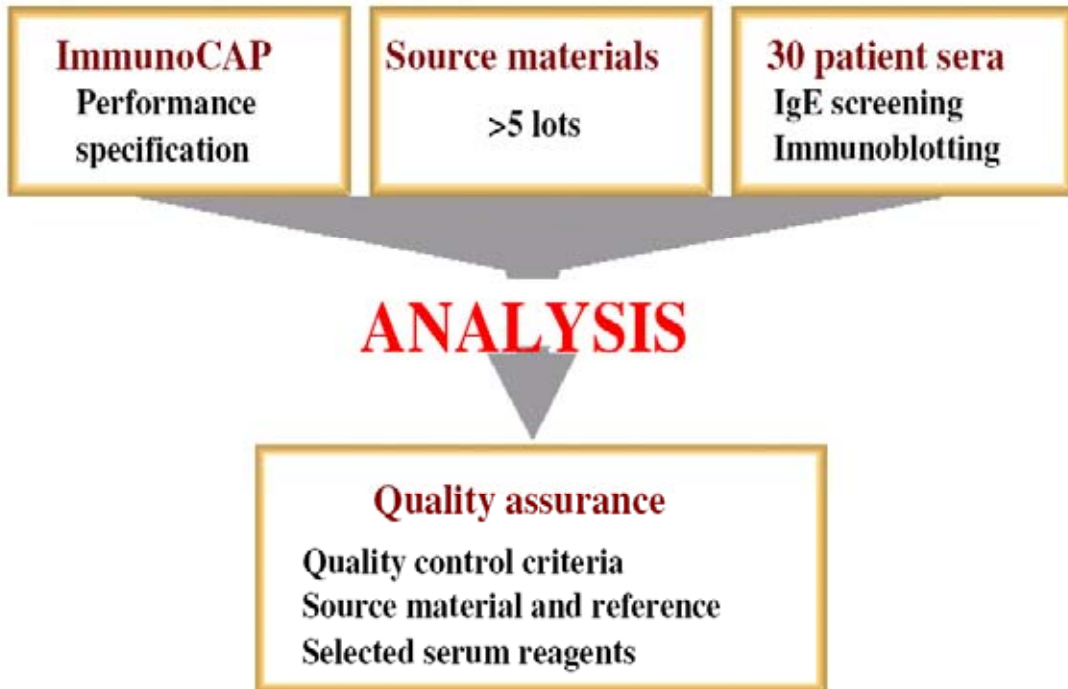
Comparison of binding capacities



The Allergen Standardization

Basis for allergen source material standardization

ImmunoCAP Technology

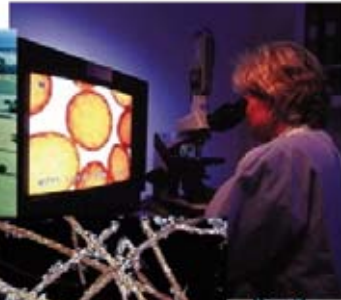






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The Allergen Standardization

Phadia Allergon AB

ImmunoCAP Technology



-  Leading producer of allergen source material
-  Subsidiary of Phadia AB
-  >30 years experience in the field
-  GMP and GLP compliance, ISO 9001 certified

The Allergen Standardization

The Biobank

at Pharmacia & Upjohn Diagnostics AB

ImmunoCAP Technology

The Biobank

Serum/plasma collection
Characterization



Clinically documented
sera/plasma of great variety



R&D

Allergen standardization



Production

Products
Quality control
Quality assurance programs



The Performance

ImmunoCAP Technology

Sensitivity and specificity

Clinical evaluation of UniCAP

UniCAP specific IgE

	Pos	Neg	Total
Allergen specific diagnosis			
Pos	1121	144	1265
Neg	360	3545	3905
Total	1481	3689	5170

