ImmunoCAP® Specific IgE

Fluoroenzymeimmunoassay

Calibrator Range 0-100 kU/l

Directions for Use 52-5255-EN/10

INTENDED USE

ImmunoCAP Specific IgE is an *in vitro* test which measures the concentration of circulating allergen specific IgE in human serum or plasma. ImmunoCAP Specific IgE is to be used with the instrument Phadia 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 to be used in clinical laboratories, as well as in 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 called reagins. These reagins have been identified as 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 sample. 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 two digit suffix (-01) on the article number may vary between countries.
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.

ImmunoCAP Specific IgE 0-100 (Art No 10-9462-01)
(Fluoroenzymeimmunoassay for 96 determinations)

Specific IgE Conjugate ß-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) Preservative* <0.003% 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) Preservative* <0.003% Color coded yellow; 0.2 ml	2 single dose vials	Ready for use Store at 2-8 °C until expiration date

ImmunoCAP Specific IgE Conjugate 0-100 (Art No 10-9463-01)
(Fluoroenzymeimmunoassay for 6 x 96 determinations)

Specific IgE Conjugate ß-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!**

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 Preservative* <0.003% Color coded yellow; 0.2 ml	6 single dose vials	Ready for use Store at 2-8 °C until expiration date

ImmunoCAP Specific IgE Curve Controls (Art No 10-9408-01)

Specific IgE Curve Control 1 (CC-1) (human IgE in buffer) Preservative* <0.003% 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) Preservative* <0.003% Color coded yellow; 0.2 ml	3 single dose vials	Ready for use Store at 2 - 8 °C until expiration date

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

ImmunoCAP Anti-IgE (a_IgE) (mouse monoclonal antibodies) Preservative* <0.003%	Carriers of 16 ImmunoCAP	Ready for use Store at 2 - 8 °C until expiration date

ImmunoCAP Allergens (Art No see Product Catalog)

ImmunoCAP Allergen Preservative* <0.003%	Carriers of 16 or 10 ImmunoCAP	Ready for use Store at 2 - 8 °C until expiration date

ImmunoCAP Phadiatop (Art No 14-4405-35)
(For 48 determinations)

ImmunoCAP Phadiatop (phad) Preservative* <0.003%	3 carriers of 16 ImmunoCAP	Ready for use Store at 2 - 8 °C until expiration date

ImmunoCAP Phadiatop Infant (Art No 14-4510-35)
(For 48 determinations)

ImmunoCAP Phadiatop Infant (phinf) Preservative* <0.003%	3 carriers of 16 ImmunoCAP	Ready for use Store at 2 - 8 °C until expiration date

Development Solution (Art No 10-9478-01)
(Reagents for 600 determinations)

Development Solution 4-Methylumbelliferyl-ß-D-galactoside 0.01% Preservative* <0.0010%; 6.0 ml	6 vials	Ready for use Store at 2–8 °C until expiration date **Do not freeze!**

Stop Solution (Art No 10-9479-01)
(Reagents for 600 determinations)

Stop Solution Sodium carbonate 4 %; 65 ml	6 bottles	Ready for use Store at 2 - 8 °C until expiration date

Washing Solution (Art No 10-9422-01/10-9202-01)
For information see separate Washing Solution package insert.

***Preservative**: Mixture of 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2*H*-isothiazol-3-one [EC no. 220-239-6] (3:1).

⚠ 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.
- Reagents containing >0.0015% mixture of 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2*H*-isothiazol-3-one [EC no. 220-239-6] (3:1) may cause sensitisation by skin contact. Avoid contact with skin. Wear suitable gloves. For more information see Safety Data Sheet.
- Reagents that contain sodium azide as a preservative must be handled with care. For more information see Safety Data Sheet.

Handling of ImmunoCAP Carrier

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

Phadia 100 software has built-in acceptance limits for the calibration curve and the curve controls. For more information see Phadia 100 User Manual.

INSTRUMENTS

Phadia 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 setup, instrumentation and software etc. see Phadia 100 User 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 °C to 8 °C up to one week, or else at –20 °C. Avoid repeated freezing and thawing.
For information about interfering substances see references (8, 9).
Note! Blood samples for testing with drugs and venom ImmunoCAP should be collected during or close to the event, preferably not later than 6 months after exposure. If the test result is negative and an IgE-mediated reaction is still strong suspected, it is advisable to draw a new sample and repeat the test at 5 to 6 weeks (10, 11).

