PATHOZYME DIAGNOSTICS

IMMUNOGLOBULIN E (IgE)

(IMMUNOTURBIDIMETRIC METHOD)

CAT No.: IGE

Reagent kit for quantitative estimation of IgE in Serum

DIAGNOSTIC SIGNIFICANCE:

Immunoglobulin E is a 190000 g/mol protein produced by type I hypersensitivity reaction to antigens such as a grass pollen, food, parasites or insect secretion. IgE binds to mast cell, which degranulate and release mediators such as a histamine upon recontact with the antigen, causing clinical signs of allergy such as a rhinitis, urticaria, asthma and eczema. High levels of IgE also occurs in parasitism and immunodeficiency syndromes, such as acquired T-cells deficiency or wiskott-Aldrich syndrome. It is also useful in the diagnosis of recurrent respiratory disease and IgE type myelomas infants and young Childrens.

PRINCIPLE:

IgE test is used for the quantitative in vitro determination of total immunoglobulin IgE in serum samples. Anti-IgE antibodies covalently bound to latex particles react with the antigen (IgE) in the sample to form an antigen-antibody reaction complex, which can be measured turbidimetrically after particle aggregation.

SPECIMEN COLLECTION:

Fresh Serum specimens should be collected by venipuncture following good laboratory practices. Suitable assay specimens are human serum samples, as fresh as possible (specimen can be stored up to 7 days at 2-8°C maximum) or deep-frozen 6 months at -20°C). Any additional clotting or precipitation, which occurs due to the freeze/thaw cycle, should be removed by centrifugation prior to assay. Very lipemic specimens, or turbid frozen specimens after freeze-thaw, must be clarified before the assay by high-speed centrifugation (15 min at approx. 15000 RPM). Heat inactivation of serum samples results in loss of IgE antigenicity and therefore must be avoided.

KIT PRESENTATION:

PACK SIZE	1 X 20 ml	2 X 20 ml
R1 - IgE (Buffer Reagent)	1 X 18 ml	2 X 18 ml
R2 - IgE (Latex Reagent)	1 X 02 ml	2 X 02 ml
IgE Calibrator	1 No	1 No

REAGENT:

R1- Buffer Reagent:

Phosphate buffer, pH:7.0, containing protein stabilizers and < 0.1% sodium azide as preservative, PEG.

R2- Latex reagent:

Anti human IgE antibodies covalently bound to latex microparticles suspended in a neutral aqueous solution and sodium azide 0.09%.

REAGENT STORAGE AND STABILITY:

Unopened reagents and calibrator are stable till the expiry date mentioned on the label when stored at 2 – 8°C. Do not freeze.

Once opened reagent and calibrator should be stored immediately after assay run, tightly closed at 2 – 8°C and used within 1 month.

REAGENT PREPARATION:

The Reagents and Calibrator are ready to use. Shake the Latex Reagent (invert the container 3 – 4 times) before each use.

ASSAY PARAMETERS:

These data are to be interpreted as a guide. Each laboratory should establish its own reference intervals.

Reaction	Fixed Time With Multi Standard
Wavelength	600 nm (570 – 630)
Flow Cell Temperature	37°C
Reaction Slope	Increasing
Lag Time (Initial Delay)	5 Sec
Read Time	240 Sec
Zero Setting (Blank)	Distilled Water
R1 + R2 Volume	500 µl (450 µl R1 + 50 µl R2)
Sample Volume	20 µl
Calibrator Concentration	Stated on Vial label
Linearity	2000 IU/ml
Unit	IU/ml

CALIBRATION CURVE:

Prepare dilutions of the Calibrator using Normal Saline (0.9gm% NaCl) as diluent. Multiply the concentration of the Calibrator by the corresponding factor indicated in the table below to obtain the IgE concentration of each point of the curve.

	A	B	C	D	E	
Dilution Factor	1	2	4	8	16	Blank
Saline (µl)	---	50	50	50	50	--
Calibrator (µl)	100	50	50	50	50	--
Multiplying Factor	0	0.500	0.250	0.125	0.0625	--

For Dilution factor 1: calibrator as it is = (A)
For Dilution factor 2: 50 µl of (A) + 50 µl normal saline = (B)
For Dilution factor 4: 50 µl of (B) + 50 µl normal saline = (C)
For Dilution factor 8: 50 µl of (C) + 50 µl normal saline = (D)
For Dilution factor 16: 50 µl of (D) + 50 µl normal saline = (E)

Normal Saline = 0.9gm% NaCl

***How to calculate the serially diluted calibrators concentrations:**

multiply the original concentration with multiplying factor or divide with dilution factor.

Please note: These dilutions should be used for measurement within 4 hours of preparation.

(P.T.O.)

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PROCEDURE:

The reagents are ready to use as supplied. Latex reagent should be gently shaken (invert the recipient 3-4 times) before each use.

Pipette into TT	Calibrator	Test
R1 - IgE (Buffer Reagent)	450 µl	450 µl
R2 - IgE (Latex reagent)	50 µl	50 µl
IgE Calibrator	20 µl	-
Sample/Test	-	20 µl

Mix immediately and read difference in absorbance at 5 second (AT1) and at 240 second (AT2) for Calibrator and sample/test.

CALCULATION:

$$\text{IgE (IU/ml)} = \frac{\Delta\text{Abs of Test X Conc. of Calibrator}}{\Delta\text{Abs of Calibrator}}$$

Where ΔAbs = (AT1) – (AT2)

NORMAL VALUES:

The serum IgE concentration in healthy, non- atopic test subjects is very age dependent.

Age	IU/ml
New-Borns	< 1.5
Infants<1 year	< 15
Children (1-5 years of age)	< 60
Children (6-9 years of age)	< 90
Children (10-15 years of age)	< 200
Adults	< 100

PERFORMANCE CHARACTERISTICS:

Detection limit: < 10 IU/ml.

Analytical sensitivity: 0.5 mAbs/(IU/ml)

Prozone effect: not at least up to 12000 IU/ml

Precision: Using three pooled samples of IgE with values ranging from 40 to 140 IU/ml

- Intra- assay CV (%) ranges from 1.3 to 3.3
- Intra- assay CV (%) ranges from 1.5 to 4.7

Accuracy: Results obtained with this reagent did not show systematic differences when compared with commercial reagents of similar characteristics. The correction coefficient (r) was 0.986.

LINEARITY:

Up to 2000 IU/ml

INTERFERENCES:

No significant interference by hemoglobin, bilirubin or rheumatoid factors. A negative interference (<10%) has been observed when an Intralipid Concentration >1% is added. Other substances may interfere.

DON'T USE THE REAGENT IF:

Visible agglutination or precipitation present in latex reagent. Buffer reagent has turbid or any visible particles. Calibrator is not clear and colorless.

NOTES:

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument.

2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES:

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