2. Introduction

Immunoglobulin E is a serum protein and the main carrier of reactive activity of type I allergic reactions (immediate type). IgE circulates in blood. The surface of mastocytes and IgE which is bound to basophil granulocytes are responsible for the clinical symptoms of the type I reaction. Binding occurs on the Fc component of the IgE molecule. If an allergen comes into contact with the corresponding (specific) cell-bound IgE, mastocytes and IgE circulate in serum. For in-vitro diagnosis, the allergen-specific IgE is released. Cell-bound specific IgE cannot be determined with the test procedure for the detection of circulating IgE. Therefore, the results should only form part of a diagnostic concept for the determination of the specific IgE in serum, which also includes a detailed history and skin and challenge tests.

3. Test Principle

The quantitative determination of the circulating specific IgE in serum is carried out by means of a non-competitive enzyme immunoassay. The solid phase consists of a chemically activated paper disc on which the corresponding allergen covalents are bound. In the first step, the patient’s serum or plasma is pipetted on this allergen disc. Here the allergen-specific IgE binds with the allergen which is bound in the solid phase. Excess serum or plasma is then removed in a washing stage. In the second step an enzyme-labeled anti-human-IgE is placed on the disc which contains the allergen-IgE complex. Here the marked anti-human-IgE is bound to the specific IgE which is bound to the solid phase. Upon binding the anti-human-IgE is removed in a washing stage. Excess serum or plasma is then removed in a washing stage. The quantity of bound and marked anti-human-IgE is proportional to the quantity of the specific IgE in the serum sample. In the next step a substrate solution (p-nitrophenyl phosphate) is added. Due to the activity of alkaline phosphatase, a coloured solution is obtained. At the end of the incubation period the enzyme reaction is terminated with a stop solution. The extensions of the coloured solutions are measured with a photometer. Evaluation is performed by means of a standard curve consisting of the extinction values of the measured reference wells.

4. Content of the specific IgE 96 test kit

- 1. CONJ Conjugate: 1 bottle with 5 ml monoclonal anti-human-IgE monoconjugated with alkaline phosphatase in a buffered protein solution; preservation agent: 0.02% sodium azide. Differences in colour do not affect the efficacy of the conjugate.
- 2. WASP: 25 ml Washing solution (concentrate); 1 bottle with 100 ml concentrated sodium chloride solution with Tween 20; preservation agent: 0.05% saponin (for production of the washing solution see 10.2).
- 3. SUBS Substrate: 1 bottle with 20 ml p-nitrophenyl phosphate (pNPP).
- 4. STOP Stop solution: 1 bottle with 10 ml 1 M sodium hydroxide solution.
- 5. MP Micro-titration plate: 1 plate with flat bases (96 wells, breakable).

5. Additional materials and devices

1. Calibration system:
   - CAL DISC: Calibration discs: two boxes each with 20 reference discs (anti-human-IgE), preservation agent: 0.02% sodium azide.
2. Test discs:
   - CAL SERUM: Calibration sera 1, 2, 3, 4, 5: Five bottles, each with 0.5 ml human serum with total IgE calibrated against WHO IRP 75/502. Preservation agent: 0.02% sodium azide. Contains bovine serum albumin (BSA). The calibrators are filled in increasing concentrations:
   - CAL SERUM: Calibrator 1 = 0.35 IU/ml;
   - CAL SERUM: Calibrator 2 = 1.0 IU/ml;
   - CAL SERUM: Calibrator 3 = 3.5 IU/ml;
   - CAL SERUM: Calibrator 4 = 10 IU/ml;
   - CAL SERUM: Calibrator 5 = 50 IU/ml.
3. Allergen discs:
   - Allergen discs are available in packages containing 10 or 25 discs. Preservation agent: 0.02% sodium azide.
4. Materials and equipment:
   - Micro-pipette with disposable tips 50 µl
   - Manual hand dispenser e.g. Eppendorf Multipette with Combitips 2.5 and 5 µl
   - Micropipettes, stop watch, printer
   - Micro-titration plate photometer 405/450/620 nm (e.g. TECAN Spectra or TECAN Sunrise)
   - Incubator Omega Diagnostics (37 °C)
   - Washer for micro-titration plates (e.g. TECAN-Columbus Washer or Omega Diagnostics manual washer)

