UMC Utrecht

Basophil activation test

Edward Knol
Dept. Immunology & Dermatology/Allergology

Basophil activation test

1) Allergy diagnostics, introducing cellular testing

2) Discovery of CD63 as basophil activation marker

3) Application of CD63 in peanut allergy diagnostics

4) Overview application of BAT in allergy diagnostics

Basophil activation test

1) Allergy diagnostics, introducing cellular testing

2) Discovery of CD63 as basophil activation marker

3) Application of CD63 in peanut allergy diagnostics

4) Overview application of BAT in allergy diagnostics

Testing for allergies

in vivo

in vitro

SPT

Challenge test

ImmunoCAP

ISAC Allergen chip

In vivo testing
Oral provocations are not without risks

Allergic Living

Alabama Boy, 3, Dies of Severe Reaction During Baked Milk Challenge Test

By: **Gwen Smith** in **Food Allergy**, **Milk & Egg**
Published: August 2, 2017

A 3-year-old Alabama boy has tragically died following a severe anaphylatic reaction. The boy named Alastair had multiple food allergies, including dairy, as well as asthma.

His mother Pamela wrote to *Allergic Living* that her son's symptoms of anaphylaxis began as he taking part in an oral challenge test to see whether he could tolerate food that contains baked milk. He passed away on July 30. The challenge test took place at Children's Hospital of Alabama in Birmingham.

The grieving parents held Alastair's funeral on Wednesday, August 2, and Pamela plans to say more soon. "I want to share his story to everyone as accurately as possible," she briefly wrote. **"It is my understanding that this has never happened before. I was not warned of this possibility."**

In his obituary, the family describes their son, who would have turned 4 years old in August, as "Alastair X, Super

In vivo testing
EU regulations limit application of skin testing

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

Explore this journal >

Original Article

Current practice of allergy diagnosis and the potential impact of regulation in Europe

Victoria Cardona ✉, Pascal Demoly, Sten Dreborg, A. Fusun Kalpaklioglu, Ludger Klimek, Antonella Muraro, Oliver Pfaar, Todor A. Popov, Hans Jürgen Hoffmann

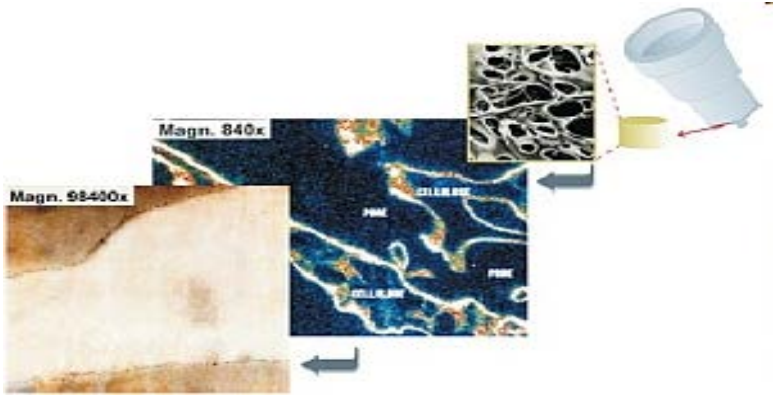
Accepted manuscript online: 14 September 2017 Full publication history

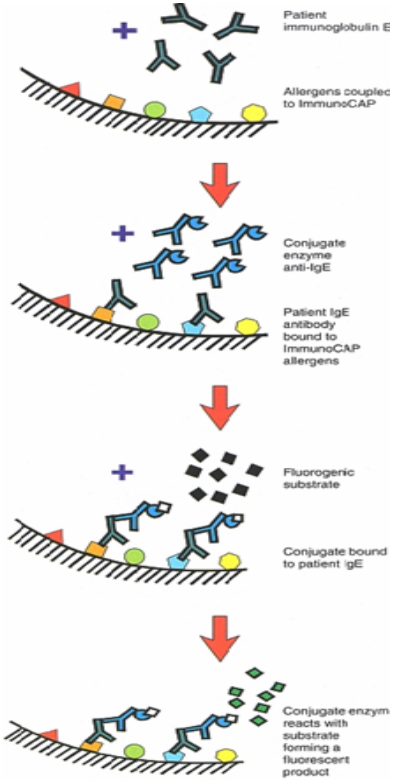
CURRENT OPINION

Impact of changed legislation on skin tests: the present and future

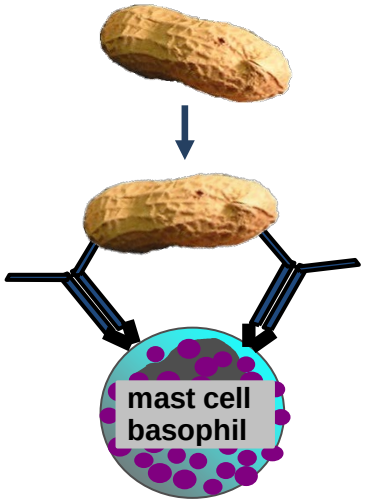
Ludger Klimek[a], *Hans Jürgen Hoffmann*[b,f], *Alexa Kugler*[a], *Antonella Muraro*[c], *and Peter W. Hellings*[d,e]

In vitro allergy diagnostics: Monovalent binding of IgE to allergens





Type I hypersensitivity reaction

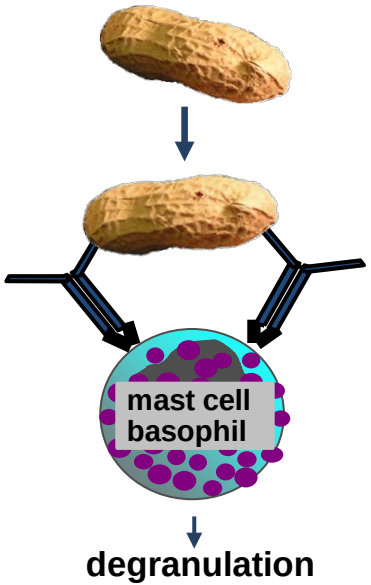


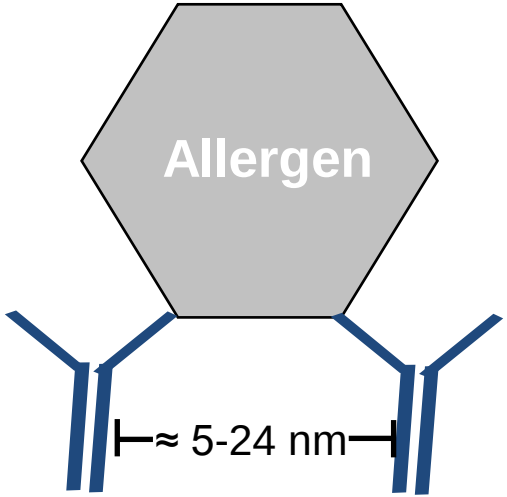
degranulation

release of inflammatory mediators

allergic symptoms

Reguirements of effective FcεRI cross-linking. Including cellular testing in allergy diagnostics





