ImmunoCAP® Specific IgE

Fluoroenzymeimmunoassay
Calibrator Range 0-100 kU/l
Directions for Use 52-5291-EN/05

INTENDED USE

ImmunoCAP Specific IgE is an in vitro test system for the quantitative measurement of allergen specific IgE in human serum or plasma. 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. ImmunoCAP Specific IgE is to be used with the instruments Phadia 100, Phadia 250, Phadia 1000, Phadia 2500 or Phadia 5000.

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 labeled antibodies against IgE are added to form a complex. Following 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 is present in the sample. To evaluate the test results, the responses for the patient samples are transformed to concentrations with the use of a calibration curve.

REAGENTS AND MATERIAL

Reagents are packaged as described below, each purchased separately. The two digit suffix (-xx) on the article number may vary between countries. All kits are not available in all countries.

The expiration date and storage temperature are stated on the labels. Do not use reagents beyond their expiration dates.

Note: It is not recommended to pool any reagents.

Keep the ImmunoCAP 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.

Reagents for Phadia 100

- **ImmunoCAP Specific IgE 0-100** (Art No 10-9462-01: for 96 determinations)
  - Specific IgE Conjugate (1 vial)
  - Specific IgE Curve Control 1 (CC-1) (2 single dose vials)
  - Specific IgE Curve Control 2 (CC-2) (2 single dose vials)
- **ImmunoCAP Specific IgE Conjugate 0-100** (Art No 10-9463-01: for 6 x 96 determinations)
- **ImmunoCAP Specific IgE Calibrators 0-100** (Cal-xx) (Art No 10-9460-01: for 1 calibration curve)
- **ImmunoCAP Specific IgE Curve Controls** (CC-1 and CC-2) (Art No 10-9408-01: 3 single dose vials)
- **ImmunoCAP Specific IgE Anti-IgE** (a_IgE) (Art No 14-4417-01: carriers of 16 ImmunoCAP)
- **ImmunoCAP Allergen** (See Product catalogue: carriers of 16 or 10 ImmunoCAP)
- **ImmunoCAP Total IgE Low Range** (low) (Art No 14-4497-35: for 48 determinations)
- **ImmunoCAP Phadiatop** (phad) (Art No 14-4405-35: for 48 determinations)
- **ImmunoCAP Phadiatop Infant** (phinf) (Art No 14-4510-35: for 48 determinations)
- **Development Solution** (Art No 10-9478-01: for 600 determinations)
- **Stop Solution** (Art No 10-9479-01: for 600 determinations)
- **Washing Solution** (Art No 10-9422-01: 6 x 1 l)
  - Washing Solution Additive, 6 x 17.2 ml
  - Washing Solution Concentrate, 6 x 80 ml
- **Washing Solution** (Art No 10-9202-01: 2 x 5 l)
  - Washing Solution Additive, 2 x 86 ml
  - Washing Solution Concentrate, 2 x 400 ml
- **ImmunoCAP Specific IgE Control** (Art No 10-9449-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE f1 Control** (Art No 10-9450-01: for 4 x 4 determinations)
- **ImmunoCAP Specific IgE Control L** (Art No 10-9528-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Control M** (Art No 10-9529-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Control H** (Art No 10-9530-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Negative Control** (Art No 10-9445-01: for 6 x 4 determinations)

