VIDAS® Total IgE (IGE)

IVD

The VIDAS® Total IgE (IGE) is intended for use with the instruments of the VIDAS family (VITEK® ImmunoDiagnostic Assay System) as an automated quantitative enzyme linked fluorescent immunoassay (ELFA) for the determination of total IgE concentration in serum or plasma. The VIDAS Total IgE assay is intended for use as an aid in the diagnosis of allergic diseases.

SUMMARY AND EXPLANATION OF THE TEST

IgE is an immunoglobulin with a molecular weight of approximately 190,000 daltons. Produced by plasma cells, IgE has a significant role in atopic diseases such as allergic rhinitis, allergic asthma, and atopic dermatitis (1,2). IgE has a high affinity for receptors on mast cells and basophils, mediating the binding of allergens to these cells. The subsequent release of vasoactive amines, such as histamine, produce the clinical manifestations associated with atopic disease (2,3). Measurement of IgE serum levels can be important in the diagnosis and treatment of these disorders.

In most nonatopic patients, IgE serum levels are relatively low. However, certain parasitic or helminth infections have been associated with elevated IgE levels due to IgE sensitization of macrophages, eosinophils, and other inflammatory cells (4,5). Elevated IgE levels have also been reported in some forms of glomerular disease and in some immunodeficiency syndromes such as Wiskott-Aldrich syndrome (6,7,8).

PRINCIPLE OF THE PROCEDURE

The VIDAS® Total IgE (IGE) assay is an enzyme-linked fluorescent immunoassay (ELFA) performed in an automated instrument.

All assay steps and assay temperature are controlled by the instrument. A pipette tip-like disposable device, the Solid Phase Receptacle (SPR®), serves as a solid phase for the assay as well as a pipetting device. The SPR is coated at the time of manufacture with mouse monoclonal anti-IgE antibodies. The VIDAS Total IgE assay configuration prevents non specific reactions with the SPR. Reagents for the assay are located in the sealed IGE Reagent Strips.

The sample is transferred into the well containing the anti-IgE antibody conjugated with alkaline phosphatase. The sample/conjugate mixture is cycled in and out of the SPR and the IgE in the sample will bind to antibodies coated on the SPR and to the conjugate forming a "sandwich". Wash steps remove unbound conjugate.

A fluorescent substrate, 4-methylumbelliferyl phosphate, is cycled through the SPR. Enzyme remaining on the SPR wall will catalyze the conversion of the substrate to the fluorescent product 4-methylumbelliferone. The intensity of fluorescence is measured by the optical scanner in the instrument; it is proportional to the IgE concentration present in the sample.

When the VIDAS Total IgE assay is completed, the results are analyzed automatically by the instrument, and a report is printed for each sample.

Kit composition (60 tests) :

60 IGE Reagent Strips	STR	Ready to use. The IGE Reagent Strip is comprised of a polypropylene strip of 10 wells covered with a foil seal and paper label. The first well in the strip is for the sample. The last well of the strip is an optically clear cuvette in which the fluorometric determination is made. The eight wells in the center section of the strip contain the various reagents needed for the assay.
60 IGE SPRs (2 x 30)	SPR®	Ready to use. SPRs coated with mouse monoclonal anti-IgE antibodies.
IGE Control (liquid) (1 x 2 ml)	C1	Ready to use. Horse serum with human IgE and metacresol 1.4 g/L. Dose value range in kIU/l is indicated on the MLE card after the following mention : "Control C1 Dose Value Range".
IGE Calibrator (liquid) (1 x 2 ml)	S1	Ready to use. Horse serum with human IgE and metacresol 1.4 g/L. Dose value range in kIU/l (2nd IRP 75/502) is indicated on the MLE card after the following mention: "Calibrator (S1) Dose Value". The confidence interval in “Relative Fluorescence Value” is indicated on the MLE card after the following mention: "Calibrator (S1) RFV Range".
IGE Diluent (liquid) (1 x 5 ml)	R1	Ready to use. Horse serum with metacresol 1.4 g/L.
1 MLE Card (Master Lot Entry)		Specifications for the factory master data required to calibrate the test: to read the MLE data, please refer to the Operator’ s Manual.
1 Package Insert		

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 VIDAS IGE Reagent Strip

Wells	Reagents
1	Sample
2-3-4	Empty
5	Conjugate: Mouse monoclonal anti-IgE antibodies conjugated to alkaline phosphatase with 1 g/L sodium azide (600 µl)
6-7	Wash buffer: Sodium phosphate (0.01 mol/l, pH 7.4) with 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
10	Reading 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).

