**QUANTITATIVE TURBIDIMETRIC IMMUNOASSAY FOR ESTIMATION OF IgE IN HUMAN SERUM**

## SUMMARY

Allergy a hypersensitivity reaction is mediated by immunological mechanisms that can be antibody or cell mediated. In majority of cases the antibody typically responsible for an allergic reaction belongs to IgE isotype and the individual is said to suffer from an IgE mediated allergic disease. IgE bind to the surface of mast cells and basophilic granulocytes. The subsequent binding of allergens to the cell-bound IgE causes the release of histamines and other vasoactive substances by these cells, thereby initiating events that are described as an allergic reaction. IgE is present in trace amounts in normal serum and has a very short half-life. IgE levels exhibit a slow increase during childhood, reaching adult levels in the second decade of life. Usually the total IgE level increases with number of allergies that a person has and with the amount of exposure to relevant allergens. Measurement of serum IgE is useful to determine if the allergic reaction is IgE mediated or non-IgE mediated. IgE levels can increase in atopic diseases, neoplasm, immunodeficiencies and parasitic infections. Estimation of total circulating IgE would also be useful in early detection of allergy in infants and as a means of predicting future atopic manifestations.

Several studies indicate an increase in IgE levels in parasitic infections and a significant fall in IgE concentration after effective therapy.

**Quantia®-IgE** is an immunoturbidimetric assay for the measurement of total IgE in human serum.

## REAGENTS

1. **Quantia®-IgE** Activation buffer (R1), ready to use
2. **Quantia®-IgE** Latex reagent (R2) - ready to use uniform suspension of polystyrene latex particles coated with anti IgE.
3. **Quantia®-IgE** Calibrator: ready to use and is equivalent to the stated amount of IgE on IU/ml bases. The **Quantia®-IgE** calibrator value is traceable to the International Reference Preparation of IgE 75/502 from WHO.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

## REAGENT STORAGE AND STABILITY

1. Store the reagents at 2 - 8 $^{\circ}$C. DO NOT FREEZE.
2. The shelf life of the reagent, activation buffer and the calibrator is as per the expiry date mentioned on the respective vial label.

## PRINCIPLE

**Quantia®-IgE** is a turbidimetric immunoassay for the determination of immunoglobulin IgE in human serum and is based on the principle of agglutination reaction. The test specimen is mixed with the Activation buffer (R1) and **Quantia®-IgE** Latex reagent (R2) and allowed to react. The presence of IgE in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at 630 nm wavelength. The extent of turbidity, corresponds to the concentration of IgE in the specimen.

## NOTE:

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use
2. All the reagents derived from human source have been tested for HBsAg and HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagent contains 0.095% Sodium Azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of reagent be verified with known controls.
5. Gently swirl each of the reagent vials to attain homogeneity prior to use for obtaining better performance.
6. Do not use vortex mixer for mixing reagents. Gently mix reagents and samples during test procedures.

7. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
8. The **Quantia®-IgE** kit is recommended only for analyzers with cuvette mode. Though any semi automated analyzer with appropriate programming facility can be used, for best results it is recommended to use **Quantiamate** analyzer. Fully automated analyzers may be used provided the reagent has been standardized on the system.
9. Calibrators of different manufacturers must not be used in this method.
10. In case of instruments where required minimum reading volume is 1.0 ml use double the quantity of reagents and samples mentioned in the test procedure.
11. Do not use damaged or leaking reagents.

**SPECIMEN COLLECTION AND PREPARATION**

No special preparation of the patient is required prior to specimen collection by approved techniques.
Only serum should be used for testing. Should delay in testing occur, store the sample at 2-8$^0$C, provided they are not contaminated. Samples can be stored for up to a week. Do not use hemolysed, icteric, or turbid serum specimen.

**ADDITIONAL MATERIAL REQUIRED**

Spectrophotometer with 630nm wavelength and cuvette mode, Stopwatch, appropriate micropipettes, disposable tips, isotonic saline, test-tube rack, incubator/ water bath set at 37$^0$C, Optically clean disposable/ glass square cuvettes.

**TEST PROCEDURE**

Bring reagents and samples to room temperature before use.

Assay conditions:

| | |
|---|---|
| Wavelength | 630nm |
| Reaction temperature | 37$^0$C |
| Cuvette path length | 1 cm |

**Method for preparation of IgE Calibration Curve**

The concentration (S) of **Quantia®-IgE** calibrator is as mentioned on the vial label and at the end of this package insert.
Dilute the **Quantia®-IgE** calibrator serially as mentioned below for preparation of calibration curve.

| Test Tube No. | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Calibrator dilution No. | D1 | D2 | D3 | D4 | D5 |
| Vol. of Calibrator | 100 µl | 50 µl D1 | 50 µl D2 | 50 µl D3 | 50 µl D4 |
| Vol. of Saline | - | 50 µl | 50 µl | 50 µl | 50 µl |
| Conc. (S) of IgE | S | S/2 | S/4 | S/8 | S/16 |

**Test procedure for preparation of calibration curve.**

(Note: Increasing concentration of calibrators must be used for preparing the calibration curve during programming on instruments)

1. Zero the instrument with distilled water.
2. Pipette 300 µl of activation buffer (R1) and 150 µl of **Quantia®-IgE** latex reagent (R2) in the measuring cuvette. Mix well and incubate for 5 minutes at 37$^0$C.
3. Add 10 µl of calibrator D1, mix gently (do not vortex) and start the stopwatch simultaneously.
4. Read absorbance (A1), **exactly** at 10 seconds, and absorbance (A2) again at the end of **exactly** five minutes.
5. Repeat steps No. 2 - 4 for each diluted calibrator (D2-D5) for preparing calibration curve.
6. Calculate ΔA (A2 - A1) for each calibrator (D1-D5). Plot a graph ΔA versus concentration of IgE on the graph paper provided with the kit.
7. "The calibration curve" so obtained is valid only for the same lot of **Quantia®-IgE** reagents.

**Test procedure for specimens**

1. For determination of IgE concentration in the test specimen please follow steps 2-4 using the test specimen in place of calibrator.
2. Calculate Δ (A2 - A1) for the test specimen.

**VALIDATION CRITERIA**

If the ΔA of the test specimen is less than ΔA obtained for the calibrator of highest concentration (D1) then the concentration of IgE in the test specimen can be determined directly by interpolating ΔA of the test specimen on the obtained calibration curve.

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If the ΔA of the test specimen is higher than ΔA of standard with highest concentration (D1) then the test has to be rerun by carrying out serial dilutions of test specimen such as 1:2, 1:4 etc. with isotonic saline till the ΔA of the diluted test specimen is less than ΔA of D1.

The IgE concentration for such samples can be determined as mentioned in **calculations.**

**CALCULATIONS**

1. Interpolate ΔA of the diluted test specimen on the calibration curve and obtain the IgE concentration 'C' of the diluted test specimen.
2. Multiply the IgE concentration 'C' with the dilution factor (F) of the test specimen for obtaining the concentration of IgE in the neat test specimen.
   Concentration of IgE in the neat test specimen in IU/ml = C x F. (Where 'F' is the dilution factor of the test specimen, for e.g. 2 for 1:2 dilutions of test specimen and so on).