

UniCAP® Specific IgE

Fluoroenzymeimmunoassay

CLIA Complexity Category =Moderately Complex

Directions for Use 52-5255-02/04

Calibrator Range 0-100 kU/l

INTENDED USE

UniCAP Specific IgE Assay is an *in vitro* quantitative assay for the measurement of allergen specific IgE in human serum or plasma. UniCAP Specific IgE Assay is to be used with the instrument ImmunoCAP 100/UniCAP 100. It is intended for *in vitro* diagnostic use as an aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings, and is intended for use in clinical laboratories, as well as physician office laboratories.

SUMMARY AND EXPLANATION OF THE TEST

In patients suffering from extrinsic asthma, hay fever or atopic eczema, symptoms develop immediately after exposure to specific allergens. This immediate (atopic or anaphylactic) type of allergy is a function of a special type of serum antibodies belonging to the IgE class of immunoglobulins (1, 2).

PRINCIPLE OF THE PROCEDURE

The allergen of interest, covalently coupled to ImmunoCAP, reacts with the specific IgE in the patient sample. After washing away non-specific IgE, enzyme labelled antibodies against IgE are added to form a complex. After incubation, unbound enzyme-anti-IgE is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured. The higher the response value the more specific IgE present in the specimen. To evaluate the test results, the response for the patient samples are transformed to concentrations with the use of a calibration curve.

REAGENTS

Reagents are packaged in separate units, each purchased separately.

The expiration date and storage temperature for each of the units are stated on the outer label. However, each component is stable until the date stated on each individual component's label.

Note! It is not recommended to pool any reagents.

UniCAP Specific IgE (Art No 10-9253-02)

(Fluoroenzymeimmunoassay for 96 determinations)

Specific IgE Conjugate β-Galactosidase-anti-IgE (mouse monoclonal antibodies) Approximately 1 µg/ml Sodium azide 0.06% Color coded blue; 5.1 ml	1 vial	Ready for use Store at 2–8 °C until expiration date Do not freeze!
Specific IgE Curve Control 1 (CC-1) (human IgE in buffer) Kathon CG 0.15% Color coded yellow; 0.2 ml	2 single dose vials	Ready for use Store at 2–8 °C until expiration date
Specific IgE Curve Control 2 (CC-2) (human IgE in buffer) Kathon CG 0.15% Color coded yellow; 0.2 ml	2 single dose vials	Ready for use Store at 2–8 °C until expiration date

UniCAP Specific IgE Conjugate (Art No 10-9419-02)

(Fluoroenzymeimmunoassay for 6 x 96 determinations)

Specific IgE Conjugate β-galactosidase-anti-IgE (mouse monoclonal antibodies). Approximately 1 µg/ml Color coded blue; 5.1 ml Sodium azide 0.06%	6 vials	Ready for use Store at 2–8 °C until expiration date Do not freeze!
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ImmunoCAP Specific IgE Calibrators 0-100 (Art No 10-9460-01) (For 1 calibration curve)

Specific IgE Calibrators (Cal-xx) (human IgE in buffer) Conc. 0; 0.35; 0.7; 3.5; 17.5 and 100 kU/l Kathon CG 0.15% Color coded yellow; 0.2 ml	6 single dose vials	Ready for use Store at 2–8 °C until expiration date
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UniCAP Specific IgE Curve Controls (Art No 10-9408-01)

Specific IgE Curve Control 1 (CC-1) (human IgE in buffer) Kathon CG 0.15% Color coded yellow; 0.2 ml	3 single dose vials	Ready for use Store at 2–8 °C until expiration date
Specific IgE Curve Control 2 (CC-2) (human IgE in buffer) Kathon CG 0.15% Color coded yellow; 0.2 ml	3 single dose vials	Ready for use Store at 2–8 °C until expiration date

Specific IgE Anti-IgE ImmunoCAP (Art No 14-4417-01)