Reagents for Phadia 250

- **ImmunoCAP Specific IgE Conjugate 100** (Art No 10-9316-01: for 6 x 100 determinations)
- **ImmunoCAP Specific IgE Conjugate 400** (Art No 10-9310-01: for 6 x 400 determinations)
- **ImmunoCAP Specific IgE Calibrator Strip 0-100** (Art No 10-9459-01: for 5 calibration curves)
- **ImmunoCAP Specific IgE Curve Control Strip** (CC-1 and CC-2) (Art No 10-9312-01: 5 x 3 sets of curve control)
- **ImmunoCAP Specific IgE Anti-IgE** (a_IgE) (Art No 14-4417-01: carriers of 16 ImmunoCAP)
- **ImmunoCAP Allergen** (See Product catalogue: carriers of 16 or 10 ImmunoCAP)
- **ImmunoCAP Total IgE Low Range** (low) (Art No 14-4497-35: for 48 determinations)
- **ImmunoCAP Phadiatop** (phad) (Art No 14-4405-35: for 48 determinations)
- **ImmunoCAP Phadiatop Infant** (phinf) (Art No 14-4510-35: for 48 determinations)
- **Development Solution** (Art No 10-9441-01: for 6 x 200 determinations; Art No 10-9440-01: for 6 x 315 determinations)
- **Stop Solution** (Art No 10-9442-01: for 6 x 185 determinations)
- **Washing Solution** (Art No 10-9422-01: 6 x 1 l)
  - Washing Solution Additive, 6 x 17.2 ml
  - Washing Solution Concentrate, 6 x 80 ml
- **Washing Solution** (Art No 10-9202-01: 2 x 5 l)
  - Washing Solution Additive, 2 x 86 ml
  - Washing Solution Concentrate, 2 x 400 ml
- **ImmunoCAP Specific IgE Control** (Art No 10-9449-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE f1 Control** (Art No 10-9450-01: for 4 x 4 determinations)
- **ImmunoCAP Specific IgE Control L** (Art No 10-9528-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Control M** (Art No 10-9529-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Control H** (Art No 10-9530-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Negative Control** (Art No 10-9445-01: for 6 x 4 determinations)

Reagents for Phadia 1000

- **ImmunoCAP Specific IgE Conjugate 100** (Art No 10-9316-01: for 6 x 100 determinations)
- **ImmunoCAP Specific IgE Conjugate 400** (Art No 10-9310-01: for 6 x 400 determinations)
- **ImmunoCAP Specific IgE Calibrator Strip 0-100** (Art No 10-9459-01: for 5 calibration curves)
- **ImmunoCAP Specific IgE Curve Control Strip** (CC-1 and CC-2) (Art No 10-9312-01: 5 x 3 sets of curve control)
- **ImmunoCAP Specific IgE Anti-IgE** (a_IgE) (Art No 14-4417-01: carriers of 16 ImmunoCAP)
- **ImmunoCAP Allergen** (See Product catalogue: carriers of 16 or 10 ImmunoCAP)
- **ImmunoCAP Total IgE Low Range** (low) (Art No 14-4497-35: for 48 determinations)
- **ImmunoCAP Phadiatop** (phad) (Art No 14-4405-35: for 48 determinations)
- **ImmunoCAP Phadiatop Infant** (phinf) (Art No 14-4510-35: for 48 determinations)
- **Development Solution** (Art No 10-9439-01: for 6 x 1200 determinations; Art No 10-9314-01: for 6 x 2000 determinations)
- **Stop Solution** (Art No 34-2271-51: for 1200 determinations)
- **Washing Solution** (Art No 10-9202-01: 2 x 5 l)
  - Washing Solution Additive, 2 x 86 ml
  - Washing Solution Concentrate, 2 x 400 ml
- **ImmunoCAP Specific IgE Control** (Art No 10-9449-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE f1 Control** (Art No 10-9450-01: for 4 x 4 determinations)
- **ImmunoCAP Specific IgE Control L** (Art No 10-9528-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Control M** (Art No 10-9529-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Control H** (Art No 10-9530-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Negative Control** (Art No 10-9445-01: for 6 x 4 determinations)

Reagents for Phadia 2500 and Phadia 5000

- **ImmunoCAP Specific IgE Conjugate 400** (Art No 10-9310-01: for 6 x 400 determinations)
- **ImmunoCAP Specific IgE Calibrator Strip 0-100** (Art No 10-9459-01: for 5 calibration curves)
- **ImmunoCAP Specific IgE Curve Control Strip** (CC-1 and CC-2) (Art No 10-9312-01: 5 x 3 sets of curve control)
- **ImmunoCAP Specific IgE Anti-IgE** (a_IgE) (Art No 14-4417-01: carriers of 16 ImmunoCAP)
- **ImmunoCAP Allergen** (See Product catalogue: carriers of 16 or 10 ImmunoCAP)
- **ImmunoCAP Total IgE Low Range** (low) (Art No 14-4497-35: for 48 determinations)
- **ImmunoCAP Phadiatop** (phad) (Art No 14-4405-35: for 48 determinations)
- **ImmunoCAP Phadiatop Infant** (phinf) (Art No 14-4510-35: for 48 determinations)
- **Development Solution** (Art No 10-9314-01: for 6 x 2000 determinations)
- **Stop Solution** (Art No 34-2337-11: for 4600 determinations)
- **Washing Solution Additive** (Art No 10-9518-01: 4 x 850 ml)
- **Washing Solution Concentrate** (Art No 34-2337-21: 1 x 2800 ml)
- **ImmunoCAP Specific IgE Control** (Art No 10-9449-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE f1 Control** (Art No 10-9450-01: for 4 x 4 determinations)
- **ImmunoCAP Specific IgE Control L** (Art No 10-9528-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Control M** (Art No 10-9529-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Control H** (Art No 10-9530-01: for 6 x 4 determinations)
- **ImmunoCAP Specific IgE Negative Control** (Art No 10-9445-01: for 6 x 4 determinations)

