Quality aspects on IgE antibody testing

Your Results – Our Priority

Thermo SCIENTIFIC

In 1967, immunoglobulin E (IgE) was identified, which was also the beginning of the company (Pharmacia Diagnostics) that developed and brought the first commercial allergy blood test (Phadebas RAST®) to market 1974.

The company is still located in Uppsala, Sweden and since the beginning has been the world leader in allergy *in vitro* testing. In 1989, the name of the test was changed to ImmunoCAP® and the company (Phadia AB) is now a part of Thermo Fisher Scientific with more than 50,000 employees worldwide.

Thermo Fisher Scientific is dedicated to allergy testing and since the start we have not only developed tests for more than 600 allergens, but also automated instruments for laboratories of all sizes. Today, our instruments are used by more than 3,000 laboratories in more than 70 countries.

The quality of your IgE antibody results is our priority. We are working, each day, dedicated to controlling every step of the production procedures to ensure that you get the allergy tests that we promise and you expect.

Stefan Wolf

President
Thermo Fisher Scientific
ImmunoDiagnostics

What exactly does quality mean when it comes to IgE antibody testing?

Quality refers to the overall performance of a test regarding the ability to deliver true and reproducible IgE antibody results. To achieve this, many aspects and facts have to be taken into account such as test precision, reproducibility, detection limit, parallelism and not least quality assurance (page 4).

Why the test quality is of importance in the diagnosis of allergy

Reliability and clinical usefulness of any assay requires high precision and reproducibility in order to give standardized and comparable values over time. This is even more important for allergy *in vitro* testing where no real clinical cut-off exists for IgE mediated allergy, but there is generally a quantitative, continuous relationship between the IgE antibody level and the risk for clinical symptoms. The higher the IgE antibody level, the higher the risk for a reaction, but having IgE antibodies always implies a risk even at low levels[1-5]. The test result reliability is strongly dependent on the assay technology and it has potential implications on the patient diagnosis and treatment.

CLSI Guideline on IgE antibody assays

Analytical Performance Characteristics and Clinical Utility of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies and Defined Allergen Specificities; Approved Guideline-Second Edition (I/LA20-A2)[6] is a document released by the Clinical Laboratory Standards Institute (CLSI) that provides guidance on the design, analytical performance, standardization, quality assurance and clinical application of laboratory assays used in the measurement of human IgE antibodies of defined allergen specificity. This document is approved, which means that the guideline has achieved consensus within the health care community. It is a unique 160-page document, the only one of its kind, written for laboratorians/clinicians (users), manufacturers (producers) and governmental regulators (inspectors, regulators, reviewers). ImmunoCAP as well as other information in this brochure comply with this CLSI Guideline (I/LA20-A2), where a more detailed description is found.

Phadia® 5000

Manufacturer of allergen-specific IgE assays

ImmunoCAP Specific IgE (ImmunoCAP) are tests commercially available, produced by Phadia AB, Uppsala, Sweden according to GMP and with procedures continuously audited by FDA and other applicable authorities to certify that defined requirements are fulfilled. Phadia AB is also ISO certified with most products CE marked (EU) and many also FDA cleared (USA). In addition to commercially available allergy *in vitro* tests such as ImmunoCAP, on certain markets there may also exist Laboratory Developed Tests (LDT) that are not registered or cleared by regulatory agencies, but developed by a laboratory that takes responsibility for the quality control and the appropriate validation of any claims made.

LNE

CERTIFICAT
CERTIFICATE OF REGISTRATION
N° 9462 rev.3

Le LNE certifie que le système de management de la qualité développé par
LNE certifies that the quality management system developed by

PHADIA AB
Rapsgatan 7P, PO. Box 6460
751 37 UPPSALA SWEDEN

pour les activités
for the activities

Conception et développement, production et ventes de tests pour le diagnostic in vitro de l'allergie, l'asthme et les maladies autoimmunes. Conception et développement, production, ventes et installation d'instruments et logiciels utilisés avec les tests de diagnostic in vitro.

Design and development, production and marketing of in vitro allergy, asthma and autoimmunity assays for diagnostic use.
Design and development, production, marketing and installation of instruments and softwares to handle in vitro assays for diagnostic use.

réalisées sur le(s) site(s) de
performed on the location(s) of

PHADIA AB
Rapsgatan 7P - P.O. Box 6460 - 751 37 UPPSALA SWEDEN

est conforme aux exigences des normes internationales
complies with the requirements of the international standards

ISO 13485 : 2003

Début de validité / *Effective date* : February 2nd, 2015 (included)
Valable jusqu'au / *Expiry date* : February 1st, 2018 (included)
Etabli le / *Issued on* : January 15th, 2015