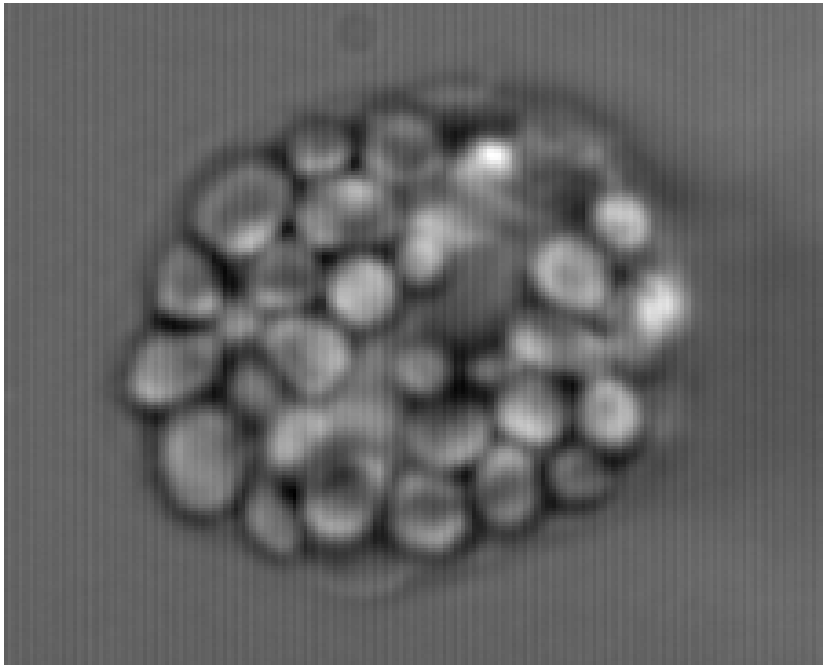
> 100 seconds crosslinking FcεRI (association/dissociation)

oligomers > dimers

> 100 cross-links per cell, ≈ 0.26% of FcεRI

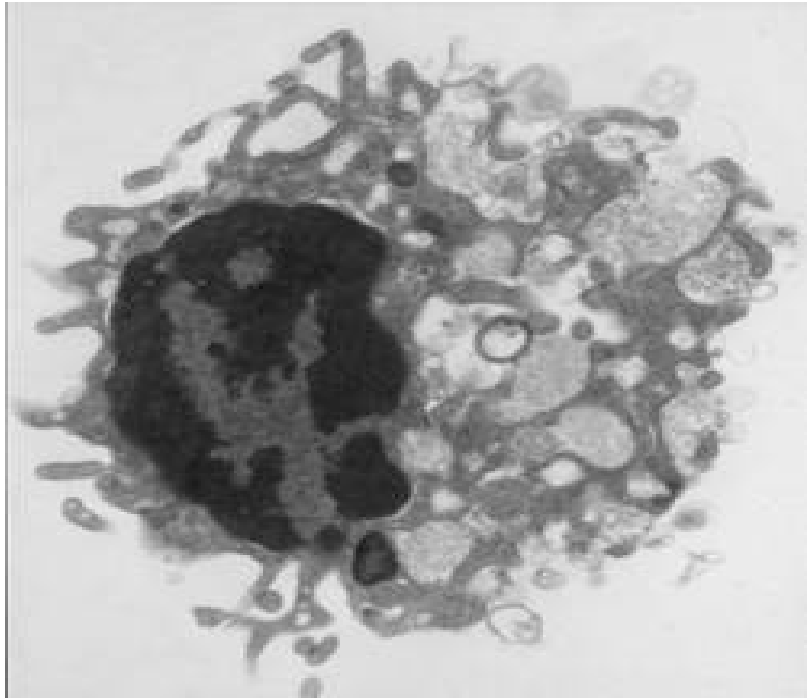
Knol, Mol Nutr Food Res. 2006;50(7):620-4

Degranulation




réaction de type I
AG
DYNAMITE
POK
BAOM

Degranulated human basophil



Allergy
EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY
EAACI
Allergy

POSITION PAPER

The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease

H. J. Hoffmann[1], A. F. Santos[2,3,4], C. Mayorga[5], A. Nopp[6], B. Eberlein[7], M. Ferrer[8], P. Rouzaire[9], D. G. Ebo[10], V. Sabato[10], M. L. Sanz[8], T. Pecaric-Petkovic[11], S. U. Patil[12], O. V. Hausmann[13,14], W. G. Shreffler[12], P. Korosec[15] & E. F. Knol[16]

Basophil activation test

1) Allergy diagnostics, introducing cellular testing

2) Discovery of CD63 as basophil activation marker

3) Application of CD63 in peanut allergy diagnostics

4) Overview application of BAT in allergy diagnostics

Basophil activation test

1) Allergy diagnostics, introducing cellular testing

2) Discovery of CD63 as basophil activation marker

3) Application of CD63 in peanut allergy diagnostics

4) Overview application of BAT in allergy diagnostics

The story of IgND, γE

Immunology, 1968, **15**, 323.

Immunoglobulin E: A New Class of Human Immunoglobulin*

H. H. Bennich: The Institute of Biochemistry, University of Uppsala, *Uppsala*, Sweden.
K. Ishizaka: Children's Asthma Research Institute and Hospital, *Denver*, Colorado, U.S.A.
S. G. O. Johansson: The Blood Centre, University Hospital, *Uppsala*, Sweden.
D. S. Rowe: Director, WHO International Reference Centre for Immunoglobulins, Institut de Biochimie, *Lausanne*, Switzerland.
D. R. Stanworth: Department of Experimental Pathology, University of Birmingham, *Birmingham*, England.
W. D. Terry: National Cancer Institute, National Institutes of Health, *Bethesda*, Maryland, U.S.A.

The Lancet · Saturday 4 November 1967

RAISED LEVELS OF A NEW IMMUNOGLOBULIN CLASS (IgND) IN ASTHMA

S. G. O. Johansson
M.B. Uppsala
OF THE BLOOD CENTRE, UNIVERSITY HOSPITAL, UPPSALA, SWEDEN

Ishizaka, and Ishizaka 1967) indicate that there is an antigenic relationship between IgND and the reaginic antibodies to ragweed allergen E, γ E-globulins, extensively studied by Ishizaka et al. (1966)