Sensitivity: 89%

Specificity: 91%

The Performance

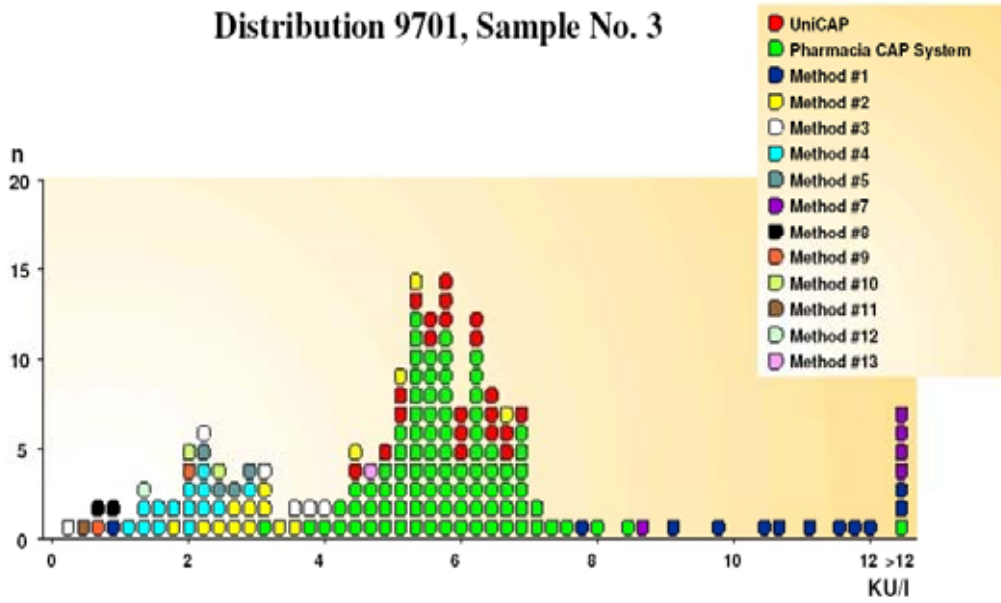
ImmunoCAP Technology



IC-34

Trueness EuroEQAS

Distribution 9701, Sample No. 3



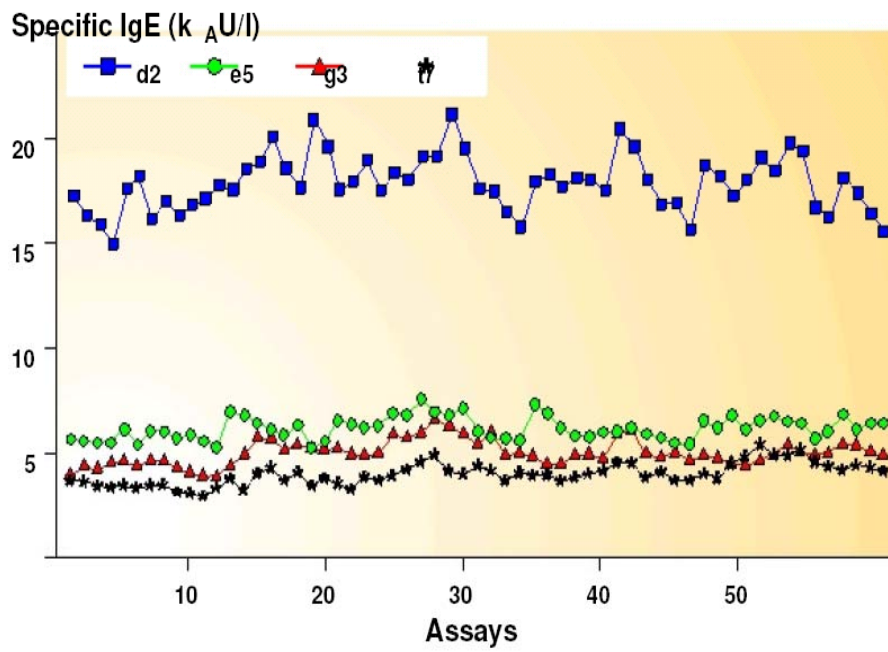
With permission from Dr R. Fifield, Protein Reference Unit, Cardiff, Wales, UK.