For the General Director
Laurence DASALLIER
Deputy Director

LNE N° 9462-3
Ce certificat est délivré selon les règles de certification G-MED / This certificate is issued according to the rules of G-MED certification
LNE/G-MED Organisme notifié pour les Dispositifs Médicaux / LNE/G-MED Notified Body for Medical Devices
Renouvelle le certificat 9462-3

Laboratoire national de métrologie et d'essais - Établissement public à caractère industriel et commercial
LNE/G-MED - Organisme notifié n° 0459
1, rue Gaston Boissier - 75724 Paris Cedex 15 - Tél. : 01 40 43 37 00 - Fax : 01 40 43 37 37 - www.lne.fr - www.gmed.fr

ISO certification of manufacturer and accreditation of laboratories

When a company is ISO certified or a laboratory is ISO accredited by an audit body, for example by CAP (College of American Pathologists), it does not per se mean that it is better than other companies or laboratories, but that it has documented routines that are followed. However, being certified or accredited is definitely a clear sign that quality is of great concern, and it is often a requirement for being competitive in a market. ImmunoCAP is shown to be a highly repeatable, reproducible, and accurate method which may be considered as a single analyte assay in view of the EN ISO 15189 accreditation procedure[7].

Classification of allergen-specific IgE assays[6]

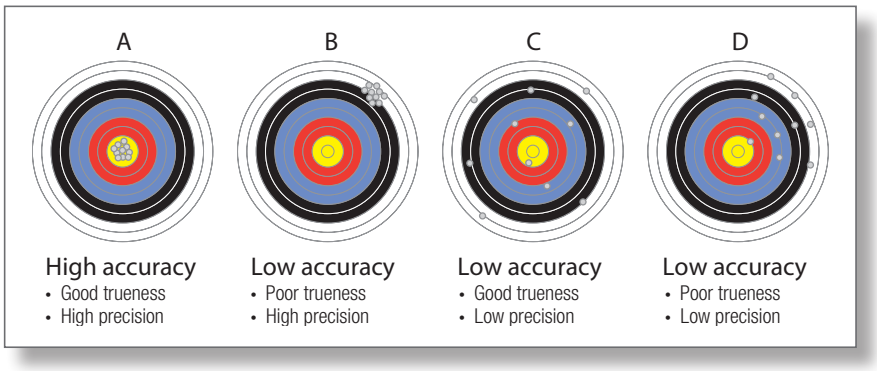
- **Qualitative assays** present the results only in positive or negative terms, and are not intended to provide a precise measurement of the concentration. Ideally, a positive assay signal indicates the presence of allergen-specific IgE antibodies in the serum tested.
- **Semi-quantitative assays** provide an additional option over the qualitative assays in terms of defining the magnitude of the response in classes (*e.g.* 1-6) or in arbitrarily defined units per milliliter (AU/ml). These assays are often unable to consistently achieve the linearity, dilution recovery, and parallelism that are typical of quantitative assays, however.
- **Quantitative assays** produce an accurate and reproducible measurement of the IgE antibody concentration (kU_A/l – traceable to to an internationally recognized standard, *e.g.* WHO 75/502). They fulfil the analytical criteria for quantitation, including parallelism (linearity), recovery, and precision across the assay's working range. Quantitative assays tend to be among the most complex tests.

Quality assurance of IgE assays[6]

Three areas of quality assurance should be considered for IgE antibody assays:

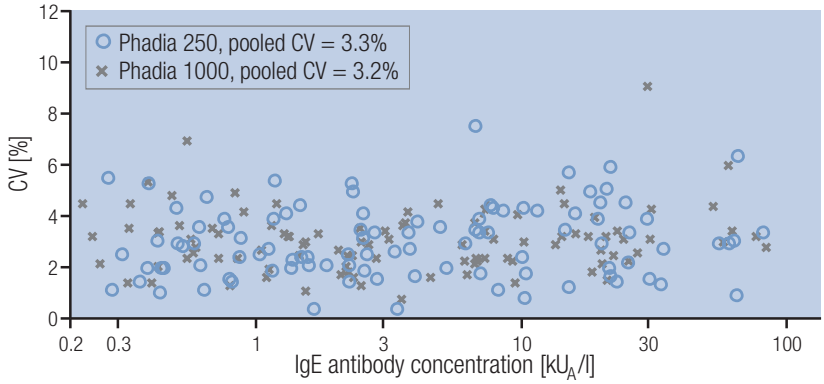
1. The first quality assurance is performed by the manufacturer at the time of reagents and assay production.
2. "Internal" intra-laboratory quality control is the second level of quality assurance. It is performed by the clinical laboratory and is intended to demonstrate that each assay is under control, *i.e.* the daily laboratory handling (daily quality controls).
3. "External" inter-laboratory proficiency testing is the third level of quality assurance and involves participation of the laboratory in an external quality assurance survey (*e.g.* UKNEQAS in Europe, CAP in the USA and Quality Club specifically for ImmunoCAP).