Details of reagents

ImmunoCAP Specific IgE Conjugate/Conjugate 100/Conjugate 400	
ß-Galactosidase-anti-IgE Approximately 1 µg/ml (mouse monoclonal antibodies) Sodium azide 0.06%	Ready for use. Store at 2 – 8 °C until expiration date. **Do not freeze!**

ImmunoCAP Specific IgE Calibrators/Calibrator Strip 0-100	
(human IgE in buffer) Conc. 0; 0.35; 0.7; 3.5; 17.5 and 100 kU/l Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

ImmunoCAP Specific IgE Curve Controls/Curve Control Strip	
(human IgE in buffer) Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

ImmunoCAP Specific IgE Anti-IgE	
(mouse monoclonal antibodies) Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

ImmunoCAP Allergen	
Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

ImmunoCAP Total IgE Low Range	
ImmunoCAP Anti-IgE (mouse monoclonal antibodies) Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

ImmunoCAP Phadiatop	
Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

ImmunoCAP Phadiatop Infant	
Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

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

Stop Solution	
Sodium carbonate 4%	Ready for use. Store at 2 – 32 °C until expiration date.

Washing Solution
For information, see separate Directions for Use for Washing Solution.

ImmunoCAP Specific IgE Control	
Sodium azide 0.05%	Ready for use. Store at 2 – 8 °C until expiration date.
ImmunoCAP Specific IgE Control is prepared from selected pooled human samples and contains IgE antibodies to a number of different allergens.	

ImmunoCAP Specific IgE f1 Control	
Sodium azide 0.05%	Ready for use. Store at 2 – 8 °C until expiration date.
ImmunoCAP Specific f1 IgE Control is prepared from selected pooled human samples and contains IgE antibodies to the allergen f1.	

ImmunoCAP Specific IgE Control L	
Sodium azide 0.05%	Ready for use. Store at 2 – 8 °C until expiration date.
ImmunoCAP Specific IgE Control L is prepared from selected pooled human samples and contains IgE antibodies to the allergen e1.	

ImmunoCAP Specific IgE Control M	
Sodium azide 0.05%	Ready for use. Store at 2 – 8 °C until expiration date.
ImmunoCAP Specific IgE Control M is prepared from selected pooled human samples and contains IgE antibodies to the allergen t3.	

ImmunoCAP Specific IgE Control H	
Sodium azide 0.05%	Ready for use. Store at 2 – 8 °C until expiration date.
ImmunoCAP Specific IgE Control H is prepared from selected pooled human samples and contains IgE antibodies to the allergen d1.	

ImmunoCAP Specific IgE Negative Control	
Sodium azide 0.05%	Ready for use. Store at 2 – 8 °C until expiration date.
ImmunoCAP Specific IgE Negative Control is prepared from selected pooled human samples.	

ImmunoCAP IgE/ECP/Tryptase Sample Diluent	
(buffer solution with Bovine Serum Albumin) Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

***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).

Additional material

Additional products available from Phadia AB:

- ImmunoCAP IgE/ECP/Tryptase Sample Diluent (10-9256-01/10-9360-01)
- Maintenance Solution Kit (10-9476-01)

Materials required but not provided by Phadia AB:

- Measuring cylinder 1000 ml
- Purified water (14, 15) or Clinical Laboratory Reagent Water (CLRW, 16)

Precautions

- For in vitro diagnostic use. Not for internal or external use in humans or animals.
- Some reagents are 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 sensitization by skin contact. Avoid contact with skin. Wear suitable gloves. For more information see Safety Data Sheet.

INSTRUMENTS

Phadia 100 processes all steps of the assay and prints results automatically after the assay is completed[(a)]. Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000 are continuous random access systems that perform all steps of the assay[(a)].