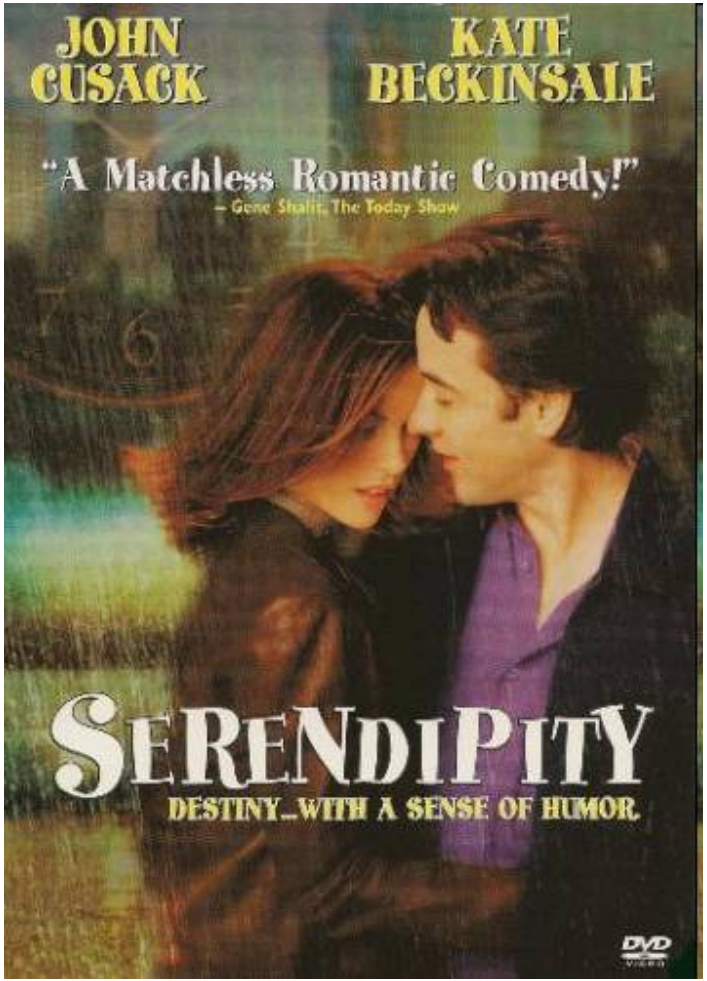
The present study deals with the IgND distribution in sera from an unselected sample of asthmatic bronchitics in relation to the clinical diagnosis.

February 1968, WHO Reference Center for immunoglobulins

Deltagarna i mötet i Lausanne (25). Från vänster: W.D. Terry, D.S. Rowe, D.R. Stanworth, K. Ishizaka och H.H. Bennich. Fotografen, Gunnar Johansson syns inte.

Dept Blood Cell Chemistry, CLB, 1989

Jero Calafat
Hans Janssen


JOHN CUSACK
KATE BECKINSALE
"A Matchless Romantic Comedy!"
– Gene Shalit, The Today Show
SERENDIPITY
DESTINY...WITH A SENSE OF HUMOR
DVD

Serendipity:

is the effect by which one accidentally discovers something fortunate, especially while looking for something else entirely

Isaac Asimov: “The most exciting phrase to hear in science, the one that heralds new discoveries, is not **'Eureka!**', but **'That's funny** …**‘”**

Pek van Andel: “Serendipity is looking for a needle in a haystack and discovering the farmer's daughter”

How [illegible] binding on activated basophils

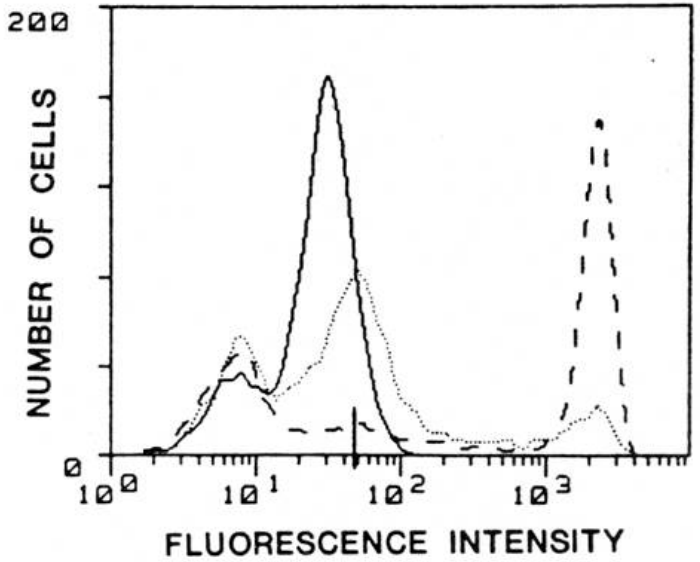
Knol, you ruined my experiment! All my activated platelets are binding the control antibody !

Wh[illegible]

[illegible]ity.

[illegible]njoying lunch in the cafetaria

Increased LM-435 binding on activated basophils (1989) (IV Leukocyte workshop: CD63)



— : resting

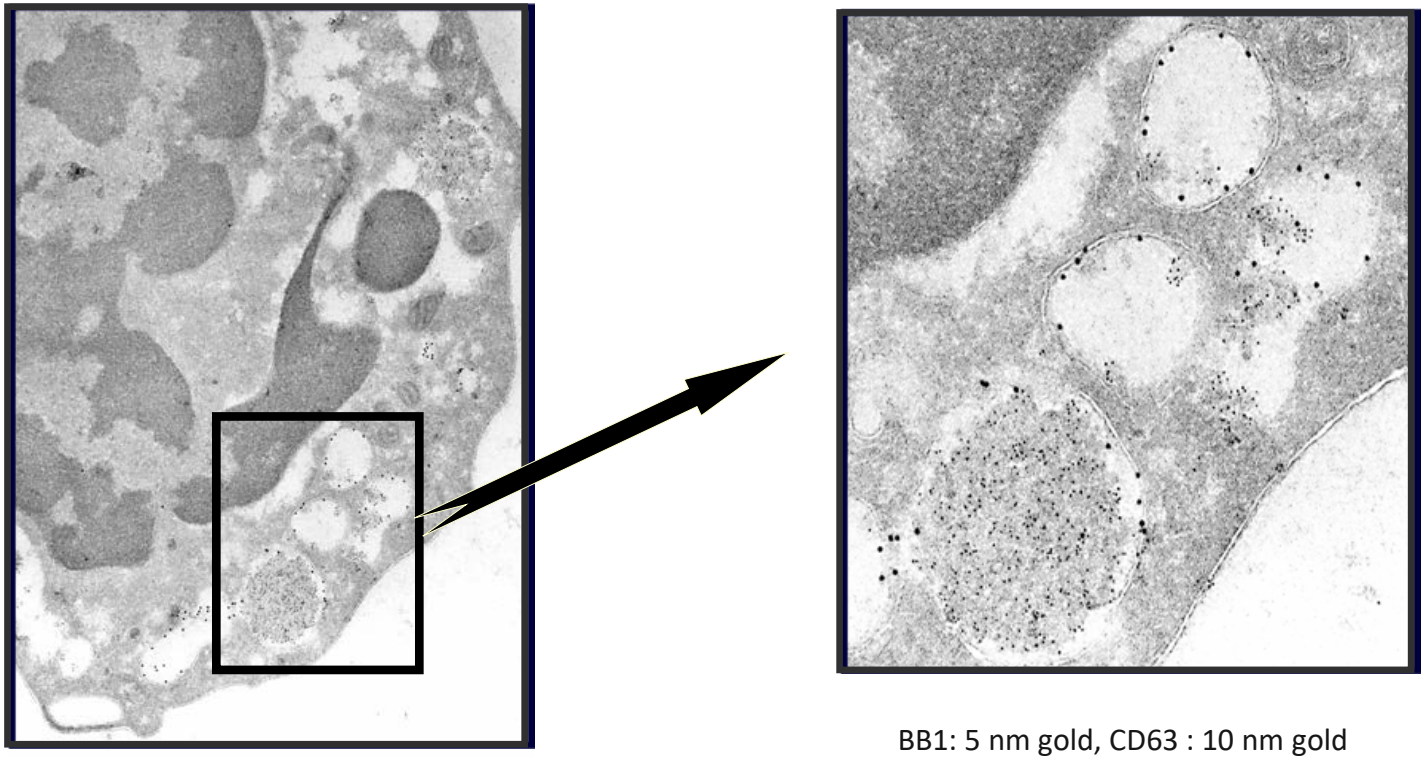
····· : 10 ng/ml α-IgE (14% HR)