The Performance

ImmunoCAP Technology

Reproducibility

Repeatability of Pharmacia CAP System

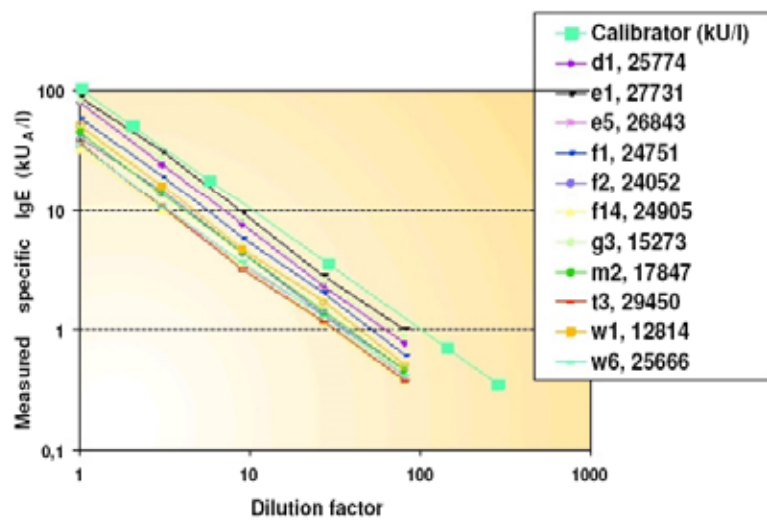


The Performance

ImmunoCAP Technology




Quantitative

Parallelism - UniCAP




Phadia AB Quality Club



-  **The largest quality assessment program in the world in allergy testing!**
-  *Quality Club Specific IgE has more than 700 members i 30 countries* 
-  **Available programs:** **Quality Club Specific IgE**
Quality Club Total IgE
Quality Club ECP

Phadia AB

The world leader in *in vitro* allergy diagnostics

-  >4000 laboratories in more than 50 countries use UniCAP/Pharmacia CAP System
-  Close to 1000 employees dedicated to *in vitro* allergy diagnostics
-  ISO 9001 certified and GMP compliant according to FDA requirements

ImmunoCAP[®]
System



ImmunoCAP[®] Systems

Optimized for allergy testing

- Precision
- Accuracy
- Consistency

Phadia

The ImmunoCAP® systems from Phadia offer an optimal allergy testing solution for every laboratory, regardless of testing volume requirements.

- ◆ From local clinics to the world's largest commercial laboratories
- ◆ From 1 to 30 000 tests per day
 - ↳ there is always an ImmunoCAP system designed for your needs.

ImmunoCAP systems all offer full automation and the same unrivalled ImmunoCAP technology, still the only technology giving true quantitative IgE measurements.

The ImmunoCAP® system is the number one choice worldwide for allergy testing both for research and general testing

A family to grow with

When your allergy testing grows you can simply add new ImmunoCAP instrumentation without having to abandon your previous system.

The unique ImmunoCAP Information Data Manager software allows you to integrate several ImmunoCAP systems into one network.

- ↳ without having to learn new software.



Technical features

Common system features

- World-leading ImmunoCAP technology providing accurate and reproducible test results
- True quantitative measurements
- Large panel of standardized high-quality allergens and autoimmunity tests



ImmunoCAP® 100F

Maximum flexibility in the small lab or clinic

- Fast and automated
- Single integrated unit
- Up to 48 different determinations in less than three hours
- Also for EIA* autoimmunity testing
- Run as stand-alone unit in a local clinic or in a cluster at a larger laboratory
- Connectable to mainframe
- Automatic dilution

ImmunoCAP® 250

Higher capacity and automation for increased productivity

- Ideal for medium-sized laboratories running 80-400 tests/day
- Fully automated, continuous random access and mainframe connection
- Throughput: 60 tests/hour
- Also for EIA* autoimmunity testing
- Positive identification and full traceability of all samples and reagents
- All reagents and up to 3 000 tests on-board
- Up to 6 different methods
- Automatic sample dilution
- Stand alone PC hosting the IDM system software
- Built-in touch screen



ImmunoCAP® 1000

The optimal solution for the large cost-conscious laboratory to meet the growing need for allergy testing

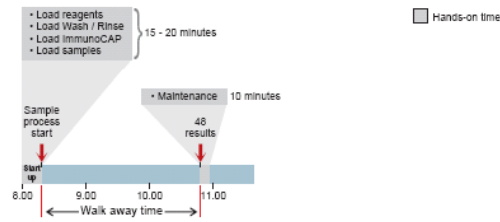
- Fully automated, continuous random access and mainframe connection
- Throughput: 240 tests/hour
- Positive identification and full traceability of all samples and reagents
- All reagents and up to 10 000 tests on-board
- Stand alone PC hosting the IDM system software
- Built-in touch screen

Automation for walk-away productivity

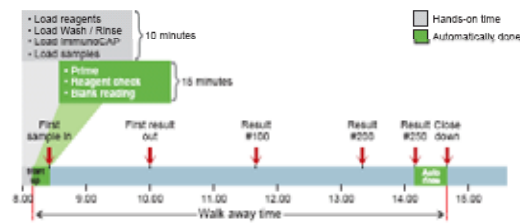
The ImmunoCAP® systems are all highly automated, requiring minimal hands-on time. Choosing the system with the capacity matching your needs for throughput ensures optimal productivity.

Typical work-day schedules for each of the systems are shown below.

