VIDAS®TOTAL IgE (IGE)

IVD

VIDAS TOTAL IgE is an automated quantitative test for use on the VIDAS family instruments, for the immunoenzymatic determination of total human IgE in human serum or plasma (lithium heparin or EDTA) using the ELFA technique (Enzyme Linked Fluorescent Assay).

SUMMARY AND EXPLANATION (1-9)

Since their discovery in 1967 by the teams of Ishizaka K. and T. and Johannsen and Bennich, IgE have been associated with type I immediate hypersensitivity. In fact, atopic individuals can produce fairly high quantities of IgE after exposure to specific allergens.
An abnormal rise in total serum IgE can also be observed in non allergic diseases such as:
- parasitosis, particularly helminthiasis,
- nephropathies,
- immune deficiency and neoplastic diseases, including among others, Wiskott-Aldrich syndrome, Hodgkin's disease, IgE myeloma,
- dermatosis: pemphigus, psoriasis, pityriasis rubra pilaris.

VIDAS TOTAL IgE assay aids in diagnosing allergic affections.

PRINCIPLE

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.
During the first step, the sample is collected and transfered into the well containing alkaline phosphatase-labeled anti-IgE antibodies. The sample/conjugate mixture is cycled in and out of the SPR several times to speed up the reaction. This operation enables the IgE to bind with the immunoglobulins coated on the interior of the SPR and with the conjugate to form a sandwich.
Unbound components are eliminated during the washing steps.
During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of IgE present in the sample. At the end of the assay, the results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT (60 TESTS):

60 IGE strips	STR	Ready-to-use.
60 IGE SPRs 2 x 30	SPR	Ready-to-use. Interior of SPRs coated with monoclonal anti-IgE immunoglobulins (mouse).
IGE control 1 x 2 ml (liquid)	C1	Horse serum + human IgE + metacresol 1.4 g/l. MLE data indicate the confidence interval in KIU/L (international kilo-units per liter) ("Control C1 Dose Value Range").
IGE calibrator 1 x 2 ml (liquid)	S1	Horse serum + human IgE + metacresol 1.4 g/l. MLE data indicate the concentration in KIU/L ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value" ("Calibrator (S1) RFV Range").
IGE diluent 1 x 5 ml	R1	Ready-to-use. Horse serum + metacresol 1.4 g/l.
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar code printed on the box label.		
1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

The SPR

The interior of the SPR is coated during production with monoclonal anti-IgE immunoglobulins (mouse). Each SPR is identified by the IGE code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening**.

The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the strip

Wells	Reagents
1	Sample well.
2 - 3 - 4	Empty wells.
5	Conjugate: alkaline phosphatase-labeled monoclonal anti-IgE immunoglobulins (mouse) + 0.9 g/l sodium azide (600 µl).
6 - 7	Wash buffer: sodium phosphate (0.01 mol/l) pH 7.4 + 1 g/l sodium azide (600 µl).
8	Wash buffer: diethanolamine* (1.1 mol/l or 11.5%) pH 9.8 + 1 g/l sodium azide (600 µl).
9	Empty well.
10	Cuvette with substrate: 4-Methyl-umbelliferyl-phosphate (0.6 mmol/l) + diethanolamine** (DEA) (0.62 mol/l or 6.6%, pH 9.2) + 1 g/l sodium azide (300 µl).

* Signal Word: **DANGER**


Hazard statement

H318 : Causes serious eye damage.
H373 : May cause damage to organs through prolonged or repeated exposure.
H315 : Causes skin irritation.
H302 : Harmful if swallowed.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P309 + P311 : IF exposed or if you feel unwell: Call a POISON CENTER or doctor/physician.

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For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip to dispense 100 µl.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's Manual.
- Instrument of the VIDAS family.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory Biosafety Manual - WHO - Geneva - latest Edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use the SPRs if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Use **powderless** gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- The wash buffer in well 8 contains a harmful agent (11.5% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- The substrate in well 10 contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

STORAGE CONDITIONS

- Store the VIDAS TOTAL IgE kit at 2-8°C.
- **Do not freeze reagents.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

SPECIMENS

Specimen type and collection:

Serum or plasma (collected in lithium heparin or EDTA).
It is recommended that each laboratory checks the compatibility of collection tubes used.
The use of heat inactivated sera has not been validated.
None of the following factors have been found to significantly influence this assay.

- hemolysis (after spiking samples with hemoglobin: 0 to 300 µmol/l (monomer)),
- lipemia (after spiking samples with lipids: 0 to 2 g/l equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin: 0 to 275 µmol/l).

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.

Specimen stability

Samples can be stored at 2-8°C in stoppered tubes for up to 5 days; if longer storage is required, freeze the sera or plasma at -25 ± 6°C.
A study performed on frozen samples over a period of 2 months, showed that the quality of results is not affected.
Avoid successive freezing and thawing.

INSTRUCTIONS FOR USE

For complete instructions, see the User's Manual.

Reading Master lot data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.

If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.

Note: the master lot data need only be entered once for each lot.

It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User's Manual).