On board stability

Phadia 100 has no provisions for on board reagent storage. Information on reagent storage for other Phadia instruments is shown below.

	Phadia 250	Phadia 1000	Phadia 2500, Phadia 5000
Calibrator/ Curve Control Strip	28 days[B].		No on board storage.
Conjugate	4 days open[A]. Recap bottles every night.	7 days open[A].	
Development Solution	5 days open[B]. Recap bottles every night.	14 days open[A].	5 days open[B].
Stop Solution	Until expiration date[B]. Recap bottles every night.	Until expiration date[B].	
Washing Solution (prepared solution)	7 days[B]. Discard every seventh day and perform weekly maintenance according to respective instrument user manual.		
Washing Solution Concentrate	N/A		6 months[B].
Washing Solution Additive	N/A		6 months[B].
Sample Diluent	7 days[B]. Recap bottles every night.	N/A	
ImmunoCAP Carrier	Until expiration date[A].		

[A]Refrigerated (2-8 °C)

[B]Room temperature (18-32 °C)

SPECIMEN COLLECTION AND PREPARATION

Specimen collection

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 (3). For further reading on interfering substances, see reference (4).

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 strongly suspected, it is advisable to draw a new sample and repeat the test at 5 to 6 weeks. (5, 6)

Preparation of samples

Sample dilution is usually not required.

For determination of values higher than 100 kU_A/l IgE, dilute the samples with ImmunoCAP IgE/ECP/Tryptase Sample Diluent.

Handling of control specimen

It is recommended to remove and recap the control vials from the instrument as soon as the pipetting of the samples is finished and the sample incubation is started. It is also recommended to gently stir the vial before use.

ImmunoCAP Specific IgE Controls should be treated in the same way as a patient sample in the procedure. ImmunoCAP Specific IgE Controls are ready for use and must not be further diluted.

PROCEDURES

Procedural steps[(a)]

For procedural steps, see **Notes** a.

Parameters of the procedure

Patient samples are run in single determinations.

Volumes per determination:

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

Incubations are performed at 37 °C by Phadia instruments.

Phadia 100: Total time for one assay is 2.5 hours.

Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000: The process time is 1 hour and 45 minutes from entering the first sample until the result is available.

Calibration[(a)]

ImmunoCAP Specific IgE Calibrators or ImmunoCAP Specific IgE Calibrator Strips are run in duplicates to obtain a calibration curve. The curve can be stored. The software for Phadia instruments have built-in acceptance limits for the calibration curve and the curve controls. Use two curve controls, CC-1 and CC-2, each in single determination to evaluate subsequent assays against the stored curve.

Calibrator range: 0-100 kU/l.

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).

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 Specimen

Good laboratory practice requires that quality control specimen 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 Control (10-9449-01)
- ImmunoCAP Specific IgE f1 Control (10-9450-01)
- ImmunoCAP Specific IgE Control L (10-9528-01)
- ImmunoCAP Specific IgE Control M (10-9529-01)
- ImmunoCAP Specific IgE Control H (10-9530-01)
- ImmunoCAP Specific IgE Negative Control (10-9445-01)

Intended use

ImmunoCAP Specific IgE Controls are used for monitoring ImmunoCAP Specific IgE measurements performance in Phadia instruments.

Expected values for positive ImmunoCAP Specific IgE Controls

As with all immunoassays the results are affected by the testing procedures and equipment used by different laboratories. It is therefore recommended that each laboratory establishes its own target value for each actual lot of control together with criteria of acceptance (recommended range ±30%).

This established target value is expected to fall within the range for the actual lot. The range is stated on the vial for:

- ImmunoCAP Specific IgE f1 Control
- ImmunoCAP Specific IgE Control L
- ImmunoCAP Specific IgE Control M
- ImmunoCAP Specific IgE Control H

The range is stated in the Directions for Use for ImmunoCAP Specific IgE Control for:

- ImmunoCAP Specific IgE Control

The range for each specific lot is calculated as a mean ±2 SD using the expected long term variation. The mean value for each specific lot has been determined from 8 consecutive control assays, each in 6 replicates using ImmunoCAP Specific IgE.

Expected values for ImmunoCAP Specific IgE Negative Control
The negative control will give results representative for non-atopic blood donors with allergen ImmunoCAP. Results will be below 0.35 kU_A/l.