Anti-IgE ImmunoCAP (a_IgE) (mouse monoclonal antibodies) Kathon CG 0.15%	Carriers of 16 ImmunoCAP	Ready for use Store at 2–8 °C until expiration date
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Allergen ImmunoCAP Carriers (Art No see separate list)

Single Allergen ImmunoCAP Kathon CG 0.15%	Carriers of 16 or 10 ImmunoCAP	Ready for use Store at 2–8 °C until expiration date
Multiple Allergen ImmunoCAP Kathon CG 0.15%	Carriers of 16 or 10 ImmunoCAP	Ready for use Store at 2–8 °C until expiration date

UniCAP/Pharmacia CAP System Phadiatop ImmunoCAP (Art No 14-4338-35)

(For 48 determinations)

Phadiatop ImmunoCAP (phad) Kathon CG 0.15 %	3 carriers of 16 ImmunoCAP	Ready for use Store at 2–8 °C until expiration date
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UniCAP Development Kit (Art No 10-9263-01) (Reagents for 400 determinations)

Development Solution 4-Methylumbelliferyl-β-D-galactoside 0.01%; 6.0 ml Kathon CG 0.05%	4 vials	Ready for use Store at 2–8 °C until expiration date
Stop Solution Sodium carbonate 4%, 65 ml	4 bottles	Ready for use Store at 2–8 °C until expiration date

UniCAP/Pharmacia CAP System Washing Solution (Art No 10-9422-01/10-9202-01)

For information see separate Washing Solution package insert .

Precautions

- For *in vitro* diagnostic use. Not for internal or external use in humans or animals.
- Do not use reagents beyond their expiration dates.
- This kit contains reagents manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or other local/national guidelines on laboratory safety procedures.

Warning! Reagents that contain sodium azide as a preservative must be handled with care. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by Centers for Disease Control and Prevention (CDC) or other local/national guidelines.

Handling of ImmunoCAP

Keep the carrier closed to avoid evaporation of buffer. Do not leave the carrier open for more than 1 day at room temperature, otherwise, discard the first ImmunoCAP.

Indication of instability

UniCAP 100 software has built-in acceptance limits for the calibration curve and the curve controls. For more information see UniCAP 100 User's Guide/Reference manual.

INSTRUMENTS

UniCAP 100 with built-in software processes all steps of the assay and prints results automatically after the assay is completed. For further information regarding assay set up, instrumentation and software etc. see UniCAP100 User's Guide/Reference Manual.

SPECIMEN COLLECTION AND PREPARATION

Serum and plasma (EDTA or heparin) samples from venous or capillary blood can be used. Collect blood samples using standard procedures. Keep specimens at room temperature (RT) for shipping purposes only. Store at 2–8 °C up to one week, or else at –20 °C. Avoid repeated freezing and thawing.

For information about interfering substances see references (12, 13).

Note! Due to the rise and fall of circulating specific IgE antibodies to insect venom and drugs, blood samples for testing with venom and drug ImmunoCAP should be collected from 2 to 3 weeks up to 6 months after an insect sting or drug exposure.

Preparation of Samples

Dilution of sample is required for determination of values higher than 100 kU_A/l IgE.

Samples are diluted with:

UniCAP IgE/ECP/Tryptase Sample Diluent (10-9256-01)

PROCEDURES

See UniCAP 100 User's Guide/Reference Manual for detailed description.

Parameters of the procedure

Volumes per determination:

Sample	40 µl
Conjugate	50 µl
Development Solution	50 µl
Stop Solution	600 µl

Total time for one assay is 2.5 hours

Incubations are performed at 37 °C by UniCAP 100.

Procedural steps

See UniCAP 100 User's Guide/Reference Manual for detailed description.

Material

Materials provided by Pharmacia Diagnostics AB:

See under REAGENTS.