* **HARMFUL reagent:**

- **R 48/22 :** Harmful **:** danger of serious damage to health by prolonged exposure if swallowed.
- **R 41 :** Risk of serious damage to eyes.
- **S 26 :** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- **S 46 :** If swallowed, seek medical advice immediately and show this container or label.

***IRRITANT reagent:**

- **R 36 :** Irritating to eyes.
- **S 26 :** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

For further information, refer to the Safety Data Sheet available on request.

MATERIALS REQUIRED BUT NOT PROVIDED

– Pipette with disposable tips that will dispense 100 µl.
– Powderless disposable gloves.
- For other specific materials, please refer to the Instrument Operator'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).**
- Consider all patient specimens potentially infectious and observe routine biosafety precautions. Dispose of all used components and other contaminated materials by acceptable procedures for potentially biohazardous human blood products.
- 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.
- Powderless gloves are recommended as powder has been reported as a cause of false results in some enzyme immunoassays.
- Kit reagents contain 1 g/L sodium azide which could 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 Optical Cuvette with Substrate (well 10) contains an irritant agent (diethanolamine). Refer to the risk sentence "R" and the precautions "S" above.

- Wipe up spills thoroughly after treatment with liquid detergent and a solution of household bleach containing at least 0.5 % sodium hypochlorite to inactivate infectious agents. See the Operator's Manual for cleaning spills on or in the instrument. Do not place solutions containing bleach in the autoclave.
- The instrument should be routinely cleaned and decontaminated. See the Operator's Manual for the appropriate procedures.

STORAGE AND HANDLING

- Store the VIDAS® Total IgE kit at 2-8ºC. **Do not freeze reagents.** Return unused components to 2-8ºC.
- The IGE SPR®s are provided in a resealable pouch. Remove only the required number of SPRs, closing the pouch completely after opening.
- All components are stable, when stored appropriately, until the expiration date printed on the label. Do not use components beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

Acceptable specimens include sera or plasma (with EDTA or heparin anticoagulant). The use of heat inactivated sera has not been established - do not heat sera. Samples can be stored at 2-8ºC in stoppered tubes for up to 1 week. If storage for longer than this is required, freeze the sera or plasma at - 25 ± 6°C for up to 2 months. Avoid repeated cycles of freezing and thawing. If necessary, clarify samples by centrifugation. Although interference linked to the presence of hemoglobin, bilirubin, or lipids has not been observed, do not use hemolyzed, icteric, or lipemic samples. If possible, collect a new specimen.

INSTRUCTIONS FOR USE

Master lot data entry

Before each new lot of reagents is used, specifications (or factory master data) must be entered 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. 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 (for complete instructions refer to the Operator'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 Operator Manual). The calibrator value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

Assay Procedure

1. Remove necessary components from the kit and return all unused components to storage at 2-8ºC.
2. Allow components to reach room temperature (approximately 30 minutes).
3. 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.**
4. 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".
5. If needed, label the “IGE” Reagent Strips with the appropriate sample identification numbers.
6. Mix the calibrator, control, and sample using a vortex-type mixer (for serum or plasma separated from the pellet).
7. **For this test, the calibrator, control, and sample test portion is 100 µl.**
8. Insert the “IGE” Reagent Strips and “IGE” SPRs into the appropriate positions on the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
9. Initiate the assay processing as directed in the Operator's Manual. All steps will be executed automatically by the instrument.
10 Reclose the vials and return them to 2–8°C after pipetting.
11 Results are obtained within approximately 30 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
10. Dispose of the used SPRs and strips into an appropriate recipient.

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.

RESULTS AND INTERPRETATION

Two instrument readings for fluorescence in the Reagent Strip's reading cuvette are taken for each specimen tested. The first reading is a background reading of the cuvette and substrate before the SPR® is introduced into the substrate.

The second reading is taken after the substrate has been exposed to the enzyme conjugate remaining on the interior of the SPR. The background reading is subtracted from the final reading to give a Relative Fluorescence Value (RFV) for the test result.

Samples with concentrations greater than 1000 kIU/l must be diluted 1/10 (1 volume of sample and 9 volumes of diluent) or 1/100 (1 volume of sample and 99 volumes of diluent). If the dilution factor has not been entered when the analysis has been requested (see Operator's Manual), multiply the result by the dilution factor to obtain the IgE sample concentration.

A report is printed which records :

- the type of test performed,
- the sample identification,
- the date and time,
- the lot number and the expiration date of the reagent kit being used,
- each sample's RFV and IgE concentration.

