INSTRUCTION MANUAL

REF 5110

April 14, 2016

sIgE Allergy LINE 1

- 24 determinations -

CE

IVD *In vitro* diagnostic device

Line Immunoassay for the determination of specific IgE antibodies to respiratory allergens / allergen mixes in human serum or plasma

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction	D	Biological risk

GA GENERIC ASSAYS GmbH

Ludwig-Erhard-Ring 3

15827 Dahlewitz, Germany

Telephone: +49 (0) 33708-9286-0
Fax: +49 (0) 33708-9286-50

www.genericassays.com

INTENDED USE

sIgE Allergy LINE 1 is used for the parallel determination of specific IgE antibodies against respiratory allergens and allergen mixes in human serum or plasma.

The test is designed to supply diagnostic information on allergies in combination with other test results and the medical history. The assay permits a large number of tests to be conducted in parallel over a short period of time. It must be performed by qualified professionals familiar with in vitro diagnostic methods.

PRINCIPLE of the TEST

sIgE Allergy LINE 1 is a Line Immunoassay based on ELISA test system (enzyme-linked immunosorbent assay) for the determination of specific IgE antibodies to allergens in human serum or plasma.

One test strip contains 27 allergen extracts and 3 standards which are embedded in the membrane.

Specific IgE antibodies (sIgE) from the specimen bind to the respective antigens on the membrane. The bound human IgE is detected by a specific anti-human IgE antibody. To intensify the signal, a secondary antibody, conjugated to the horseradish peroxidase enzyme, is added.

After adding a colour substrate, bound IgE antibodies are identified by an indigo precipitate.

The individual degree of sensitisation can be determined quantitatively by using a separately available reader or semiquantitatively with the naked eye in the case of all allergen extracts/allergen mixtures, and assigned to sIgE classes. The result is read out by comparing the colour intensity of the signal against the standard signals on the strip assay.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can also be used.

The samples may be kept at 2 - 8 °C for up to 14 days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Dilute each of the specimens at a ratio of **1:10**, i.e. 150 µl serum or plasma with 1.35 ml wash buffer (made from B) directly in the incubation tray. The dilution can also prepared before, the assay than is started using 1.5 ml of prediluted sample.

TEST COMPONENTS for 24 determinations

A Ag 24	**LINE strips** 24 numbered strips, coated with 30 reaction lines and red colour code: - 27 lines coated with allergens or allergen mixes - 3 Standard lines	24 strips for the determination of 27 antibody specificities each
B WASH 20x	**Wash buffer** sufficient for 800 ml solution	40 ml concentrate capped white
C CON 1E	**Conjugat 1** anti-human IgE, coupled with Digoxigenin	40 ml ready for use capped yellow
D CON 2E	**Conjugat 2** anti-Digoxigenin, coupled with HRP	40 ml ready for use capped green
E SUB	**Substrate** 3,3',5,5'-tetramethylbenzidine	40 ml ready for use capped blue
F STOP	**Stop solution** Tris/Phosphate buffered solution, pH 3.2	40 ml ready for use capped black
G	**Incubation tray** for 8 test strips	3
H	**Plastic tweezers**	1
I	**Interpretation template** for glueing of processed strips	1

Allergens coated on Allergy LINE sIgE Panel 1:

w1	Ragweed	t216	Alder
w6	Mugwort	t3	Silver birch
w9	Plantain	t4	Hazelnut
w20	Stinging nettle	t9	Olive
w21	Wall pelitory	t14	Cotton wood
m2	Cladosporium herbarum	t23	Cypress
e1	Cat dander	t25	European ash
e3	Horse dander	g2	Bermuda grass
e5	Dog dander	g17	Bahia grass
i6	German cockroach	g6	Timothy grass
i903	Am. cockroach	g12	Rye
-	total IgE	gx17	Grass mix** (g1, g3, g5, g8)
d1,d2	House dust mite mix *		
k81	Benjamin fig		
k82	Latex milk		

* Dermatophagoides pteronyssinus (d1), Dermatophagoides farinae (d2)

** Sweet vernal grass (g1), Orchard grass (g3), Ryegrass (g5), Kentucky blue grass (g8)

Materials required in addition

- adjustable micropipettes
- pipette tips
- horizontal or rocking shaker
- graduated cylinders
- distilled or deionised water
- timer
- paper towel

Size and storage

sIgE Allergy LINE 1 has been designed for 20 x 2 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label.

Upon receipt all components of the sIgE Allergy LINE 1 have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

Take strips with a plastic pincer only.

Prepare a sufficient amount of wash solution by diluting the concentrated washing buffer 20 times (1 + 19) with deionised or distilled water. For example, dilute 40 ml of the concentrate with 760 ml of deionised or distilled water.

For each test strip 10.5 ml of washing buffer are requested.

The wash solution prepared is stable at 2 - 8 °C up to 30 days.

All other components are ready for use and so stable until the expiry date.

