CRM® *diagnostic systems*

IgE (Total Immunoglobulin E)

In vitro diagnostic reagents for the quantitative determination of human IgE in serum by means of particle-enhanced turbidimetric immunoassay.

Diagnostic Relevance

The Immunoglobulin E (IgE) has a molecular weight of aprox. 190000 g/mol and is produced by the organism in small quantities. Allergic diseases are a sign of hypersensitivity of the body. The type I hypersensitivity reaction, also called immediate hypersensitivity, is IgE mediated and is characterised by an immediate reaction following contact with the antigen. Antigens facilitating an IgE response include components of grass pollen, components of food, parasites and secretions from insects. This antigen induces the mucosal-B-cells, in conjunction with T-helper cells, to produce specific IgE. The IgE molecules bind via Fc receptors to mast cells, which thus becomes sensitized. The next time when the antigen comes into contact with the sensitised mast cells, the bound IgE antibodies become cross-linked, leading to degranulation of the mast cells and release of mediators (as Histamine). The mediators bring about clinical sings typical for allergy, such as rhinitis, urtecaria, asthma and eczema. IgE is formed mainly in the lymph nodes and mucous membranes of the respiratory and gastrointestinal tracts. IgE molecules cannot pass through the placental barrier and do not activate complement. IgE determinations are indicated in the diagnosis and monitoring of allergic diseases. Elevated IgE levels also occur in parasitosis and immunodeficiency syndromes, such as acquired T-cell deficiency or the Wiskott-Aldrich syndrome. In infants and small children with recurrent respiratory tract diseases (bronchitis, pseudocroup attacks), the determination of IgE is of prognostic relevance, also in some mielomas of IgE type.

Principle

The Biolatex IgE test is used for the quantitative in vitro determination of total immunoglobulin IgE in serum and plasma 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.

Reagents

It is a bi-reagent technique that uses liquid reagent ready to use.

A.- Buffer - 25 ml of phosphate buffer, pH:7,0 , containing protein stabilizers and 0,09 % sodium azide as preservative**. Free of polyethyleneglicol**.

B.- Latex reagent – 7,5 ml of a suspension of latex microparticules covalently bound anti-IgE antibodies suspended in a neutral aqueous solution, with 0,09 % sodium azide as preservative.

Precautions

For in vitro diagnostic use only. Do not pippete by mouth. Reagents containing sodium azide must be handled with precaution. Sodium azide can form explosive azides with lead and copper plumbing. Since absence of infectious agents cannot be proven, all specimens and reagents obtained from human blood should always be handled with precaution using established good laboratory practices. Disposal of all waste material should be in accordance with local guidelines.

As with other diagnostic tests, results should be interpreted considering all other test results and the clinical situation of the patient.

Materials required but not provided

Automatic analyser.
Saline solution.
Calibrator.
Controls.

Storage and Stability

The IgE reagents should be stored tightly capped at +2..+8ºC when not in use. **Do not freeze**. Reagents in the original vials are stable to the expiration date on the vial label when capped and stored at +2...+8ºC. Immediately following the completion of an assay run, the reagent vials should be capped until next use in order to maximize curve stability. Once opened the reagent can be used within 1 month if stored tightly closed at +2...+8ºC after use.

The IgE buffer reagent should be clear and colourless. Any turbidity may be sign of deterioration and reagent should be discarted.

The IgE latex reagent should have a white, turbid appearance free of granular particulates. Visible agglutination or precipitation may be a sign of deterioration, and the reagent should be discarted.

Specimens

Serum specimens should be collected by venipuncture following good laboratory practices. Suitable assay specimens are human serum samples, as fresh as possible (stored up to 7 days at +2...+8ºC) or deep-frozen (6 months at –20ºC). 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 thawing, must be clarified before the assay by high-speed centrifugation(15 min at approx. 15.000 xg). Heat inactivation of serum samples results in loss of IgE antigenicity and therefore must be avoided.

Procedure

The reagents are ready to use as supplied. Reagents should be gently shaken before each use.

Follow the instructions of the operator's manual to load the cartridge, technique programation, calibration, sample measurement and control.

Calibration. Quality control

Standardization: use CRM® *diagnostic systems* Calibrators. The method was standardized against to IRP 75/502.

The IgE concentration of the Standard and Control is given on the label.

This curve is stored in memory by the analyser and recalled for later use. Calibration curves are stable for up to 14 days, after which a new curve must be generated. Additionally, recalibration must be performed whenever reagent lots are changed.

For quality control use CRM® *diagnostic systems* Control or other suitable control material. The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Control must be assayed and evaluated as for patient samples.

Calculation

The turbidimetric analysers automatically calculate the IgE concentration of each sample.

Reference Intervals

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

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

Assay range and Sensitivity

The assay range is established from 10 to 1200 IU/ml.

Calculating the mean plus 3SD of twenty replicates of zero standard resulted in a lower limit of detection of 10 IU/ml.

Specificity

The assay is specific for IgE determination. Interference from billirrubin, haemoglobin and rheumatoid factors has not been observed. A negative interference (<10%) has been observed when an intralipid ™ concentration >1% is added.

Other substances can interfere. For a comprehensive review of interfering substances, refer to the publication by Young.

Assay Precision

Intra-assay coefficients of variation (CV) for three pooled samples (IgE values ranging from 40 to 140 IU/ml) were between 1,3 and 3,3%; daily calibrated inter-assay CVs were between 1,5 and 4,7%.

