Arlington Scientific, Inc.

IVT Allergy™ 3 Screen

Diagnostic Enzyme Immunoassay for the Determination of Specific IgE in Human Serum

INTENDED USE

Diagnostic enzyme immunoassay for the qualitative determination of allergen specific IgE antibody in human serum or plasma. These materials are intended to be acquired, possessed and used only by health professionals for in vitro diagnostic use.

SUMMARY AND EXPLANATION OF THE TEST

IgE antibody is the class of antibodies in the serum of atopic individuals which are mainly responsible for the immediate type allergy symptoms after exposure to specific allergens[1,2]. The Radioallergosorbent Test or RAST has been developed for the quantitation of the level of IgE antibodies to various allergens[3]. In addition, quantitation of Total IgE levels in serum can provide valuable information for the identification of individuals predisposed to allergies. The serum concentration of Total IgE is elevated above normal in the majority of allergic patients[4] and in hereditary predisposed children[5]. Screening for allergies by testing against the most common allergens and for Total IgE has been recommended as a cost effective method of identifying those atopic individuals who are most likely to benefit from a detailed allergy investigation[6,7]. Clinical manifestations of allergy may include anaphylaxis, hay fever, asthma, atopic eczema, dermatitis, urticaria, respiratory distress and rhinitis. The medical history often provides valuable clues and occasionally definitive information on the offending allergen[8].

The IVT ALLERGY PROFILE is an *in vitro* qualitative enzyme immunoassay for the simultaneous, but separate, identification of various common allergies and for the estimations of Total IgE levels in human serum or plasma. The unique device consists of a capillary tube containing a series of plastic segments. On the surface of the plastic segments are insolubilized single allergens or mixes of allergens or monoclonal antibody for immune complexing of IgE in the patient sample. The IVT ALLERGY PROFILE device is available in various formats differing in the selection of allergens from grasses, weeds and trees indigenous to defined geographical regions, mites, molds, epidermals and the most common food allergens for the determination of circulating allergen specific IgE antibody. Some profiles contain an additional segment for the estimation of the levels of Total IgE. Negative and positive control segments are incorporated in each device to assist with the visual interpretation of results.

PRINCIPLE OF THE PROCEDURE

The IVT ALLERGY PROFILE is a solid phase, enzyme linked immunosorbent assay designed to detect simultaneously, but separately in a single test specific IgE antibodies to common allergens. Some test formats provide an estimate of Total IgE levels in patient samples. IgE immunoglobulin or antibody in the sample is allowed to react for 60 ± 5 minutes in the multiple immunoassay device. After rinsing, the device is filled with an anti-IgE-Urease CONJUGATE and reacted for 40 ± 5 minutes or longer. The device is then thoroughly rinsed again and filled with SUBSTRATE/INDICATOR. IgE bound to a particular reactive segment will bind CONJUGATE which triggers a visually detectable yellow to purple color change about that segment within the next 15 minute period, permitting the positive identification of allergies to one or more of the specific allergens in the test.

REAGENTS

Components in each IVT ALLERGY PROFILE kit. See individual component labels for expiration dates.

- **5 Multiple Immunoassay Devices** - Capillary board containing alternating inert white spacers and reactive clear segments. Coupled to the surface of the reactive segments are insolubilized allergens for the detection of IgE antibodies or anti-IgE antibody for the estimation of Total IgE. Within each device is incorporated an inert negative control segment and a positive control segment with insolubilized human IgE to monitor the proper functioning of the CONJUGATE and SUBSTRATE/INDICATOR reagents.
- **1 Vial Conjugate** - 4 ml of Goat anti-IgE-Urease enzyme conjugate in physiological saline with protein stabilizers and 0.1% sodium azide as a preservative. Contains green dye as a coloring agent.
- **5 Vials Substrate/Indicator** - 2.5 ml each, containing bromocresol purple indicator and urea substrate provided at pH 4.8. Yellow color is inherent to this reagent at the proper pH.
- **1 Bottle Wash Solution** - 55 ml of detergent in saline solution.
- **5 Syringes** - 1ml Syringe - Non-sterile syringe is connected to device in order to facilitate loading of sample, reagents and wash solution.
- **10 ml Plastic Beaker** - Small plastic cup for sample transfer and/or use in wash procedure.

STORAGE INSTRUCTIONS FOR KIT - Upon receipt, store contents of the IVT Allergy 3 Screen at 2-8° C.

WARNINGS AND PRECAUTIONS - For In Vitro Diagnostic Use Only. Disposal of solutions containing sodium azide:

CAUTION: CONJUGATE reagent contains sodium azide which may react with lead and copper plumbing to form potentially explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.

CAUTION: The positive control in the Multiple Immunoassay Device is prepared from human blood products which have been tested and found negative to HIV and HBsAg. All blood products should be treated as if capable of transmitting infectious agents.

SPECIMEN COLLECTION/PREPARATION
No special preparation of the patient is necessary and fasting is not required. Serum is preferred; however, plasma derived from the use of ethylenediamine-tetra-acetate (EDTA) or heparin may be employed. Handle specimens as if capable of transmitting infectious agents.