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified by S1, must be tested in **duplicate** (see User's Manual). The calibrator value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

Procedure

1. **Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.**
2. Use one "IGE" strip and one "IGE" SPR for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed**.
3. The test is identified by the "IGE" code on the instrument. The calibrator must be identified by "S1", and tested in **duplicate**. If the control is to be tested, it should be identified by "C1".
4. If necessary, clarify samples by centrifugation.
5. Mix the calibrator, control and samples using a vortex-type mixer (for serum or plasma separated from the pellet) in order to improve result reproducibility.
6. **For this test, the calibrator, control, and sample test portion is 100 µl.**
7. Insert the "IGE" SPRs and "IGE" strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
8. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
9. Reclose the vials and return them to 2–8°C after pipetting.
10. The assay will be completed within approximately 30 minutes. After the assay is completed, remove the SPRs and strips from the instrument.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate.

The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

The results are automatically calculated by the instrument using calibration curves which are stored by the instrument (mathematical model: 4 parameter logistic model) and the concentrations are expressed in KIU/l according to the international standard: 2nd IRP 75/502.

Samples with IgE concentrations greater than 1,000 KIU/l should be retested after being diluted by 1/10 or 1/100 in IGE diluent (R1).

If the dilution factor has not been entered when the Work List was created (see User's Manual), multiply the result by the dilution factor to obtain the sample concentration.

Interpretation of test results should be made taking into consideration the patient's history, and the results of any other tests performed.

QUALITY CONTROL

A control is included in each VIDAS TOTAL IgE kit. This control must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using this control. The instrument will only be able to check the control value if it is identified by C1.

Results cannot be validated if the control value deviates from the expected values.

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted taking into consideration the patient's history, and the results of any other tests performed.

RANGE OF EXPECTED VALUES

In adults: **(10, 11)**

Published studies show that it is difficult to determine "normal" values for the IgE serum concentration. The choice of sample is very important. Furthermore, the circulation of IgE is brief, as their life span is short.
As a guideline, 80% of the values corresponding to a non atopic population are less than 150 KIU/l.
These figures are given as a guide; it is recommended that each laboratory establishes its own reference values from a rigorously selected population.

In children:

The usual values determined during an evaluation at an external site coincide with publications and are presented in the following table:

	IgE concentrations							
	0 → < 20 KIU/l		20 → < 50 KIU/l		50 → < 100 KIU/l		100 → > 100 KIU/l	
Age	Number	%	Number	%	Number	%	Number	%
10 months (n = 93)	65	69.9	19	20.4	6	6.5	3	3.2
2 years (n = 86)	27	31.4	20	23.3	16	18.6	23	26.7
4 years (n = 49)	10	20.4	10	20.4	8	16.3	21	42.9
5-10 years (n = 27)	3	11.1	6	22.2	6	22.2	12	44.5
> 10 years (n = 11)	3	27.3	1	9.1	3	27.3	4	36.3

PERFORMANCE

Studies performed using VIDAS TOTAL IgE gave the following results:

Analytical detection limit

Defined as the smallest concentration of IgE which is significantly different from the zero concentration with a probability of 95%: **0.5 KIU/l.**

Hook effect

No hook effect was found up to IgE concentrations of 100 000 KIU/l

Precision

Within-run reproducibility:

Five samples were tested 30 times in the same run.

Sample	1	2	3	4	5
Mean concentration (KIU/l)	56.3	267.2	429.8	609.9	918.2
CV %	4.5	5.8	3.8	4.7	3.7

Between-run reproducibility:

Five samples were tested singly on the same VIDAS over a period of 7 weeks.

Sample	1	2	3	4	5
Mean concentration (KIU/l)	56.2	280.8	445.0	639.6	895.6
CV %	3.7	3.2	3.0	5.1	5.0

Accuracy

Dilution test

Three samples were diluted in the IGE diluent and tested singly in 3 runs. The ratio of the mean concentration measured over the expected concentration is expressed as a mean recovery percentage.

Sample no.	Dilution factor	Expected mean concentration (KIU/l)	Measured mean concentration (KIU/l)	Mean recovery percentage (%)
1	1/1	299.4	299.4	100.0
	1/2	149.7	154.9	103.5
	1/4	74.9	83.0	110.8
	1/8	37.4	43.6	116.5
	1/16	18.7	22.5	120.3
	1/32	9.4	11.1	118.1
2	1/1	495.9	495.9	100.0
	1/2	248.0	234.3	94.5
	1/4	124.0	126.8	102.3
	1/8	62.0	69.3	111.8
	1/16	31.0	36.8	118.7
	1/32	15.5	19.1	123.2
3	1/1	714.9	714.9	100.0
	1/2	357.5	331.2	92.6
	1/4	178.7	168.4	94.2
	1/8	89.4	89.9	100.6
	1/16	44.7	49.3	110.3
	1/32	22.3	25.1	112.6

Comparison with other test methods

Correlations were established between the VIDAS TOTAL IgE kit, and another commercialized kit.

VIDAS TOTAL IgE = 1.209 X - 15.9 r = 0.995 (n = 196)

CROSS REACTIVITY AND RELEVANT INTERFERENTS

There is no cross reactivity with other immunoglobulins: with IgG up to 19 g/l, with IgM up to 30 g/l, with IgA up to 40 g/l.

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their type and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	06932I	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (60 TESTS) WARNINGS AND PRECAUTIONS INSTRUCTIONS FOR USE

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