Preparation of Samples
Dilution of sample is required for determination of values higher than 100 kU_A/l IgE. Samples are diluted with:

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

PROCEDURES
See Phadia 100 User Manual for more information.

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 Phadia 100.

Procedural steps
See Phadia 100 User Manual for more information.

Material
Materials provided by Phadia AB:
See under REAGENTS.
Materials required but not provided by Phadia AB:
- Measuring cylinder 1000 ml
- Purified water

Additional products available from Phadia AB:

Phadia 100 Instrument (12-3500-01)

Phadia Information Data Manager Software Package (12-3801-11)

FluoroC (10-9264-01)

Maintenance Solution Kit (10-9476-01)

Calibration
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 Phadia 100 User 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 Phadia AB for day to day quality control:

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

ImmunoCAP Specific IgE Control (10-9449-01)

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

Proficiency Testing: An external quality assessment program (proficiency testing) is available from Phadia AB for quality assurance purposes (Quality Club):

Quality Club Specific IgE (10-9298-01)

CALCULATION OF RESULTS
Phadia 100 is programmed to automatically calculate all results.
For more information, see Phadia 100 User Manual.

Individual Allergens

ImmunoCAP Specific IgE antibody concentration (kU_A/l)
IgE 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 relative concentration of allergen-specific antibodies (3).
Calculations of results for other applications of specific IgE are provided with:

ImmunoCAP Phadiatop (Art No 14-4405-35)
ImmunoCAP Phadiatop Infant (Art No 14-4510-35)

ImmunoCAP Multiple Allergen
ImmunoCAP Multiple Allergen results are reported as qualitative values (positive or negative). For ImmunoCAP multiple allergen 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 ImmunoCAP multiple allergen.
Reinvestigation with appropriate ImmunoCAP single allergen 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 ImmunoCAP multiple allergen but deviations from results obtained with ImmunoCAP single allergens may occur.
The interpretation of results obtained with ImmunoCAP multiple allergen cannot be compared with the results with ImmunoCAP single allergen. The degree of positivity of ImmunoCAP multiple allergen cannot be considered the cumulative degree of positivity of the respective ImmunoCAP single allergen.

LIMITATIONS OF THE PROCEDURE
A definitive clinical diagnosis should not be based on the results of any single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
- 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.
- Results below limit of quantitation obtained for a drug-specific IgE determination indicates the absence of specific IgE antibodies to the drug. Such results are found in nonsensitive individuals. However, they can also be found in patients hypersensitive to drugs e.g. when:
  a. the symptoms are mediated without IgE involvement.
  b. 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 (4).
  c. 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 (5). This can lead to negative results for drug-specific IgE. Such results can be checked by collecting a new blood sample and repeating the test 5 to 6 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 sting.

EXPECTED VALUES
Good practice recommends that each laboratory establish its own expected range of values.
When a pool from 31 healthy non-allergic blood donors was tested against the existing panel of ImmunoCAP 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 ImmunoCAP 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 (6, 7, 12).
Comparison studies[1] between Pharmacia CAP System Specific IgE FEIA and ImmunoCAP Specific IgE have been performed with 510 samples and 74 different allergens. Results for patient samples obtained with Pharmacia CAP System Specific IgE FEIA and ImmunoCAP Specific IgE show good agreement.
Expected values for other applications of specific IgE measurements are provided with:

ImmunoCAP Phadiatop (Art No 14-4405-35)
ImmunoCAP Phadiatop Infant (Art No 14-4510-35)

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) (13) 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.

WARRANTY
The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Phadia AB may affect the results, in which event Phadia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. Phadia AB and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

SYMBOLS USED

LOT	Batch Code	IVD	*In Vitro* Diagnostic Medical Device
	Biological Risks		Caution
	Temperature Limitation	Σ	Contains Sufficient for <n> Tests
	Use By		Consult Instructions for Use
	Manufacturer		

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The ImmunoCAP brand name has replaced the UniCAP brand name.
The Phadia brand name is applied to instrument platforms and related system items.

Notes

(1)Studies performed at Phadia AB, Uppsala, Sweden.

Patents/Trademarks

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