-- : 100 ng/ml α-IgE (70% HR)

Single-color staining: only on “purified” basophils (500ml blood, purification 4-6 hours)

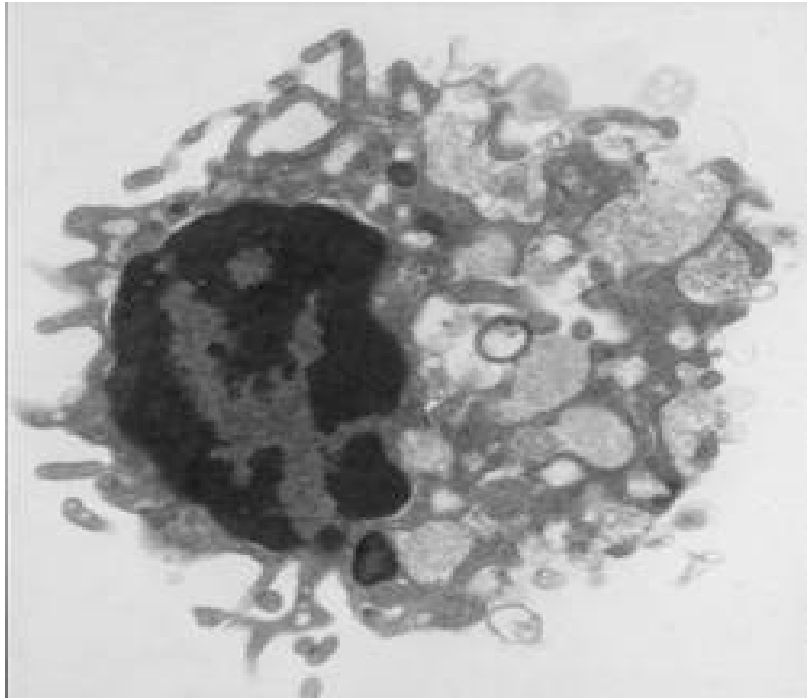
Knol et al. J.Allergy Clin.Immunol, (1991) 88:238-38

CD63 binds the membrane and BB1 binds the matrix of basophils granules



BB1: 5 nm gold, CD63 : 10 nm gold

Degranulated human basophil



CD63 assay on non-purified basophils

Saint Laudy et al, 1994

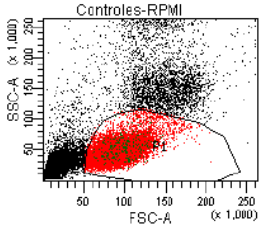
Analyse de l'expression membranaire du mar-queur CD63 par activation du basophile humain. Application au diagnostic allergologique

ALLERGIE ET IMMUNOLOGIE vol.26 iss.6 pg.211 -4

Basophil activation test, commercial kits

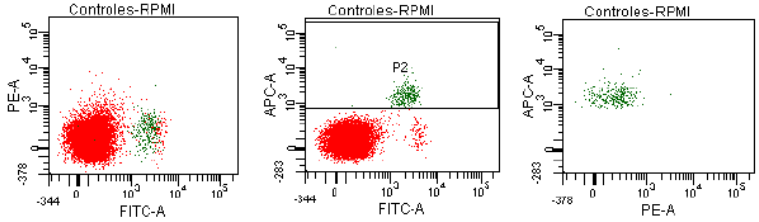
Brand name	Assay principle	Manufacturer
Flow-CAST, Flow^{2}CAST	CD63, flow cytometry	Bühlmann Laboratories AG, Switzerland
Basotest	CD63, flow cytometry	Orpegen Pharma, Heidelberg, Germany
Allergenicity kit	CD63, CD203c, flow cytometry	Beckman Coulter
Fast immune-Basotest	CD63, flow cytometry	Becton Dickinson
Baso Flow-Ex kit	CD63, flow cytometry	Exbio

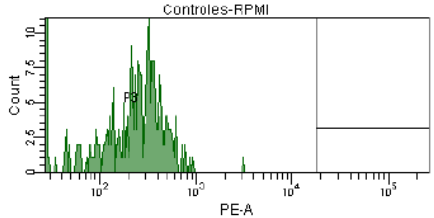
Strategy to monitor basophil activation, simple protocol/cheap