Materials required but not provided by Pharmacia Diagnostics AB:

- Measuring cylinder 1000 ml
- Purified water

Additional products available from Pharmacia Diagnostics AB:

UniCAP 100 Instrument (12-3500-01)

UniCAP 100 RM Software (12-3505-05)

UniCAP 100 RM Reference Manual (12-3505-06)

UniCAP FluoroC (10-9264-01)

Calibration

ImmunoCAP Specific IgE Calibrators are run in duplicate to obtain a calibration curve. The curve can be stored. Curve Controls run in single determinations in subsequent assays validate the stored curve. Patient samples are run in single determinations. For more information see UniCAP 100 User's Guide/Reference manual.

Reference Material

The IgE calibrators are traceable (via an unbroken chain of calibrations) to the 2nd International Reference Preparation (IRP) 75/502 of Human Serum Immunoglobulin E from World Health Organisation (WHO).

Calibrator Range

0 - 100 kU/l.

Quality Control

Record keeping for each assay: It is good laboratory practice to record the lot numbers of the components used, the dates when they were first opened and the remaining volumes.

Control Specimens: Good laboratory practice requires that quality control specimens should be included in every run. Any material used should be assayed repeatedly to establish mean values and acceptable ranges.

Controls available from Pharmacia Diagnostics AB for day to day quality control:

UniCAP Specific IgE Negative Control (10-9445-01)

UniCAP Specific IgE Control (10-9449-01)

UniCAP Specific IgE f1 Control (10-9450-01)

Proficiency Testing: External quality assessment programs (proficiency testing) are available from various independent organizations. Pharmacia Diagnostics has available a Quality Club program for internal Quality Assessment needs:

Quality Club Specific IgE (10-9298-01)

CALCULATION OF RESULTS

UniCAP 100 is programmed to automatically calculate all results. For complete details, see UniCAP 100 User's Guide/Reference Manual.

Individual Allergen

Quantitative evaluation of Specific IgE antibody concentration (kU_A/l)

IgE calibrators traceable to the WHO preparation 75/502 for Human IgE are used for determination of total IgE and values are expressed in kU/l. In UniCAP Specific IgE assay these calibrators are used for determination of specific IgE antibodies, and values are expressed in kU_A/l, where A represents allergen-specific antibodies. Values above limit of quantitation represent a progressive increase in the concentration of allergen-specific IgE antibodies.

ASM Evaluation and Class Numbers

UniCAP Specific IgE ASM (Alternate Scoring Method) provides semiquantitative result presentation in classes equivalent to those obtained in the Modified RAST procedure. The ImmunoCAP Information Data Manager software automatically performs this calculation. The concentrations of IgE antibodies in kU_A/l corresponding to the ASM classes are shown below.

ASM Classes

ASM class	Specific IgE antibody concentration (kU _a /l)	Level of allergen specific IgE antibody
0/1	0.22 - 0.31	Equivocal/very low
1	0.31 - 0.55	Low
2	0.55 - 1.4	Moderate
3	1.4 - 3.9	High
4	3.9 - 19	Very high
5	19 - 100	Very high
6	>100	Very high

Calculations of results for other applications of specific IgE are provided with:

UniCAP/Pharmacia CAP System Phadiatop ImmunoCAP ArtNo. 14-4338-35

Interpretation of results

Phadiatop ImmunoCAP

Phadiatop results are expressed as positive or negative, using the 0.35 kU/l calibrator as a cut-off.

A positive Phadiatop result indicates that the patient is atopic, a negative result indicates that the patient is non-atopic, i.e. not sensitized to inhalant allergens.

In order to determine the concentration of allergen specific IgE antibodies, it is recommended to retest the sample with the appropriate Specific IgE ImmunoCAP allergens.