Possible test result variations



The daily quality controls (QC) in a test run make it possible to keep control of the result variation (precision) within a run but also between runs (internal inter-laboratory quality control). Good trueness, as well as high precision is the optimal situation for every laboratory (**A**), but to find out when the results are at the wrong level (**B**) the laboratory has to compare its results with the results from other laboratories, which is exactly what is done in external proficiency surveys (external intra-laboratory quality control). The third example (**C**) shows bad performance with high variability of results that may occur *e.g.* if maintenance is not regularly performed, while the fourth situation (**D**) is indicating high variability of results, as well as a systematically biased distribution.

ImmunoCAP shows good precision across the whole measuring range (0-100 kU_A/l)

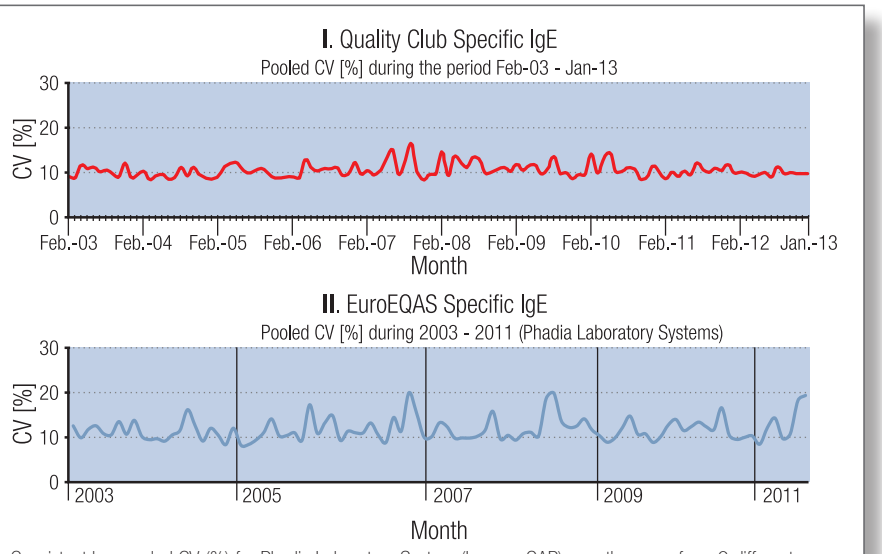


Precision profile (within assay) of Phadia 250/1000[8].

	Pooled CV% at a sample range of 0.08 - 3.8 kU_A/l		
	Within	**Between**	**Total**
Phadia 100	3.2	4.6	5.8
Phadia 250	3.9	4.0	5.6
Phadia 1000	3.6	5.7	6.8

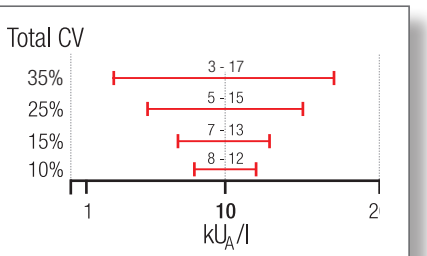
ImmunoCAP shows excellent precision even at very low IgE antibody levels[9].

ImmunoCAP reaches reliable results comparable all over the world



Consistent low pooled CV (%) for Phadia Laboratory System (ImmunoCAP) over the years from 2 different proficiency surveys: (**I**) Quality Club Specific IgE with around 500 and (**II**) EuroEQAS Specific IgE with around 200 participating laboratories.

Implication of different coefficients of variation (CV)

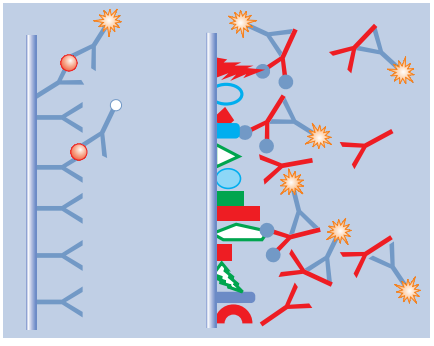


Implication of CV(%) at different levels, effect of ± 2SD.

- It is important to fully understand what different CV (%) means in kU$_A$/l. This figure shows the confidence ranges (95%) of some different CV (%) at an IgE antibody level of 10 kU$_A$/l as the target value.
- Total CV ≤10% in the laboratory and ≤15% in proficiency testing (*e.g.* Quality Club) are preferred targets for ImmunoCAP.
- The higher the reproducibility, the better the clinical performance and less risk of incorrect test results.

IgE antibody testing is more complex than most other immunoassays

There are many factors to consider when the goal is to achieve a high quality allergy test. True and reproducible are obvious, but to accomplish this other facts must be taken into account as well. ImmunoCAP is not just another test but the only system developed with this entirely in mind and specifically made for IgE antibody measurement.