Move the incubation tray on a shaker at 50 rpm. Ensure that the strips are immersed completely.

Avoid exposure of the substrate solution to light.

ASSAY PROCEDURE

Predilute samples at a ratio of **1:10**, i.e. 150 µl sample with 1.35 ml wash buffer (made from B), or preparation of sample dilution directly within the incubation tray.

1. Bring all reagents to room temperature (RT) (18-25°C) before use. Mix gently without causing foam.
2. Place the strips (A) with the reactive side up (labels on top) into the respective well. Dispense **1.5 ml** of diluted samples into the respective wells (or prepare sample dilution directly in the tray). Completely immerse the test strips.
3. Incubate **30 min** at RT (18-25°C) while shaking (about 50 rpm)..
4. Decant or aspirate, wash each well **3 times** for **3 min** with **1.5 ml** wash solution (made of B) while shaking. (Discard the solution in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper in order to remove the remaining fluid.)
5. Add **1.5 ml** conjugate 1 (C) to each well
6. Incubate **30 min** at RT (18-25°C) while shaking (about 50 rpm).
7. Decant or aspirate (see 4.) and wash each well **3 times** for **3 min** with **1.5 ml** wash solution (made of B) while shaking.
8. Add **1.5 ml** conjugate 2 (D) to each well
9. Incubate **30 min** at RT (18-25°C) while shaking (about 50 rpm).
10. Decant or aspirate (see 4.) and wash each well **3 times** for **3 min** with **1.5 ml** wash solution (made of B) while shaking.
11. Add **1.5 ml** of substrate (E) to each well.
12. Incubate **10 min** at RT (18-25°C) while shaking (about 50 rpm).
13. Add 1.5 ml of stop solution (F) to each well.
14. Incubate **5 min** at RT (18-25°C) while shaking (about 50 rpm).
15. Dry the strips: Collect the strips from the wells and put them onto absorbent paper with the **reactive side upwards**. After approximately **15 - 30 min** the strips are to be interpreted using the interpretation template.

EVALUATION OF RESULTS

Evaluation:

Results should be interpreted only after strips have been dried for about 15 to 30 minutes.

Each test strip contains methodological controls (standards). They appear as coloured bands after the test has been performed. If standards are missing, the result for the given test strip will be invalid and the test will need to be repeated.

If using a separately available reader, the results can be analyzed quantitatively. To do so, place the test strips in the reader and read out the results using the accompanying software. By comparing the intensities of the test and standard bands, the results can be determined in kUA/L and by class. Note the instructions in the corresponding manual. To evaluate the results semi-quantitatively, compare the intensities of the standards against those of the individual allergens / allergen mixes. See Table 1.

Correlation between Allergy LINE class and allergen-specific IgE:

Class *	kUA/L	Result
0	< 0,35	negative, no clinical significance
Standard 1		
1 – 2 (+)	≥ 0,35 < 3,5	low allergen-specific IgE concentration, of partial clinical significance
Standard 2		
3 – 4 (++)	≥ 3,5 < 50	moderate allergen-specific IgE concentration, often accompanied by clinical symptoms
Standard 3		
5 – 6 (+++)	≥ 50	high allergen-specific IgE concentration, clinical symptoms in most cases

* To measure total IgE, a different qualitative classification applies:
Class 0 – 2: < 100 kUA/L (result: seldom clinically relevant),
Class 3 – 6: ≥ 100 kUA/L (result: often clinically relevant)

Please note: A negative result for total IgE does not preclude positive results for allergen-specific IgE.

Interpretation of results:

In diagnostic testing for allergies, it is not customary to interpret measurements within stringently defined limits. The given reaction to a particular level of sensitization is very individual and cannot be fixed within precise limits. The higher the concentration of IgE, the greater the likelihood of a direct correlation with symptoms. To make a diagnosis, the test results must always be considered in conjunction with the symptoms and medical history.
Note! Allergy LINE is used to test allergens which permit potential cross-reactions to be predicted. This must be considered when evaluating the results, deciding on further diagnostic examinations, and advising the patient.

Limitations of Method

1. A conclusive clinical diagnosis should not be based solely on the results of a single diagnostic procedure. In addition to the in vitro determination of allergen-specific IgE, a thorough medical history should be taken and the various symptoms investigated.
2. Every patient reacts differently. Hence, identical results delivered by the test will not automatically lead to the same diagnosis. Diverse allergens with similar molecular structures or epitopes can trigger weak or strong cross-reactions which must always be taken into account.
3. Occasionally, negative in vitro results can also be obtained in patients with symptoms which are clearly correlated to contact with certain allergens.
4. Sensitization to other allergens not contained in the Allergy LINE test cannot be ruled out.
5. Patients with clinical symptoms and Allergy LINE class 0 reactions to the respective allergens/allergen mixtures should be referred to a specialist for further investigation.
6. The binding capacities of allergen-specific IgE antibodies can vary from allergen to allergen. Therefore, identical classes for various allergens will not necessarily correspond to the same sIgE content.
7. Reliable and reproducible results are achieved if the test is performed in accordance with the methodological instructions and good laboratory practice.