LIMITATIONS OF THE TEST

1. Specimens from patients receiving preparations of mouse monoclonal antibodies for diagnostic or therapeutic purposes may contain human anti-mouse antibodies (HAMA).These specimens may exhibit elevated or depressed values when tested with assays that utilize mouse monoclonal antibodies. These specimens should not be tested in the VIDAS® Total IgE assay.
2. Low total IgE concentrations do not necessarily indicate the absence of allergies. Some patients may have a low total IgE but a high concentration of specific IgE antibody.

PERFORMANCE DATA

Immunological Specificity:

Cross reactions with immunoglobulins G, M and A have been tested by adding these immunoglobulins to two sera containing IgE. No cross reactivity or interference was observed for the concentrations tested (30 g/l IgM, 40 g/l IgA, and 19 g/l IgG).

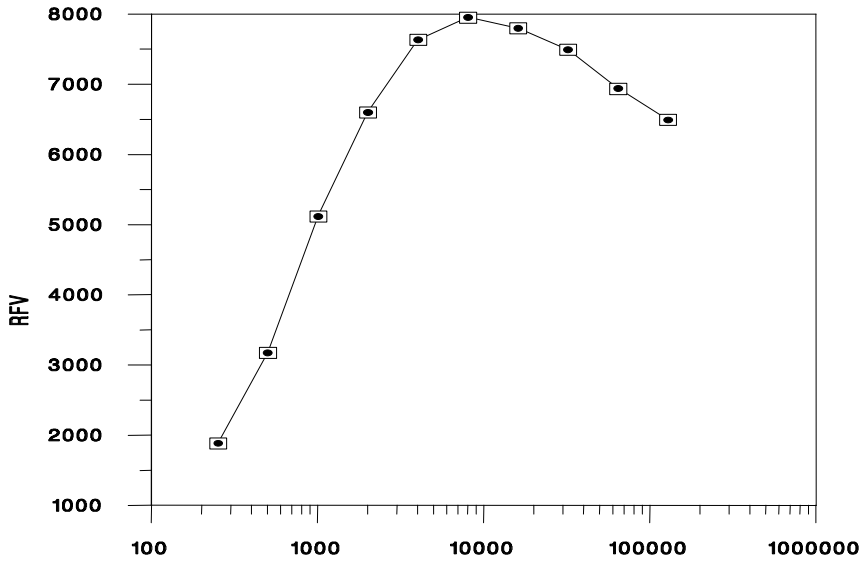
Detection limit

The detection limit (assay sensitivity) is defined as the lowest concentration that can be distinguished from zero with 95 % probability. The detection limit for the VIDAS Total IgE assay is 0.5 kIU/l.

Hook Effect

The Hook effect was tested using human IgE solutions at concentrations from 250 to 130,000 kIU/l. No hook effect was seen up to 8000 kIU/l. All concentrations tested between 8000 kIU/l and 130,000 kIU/l produced results with the statement "> 1000 kIU/l OFF CURVE".

HOOK EFFECT FOR TOTAL IgE



<----------> **kIU/L**

Calibration Range

PRECISION//REPRODUCIBILITY

Intra-assay precision

Five samples were tested for intra-assay precision. Thirty replicates of each sample were tested 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

Inter-assay reproducibility on the same instrument

Five samples were tested in 24 runs on the same instrument over a 7-week period (recalibration was performed every 14 days as described in the Operator's Manual).

Sample	1	2	3	4	5
Mean concentration (kIU/l)	56.2	280.8	445.0	639.6	895.6
% CV	3.7	3.1	2.9	5.0	4.9

Inter-instrument, inter-assay reproducibility

Five samples were tested in singlet in 8 runs on different instruments.

Sample	1	2	3	4	5
Mean concentration (kIU/l)	58.1	271.1	452.0	643.5	898.9
% CV	4.7	6.7	4.1	3.4	6.0

PARALLELISM (Dilution Tests)

Three samples were diluted in IGE diluent and tested in singlet in 3 runs.

Sample	Dilution factor	Expected values (kIU/l)	Measured values (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	69.4	111.9
	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	331.3	92.7
	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

RECOVERY TESTS

Three samples were spiked with known quantities of human IgE and tested in singlet in 3 runs. The measured mean concentration compared to the expected mean concentration is shown below.