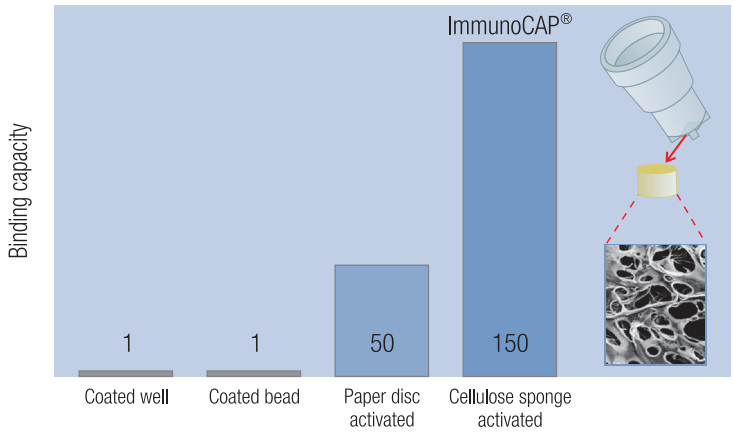
a) Total analyte concentration *e.g.*TSH

b) IgE antibodies to all different allergen components

- Extremely low concentration of IgE in serum (µg/l).
- Each allergen contains a large number of proteins/ components that may provoke an IgE antibody response.
- For each allergen the test has to be sensitive enough to capture all IgE antibodies to all relevant components.
- The test must have a capacity high enough to bind all IgE antibodies in competition with other immunoglobulin classes.
- A multitude of allergen sources.

ImmunoCAP – a unique high capacity solid phase

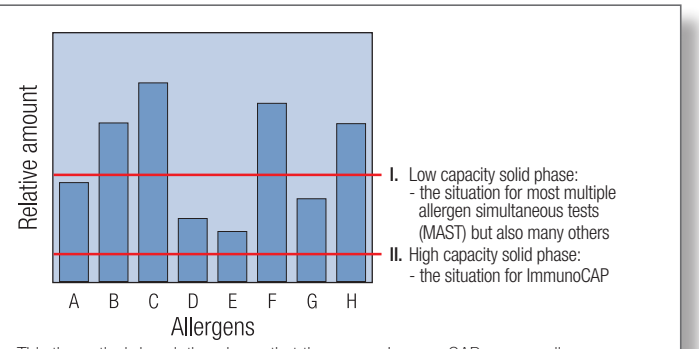
The allergen-containing reagent is considered the most complex assay component, and if a test is not capable of representing all the different IgE-provoking proteins in an allergen source (*e.g.* peanut), it doesn't matter if or how well this system is calibrated to the WHO standard.



Binding capacity: plastic well, coated bead, paper disc and cellulose sponge[10].

The consequence of a low capacity solid phase

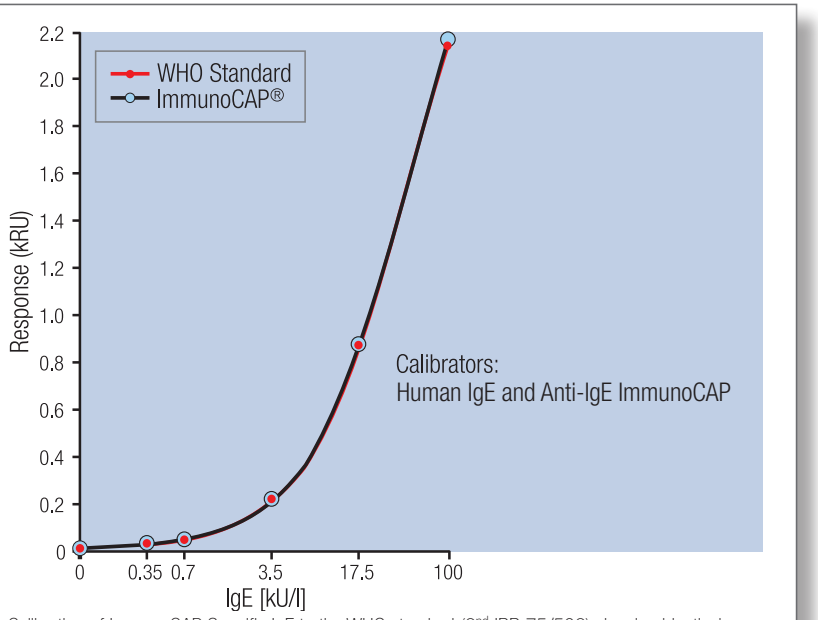
A low capacity solid phase has a restricted area for all the allergens/proteins (situation I) and the consequence may, for some patients, be a too low IgE antibody level or even a false negative test result (low sensitivity). The negative impact from competition with other immunoglobulin classes in higher concentrations is also a well-known phenomenon. Moreover, false positive test results (low specificity) are also common, which may complicate the diagnosis and thus also the patient management.