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)

RESULTS

For Phadia instruments using Phadia Information Data Manager Software, all calculations are automatically performed[(a)]. Phadia 100 is programmed to automatically calculate all results[(a)]. It can also be used with Phadia Information Data Manager Software.

ImmunoCAP Specific IgE antibody concentration (kU_A/l)
ImmunoCAP Specific 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 the limit of quantitation represent a progressive increase in the concentration of allergen-specific antibodies (7).

Calculations of results for other applications of specific IgE are provided in the DFUs for:
- ImmunoCAP Phadiatop (Art No 14-4405-35)
- ImmunoCAP Phadiatop Infant (Art No 14-4510-35)
- ImmunoCAP Total IgE Low Range (Art No 14-4497-35)

Interpretation of results for ImmunoCAP Allergen mixes
Results for ImmunoCAP Allergen mixes are qualitative values and 0.35 kU/l is recommended as a cut-off value.

Values ≥0.35 kU/l indicate specific IgE antibodies to one or more of the allergens coupled to ImmunoCAP Allergen mixes.

A value below 0.35 kU/l indicates undetectable levels or very low levels, of allergen specific IgE antibodies. Deviations from results obtained with single ImmunoCAP Allergen(s) may occur.

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

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

LIMITATIONS OF THE PROCEDURE

A definitive clinical diagnosis should only 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.
- Very low levels of allergen specific IgE antibodies should be evaluated with caution when:
  a. total IgE values are above 1000 kU/l
  b. total IgE values are above 500 kU/l when testing for specific IgE antibodies to beta-lactames and chlorhexidine (ImmunoCAP Allergen c1, c2, c5, c6, c7 & c8)
- In food allergy, circulating IgE antibodies may remain undetectable despite a convincing clinical history. The 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- or venom specific IgE determination indicates the absence of specific IgE antibodies to that drug or venom. Such results do not preclude existence of current or future clinical hypersensitivity to drugs or venoms (5, 6, 8, 9).
- Samples with results below limit of quantitation obtained with ImmunoCAP Allergen Components are recommended to be tested with the corresponding extract based ImmunoCAP Allergen, if not already performed. Additional extract based testing will cover additional allergen components present in the allergen source material to which the patient may be sensitized.
- A result below limit of quantitation obtained with an extract based ImmunoCAP Allergen never excludes the possibility of obtaining measurable concentrations of specific IgE when testing with ImmunoCAP Allergen Components from the same allergen source. This is due to the fact that some components may be present in very low amounts in the natural extract.

EXPECTED VALUES[(b)]

Good laboratory 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 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 (10, 11, 12).

Expected values for other applications of specific IgE are provided in the DFUs for:
- ImmunoCAP Phadiatop (Art No 14-4405-35)
- ImmunoCAP Phadiatop Infant (Art No 14-4510-35)
- ImmunoCAP Total IgE Low Range (Art No 14-4497-35)

PERFORMANCE CHARACTERISTICS

Instrument comparison
Comparison studies[(b)] have been performed with different combinations of Phadia instruments, including more than 180 patient samples and more than 45 ImmunoCAP Allergens. The results obtained show good concordance between instruments.

Precision[(b)]
The following pooled coefficients of variation have been obtained with Phadia 1000 when testing representative allergens from 7 allergen groups. Each sample has been assayed in 2 replicates on 18 different occasions using stored calibration curves. The values are representative for all Phadia instruments.

Sample level (kU_A/l)	Coefficients of variation (%)	
	Within assay	Between assay
0.35 – 1.5	4	4
1.5 – 50	5	5
50 – 100	5	9

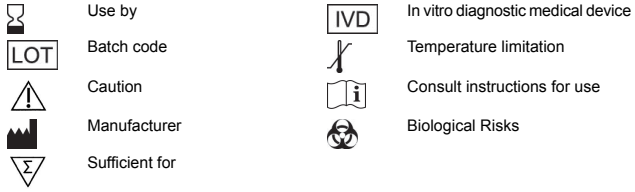
Analytical sensitivity[(b)]
The overall limit of quantitation (13) for allergen specific IgE antibodies is 0.1 kU_A/l.