STORAGE
Serum or plasma may be capped and stored at 2-8° C for up to 5 days prior to testing. Specimens may be capped and frozen at -20° C for 3 months, but repeated freezing and thawing should be avoided.

KNOWN INTERFERING SUBSTANCES
Specimens which exhibit gross hemolysis and severe lipemia are not recommended. Particulate material in samples should be avoided. Steroid intake by the patient may affect serum levels of antibodies. Parasitism may produce elevated levels of IgE which might result in false positives.

PERFORMANCE OF THE TEST

Materials Provided: Quantities sufficient for 5 tests:
- Multiple immunoassay devices
- Conjugate
- Substrate/Indicator
- Wash solution
- 1cc syringes
- Plastic beaker

Additional Materials provided:
- Scissors
- Sink with tap water
- Tissue
- Timing device

PRELIMINARY COMMENTS
All components in the test kit must be of the same master lot number. Components should not be used following the indicated expiration date. All components should be allowed to come to the temperature range of 20-30° C before use. Store all components at 2-8° C when not in use.

TEST PROCEDURE:
1. Cut off the extreme sealed tip and remove the red plug from properly labeled multiple immunoassay device, allowing storage buffer to drain into sink. Blot tip with tissue.
2, Attach a syringe to the connector on the device and draw up the serum sample or control sample through the device to the syringe connector.
3. Lay the device on the workbench and allow to react for 60 ± 5 minutes at room temperature (20-30° C).
4. Expel the sample from the device, remove the syringe plunger, fill the syringe barrel with 1 ml WASH SOLUTION and allow to drain through the device. Insert the syringe plunger completely to the bottom of the syringe and wipe tip using a tissue.
5. Draw up the CONJUGATE reagent (green reagent) through the device to the syringe connector.
6. Lay the device on the workbench and allow to react for 40 ± 5 minutes at room temperature (20-30° C).
7. Expel the CONJUGATE reagent from the device, remove the syringe plunger. Add 5 DROPS of WASH SOLUTION into syringe barrel. Allow to drain. Fill the syringe with 1 ml WASH SOLUTION and allow to drain through the device. Insert the syringe plunger completely to the bottom of the syringe and wipe tip using a tissue.
8. Put a small amount (about 2 ml) of WASH SOLUTION into supplied plastic cup. Draw up WASH SOLUTION through the device substantially into the syringe. Hold the device over the sink, remove the syringe plunger and allow to drain.
9. Fill the syringe barrel with 1 ml WASH SOLUTION and allow to drain. Repeat once. Insert the syringe plunger completely and wipe tip using a tissue.
10. Draw up the SUBSTRATE/INDICATOR (yellow reagent) through the device substantially into the syringe.
11. Carefully lay the device on the workbench and observe for the characteristic yellow to purple color change within the next 15 minute period. ***NOTE: To ensure sharp, specific color changes, the device, once filled with SUBSTRATE/INDICATOR, should not be disturbed so as to cause fluid movement within the device.***

PROCEDURAL NOTES:
1. Since the reaction occurs only in the device tubing, to conserve on serum and CONJUGATE reagent, fill the device up to the syringe connector only, not into the syringe. Draw up SUBSTRATE/INDICATOR substantially into the syringe.
2. When filling the device with reagent (sample, CONJUGATE or SUBSTRATE/INDICATOR), draw the reagent up quickly but smoothly to displace air in the capillary.
3. When filling the device with sample or CONJUGATE, in the event of excessive trapped air bubbles in the capillary, the device can be emptied and refilled without intermediate washing.
4. When the device is filled with SUBSTRATE/INDICATOR, enzyme-catalyzed conversion of substrate to product (seen as a yellow to purple change) begins immediately. To ensure sharp specific color changes, the apparatus, once filled with SUBSTRATE/INDICATOR, should not be disturbed so as to cause fluid movement within the capillary.

5. Should the device be inadvertently disturbed during the color reaction, or an inadequate wash in steps 7-9 be suspected, repeat of steps 9-11 can be done within 10 minutes of filling without a sacrifice in test results.

6. When removing the syringe plunger for washing, it should be withdrawn slowly to avoid foaming in the syringe barrel.

CAUTION: Individuals with visual impairments which could inhibit the interpretation of results should not perform this test.

INTERPRETATION OF RESULTS

The IVT ALLERGY PROFILE is a qualitative test designed to identify IgE levels in the test samples. A positive result is identified visually by a gradual yellow to purple color change around a reactive segment within the capillary. The test is also semiquantitative in that the rate of conversion from yellow to purple and the intensity of the purple color is proportional to the level of IgE antibody or immunoglobulin in the patient sample. Highly allergic reactions to a particular allergen will cause a rapid, intense purple coloration around that specific segment within 5 minutes. Weaker allergies will take progressively longer to effect the color change. Negative reactions at reactive segments will show equivalent color to the inert spacers on either side of the reactive segment.