This theoretical description shows that the porous ImmunoCAP sponge allows excess of allergens to be coupled (protein A-H), while in a low capacity solid phase test the high concentration proteins will bind first and leave no room for the ones at lower concentration (A, D, E, G)[11].

Calibration of ImmunoCAP

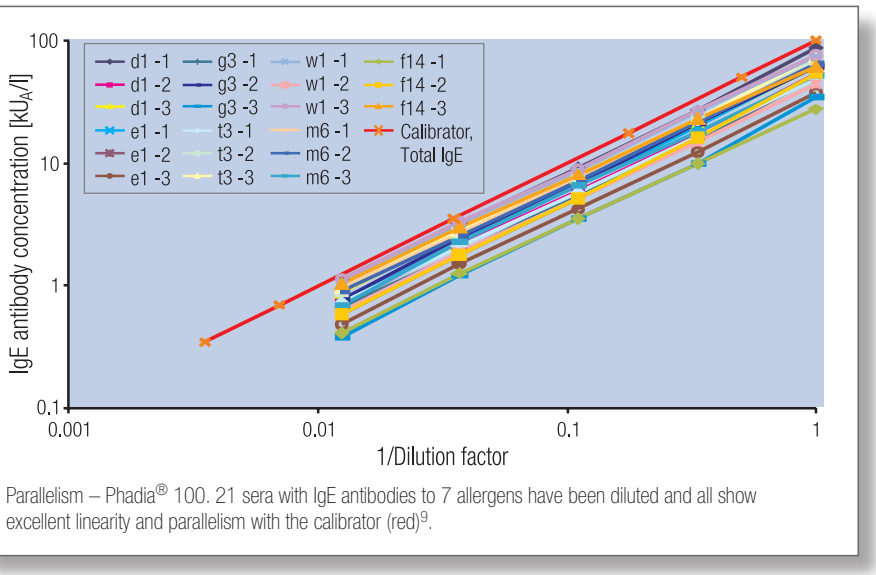
The 2nd World Health Organization (WHO) International Reference Reagent (IRR) for serum IgE (75/502) is widely used to calibrate assays for serum IgE. Calibration traceable to this international standard (IS) is a prerequisite for a quantitative measurement of IgE antibodies and it allows test result reporting in kU_A/l (alt. IU/ml). Exhaustion of stocks of the 2nd IRR has necessitate a replacement by IRR 11/234, established by the WHO Expert Committee on Biological Standardization as the 3rd IS for serum IgE[12].



Calibration of ImmunoCAP Specific IgE to the WHO standard (2nd IRR 75/502) showing identical overlapping curves[9].

Parallelism indicates true quantitation

Parallelism is another property and a requirement of quantitative immunoassays, in which the calibrator and test sera produce parallel dose-response curves. Making a sample dilution series is a simple way of checking the quantitative performance of a test.



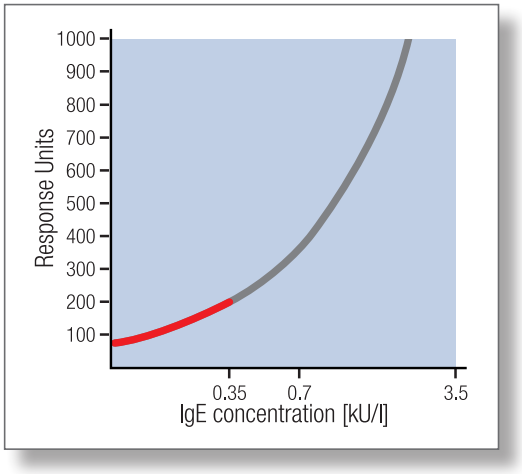
Parallelism – Phadia® 100. 21 sera with IgE antibodies to 7 allergens have been diluted and all show excellent linearity and parallelism with the calibrator (red)[9].

Limit of quantitation (LoQ) of ImmunoCAP[9,13]

In Phadia Laboratory Systems the lowest level of quantitation of ImmunoCAP Allergens is 0.1 kU_A/l. This limit is set to ensure that reported low quantitative results are separated from the background signal from the allergens, and not exceeded by sera from non-sensitized subjects. All test results for ImmunoCAP Allergens from 0.1 kU_A/l should thus be regarded as true IgE antibodies with a possible clinical impact.