Analytical specificity[(b)]
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



REFERENCES

1. Wide L, Bennich H,Johansson SGO. Diagnosis of allergy by an in vitro test for allergen antibodies. Lancet 1967;2:1105-7.
2. Johansson SGO, Yman L. In vitro assays for immunoglobulin E. Clin Rev Allergy 1988;6(2):93-139.
3. CSLI. 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 Vol. 29 No. 9.
4. Tate J, Ward G: Interference in Immunoassay. Clin Biochem Rev 2004:25:105-120.
5. Guttormsen A.B, Johansson S.G.O, Öman H., Wilhelmsen V., Nopp A. No consumption of IgE antibody in serum during allergic drug anaphylaxis. Allergy 2007; 62: 1326-1330.
6. Goldberg A., Confino-Cohen R. Timing of venom skin tests and IgE determinations after insect sting anaphylaxis. J Allergy Clin Immunol 1997; 100:182-184.
7. Yman L. The new generation of allergy testing. Data on file.
8. Wide L, Juhlin L. Detection of penicillin allergy of the immediate type by radio-immunoassay of reagins (IgE) to penicilloyl conjugates. Clin Allergy 1971; 1: 171-7.
9. Kraft D, Wide L. Clinical patterns and results of radioallergosorbent test (RAST) and skin tests in penicillin allergy. Br J Dermatol 1976;94:593-601.
10. Johansson SGO, ed. Clinical Workshop. IgE antibodies and the Pharmacia CAP System in allergy diagnosis. Lidköping: Landströms 1988.
11. Pastorello EA, Incorvaia C, Pravettoni V, Bonini S, Canonica GV, Ortoloni C et. al. A multicentric study on sensitivity and specificity of a new in vitro test for measurement of IgE antibodies. Ann Allergy 1991;67:365-70.
12. Paganelli R, Ansotegui IJ, Sastre J, Lange CE, Roovers MH, de Groot H, et al. Specific IgE antibodies in the diagnosis of atopic disease. Clinical evaluation of a new in vitro test system, UniCAP, in six European allergy clinics. Allergy 1998;53(8):763-8.
13. NCCLS EP17-A. Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline – First Edition, National Committee for Clinical Laboratory Standards, 2004.
14. US Pharmacopeia & National Formulary, current edition.
15. European Pharmacopoeia, current edition.
16. CLSI C3-A4, Preparation and Testing of Reagent Water in the Clinical Laboratory, Approved Guideline-Fourth Edition, Clinical and Laboratory Standards Institute, 2006.

Notes
[(a)]For more information, see Phadia 100, Phadia 250, Phadia 1000 and/or Phadia 2500/Phadia 5000 User Manual.

[(b)]Studies performed at Phadia AB, Uppsala, Sweden.

Patents/Trademarks
The following designations are trademarks belonging to Phadia AB:

ImmunoCAP, Phadia, Phadiatop, Quality Club.

Trademark change: Phadia AB has changed the trademarks of the instrument platforms from "UniCAP®" and "ImmunoCAP®" to "Phadia®". The new name has been applied to the instruments and related items, e.g. Software and User Manuals. The trademark "ImmunoCAP®" has been removed from the System Reagents. This is a trademark change only; the change has no impact on performance or safety.

Addresses

AUSTRIA Phadia Austria GmbH
Donau-City-Straße 1
AT-1220 Vienna
Tel: +43-1 270 2020 Fax: +43-1 270 202020
BELGIUM Phadia NV/SA
Pontbeekstraat 2
BE-1702 GROOT-BIJGAARDEN
Tel: +32-2 749 55 15 Fax: +32-2 749 55 23
BRAZIL Phadia Diagnósticos Ltda.
Rua Luigi Galvani, 70-10° andar - conj. 101
Cidade Monções - São Paulo – SP
Cep: 04575-020
Tel: +55-11 3345 5050 Fax: +55-11 3345 5060
CHINA Thermo Fisher Scientific (China) Co., Ltd.
Building 6-7
No. 27 Xin Jin Qiao Road
Shanghai 201102
Tel. +86 800 810 5118 Fax. +86 400 650 5118
CZECH REPUBLIC Phadia s.r.o.
Drahobejlova 1019/27
19000 PRAHA 9
Tel: +420 220 518 743 Fax: +420 220 518 743
DENMARK Phadia ApS
Gydevang 33
DK-3450 ALLERØD
Tel: +45-70 23 33 06 Fax: +45-70 23 33 07
FINLAND Phadia Oy
Ratastie 2
P.O. Box 100
FIN-01621 VANTAA
Tel: +358-9 3291 0110 Fax: +358-9 3291 0531
FRANCE Phadia S.A.S.