Unstimulated, RPMI

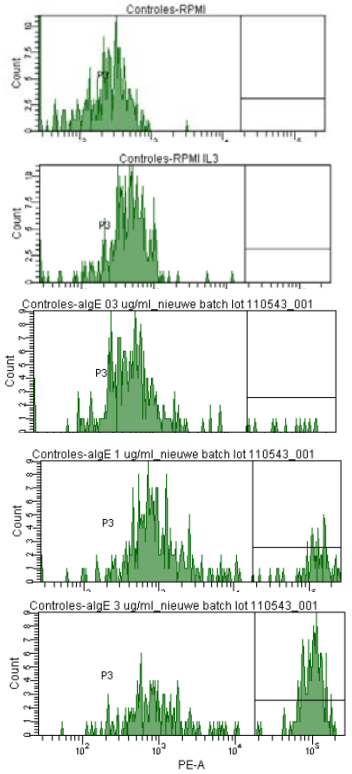
PE: CD63
FITC: CD123
APC-A: CD203C



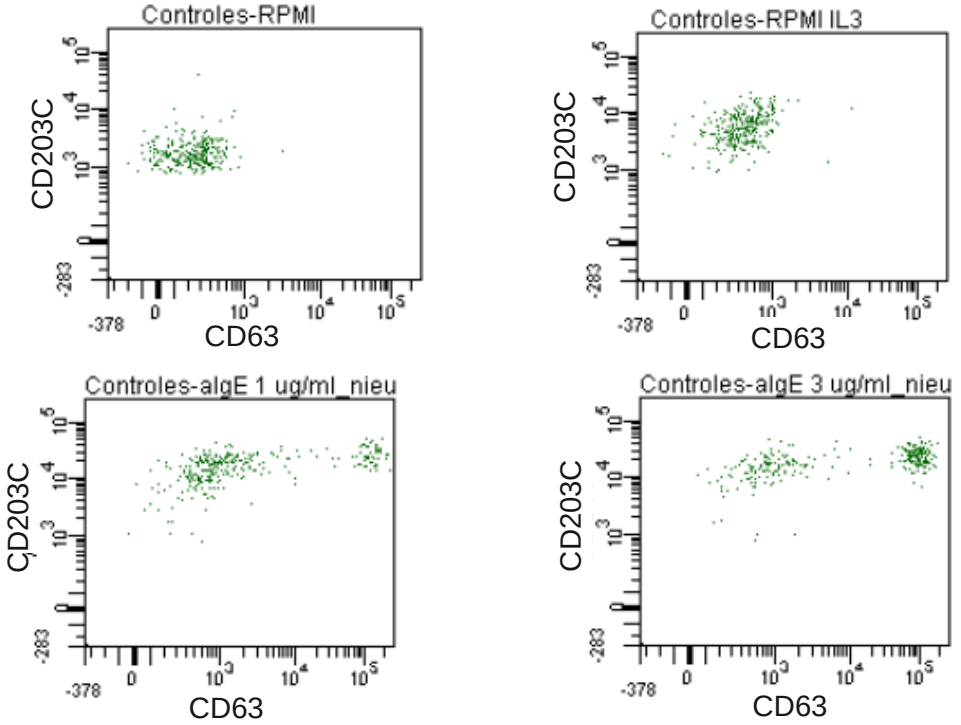


Different strengths of activation

CD63



CD63 vs. CD203C



Basophil activation test

1) Allergy diagnostics, introducing cellular testing

2) Discovery of CD63 as basophil activation marker

3) Application of CD63 in peanut allergy diagnostics

4) Overview application of BAT in allergy diagnostics

Basophil activation test

1) Allergy diagnostics, introducing cellular testing

2) Discovery of CD63 as basophil activation marker

3) Application of CD63 in peanut allergy diagnostics

4) Overview application of BAT in allergy diagnostics

- per year 2,4000,000,000 pounds of peanut are consumed in US

100 to 150 persons die each year from a peanut allergy in US only

The Complete

Handbook

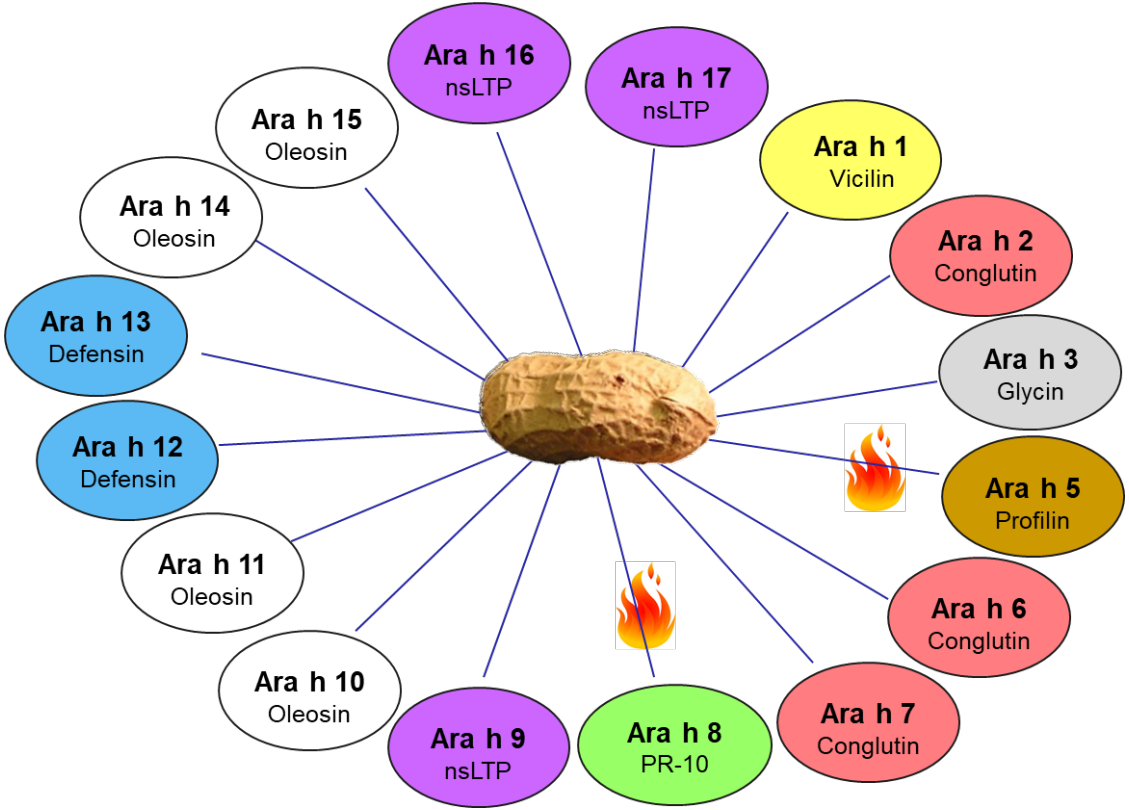
the Most Deadly Food Allergy

Scott H. Sicherer, M.D.,
and Terry Malloy

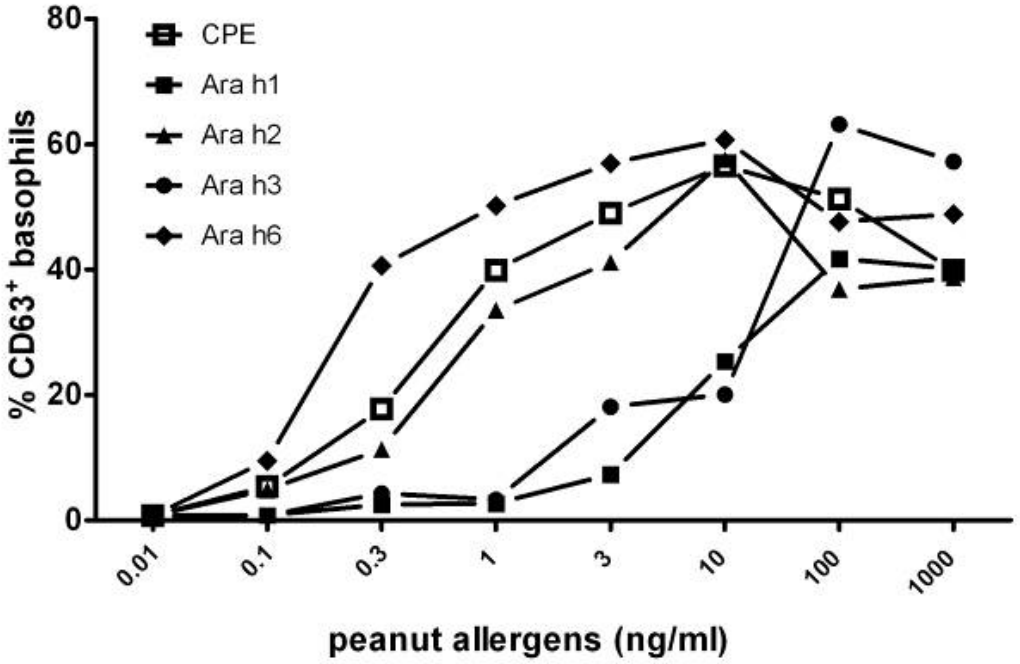
Golden standard in food allergy diagnostics: double-blinded placebo controlled provocation. > 2 days in-house