ImmunoCAP allows measurements of low levels of IgE antibodies[8-9,13]

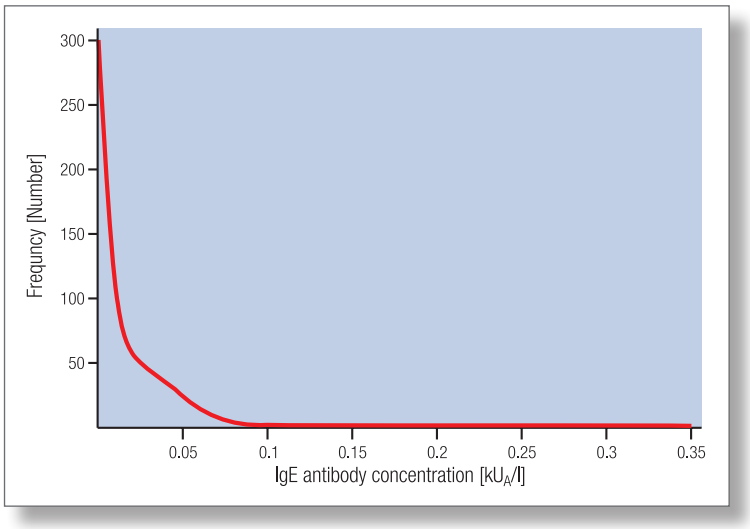
ImmunoCAP has the technical ability to accurately detect and quantitatively measure low levels of allergen-specific IgE antibodies due to:



- The limit of quantitation for ImmunoCAP Specific IgE is the same as the limit of detection, *i.e.* 0.1 kU_A/l.
- Dilution curves are parallel to the calibrators also in the lowest measuring range, giving the same high recovery independently of IgE antibody concentration and allergen specificity.
- Good precision across the whole measuring range from the limit of quantitation to 100 kU_A/l.

A recommendation from CLSI is that all clinical laboratories report their IgE antibody results as analytical measurements down to the regulatory accepted lower limit of quantitation of the assay[14].

No non-specific binding of samples from healthy individuals



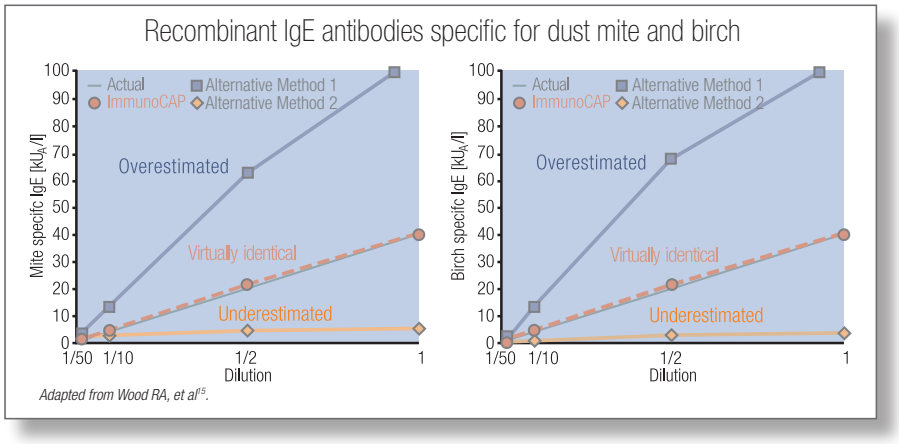
Regular production QC data of a negative serum pool tested with all ImmunoCAP allergen lots of 584 allergens produced at Phadia 2007-2010. The levels of IgE antibodies are below 0.1 kU_A/l (LoQ) for virtually all results (>99%)[9].

Despite being traceable to WHO, test results are not interchangeable

Specific IgE assays with quantitative reporting in kU_A/l or IU/ml must be calibrated to the World Health Organization (WHO) International Reference Reagent (IRR) for serum IgE since no other standard exists. However, several studies have reported that results from different IgE test systems can vary and the evident question is – which system is giving the correct results?

Three studies have been published where chimeric antibodies (humanized monoclonal mouse antibodies to allergens) have been used and sent out blinded as normal sera to some laboratories using different IgE test systems. They all revealed the same pattern, which confirmed what has been seen earlier; one system was overestimating and the other underestimating the IgE antibody concentration compared with ImmunoCAP. All three studies showed that only ImmunoCAP delivered accurate results[15-17].

These findings have potentially serious clinical implications since each of these systems is widely used. Just because two systems present their results in the same units, this does not mean that the results are necessarily correct or interchangeable.



Adapted from Wood RA, et al[15].

Unrestricted testing can lead to over-diagnosis of allergy[18-20]

Nonselective testing with large panels of allergens should be avoided since it increases the risk that a number of clinically irrelevant positive results will occur, and if assays with low specificity are used (such as many MAST systems) it may end up in false positive test results as well. This is a problem especially in food allergy where unnecessary avoidance of foods can lead to restricted diets and in some cases to malnutrition.

Specific IgE tests yield information on sensitization, which is not always equivalent to clinical allergy. The clinical history should guide which allergens are selected for testing. Because most allergic patients are sensitized to multiple allergens, the task of determining which ones are of major importance is not a simple one. Panels of tests designed for specific symptoms, seasons and geographical locations are available for this purpose and the suggested number of allergens used should be in the range of 8 to 12 and should include the most representative ones.