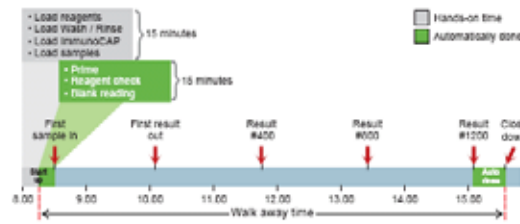
ImmunoCAP® 100E



ImmunoCAP® 250



ImmunoCAP® 1000



**ImmunoCAP 100****ImmunoCAP 250****ImmunoCAP 1000**

	ImmunoCAP 100	ImmunoCAP 250	ImmunoCAP 1000
Test/assay	48	250-400	1,200-2,500
Methods/assay	4	6	3
Maximum test types on-board	N. A.	180+36	600+8
Test on-board	N. A.	~3,000	~10,000
Sample tube on-board	48	50	200
Sample loading during assay	No	Yes	Yes
ImmunoCAP technology	Yes	Yes	Yes
EliA technology	Yes	Yes	No
Barcode reading samples	Optional	Yes	Yes
Barcode reading reagents	Optional	Yes	Yes
Foot print	70 x 55cm	127 x 75cm	180 x 100cm
Weight	47kg	250kg	700kg
Power Supply	100-240V 50/60Hz	100-240V 50-/60Hz	230V 50/60Hz
Environmental temperature	18-32°C	18-32°C	18-32°C
ImmunoCAP Information Data Manager	Optional	Yes	Yes
LIS connection	Yes	Yes	Yes
Clustering	Yes	Yes	Yes

N. A. = Not Applicable

The way to precise and correct IgE measurements

The ImmunoCAP® systems are developed specifically for allergy testing. The instruments, reagents and test procedures are optimized for the special requirements involved in the task of making precise measurements of IgE antibodies.

- ◆ ImmunoCAP consistently shows better performance than any other allergy test system in independent quality assessment programs like the EuroEQAS allergy program.
- ◆ The precision achieved with ImmunoCAP is in fact comparable to standard clinical chemistry immunoassays, whereas other allergy test methods show considerably higher coefficient of variation (CV).
- In other words, with other methods the same sample will give varying results at different laboratories and at different points in time and the clinical usefulness of the results will therefore be limited.

ImmunoCAP gives precise and reproducible results, regardless of laboratory, operator time, time occasion, lot number, instrument and country.

Quality Club™ is the largest external quality assessment program for allergy testing, with approximately 900 members in 37 countries.

This gives the member laboratories the possibility to compare their results with other laboratories world-wide. Quality Club data verify the very low variation in test results, covering all the possible sources of variation, including different laboratories, users, instruments, reagents, and dozens of ImmunoCAP kits.



"UniCAP 100 fulfils the requirements for the quality assurance of quantitative immunological determinations in medical laboratories and can thus be recommended for quantitative *in vitro* allergy diagnosis."

"UniCAP 100 is ergonomic, easy to handle, quick, robust and precise and can be recommended for quantitative *in vitro* allergy diagnosis."

Reference: *Investigation of Pharmacia UniCAP 100 for in vitro allergy diagnosis, Clin Lab 1999;45:229-35, Author: Liappis, Lantto, et al.*

"UniCAP is an efficient laboratory system for routine diagnostic testing allergy and a valuable tool for basic studies on allergens and antibodies."

"In conclusion, the studies verify the diagnostic efficiency of the new generation of ImmunoCAP technology and the automated UniCAP immunoassay system."

Reference: *Specific IgE antibodies in the diagnosis of atopic disease, Allergy 1998; 53: 763-8, Author: Paganelli, Sastre, Lindholm, et al.*

"The imprecision levels appear satisfactory for UniCAP 100, being within a CV 10 %"

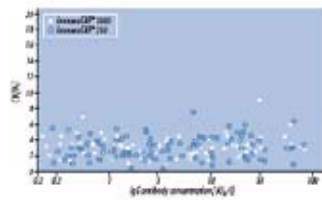
"The imprecision found in Immulite 2000 for the positive control serum was markedly higher, the CV being in the region of 25 %, a level which appears excessive for this type of determination, and which, for unknown reasons, is much higher than the average found with the same instrument in our laboratory for routine immunochemical determinations (where the CV ranges from 4 % for tPSA to 11 % for intact PTH)"

Reference: *In Vitro allergy diagnosis: comparison of a new method of fully automated determination of Specific IgE using Immulite 2000 compared with UniCAP 100, European Annals of Allergy and Clinical Immunology 2003; Vol 35(8), Author: G Vignati, E Pastori, S Portolupi, A Tenporini.*

The truly quantitative allergy test

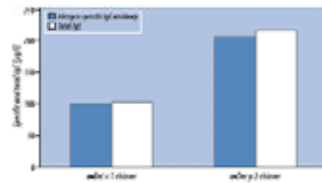
ImmunoCAP is the test system giving results with enough precision to be called truly quantitative. An inferior test system, where the values obtained from the same sample may vary from one test occasion to another, is of course not a reliable tool for following the fine-scale development of IgE sensitization in a patient over time.