Peanut components

Peanut = *Arachis hypogea*



Peanut allergen-induced basophil degranulation. Ara h 2 and Ara h 6 are most potent

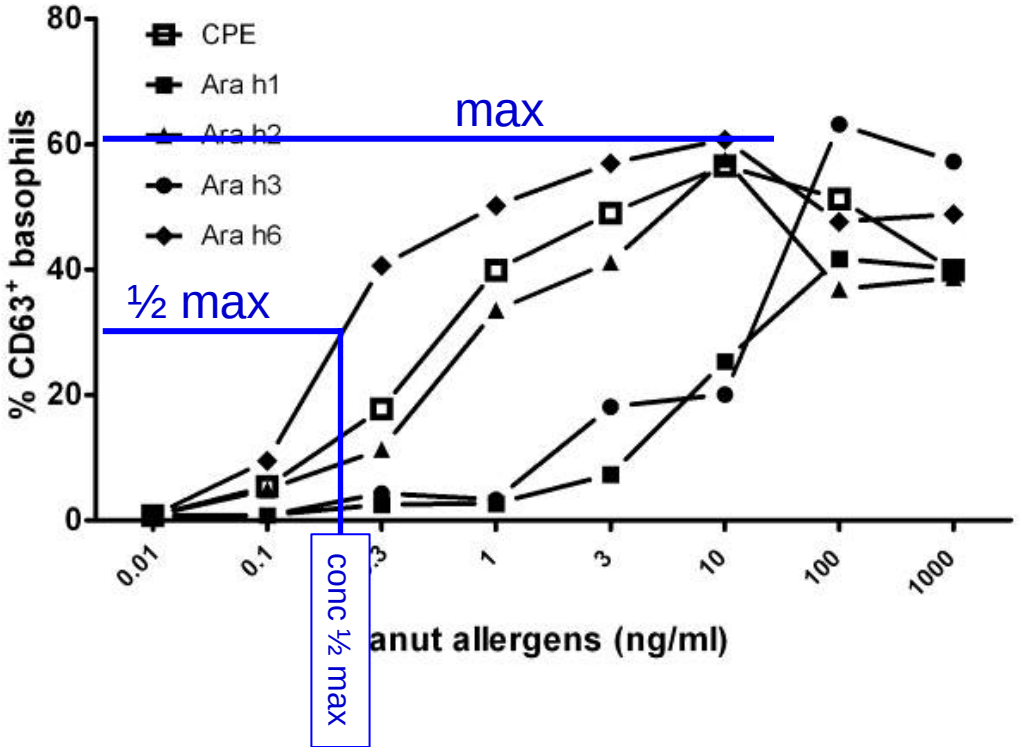


S. Koppelman, C.E.A. 2004

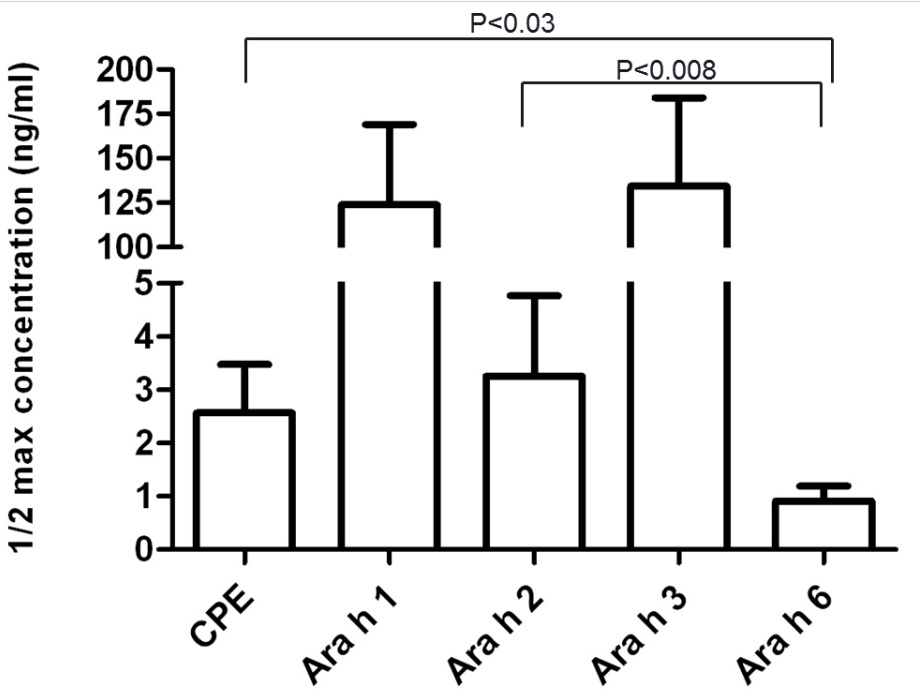
S. Koppelman, C.E.A. 2005

K. Peeters, C.E.A. 2007

Peanut allergen-induced basophil degranulation. Calculation ½ max concentration



Ara h6 is the most potent peanut allergen in inducing basophil degranulation of 9 patients



CD-sens

Allergy 2005: 60: 1192–1199

ALLERGY
DOI: 10.1111/j.1398-9995.2005.00870.x

Original article

Passive IgE-sensitization by blood transfusion

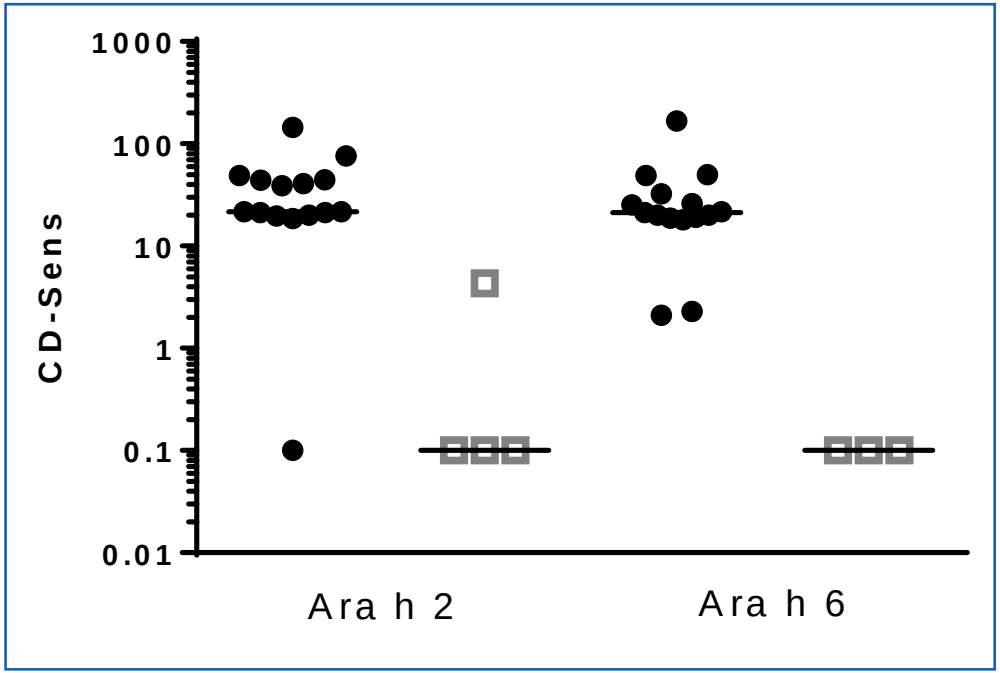
Background: To study the mechanisms of passive sensitization of patients receiving plasma containing IgE antibodies to a defined allergen.
Methods: When required for medical reasons, regular donor plasma with IgE antibodies to timothy grass allergen (8–205 kU_A/l), was given. Kinetics of IgE antibodies in the recipients' serum and his/her basophil allergen threshold sensitivity, CD-sens, was monitored up to 2–3 weeks after transfusion. The IgE antibodies were quantitated by ImmunoCAP. The CD-sens in plasma recipients, determined by CD63 up-regulation, was measured by flow cytometry and compared to CD-sens of patients with allergic asthma and/or rhinitis.
Results: There was a significant correlation ($r = 0.98$; $P < 0.001$) between amount of IgE antibody given and recipient serum peak concentration. The $T_{1/2}$