BP 610
FR-78056 ST QUENTIN-EN-YVELINES CEDEX
Tel: +33-1 61 37 34 30 Fax: +33-1 30 64 62 37
GERMANY Phadia GmbH
Postfach 1050 DE-790 10 FREIBURG
Tel: +49-761 47 805-0 Fax: +49-761 47805-338
HONG KONG
Thermo Fisher Scientific (Hong Kong) Limited
Unit 11-15, 9/F Tower 1
Grand Central Plaza
138 Shatin Rural Committee Road, Shatin
New Territories, Hong Kong
Tel: +852 2885 4613 Fax: +852 2567 4447
INDIA Phadia India Pvt. Ltd
Unit No.07, 10 & 11, ground floor,
Splendor forum, plot no 03
Jasola, District Centre,
NEW DEHLI-110025
Tel: +91 11 461 075 55 / 56 Fax: +91 11 461 075 57
IRELAND Phadia Ltd. (Irish Branch)
27 Oakhill
Moate
Co. Westmeath
Tel: +44 1800 625 167 Fax: +44 1800 625 168
ITALY Phadia S.r.l.
Via Libero Temolo, 4
IT-201 26 MILANO
Tel: +39-0264 163 411 Fax: +39-0264 163 415
JAPAN Phadia K.K.
Tokyo Opera City Tower
3-20-2, Nishi-shinjuku,
Shinjuku-ku TOKYO JP-163-1431
Tel: +81-3 5365 83 32 Fax: +81-3 5365 83 36
KOREA Phadia Korea Co. LTD.,
20 Fl, IT Mirea Tower
60-21, Gasan-dong Geumcheon-gu
Seoul 153-801
Tel: +82-2-2027-5400 Fax: +82-2-2027-5404
THE NETHERLANDS Phadia B.V.
Postbus 696
NL-3430 AR NIEUWEGEIN
Tel: +31-30 602 37 00 Fax: +31-30 602 37 09
NORWAY Thermo Fisher Scientific
Phadia AS
Postboks 4756, Nydalen
NO-0421 OSLO
Tel: +47-21 67 32 80 Fax: +47-21 67 32 81
PORTUGAL Phadia Sociedade Unipessoal Lda
Lagoas Park - Edifício n°11 - Piso 0
PT-2740-270 PORTO SALVO
Tel: +351-214 23 53 50 Fax: +351-214 21 60 36
SOUTH AFRICA Laboratory Specialities (PTY)
A Phadia Company
P.O. Box 1259
Ferndale 2160
Tel: +27 11 792 6790 Fax: +27 11 793 1064
SPAIN Phadia Spain SL.
Avda. Alcalde Barnils nº 70 Planta 2
Edificio Onada
08174 Sant Cugat del Vallés, Barcelona
Tel: +34-935 765 800 Fax: +34-935 765 820
SWEDEN Phadia AB,
Marknadsbolag Sverige
P O Box 6460 SE-751 37 UPPSALA
Tel: +46-18 16 60 60 Fax: +46-18 16 63 24
SWITZERLAND Phadia AG
Sennweidstrasse 46
CH-6312 STEINHAUSEN
Tel: +41-43 343 40 50 Fax: +41-43 343 40 51
TAIWAN Thermo Fisher Scientific Taiwan Co., Ltd.
6F-1, No. 85, Jhouzih St., NeiHu District
Taipei City 11493
Tel. +886 2 8751 6655 Fax. +886 2 8751 5353
UNITED KINGDOM Phadia Ltd
16 Shenley Pavilions, Chalkdell Drive
Shenley Wood, Milton Keynes, MK5 6LB
Tel: +44-1908 76 91 10 Fax: +44-844 324 94 95
USA Phadia US Inc.
4169 Commercial Avenue
Portage, Michigan 49002
Tel: +1 800-346-4364 (Toll Free) Fax: +1 269 492-7541
OTHER COUNTRIES Phadia AB,
Distributor Sales
P O Box 6460, SE-751 37 UPPSALA
Tel: +46 18 16 50 00 Fax: +46 18 16 63 65

Phadia AB,
Rapsgatan 7P, P. O. Box 6460, 751 37 Uppsala, Sweden
Tel: +46 18 16 50 00 Fax: +46 18 14 03 58

Issued October 2011. Revised June 2014.