Multiple Allergen ImmunoCAP

Multiple Allergen-ImmunoCAP results are reported as qualitative values (positive or negative). For multiple allergen ImmunoCAP 0.35 kU/l is recommended as a cut-off value. Values between limit of quantitation and 0.35 kU/l may represent very low levels of IgE antibodies. Values ≥ 0.35 kU/l indicate specific IgE antibodies to one or more of the allergens coupled to multiple-allergen ImmunoCAP.

Reinvestigation with appropriate single allergen ImmunoCAP is recommended when there is a need to further identify and obtain a quantitative result for the specific allergen(s).

A value below 0.35 kU_a/l indicates undetectable or very low levels of allergen specific IgE antibodies towards all of the allergens bound to the multiple allergen ImmunoCAP but deviations from results obtained with single ImmunoCAP allergens may occur.

The interpretation of results obtained with multiple allergen ImmunoCAP cannot be compared with the results with single allergen ImmunoCAP. The degree of positivity of multiple allergen ImmunoCAP cannot be considered the cumulative degree of positivity of the respective single allergen ImmunoCAP.

LIMITATIONS OF THE PROCEDURE

A definitive clinical diagnosis should be made by the physician after all clinical and laboratory findings have been evaluated. It should not be based on the results of any single diagnostic method.

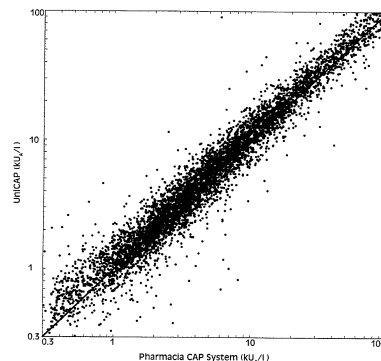
- Allergen specific IgE antibody levels as measured by *in vitro* assays are sometimes used as grounds for instituting immunotherapy, however the results of a Specific IgE test should not be the only consideration when selecting an initial dose for immunotherapy. Prior to implementing immunotherapy a skin test with the planned initial dilution of the immunotherapy solution should be performed to prove that the patient tolerates *in vivo* administration of this allergenic extract.
- Very low levels of allergen specific IgE antibodies should be evaluated with caution when total IgE values are above 1000 kU/l.
- In food allergy, circulating IgE antibodies may remain undetectable despite a convincing clinical history because these antibodies may be directed towards allergens that are revealed or altered during industrial processing, cooking or digestion and therefore do not exist in the original food for which the patient is tested.
- False positive test results in persons who are tested for food allergies may lead to inappropriate dietary restrictions while false negative results in food sensitive persons may result in anaphylactic reactions of varying severity.
- For penicillin-specific IgE determinations with UniCAP Specific IgE a result above limit of quantitation indicates the presence of specific IgE antibodies to penicilloyl, the major determinant of therapeutic penicillin.
- Results below limit of quantitation obtained for a drug-specific IgE determination with UniCAP Specific IgE indicates the absence of specific IgE antibodies to the drug. Such results are found in nonsensitized individuals. However, they can also be found in patients hypersensitive to drugs, for example when:
 - The symptoms are mediated without IgE involvement.
 - The blood sample has been collected a long time after the latest adverse reaction of a therapeutic treatment procedure. It has been shown that the concentration of IgE antibodies decreases with time after the allergic reactions (3).
 - The blood sample has been collected very soon after the allergic reaction. An interval between the time of the allergic reaction and the appearance of measurable specific IgE antibodies has been observed in some cases (4). This can lead to negative results for drug-specific IgE determinations with UniCAP Specific IgE. Such results can be checked by collecting a new blood sample and repeating the test two weeks after the allergic reaction.
- With ImmunoCAP venoms results below limit of quantitation indicate absent or undetectable levels of circulating venom-specific IgE antibodies. Such results do not preclude existence of current or future clinical hypersensitivity to insect stinging.