S. G. O. Johansson[1,2], A. Nopp[1], M. van Hage[1,2], N. Olofsson[3], J. Lundahl[1,2], L. Wehlin[1], L.Söderström[4], V. Stiller[2], H. Öman[5]

[1]Department of Medicine, Clinical Immunology and Allergy Unit, Karolinska Institute, Stockholm, Sweden; [2]Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden; [3]Department of Anaesthesiology, Karolinska University Hospital, Stockholm, Sweden; [4]Pharmacia Diagnostics AB, Uppsala, Sweden; [5]MIAB, Uppsala, Sweden

CD-sens to Ara h 2 and Ara h 6 in children in UMC Utrecht

CD-sens = 1 / ½ max [allergen]



Jasmijn Lagrouw and Francine van Erp

The IgE and basophil responses to Ara h 2 and Ara h 6 to predict peanut allergy in children

Francine van Erp

Study population

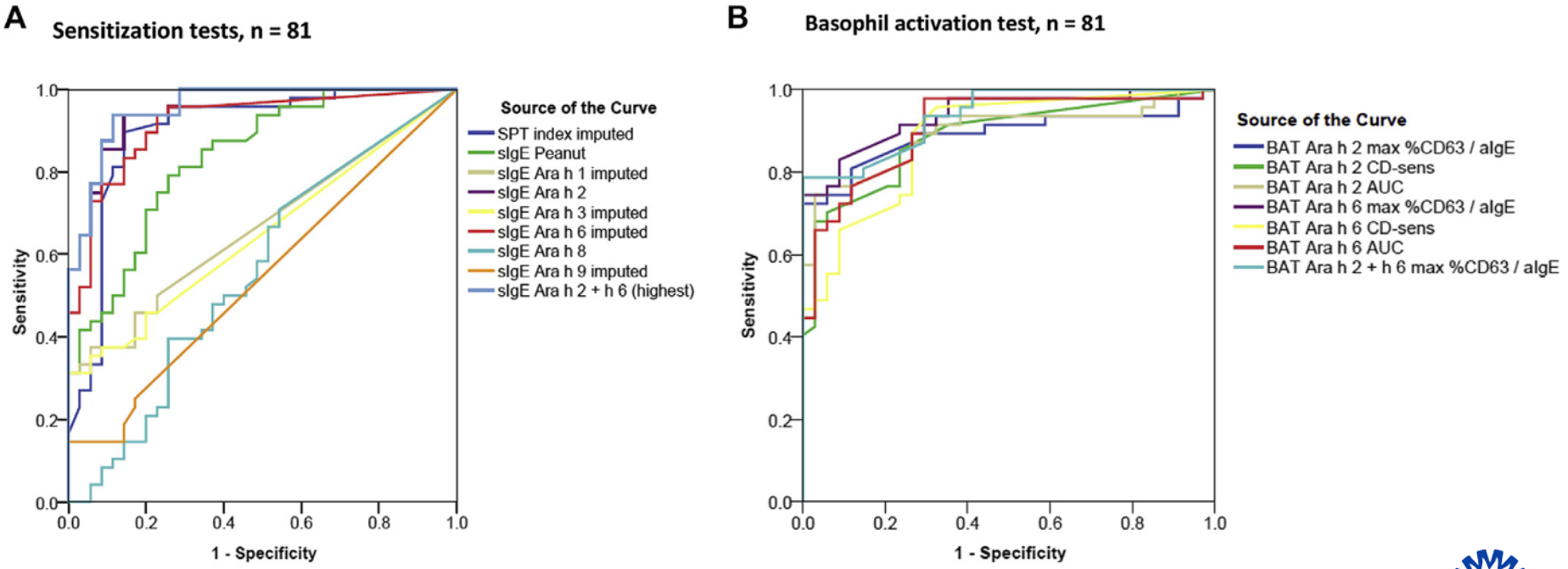
- Children of 3 – 18 years with suspected peanut allergy
  - Clinical history and / or
  - Peanut sensitization (SPT > 3 mm, sIgE peanut > 0.35 kU/L)

Study procedures

- Index tests
  - Clinical evaluation and sensitization tests (SPT, sIgE peanut, h 2, h 6)
  - Basophil Activation Test (h 2, h 6)

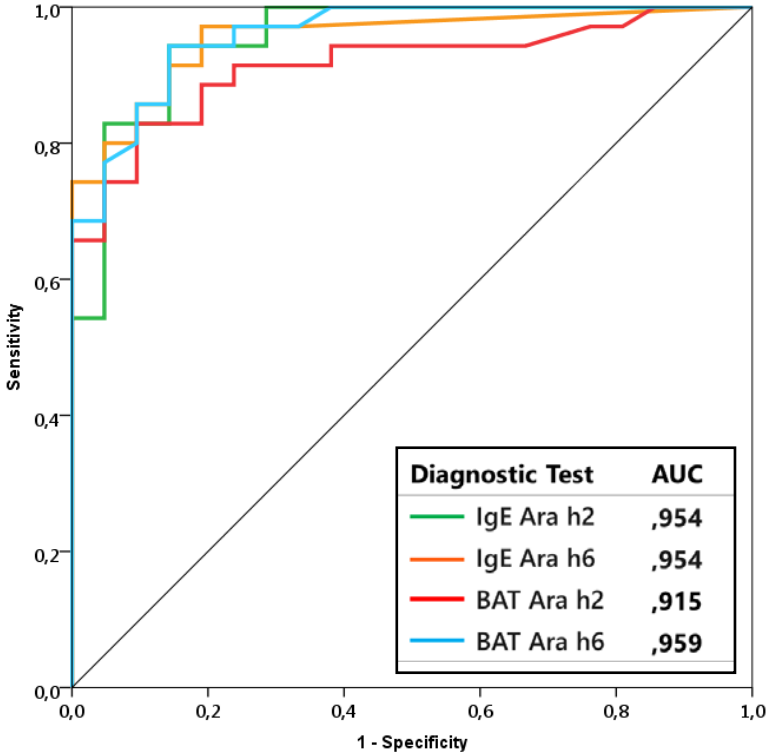
- Outcome
  - Double Blind Placebo Controlled Food Challenge
    - Follow-up
    - Panel diagnosis