On the Total IgE segment, low Total IgE levels (<30 IU/ml) will be evident by a yellow to gray coloration, distinguishable from normal IgE levels (60 +/-30 IU/ml) which yield moderately purple, and elevated IgE levels (>90 IU/ml), which yield intense purple coloration after 15 minutes incubation with SUBSTRATE/INDICATOR.

The optimum time of scoring is 15 minutes after filling with SUBSTRATE/INDICATOR. Sera containing IgE antibodies at moderate to elevated levels to one or more allergens on any segment will elicit a positive specific purple coloration within this period. IgE antibodies at low levels may be detected by allowing color development to proceed up to 30 minutes as long as specific reactions are clearly and visually distinguishable from nonspecific background coloration throughout the capillary.

LIMITATIONS OF THE PROCEDURE

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

2. Reliable and reproducible results will be obtained when the test procedure is carried out in accordance with the package insert instructions.

3. Parasitism may produce elevated levels of IgE which might result in false positives.

4. The kinetics of reaction of reagents in the test will increase with temperature. Reagents should be allowed to warm to room temperature before testing. The intensity of purple coloration with positive allergic samples may also vary with wide fluctuations in room temperature.

5. The SUBSTRATE/INDICATOR decomposes slowly on heating, evident by a gradual change in color from yellow to purple. Store capped at 2-8° C when not in use. Do not use if significantly colored purple.

6. Insufficient washing of free CONJUGATE in steps 7-9 can produce false positive results and is evident as diffuse or spotty yellow to purple coloration throughout the device, including the inert spacers separating reactive segments.

7. The IgE immune response to any given allergen differs in specificity and concentration among atopic individuals. In addition, the IgE-binding capacity may differ from allergen to allergen and among the allergen mixes. Consequently, identical results for different allergens or allergen mixes do not necessarily imply clinical equivalence.

8. A case history suggestive of allergen sensitivity, and the positive identification of IgE antibodies to allergens in the IVT ALLERGY PROFILE are strong indicators of IgE-mediated allergy. In such a case, the patient should be submitted to a detailed investigation prior to the implementation of therapeutic measures.

9. The IVT ALLERGY PROFILE incorporates numerous allergens to provide a high probability of identifying patients with IgE-mediated allergies[6]. A positive result on any segment does not exclude the patient's sensitivity to other allergens not incorporated in the test, particularly tree allergens which have limited allergenic crossreactivity[9]. Similarly, a negative result does not exclude the possibility of restricted sensitivity to an allergen not included.

10. Total IgE levels in 63% of healthy, nonatopic individuals are low (<20 IU/ml), and in 64% of atopic patients they are elevated at levels >50 IU/ml[4,10]. Elevated Total IgE levels are usually associated with atopic patients with mite, mold and multiple allergen sensitivity[10]. However, Total IgE levels may also be elevated as a result of parasitic infestations, immunological and dermatological deficiencies or disorders. Consequently, an elevated IgE level per se is not conclusive evidence of an allergy disorder but should be interpreted by a trained physician in conjunction with case history and testing against specific allergens. The IVT ALLERGY PROFILE provides an initial evaluation of the patient's serum for the Total IgE level and the presence of allergen specific IgE antibodies. The serum concentration of Total IgE is also age related, the geometric mean in newborns being 2 IU/ml, rising to adult levels over the first 5 years of childhood[11].

QUALITY CONTROL

Good laboratory practice employs the use of control sera to ensure proper assay performance.

PERFORMANCE CHARACTERISTICS

The overall performance of the IVT ALLERGY PROFILE has been evaluated by comparison against RAST.

IgE sensitivity, defined as the total number of positives in the IVT ALLERGY PROFILE divided by the number of positives in the reference method, was found to be greater than 90%.

Specificity, defined as the number of negatives in the IVT ALLERGY PROFILE divided by the number of negatives in the reference method, was found to be greater than 88%.

Reproducibility of the IVT multiple immunoassay system has been estimated by substituting 125I-labeled anti-IgE for the enzyme conjugate in the test and measuring the radioactivity bound to each reactive segment within the device. Data from 5 runs over 5 days

at 5 replicates per run indicate a pooled within run precision in the range of 7-15% coefficient of variation (CV) and a between run precision in the range of 5-13% CV for an overall precision in the range of 12-19% CV. The IVT ALLERGY PROFILE should provide a precision in the range of 10-15% CV due to lower sources of error than in the RIA method of estimation applied.

Correlation of Total IgE levels with allergen sensitivity: Total IgE levels (IU/ml) in serum samples have been quantitated relative to IgE Standards calibrated against the First British Standard for Human Serum IgE.

Total IgE level (per ml)	≤ 30 IU	31-90 IU	≥ 91 IU
90 Allergic Patients	3.1	10.8	86.1
90 Non allergic Patients	41.7	33.3	25.0

WARRANTY

This product is warranted to perform as described in its labeling and In Vitro Technologies, Inc. literature. ASI DISCLAIMS ANY IMPLIED WARRANTY OR MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE and in no event shall ASI be liable for consequential damages.

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