6. Limitations of the procedure

- Reliable and reproducible results can only be obtained if the test is performed adequately (see test procedure, Section 10).
- If several micro-titration plates are used in a test, the incubation times of the individual plates must be observed.
- The use of samples other than human serum or plasma has not been validated in this test.
- There is no reuse protocol for this product.
- The clinical diagnosis should not simply be based on the sole evidence of specific antibodies, but rather on other test results and test results. The enzyme immunoassay determination of specific IgE should never be used as the sole diagnostic decision criteria for starting any hypersensitive treatment. In addition, the above-mentioned test results and – if possible - challenge tests should be performed to provide evidence of clinical relevance (see literature 1).
- Especially in the case of allergies to foodstuffs, there may be a negative in-vitro result although there are severe clinical symptoms. This can be explained by the fact that foodstuffs undergo significant changes due to maturing, industrial processing, boiling or frying etc., as well as due to the digestion process, so that under certain circumstances protein structures completely different from those on the solid phase of the allergen substrate may be present. Furthermore, several foodstuffs are highly delicate, so that not all of the allergens which are present in the native state can be bound to the solid phase.
- Human serum albumin is used as a spacer substance for the in-vitro determination of haptenes. With this, a reproducible pseudo-antigen for in-vitro determination is obtained. Of course, this process cannot completely depict the possible reactions of a hapten in the human body. Because of this, the in-vitro test cannot produce a positive result in all cases where there are positive clinical symptoms.
- In general, negative results for insect toxins only provide evidence that at the time, no circulating specific IgE against the tested insect toxins can be detected in serum or plasma. This does not lead to the conclusion that the patient will not at present, or in the future, develop clinical symptoms in case of an insect sting. In the case of insect toxins, there may be a temporary consumption of the antibodies some time after exposure, so that no specific IgE antibody titre can be detected at the time of the measurement.
- Positive in-vitro results may occur if, among other things:
   - the symptoms are not caused by IgE;
   - the sample was taken before the body was able to produce specific IgE against the antigen;
   - the IgE level has returned to a low level a long time after sensitisation.
- Different results with different patients do not cause the same reaction, as this varies according to the individual.
- Positive results for specific IgE in-vitro test need not automatically cause the same clinical symptoms.

Many IgE antibodies show a cross-reaction with other IgE reacting substances, such as pollen/celery, latex/banana. The diagnosis must take this into account.

7. Specific performance data

- Analytical specificity
  - No evidence of influence due to immunologically interacting and/or cross-reacting substances was found.
- Analytical sensitivity
  - Lower measurement range in the lower measurement range/between Standard 1 and 2: > 0,060 U/ml
  - Upper measurement range/between Standard 4 and 5: > 0,009 U/ml
- Diagnostic specificity
  - > 90 %
- Diagnostic sensitivity
  - 72 – 116 %
- Reproducibility
  - Inter-Assay [basic classes]: CV < 8 %
  - Intra-assay [basic classes]: CV < 5 %
  - Lowest detection level: < 0.35 U/ml
- Measurement range: 0.35 – 50 U/ml
- Traceability of the specific IgE calibrators: WHO IRP 75/502

8. Relevant interferences

- Icterus
  - < 0.1 mg/ml bilirubin
  - No impairment
- Haemolysis
  - < 8 mg/ml haemoglobin
  - No impairment
- Lipidaemia
  - < 5 mg/ml triglyceride
  - No impairment
- Non-specific IgE
  - < 1000 U/ml

9. Preparation and storage of specimen

Serum and plasma which has been stored for up to 5 days at 2 to 8 °C can be used. If the test is not performed within this time, it is recommended that the sample is frozen at –20 °C (storage time at –20 °C at least 6 months). Avoid repeated thawing and freezing.

10. Test procedure

Warning. Only use a maximum of 4 micro-titration plates for each test run.

This test can be carried out with 2 different incubation versions. [VERSION A]: Short incubation / [VERSION B]: Long incubation.

Either [VERSION A] or [VERSION B] must be carried out. Combining the two versions is not permitted and leads to incorrect results.

1. Before starting the test, all components must be heated to room temperature (RT, 20 to 25 °C).
2. Preparation of the washing solution: 75 ml of the washing solution concentrate to 1500 ml with distilled water (for reasons of simplicity, 100 ml of concentrated washing solution can be diluted to 2000 ml of distilled water). After dilution, e.g. birch pollen/aggregate, mugwort, ragweed, pollin/celery, latex/banana. The diagnosis must take this into account.

Many IgE antibodies show a cross-reaction with other IgE reacting substances, such as pollen/celery, latex/banana. The diagnosis must take this into account.

Please note: A double determination of the reference values is necessary.
4. With a pair of tweezers, first place the reference discs and then the allergen discs with the specific allergens in the wells provided. Well A1 (substrate blank value) remains empty.

5. Pipette 50 µl of each of the reference serums 1 – 5 onto the corresponding reference discs. Then pipette 50 µl of the serum or plasma samples into the wells provided. Well A1 (substrate blank value) remains empty.

6. Cover the micro-titration plate and [Version A] incubate for 1 hour at 37 °C or [Version B] incubate for 3 hours at room temperature.

7. Wash the wells of the micro-titration plates either with the automatic washer (4x) or with the manual washer (5x, please observe the operating instructions) Only washing procedures approved by Omega Diagnostics must be used.

8. Pipette 50 µl of the conjugate solution directly onto each of the allergen discs, however not into the blank substrate value. Then cover the micro-titration plate again. [VERSION A] incubate for 1.5 hours at 37 °C or [VERSION B] incubate for 6 to 24 hours at room temperature.

9. Wash as described under 10.7.

10. Pipette 200 µl substrate solution into all of the wells (including the blank substrate value). Cover the micro-titration plate and with exclusion of light incubate for 1 hour at 37 °C [Version A] or [Version B] incubate for 1 hour at room temperature.