Linearity

Linearity was evaluated using serial dilutions, prepared with saline solution, of two samples, which contained values of IgE in the range of analysis ranging from 60 to 350 IU/ml. Linear regression values of IgE IU/ml vs concentration yielded correlation coefficients, r> 0,999 for all samples. Within the assay's measuring range, the deviations of measurement from theoretical values did not exceed the 10 % level. In addition, the system did not show prozone phenomenon at least up to 12000 IU/ml.

Method comparison

60 samples were correlated with a chimioluminiscence commercial procedure. When comparing the results by lineal regression the result was: y = 0,9 x + 11,3 and r=0,996.

Analytical characteristics have been obtained in a single experiment in a Cobas-Mira plus analyser. As is well known the analytical characteristics of a clinical chemistry reagent depend on both the reagents and the instrument used. Multicenter studies indicate important differences in analytical characteristics among similar instruments. Therefore, the data expressed in the present document should be interpreted as a guide example.

Literature

Kjellman NIM, Johansson SGO, Roth A. Clinical Allergy 1976; 6:51-59
Debelic,M. Clinical Significance of total and specific IgE in bronchial asthma. Allergol Immunopathol 1976;4: 361-70.
Grundbacher, F,J, Causes of variation in serum IgE levels, in normal population. J All Clin Immunol. 1975;56:104-11.
Dati, F. Ringel, K. Refernce values for serum IgE in healthy non atopic children and adults. Clin Chem. 1982; 28:1556.
Young DS. Effects of Drugs on Clinical Laboratory Test. 5th Edition, AACC Press, 2000.
Sonderdruck aus DG Klinische Chemie Mitteilungen 1995; 26: 207 – 224.

Significados de los símbolos indicados en las etiquetas. Explanation of symbols used on labelling. Explication des symbols figurant sur les etiquettes. Spiegazione dei simboli utilizzati sull'eticheta. Significado dos símbolos indicados nas etiquetas. Erläuterung der symbole auf den etiketten.

							REAG	CAL	Buffer	LYOPH	Conc.	Control H / Control L
Fecha de Caducidad Expirate Date Date de Péremption Data di Scadenza Data Expiração Verwendbar bis	Temperatura de almacén Storage Temperature Temperature de Conservation Temperatura de Conservazione Temperatura de Conservação Lagertemperatur	Número de Lote Lot Number Número de Lot Numero di Lotto Número de Lote Chargen-Nr	Para Diagnóstico In Vitro For In Vitro Diagnostic Usage In Vitro Per Uso Diagnostico In Vitro Utilizar em Diagnostico In Vitro In Vitro Diagnosticum	Número de catálogo Catalog Number Numéro de catalogue Numero di catalogo Número de catálogo Katalognummer	Conformidad Europea European Conformity Conformité aux normes européennes Conformità europea Comformidade com as normas europeias CE-Konformitätskennzeichnung	Fabricado por Manufactured by Fabriqué par Fabbricato da Fabricado por Hergestellt	Reactivo Reagent Réactif Reagenti Reagente Reagenz	Calibrador Calibrator Calibrateur Calibradore Calibrador Kalibrator	Tampón Buffer Tampon Tampone Buffer Puffer	Liofilizado Lyophilised Lyophilisé Liofilo Liofilizado Lyophilisiert	Concentración Concentration Concentration Concentrazione Concentraçao Koncentration	Control Alto / Control Bajo Control High / Control Low Contrôle élevé / Contrôle Bas Controllo Alto / Controllo Basso Controlo Alto / Controlo Baixo Kontrolle Hoch / Kontrolle Niegrid

CE

Total IgE / Ige Total
Procedure for /Procedimiento para
COBAS MIRA PLUS

GENERAL	
Measurement mode:	Absorb
Reaction mode:	R-S-SR1
Calibration mode:	LOGIT/LOG5
Reagent blank:	NO
Cleaner:	BEFORE
Wavelenght:	600 nm
Decimal position:	1
Units:	IU/mL
ANALYSIS	
Post. Dil. Factor:	10.00
Conc. factor:	NO
SAMPLE	Vol: 13 µL
	Cycle: 3
DILUENT	Name: Saline
	Vol: 20 µL
REAGENT	Vol: 200 µL
	Cycle: 1
START REAGENT	Vol: 75 µL
	Cycle: 1
DILUENT	Name: Saline
	Vol: 0.0 µL
CALCULATION	
Sample limit:	NO
Reaction Direction:	INCREASE
Check:	ON

CALCULATION (continued)	
Antigen excess	NO
Conversion factor:	1.00000
Offset:	0.00000
Normal range low:	NO
Normal range high:	NO
Number of steps:	1
Calc. Step A:	KINETIC
Reading first:	3
Reading last:	12
Reaction limit:	3.5000 ΔA
Point:	T2
CALIBRATION	
Calibr. Interval:	ON REQUEST
Reagent blank:	
Reag. Range low: NO	High: NO
Blank range low: NO	High: NO
STD POS(**):	
1: ***	2: ***
3: ***	4: ***
5: ***	6: ***
7: NO	8: NO
REPLICATE: SINGLE	
Deviation:	
Correction std:	

(**) Select one position on the standard rack
(***) Standard values on the labels / insert

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