abia IgE total

REF DK.048.01.3

IVD

CE

Note: Changes highlighted ★

abia

Intended use

abia IgE total enzyme immunoassay for the quantitative determination of total immunoglobulin E (IgE) concentration in human serum.

For professional use only.

Clinical value

IgE is the class of immunoglobulins which are found out in norm in insignificant quantities in serum and secrets (less than 0.001 % from all immunoglobulins of serum). The newborn level of the total IgE is less than 1 IU/ml. IgE levels show a slow increase during childhood, reaching adult levels in the second decade of life. In general, elevated levels of IgE indicate an increased probability of an IgE-mediated hypersensitivity, being responsible for allergic reactions. However it is necessary to mean that approximately at 30 % of patients with atopic manifestations can have normal level of the total IgE; on the contrary, raised IgE levels can be revealed at a person without an allergy.

These substances cause smooth muscle constriction and lead ultimately to allergic conditions such as wheal and flare reactions, hives, dermatitis, rhinitis, hay fever, asthma and anaphylactic shock. Infants and children with family history of atopic allergy are at increased risk of developing disease and constitute a prime population for screening.

Significant elevations may be seen in the sensitized individuals, but also in cases of myeloma, pulmonary aspergillosis, and during the active stages parasitic infections.

Principle of the test

abia IgE total is a one-step immunoassay, based on the principle of the "sandwich" method.

IgE molecules present in the sample and the labeled enzyme-anti-IgE monoclonal antibodies in the conjugate compete for binding to the capture antibody on the anti-IgE coated microplate. The enzyme activity in the antibody-bound fraction is directly proportional to the IgE concentration.

The unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue color which is converted to yellow after the reaction has been stopped with sulphuric acid.

The color intensity is directly proportional to the concentration of the IgE molecules in the specimen and can be read at 450 nm.

Kit contents

	S	
IgE Ab coated plate	1	polystyrene plate 12 × breakable 8-well strips coated with monoclonal anti-IgE antibodies
Conjugate	1 × 18 ml	ready to use; HRP-labeled monoclonal anti-IgE antibodies; transparent or slightly opalescent pink liquid
Calibrator 0	1 × 2.0 ml	human serum based buffer not containing IgE; pale yellow liquid
Calibrator 1	1 × 0.5 ml	human serum based calibrator containing IgE in concentration approx. 62.5 IU/ml; pale yellow liquid
Calibrator 2	1 × 0.5 ml	human serum based calibrator containing IgE in concentration approx. 125 IU/ml; pale yellow liquid
Calibrator 3	1 × 0.5 ml	human serum based calibrator containing IgE in concentration approx. 250 IU/ml; pale yellow liquid
Calibrator 4	1 × 0.5 ml	human serum based calibrator containing IgE in concentration approx. 500 IU/ml; pale yellow liquid
Calibrator 5	1 × 0.5 ml	human serum based calibrator containing IgE in concentration approx. 1.000 IU/ml; pale yellow liquid
Control serum	1 × 0.5 ml	human serum based control containing IgE; pale yellow liquid
Washing solution (concentrated 25-fold)	1 × 50 ml	phosphate saline buffer; colorless or pale yellow liquid
TMB/substrate solution	1 × 12 ml	ready to use; citric acid buffer containing TMB and H_2O_2; colorless liquid
Stopping reagent 0.2M H_2SO_4	1 × 25 ml	ready to use; 0.20 mol/l sulphuric acid solution; colorless liquid
Protective film	1	
Plastic dish	2	
Plastic zip-lock bag	1	

The calibrators were calibrated using a WHO 2nd 75/202. Exact concentration levels for calibrators and control serum are given on the labels on a lot specific basis. For conventional units: IU/ml x 2.4 = mg/ml.

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package.
Once opened, the components should be used within 2 months. Concentration of preserving agents: <=0.1 %.

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- microplate shaker
- automatic microplate washer
- microplate reader equipped with 450 nm filter

Safety notes

- human origin material used in the preparation of the calibrators and control serum has been tested and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2)
- as no known test method can offer complete assurance that infections agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum as soon as possible to avoid any haemolysis
- do not use sera preserved with sodium azide
- do not use contaminated, hyperlipaemic and hyperhaemolysed specimens
- the samples with hyperproteinaemia and hyperbilirubinaemia were not specially tested
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- samples can be stored at 2–8 °C within 72 hours or deep-frozen at -20 °C
- no more than one freeze/thaw cycle is allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (20–25 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section "Washing procedure"
- for the description of test procedure with the automated analyzers see section "Automated analyzers"

Washing procedure

Please contact your representative for protocols for recommended washers and procedures.
In general the following protocol is recommended:

- flow-through washing with a volume not less than 300 µl per well is used
- repeat 5 times
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion **Stability:** 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0

Test procedure

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1	Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in plastic zip-lock bag. Do not remove desiccant.
2	Analyse each calibrator, control serum, and sample duplicate. Add 20 µl of calibrators 0 - 5 into appropriate wells. Add 20 µl of control serum into appropriate wells. Add 20 µl of samples to be tested in rest of the wells. The total time should not exceed 10 min.
3	Add 150 µl conjugate into each well. Cover the plate with protective film.
4	Incubate in microplate shaker (approximately 500-800 rpm) for 45 minutes at 37.0 ± 1.0 °C.
5	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 300 µl of working washing solution into each well and aspirate. Perform this procedure 5 times. Use double aspiration in the final step where possible.
6	Add 100 µl of TMB/substrate solution to all the wells. Keep the plates in a dark place for 20 minutes at 20–25 °C.
7	Add 150 µl of stopping reagent into each well. Mix gently for 5–10 sec.
8	Read the optical density at 450 nm using a plate reader within 20 minutes after stopping reaction.

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative.
For the instrumentation without established validated protocol follow section "Test procedure" and ensure all requirements described in section "Precautions" are followed.
All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of blank value should be not more than 0.100 at 450 nm.
The absorbance (OD) of calibrator 5 should be greater than 1.300.
The absorbance (OD) of control serum should be within established range.

Calculation procedure

1 Calculate the mean optical density of each calibrator duplicate at 450 nm.

2 Calculate the mean optical density of each sample duplicate.

3 Subtract the mean absorbance value of the "blank" from the mean absorbance values of the calibrators, control and serum samples.

4 Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.

5 Read the values of the samples directly off the calibration curve.
If immunoassay software is being used, a 4-parameter curve is recommended.

If a sample reads more than 1 000 IU/ml then dilute it with calibrator 0. The result obtained should be multiplied by the dilution factor.

Example	OD 1	OD 2	Mean OD	Value, IU/ml
Calibrator 0	0.054	0.054	0.000	0.000
Calibrator 1	0.351	0.348	0.296	62.50
Calibrator 2	0.612	0.628	0.566	125.00
Calibrator 3	1.074	1.104	1.035	250.00
Calibrator 4	1.796	1.865	1.777	500.00
Calibrator 5	2.606	2.541	2.520	1 000.00
Sample	0.865	0.862	0.810	183.50

This data is for illustration only and should **not be used** to calculate of samples. Each user should obtain his or her own data and standard curve.

Performance characteristics

Analytical sensitivity

The analytical sensitivity (limit of detection) was calculated by determining the variability of the calibrator 0 based on 12 analyses runs additional 2 x SD. Limit of detection defined at 2.50 IU/ml.

Specificity

No cross-reactivity to human IgA, IgG, IgM was observed with this assay.

Precision	Mean value, IU/ml	SD	CV, %
Intra-assay, sample 1	183.00	7.450	4.10
Inter-assay, sample 1	178.20	12.180	6.80

Accuracy

The assay was compared with an enzyme immunoassay as a reference test. The total number of specimens was 266. The values ranged from 0 to 4 469 IU/ml. The least square regression equation and the correlation coefficient were computed for abia IgE total in comparison with the reference method.

The least square regression analysis was y = 1.015(x) + 14.615 with correlation coefficient of 0.97.

Expected normal value	Range, IU/ml	
Adult allergy-free population	0.00	180.00

Normal value ranges may vary slightly among different laboratories. It is strongly recommended that each laboratory should determine its own range of expected normal values.

Limitations of test

- the assay was validated only for the determination of total immunoglobulin E (IgE) in human serum
- the results obtained with this assay should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient
- some individuals may have heterophilic antibodies to mouse or other animal proteins that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution
- the assay was tested for a high dose hook effect. Up to at IgE concentration of 4 000 IU/ml no hook effect was observed.

References

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3. Stern A., van Hage-Hamsten M., Sondell K., Johansson S.G.O. Is allergy screening of blood donors necessary. Vox.Sang. 69:114-119; 1995.
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Key to symbols used

	Manufacturer
IVD	For in vitro diagnostic use
REF	Catalogue number
LOT	Batch code
YYYY-MM-DD	Expiry date
2°C 8°C	Storage temperature limitation
	Do not use if package is damaged
	Do not reuse
Σ n	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
★	Changes highlighted

Hazard and precautionary statements for certain kit components

Warning

Stopping reagent

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Warning

Conjugate, calibrators 0 - 5, control serum

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.

Attention!
For complete precautionary statements and detailed information see safety data sheets (SDS).

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