11. In the same manner and sequence as for pipetting of the substrate solution, now add 100 µl of stop solution to all of the wells (incl. the blank substrate value).

12. After stopping the reaction with the stop solution, the colour complex must be measured within 30 minutes. Place the micro-titration plates with the stopped coloured solution in the photometer. The measurement is made through the disc on the base of the micro-titration well.

For Omega Diagnostics device systems with Allervance software:

The measurement is made with a 3-wavelength method (405, 450 nm as measurement wavelengths and 620 nm as the reference wavelength). This enables the calculation of the values over a larger measurement range.

For Omega Diagnostics device systems without Allervance software:

The measurement is made at 405 nm and the reference wavelength 620 nm. The combined measurement with 405/620 nm must be adhered to. If the evaluation of the 5th reference is not calculated, i.e. the value is not printed out, the measurement range of the physio has been exceeded. In this rare case, over-pipetting must be carried out. To do this, transfer 250 µl from each well into an empty micro-titration plate (same scheme) and measure again at 405/620 nm.

Once the test has been started it must be continued without interruption, and all individual steps, temperatures and reaction times must be complied with.

Warning! If significant changes are made to the test procedure (e.g. time, sequence, temperature etc.) or if significant impairment of the analysis performance is seen, even with correct use (e.g. control values out of specifications, significant differences in double values etc.) the values which are obtained must not be used. A check of the system or the procedure is essential before continuing work. In case of doubt please contact the specialists at Omega Diagnostics.

11. Calculation

With Omega Diagnostics devices calculation of the reference curve and the evaluation of the measurement results are carried out automatically.

The standard curve can be calculated manually by entering the extents determined for the calibrators against the standard unit values on semi-logarithmic graph paper and connecting the individual points with a ruler. This standard curve is used to determine the values of the serum or plasma samples.

The following relationship exists between U/ml and allergosorbent test (EAST) classes:

- Values < 0.35 /ml = EAST class 0
- Values > 0.35 U/ml = EAST class 1
- Values > 0.7 U/ml = EAST class 2
- Values > 3.5 U/ml = EAST class 3
- Values > 17.5 U/ml = EAST class 4
- Values > 50 U/ml = EAST class 5

12. Normal values

- Values < 0.35 U/ml = EAST class 0 are considered as negative
- Values > 0.35 U/ml = EAST class 1 are considered as positive

See also Section 6 “Limitations of the procedure” and references 1 and 2.

13. Warnings and precautions

The following rules must be observed:

1. The relevant safety regulations must be observed when handling the test components.

2. References and examination samples are potentially infectious substances. Suitable agents or methods must be used to disinfect contaminated areas. The references do not show any reactivity to HBsAg, HCV and HIV-1/2.

3. The stop solution contains sodium hydroxide. Wear protective gloves / protective clothing / eye protection / face protection. In case of contact with the skin (or hair), take all contaminated clothing immediately. Wash or shower the skin with water. In case of contact with the eyes: carefully rinse with water for several minutes. If possible, remove any contact lenses. Continue rinsing. Inform the poison centre or doctor immediately. Wash contaminated clothing before wearing it again.

4. Smoking, eating and drinking are prohibited in the laboratory. Do not ingest!

5. Do not suck the pipette with your mouth!

6. Close all reagents after use. The closures must not be mixed.

7. Do not use damaged or contaminated kit components.

8. Avoid cross-contamination when pipetting!

9. Test components from different batches must not be mixed.

10. Reagents must not be used after the expiry date.

11. Reference samples and kit controls must be included with every assay array performed to ensure correct results.

12. The functionality and accuracy of the equipment used (pipettes, photometer etc.) must be checked at regular intervals. Observe the manufacturer’s instructions!

13. Reagents and chemicals must be handled and disposed of according to the applicable regulations.

List of supplied substances which may require special treatment for disposal:

- Concentrated (sodium azide <0.1% w/w CAS 26628-73-2)
- Bovine serum albumin CAS 90604-29-8
- Washing solution (sodium hydroxide 1 M CAS 26628-22-8)
- Stop solution (sodium hydroxide 1 M CAS 1310-73-2)
- Substrate (p-Nitrophenylphosphate CAS 622-85-6)

14. Quality control

- **Internal quality control**

  It is recommended that for each test set at least one positive serum and patient serum are used in the test. Omega Diagnostics provides such control sera. For the positive control, the normal ranges are stated by Omega Diagnostics. If the positive control is within the normal ranges, it can be assumed that the test method is functioning correctly.

- **External quality control**

  Participation in external quality controls (ring tests) is recommended. Here, samples with unknown analytical concentrations are not known to the laboratory participating in the external quality control are sent by a ring test provider. After collection of the results, the ring test provider evaluates and assesses the results from all senders. Details must be obtained from the ring test provider. Please contact Omega Diagnostics or your in-vitro sales representative.

15. Storage of the test kit

- 2 to 8 °C

16. Expiry date

The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Expiry date is the last day of the month on the bottle and the kit label. Do not use reagents after the expiry date.