- Identical results for different allergens may not be associated with clinically equivalent manifestations, due to differences in patient sensitivities.
- Latex specific IgE antibodies may show cross reactivity with ragweed and certain food allergens such as banana, avocado, kiwi and chestnut (8, 9, 10). Clinical diagnosis should be made by a physician only after all clinical and laboratory findings have been evaluated and not solely on the results of an *in vitro* diagnostic test (11).

EXPECTED VALUES

Good practice recommends that each laboratory establishes its own expected range of values. When a pool from 31 healthy non-allergic blood donors was tested against the existing panel of UniCAP Specific IgE allergens, the 95 percentile was below 0.1 kU_a/l. In clinical practice, 0.35 kU_a/l has commonly been used as a cut off and a large number of studies have been performed in which the clinical performance of UniCAP Specific IgE tests in allergy diagnosis has been evaluated. Clinical performance expressed as sensitivity, ranging from 84-95 %, and specificity, ranging from 85-94 %, has been reported from multi-center studies including several hundred patients tested for a range of different allergens (5,6,14).

Comparison studies⁽¹⁾ between Pharmacia CAP System Specific IgE FEIA and UniCAP Specific IgE have been performed with 6458 patient samples and 170 different single allergen ImmunoCAP, and 613 patient samples and 16 different multiple allergen ImmunoCAP. Results for patient samples obtained with Pharmacia CAP System Specific IgE FEIA and UniCAP Specific IgE Assay show good agreement, see figure. In the comparison studies done with 6458 samples, agreement in positive and negative results for the two systems was 99%.



PERFORMANCE CHARACTERISTICS

Precision⁽¹⁾

The following mean coefficients of variation (CV) have been obtained when testing representative allergens from seven allergen groups. Each sample has been assayed in 4 replicates on 18 different occasions using stored calibration curves.

Sample level (kU _a /l)	Coefficients of variation (%)	
	Within assay	Between assay
0.7 – 3.5	5	11
3.5 – 17.5	6	10
17.5 – 100	5	10

Sensitivity⁽¹⁾

The overall limit of quantitation (NCCLS EP17-A) (15) for allergen specific IgE antibodies is 0.1 kU_a/l.

Specificity⁽¹⁾

The cross-reactivity with other human immunoglobulins is non-detectable at physiological concentrations of IgA, IgD, IgM and IgG.

INFORMATION SPECIFIC TO LATEX ALLERGEN (k82)

(Data generated using 0.35 kU_a/l as cut-off)

Indication for use:

UniCAP Specific IgE, Latex (k82) test result may be used as an aid in the clinical diagnosis of patients with suspected latex allergy.

Specific Performance Characteristics to Latex Allergen (k82)

Results of Clinical Study^a

Clinical Sensitivity and Specificity

N= 182

Clinical Diagnosis ^b	UniCAP Specific IgE Latex ImmunoCAP k82			
	Pos	Neg	Clinical Sensitivity	Clinical Specificity
Positive	70	26	72.9 %	
Negative	1	85		98.8 %

^a = Data on file at Pharmacia Diagnostics AB

^b = Clinical Diagnosis as defined by clinical history and latex skin test results

Agreement: 85.1 %

(Concordance between all positive and negative test results with clinical diagnosis 155 /182)

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Pharmacia Diagnostics AB may affect the results, in which event Pharmacia Diagnostics AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Pharmacia Diagnostics AB and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

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Notes

⁽¹⁾Studies performed at Pharmacia Diagnostics AB, Uppsala, Sweden.

Patents

This system is covered by the following patents:
 US Patent 4,647,655; 4,708,932; 5,822,069 and 5,895,630
 European Patent 134 236 and 128 885
 Japanese patent 194 288 1 and 185 589 1

In addition patents pending on different aspects of UniCAP.

The following designations are trademarks belonging to Pharmacia Diagnostics AB: ImmunoCAP, Phadebas, Pharmacia CAP System, PRIST, RAST, UniCAP, Phadiatop, Quality Club.

Kathon is a trademark of Rohm and Haas Company.

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