ImmunoCAP testing only with clinically relevant allergens

Every batch of ImmunoCAP Allergens is quality controlled with positive sera from a number of patients with different IgE antibody profiles covering the diversity of IgE antibody specificities seen in allergic patients. Sera from different geographical regions with different allergen exposure must also be taken into account for this purpose. For some allergen groups, *e.g.* drugs, the number of ImmunoCAP tests may appear few in comparison with offerings from other companies. However, in reality, very few drug reactions are provoked by IgE antibodies, while other immunological reactions or mechanisms more frequently are causing the allergy-like symptoms.
IgE antibody testing for allergens, not proven to cause IgE mediated allergy should be avoided since it can lead to serious implications for the patient.

Examples of substances not proven to elicit IgE antibodies

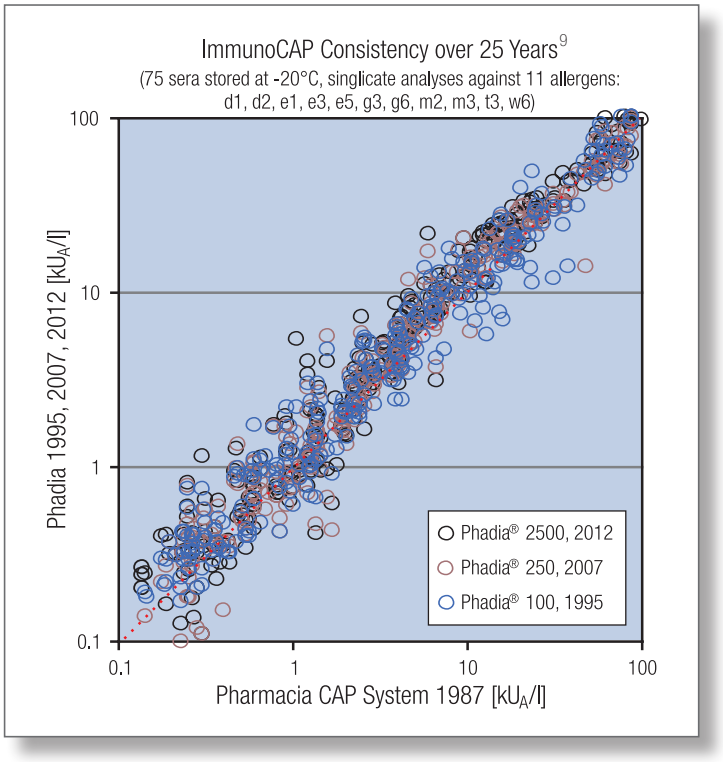
Provoking substance	Possible type of non IgE-mediated reaction	Suggested analysis/analyses
Acetylsalicylic acid (=ASA, Aspirin)	Pharmacological reaction	Challenge test *in vivo*
Alcohol, wine: - aldehyde dehydrogenase - sulfites - histamine-liberators	Toxic reaction Enzymatic defect (intolerance) Bronchial hyperresponsiveness Toxic reaction/intolerance	Blood- alcohol concentration No suggestion Lung function test (not specific) Challenge test *in vivo*
Chrome	Type IV hypersensitivity	Patch test *in vivo*
Cold air	Bronchial hyperresponsiveness	Lung function test (not specific)
Cosmetics	Type IV hypersensitivity common	Patch test *in vivo*
Cotton/Synthetic fibres *(Not causing reactions itself)*	May be linked to *e.g.* rubber, chemicals & dyes	Patch test *in vivo*
Dextran	Delayed reaction	Specific IgG ab
Diclofenac (NSAID)	Pharmacological reaction	Challenge test *in vivo*
Erythromycin	Essentially unknown	Challenge test *in vivo*
Heparin	Cytotoxic reaction	Platlet count, skin biopsy
Lactose	Enzymatic defect (intolerance)	Challenge + Hydrogen breath test / Lactose tolerance test
Nickel	Type IV hypersensitivity	Patch test *in vivo*
Paint	Bronchial hyperresponsiveness	Lung function test (not specific)
Perfume	Bronchial hyperresponsiveness Type IV hypersensitivity	Lung function test (not specific) Patch test *in vivo*
Plastic	Type IV hypersensitivity	Patch test *in vivo*
Printing ink	Bronchial hyperresponsiveness	Lung function test (not specific)
Tobacco smoke	Bronchial hyperresponsiveness	Lung function test (not specific)
Wool	Skin hyperresponsiveness Type IV hypersensitivity	No suggestion Patch test *in vivo*

Irrelevant testing of IgG4 antibodies against foods

Serological tests for IgG4 (or IgG) against foods are persistently promoted for the diagnosis of food-induced hypersensitivity and represent a growing market. Food-specific IgG4 does not indicate food allergy nor intolerance, but rather a physiological response of the immune system after exposure to food components. Therefore, testing of IgG4 to foods is considered as irrelevant for the laboratory workup of food allergy or intolerance, and should not be performed in cases of food-related complaints. Many allergy organizations worldwide have either published their own position paper or are officially supportive of this statement from the European Academy of Allergy and Clinical Immunology (EAACI)[21].