The ImmunoCAP systems consistently give precise, quantitative results reported in kU_A/L. The calibrator is directly traceable and parallel to the WHO reference preparation 75/502 for IgE. Dilution curves shows accurate results for the whole measuring range.



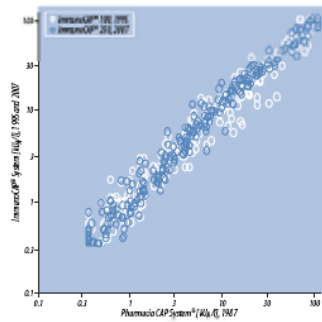
Precision

The intra-assay precision (CV%) of ImmunoCAP is as good as standard clinical chemistry immunoassays, which is quite remarkable considering the extremely low concentrations of IgE antibodies found in the serum of even severely allergic patients and the possible interference from immunoglobulins of other classes.



Accuracy

ImmunoCAP IgE antibody results are truly quantitative and comparable to IgE protein mass units, where 1 U_x IgE antibody = 1 IU IgE = 2.42 ng of IgE.

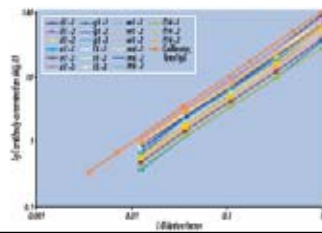


Consistency

Seventy samples from a clinical trial were first assayed for specific IgE antibodies to eleven allergens in 1987, using Pharmacia CAP System[®]. The samples were stored at -20°C and eight years later reassayed with ImmunoCAP 100.

After 20 years storage the samples were again reassayed, this time using ImmunoCAP 250.

The results showed very good agreement; r=0.97 1995/1987 and r=0.99 2007/1987.



Parallelism

Parallelism between calibrators and dilutions of sample has been repeatedly verified on the different instruments based on the ImmunoCAP technology.

Thirty years of market leadership

Phadia developed *in vitro* allergy testing in the early 1970's. We were the pioneers in allergy test development and also developed the allergen code standard. Today's ImmunoCAP® systems are the result of thirty years of technology, chemistry and instrument development. ImmunoCAP systems are the most frequently used in routine testing and in clinical studies – the reference systems in allergy testing.

The development history includes several important milestones:

1st generation

1974 – Phadebas RAST

The first laboratory test for specific IgE-antibodies. The paper disc technology combining quality with a large panel of allergens became the "gold standard" of allergy testing.

2nd generation

1989 – Pharmacia CAP System®

The ImmunoCAP technology brought new standards of quality and capacity to the market, also introducing semi-automation to increase laboratory efficiency.

3rd generation

1996 – UniCAP® 100

Introducing full automation and quick assay procedure. Further improvements in precision and reproducibility through improved chemistry, standardized handling and environmental control laid the foundation for truly quantitative measurements. Already 4000 instruments on the market.

4th generation

2001/2004 – ImmunoCAP® 1000 and 250

The unsurpassed quality of ImmunoCAP® 1000^e combined with even higher automation, speed, capacity and continuous random access ability availability.

All systems are CE-marked according to 98/79/EC; *In vitro* diagnostic medical device directive for all EU countries and including Norway and Switzerland.

Phadia

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Great Britain/Ireland +44 1 968 84 70 34 Italy +39 02 641 64 11 Japan +81 3 5385 8332 Netherlands +31 248 404 840 Norway +47 21 67 32 80 Portugal +351 21 423 5359 Spain +34 915 765 000 Sweden +46 18 16 50 00
Switzerland +41 63 343 4038 Taiwan +886 2 2516 8925 United States +1 800 346 4364 Other countries +46 18 16 50 16

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Welcome to the 21st Century

Components resolved Diagnosis (CRD) in Food Allergy

- Identifying patients at risk of severe reactions
- Explaining Cross-Reactivity's

ImmunoTherapy

- Selecting Patients
- Monitoring Immunological Response

In Identifying protein groups and where critical components
(e.g: Omega-5 Gliadia)

Are present in very small amounts.