Sample	Amount spiked (kIU/l)	Expected mean concentration (kIU/l)	Measured mean concentration (kIU/l)	Mean recovery percentage
1	0	280.9	280.9	100.0
	117.0	198.9	197.3	99.2
	225.0	252.9	257.3	101.7
	448.0	364.4	380.9	104.5
	710.0	495.4	508.4	102.6
	948.0	614.4	651.6	106.0
2	0	435.4	435.4	100.0
	117.0	276.2	274.2	99.3
	225.0	330.2	324.3	98.2
	448.0	441.7	450.6	102.0
	710.0	572.7	576.4	100.6
	948.0	691.7	706.7	102.2
3	0	696.7	696.7	100.0
	117.0	406.9	380.9	93.6
	225.0	460.9	450.4	97.7
	448.0	572.4	580.8	101.5
	710.0	703.4	687.9	97.8
	948.0	822.4	852.3	103.6

INFLUENCE OF SPECIMEN COLLECTION

Blood samples were collected from forty-four patients. For each patient, three specimens were collected at the same time in a dry glass tube, in a tube with separating gel, and in a heparinized tube. Each sample collected was tested in duplicate and sera from the same donor was tested in the same run. The dry glass tube was the reference to which the other methods were compared. The statistical ratio method that was used to analyze the results showed no significant difference between the methods tested.

INTERFERENCE STUDIES

Heparin

Three pools of human sera were spiked with increasing quantities of heparin.

		Amount of heparin spiked (U/ml)			
		0	0.5	5	50
IgE (kIU/l)	Pool 1	54.1	54.7	53.6	55.3
	Pool 2	237.1	245.7	243.8	256.2
	Pool 3	921.7	861.6	919.6	894.8

EDTA

Three pools of human sera were spiked with increasing quantities of EDTA.

		Amount of EDTA spiked (mg/ml)			
		0	1	5	10
IgE (kIU/l)	Pool 1	54.1	51.2	52.7	53.9
	Pool 2	237.1	238.2	240.7	245.0
	Pool 3	921.7	814.0	868.3	825.5

These data indicate that EDTA or heparin plasma can be used in the VIDAS® Total IgE assay.

Hemoglobin

Two pools of human sera were spiked with increasing quantities of hemoglobin obtained from a lysate of human red blood cells.

		Amount of hemoglobin spiked (µmol/l)						
		0	15	30	60	150	210	300
IgE (kIU/l)	Pool 1	53.9	52.8	53.4	54.2	51.7	52.7	55.0
	Pool 2	570.2	606.6	575.7	605.9	635.3	603.8	630.0

Lipids

Two pools of human sera were spiked with increasing quantities of a lipid solution.

		Amount of triglycerides spiked (mmol/l)				
		0	1.0	2.6	3.0	5.0
IgE (kIU/l)	Pool 1	55.9	55.2	54.1	53.1	51.3
	Pool 2	611.0	630.7	604.7	679.8	617.3
Appearance		Clear	Opalescent		Turbid	

Bilirubin

Two pools of human sera were spiked with increasing quantities of bilirubin.

		Amount of bilirubin spiked (µmol/l)						
		0	17	31	56	140	215	280
IgE (kIU/l)	Pool 1	53.5	51.8	53.1	52.6	52.5	52.0	53.4
	Pool 2	583.0	596.0	582.0	615.0	597.0	625.0	618.0

Although interference linked to the presence of hemoglobin, bilirubin or lipids has not been observed, using hemolyzed, icteric or lipemic samples is not recommended. If possible, collect a new specimen.

EXPECTED VALUES

Results are given in kIU/l (2nd IRP 75/502).
- Adults : Among a group of healthy people free from any allergic manifestation, 95 % of obtained values are under 120 kIU/l.
- Children : these values have been determined on sera from a pediatric center. These results are given by age (see below) :

It is advisable for each laboratory to establish its own reference values on a well defined population.

	0 to 20 kIU/l	21 to 50 kIU/l	51 to 100 kIU/l	> 100 kIU/l
≤10 months n = 93	70.0 %	20.4 %	6.4 %	3.2 %
2 years n = 86	31.4 %	23.2 %	18.6 %	26.7 %
4 years n = 49	20.4 %	20.4 %	16.3 %	42.9 %
5 - 10 years n = 27	11.1 %	22.2 %	22.2 %	44.5 %
> 10 years n = 11	27.3 %	9.0 %	27.3 %	36.4 %

CORRELATION

One hundred and ninety six serum specimens with IgE concentrations ranging from 0 kIU/l to 3300 kIU/l were tested using the VIDAS® Total IgE assay and a commercially available IgE EIA. The results of linear regression analysis of the correlation are summarized below:

# of samples	Slope	Intercept	Correlation Coefficient
196	1.21	- 15.92	0.995

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 nature 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	GB : Catalogue number US : Catalog number
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests

bioMérieux, Inc. Insert No. 13709B
(Rev 2010/08) Supersedes All Other Inserts

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