ROC curves of ImmunoCAP and BAT

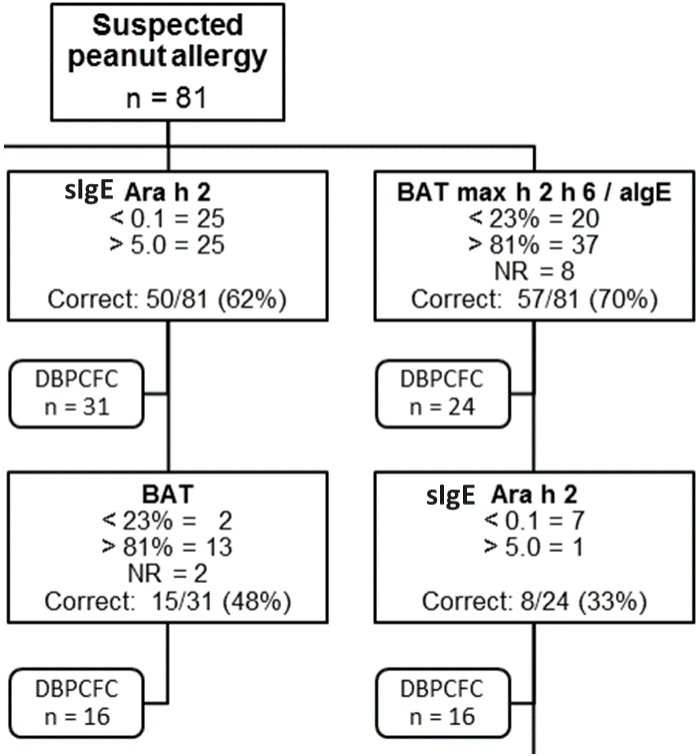


Van Erp, JACI, 2017; 139:358-60

Ara h 2 and Ara h 6 in CAP and BAT ROC and AUC



Combining sIgE and BAT for Ara h2 and Ara h6 reduces peanut provocations by 80%



Van Erp, JACI, 2017; 139:358-60

Basophil activation test

1) Allergy diagnostics, introducing cellular testing

2) Discovery of CD63 as basophil activation marker

3) Application of CD63 in peanut allergy diagnostics

4) Overview application of BAT in allergy diagnostics

Basophil activation test

1) Allergy diagnostics, introducing cellular testing

2) Discovery of CD63 as basophil activation marker

3) Application of CD63 in peanut allergy diagnostics

4) Overview application of BAT in allergy diagnostics

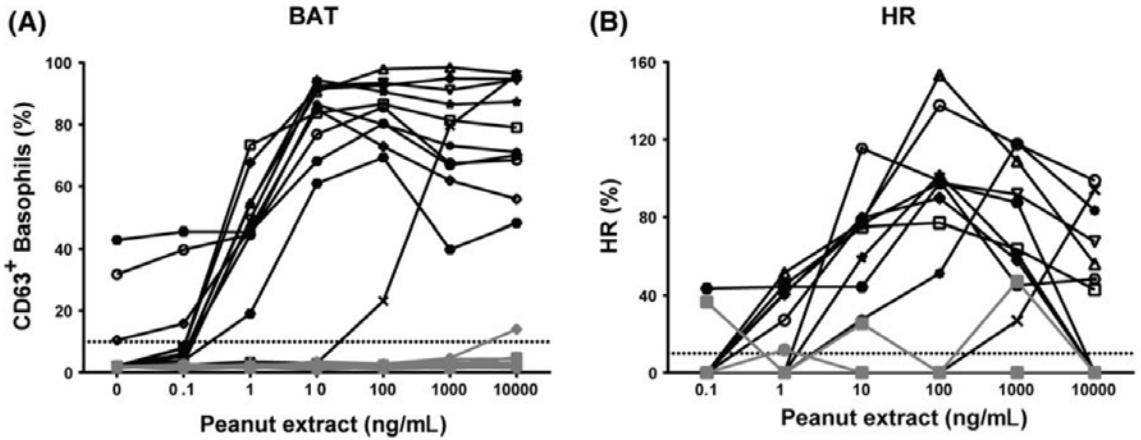
ORIGINAL ARTICLE

Biotechnology and in vitro Diagnostics

WILEY Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

A comparative study on basophil activation test, histamine release assay, and passive sensitization histamine release assay in the diagnosis of peanut allergy

L. F. Larsen[1] | N. Juel-Berg[1] | K. S. Hansen[1] | E. N. Clare Mills[2] | R. van Ree[3] | L. K. Poulsen[1] | B. M. Jensen[1]



Allergy diagnostics by BAT

Food allergy: Peanut, Milk, Hazelnut, Apple, Hen’s egg, Peach, Wheat

Drug allergy

Insect venom: Bee and wasp venoms

Patient-own products

Chronic urticaria: autoimmune

Allergy, 2015; 70:1393-405

Monitoring of allergy by BAT

AIT: Grass pollen, birch pollen, bee venom, wasp venom

AIT food: Peanut, hen's egg, milk

Spontaneous tolerance: Milk

Other treatments including anti-IgE

! BAT is performed on whole blood, so blocking factors, i.e. IgGs, affect outcome.

BAT: Issues

1. BAT should be done on fresh blood (preferably less than 24 hours old)
2. Anti-IgE non-releasers cannot be tested by BAT.
3. BAT is complex and requires an experienced laboratory and interpretation.
4. BAT and ASST do not correspond exactly in the diagnosis of CSU.
5. Drug BAT should be performed within a year of the most recent allergic response that elicits the diagnostic visit.
6. The testing setup and interpretation of BAT is complex and still lacks standardisation (Patil/Shreffler, companies)

Curr Allergy Asthma Rep 2016

Collaborators

Stans den Hartog-Jager

Helma van Doorn

Immunology

Andries Bloem
Carry Steenhuis
Dorine van den Blink
Jasmijn Lagrouw

Thermo Fisher, SE

Jonas Lidholm
Bo Pontoppidan

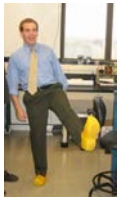
CLB, Amsterdam

Dirk Roos
Erik Mul
Hans Jansen
Jero Calafat

Francine van Erp

Mark Blankestijn

Els van Hoffen



Wayne Shreffler
Mount Sinai, NY
MGH, Boston

Andre Knulst

Edward Knol

Carla Bruijnzeel-Koomen

EUROBAT

CFA
UTRECHT CENTER FOR FOOD ALLERGY