ImmunoCAP is considered the “Gold Standard” and is the most commonly used IgE antibody test[22]

- Best performance in different proficiency surveys
- More than 3,000 laboratories use ImmunoCAP in more than 70 countries
- Recognized by EAACI, NIH and many others when recommending validated test in publications and guidelines
- More than 4,000 publications in PubMed where ImmunoCAP is used
- 2014 marked the 40 year anniversary of the first IgE antibody test (Phadebas RAST)



Celebrating 40 years
of excellence in
allergy blood testing

References

1. *Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 2001;107:891-6.*
2. *Simpson A, Soderstrom L, Ahlstedt S, Murray CS, Woodcock A, Custovic A. IgE antibody quantification and the probability of wheeze in preschool children. J Allergy Clin Immunol 2005;116:744-9.*
3. *Maloney JM, Rudengren M, Ahlstedt S, Bock SA, Sampson HA. The use of serum-specific IgE measurements for the diagnosis of peanut, tree nut, and seed allergy. J Allergy Clin Immunol 2008;122:145-51.*
4. *Beyer K, Grabenhenrich L, Hartl M, Beder A, Kalb B, Ziegert M, Finger A, Harandi N, Schlags R, Gappa M, Puzzo L, Roblitz H, Millner-Uhlemann M, Busing S, Ott H, Lange L, Niggemann B. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. Allergy 2015;70:90-8.*
5. *Olivieri M et al. The risk of respiratory symptoms on allergen exposure increases with increasing specific IgE levels. Allergy 2016; DOI 10.1111/all.12841.*
6. *Analytical Performance Characteristics and Clinical Utility of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies and Defined Allergen Specificities; Approved Guideline-Second Edition. CLSI document I/LA20-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.*
7. *Lambert C. The importance of EN ISO 15189 accreditation of allergen-specific IgE determination for reliable in vitro allergy diagnosis. Allergy 2015;70:180–6.*
8. *Lantto O, Rydén B. Automation and analytical performance in in-vitro allergy testing using ImmunoCAP™ 250 and ImmunoCAP™ 1000 Instruments. Poster presented at World Allergy Congress 2005 in Munich, Germany.*
9. *Studies performed at Phadia AB, Uppsala, Sweden (DocID 611628).*
10. *Yman L. Standardization of IgE Antibody Assays. J Int Fed Clin Chem 1991;3(5):198-203.*
11. *Yman L. Standardization of in vitro methods. Allergy 2001;56 Suppl 67:70-4.*
12. *Thorpe SJ, Heath A, Fox B, Patel D, Egner W. The 3rd International Standard for serum IgE: international collaborative study to evaluate a candidate preparation. Clin Chem Lab Med 2014 Sep;52(9):1283-9.*
13. *ImmunoCAP Specific IgE Directions for Use (52-5291-EN/05).*
14. *Hamilton RG. Clinical laboratories worldwide need to report IgE antibody results on clinical specimens as analytical results and not use differential positive thresholds. J Allergy Clin Immunol 2015 Sep;136(3):811-2.*
15. *Wood RA. Accuracy of IgE antibody laboratory result. Ann Allergy Asthma Immunol 2007;99:34–41.*
16. *Sato K. Evaluation of two serum specific IgE measurement kits for patients with an allergic disease. Igakukensa (Japanese Journal of Medical Technology) 2010;59(8);929-35.*
17. *Szecsi PB, Stender S. Comparison of Immunoglobulin E Measurements on IMMULITE and ImmunoCAP in Samples Consisting of Allergen-Specific Mouse-Human Chimeric Monoclonal Antibodies towards Allergen Extracts and Four Recombinant Allergens. Int Arch Allergy Immunol 2013;162:131–4.*
18. *Krouse JH, Stachler RJ, Shah A. Current in vivo and in vitro screens for inhalant allergy. Otolaryngol Clin North Am 2003;36(5):855-68.*
19. *Cox L et al. Pearls and pitfalls of allergy diagnostic testing: report from the American College of allergy, Asthma and Immunology/American Academy of Allergy, Asthma and Immunology Specific IgE Test Task Force. Ann Allergy Asthma Immunol 2008;101:580–92.*
20. *Portnoy JM. Appropriate Allergy Testing and Interpretation. Missouri Medicine 2011;108(5):339-43.*
21. *Stapel SO et al. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report. Allergy 2008:63:793–6.*
22. *Crameri R. In vitro allergy diagnosis – allergen-specific IgE. In: Akdis CA, editor. Global Atlas of Allergy EAACI; 2014 p.166-7.*

January 2015 RAK Design AB