Expected results:

As a rule, the higher the class that is determined the higher the concentration of allergen-specific IgE against the respective allergen in Allergy LINE. As with all sIgE tests, cross-reactions can occur between related and unrelated allergens containing similar or homologous molecules (antigens). The closer the biological relationship between the different types, the greater the degree of structural and immunologic similarity between the allergens of both species.

In light of the immunologic cross-reactions of structurally related allergens, therefore, a patient exhibiting a clinical reaction to one allergen is likely to also react to allergens of closely related species. On the other hand, cross-reactions can also occur between species that biologically are distantly related. Some protein families are widespread and contain highly preserved structures that can function as largely consistent epitopes. The performance data of the test were collected in the range of <0.35 kUA/L to >100 kUA/L, which corresponds to the biological reference range.

CHARACTERISTIC ASSAY DATA

Analytical Sensitivity

sIgE Allergy LINE 1 detects allergen-specific IgE antibodies. No cross-reactivity with other immunoglobulin species is known.

Diagnostic Specifity and Sensitivity

The test results obtained with Allergy LINE have been evaluated in comparison with the Thermo Fisher Scientific ImmunoCAP system. Indirect traceability to WHO standard 75/502 for IgE has been demonstrated by the above comparison. There are no known limitations or interferences.

Precision

Inter-assay precision: Duplicate assays were performed on control serum to determine inter-assay precision. The tests were performed by five different individuals on two consecutive days. The mean values (MV) and coefficients of variation (CV) were determined separately for each allergen. Values below 10% are accepted for the inter-assay precision of all allergens with positive signals. This specification criterion is not appropriate for describing the precision of allergens with negative signals. The result is accepted if the analysis is consistent across all 20 measurements.

Allergen code	Number of Measurements	MV (RAST)	CV (%)	Analysis
e1	**20**	**3,2**	**6,4**	**Positiv**
g2	**20**	**0,3**	**9,0**	**Negativ**
g17	**20**	**5,5**	**8,4**	**Positiv**
k82	**20**	**4,4**	**5,7**	**Positiv**
m2	**20**	**3,2**	**3,3**	**Positiv**
t9	**20**	**3,8**	**4,4**	**Positiv**
t14	**20**	**4,0**	**7,9**	**Positiv**
t25	**20**	**4,7**	**6,1**	**Positiv**
w1	**20**	**5,7**	**6,4**	**Positiv**
w9	**20**	**4,8**	**6,7**	**Positiv**

INCUBATION SCHEME

sIgE Allergy LINE 1 (5110)

Dilution of patient samples 1:10, e.g. 150 µl sample with 1.35 ml wash buffer (made from B), or sample dilution directly in the incubation tray (step 2)

1.	Bring all reagents and the requested number of strips to room temperature (18-25°C)	
2.	Place the strips with the reactive side upside in the respective tray and dispense 1.5 ml of diluted sample	
3.	Incubate while shaking	30 minutes RT (18-25°C)
4.	Decant, wash strips while shaking	3 x 3 min with 1.5 ml (made of B)
5.	Pipette 1.5 ml conjugate 1 (C) in the respective well	
6.	Incubate while shaking	30 minutes RT (18-25°C)
7.	Decant, wash strips while shaking	3 x 3 min with 1.5 ml (made of B)
8.	Pipette 1.5 ml conjugate 2 (D) in the respective well	
9.	Incubate while shaking	30 minutes RT (18-25°C)
10.	Decant, wash strips while shaking	3 x 3 min with 1.5 ml (made of B)
11.	Pipette 1.5 ml substrate (E)	
12.	Incubate while shaking	10 minutes RT (18-25°C)
13.	Pipette 1.5 ml stop solution (F)	
14.	Incubate while shaking	5 minutes RT (18-25°C)
15.	Put the strips **reaction side up** onto absorbent paper to dry. After approximately 15 - 30 min the strips are ready to be interpreted	

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for re-constituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C prior use in the original shipping container.
- Some of the reagents contain small amounts of Neolone M10 (< 1.0 % v/vw) as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Since the kit contains potentially hazardous materials the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

REFERENCES

1. Williams PB, et al. J. Efficacy of a single diagnostic test for sensitization to common inhalant allergens. Ann Allergy Asthma Immunol 2001;86:196-202.
2. Ahlstedt S. Understanding the usefulness of specific IgE blood tests in allergy. Clin Exp Allergy 2002;32:11-6.
3. Martinez FD, et al. Asthma and wheezing in the first six years of life. N Engl J Med 1995;332:133-8.
4. Heinzerling L et al. Standard skin prick testing and sensitization to inhalant allergens across Europe – a survey from the GA2LEN network. Allergy 2005;60:1287-1300.
5. Wever-Hess J, et al., Prognostic characteristics of asthma diagnosis in early childhood in clinical practice. Acta Paediatr 1999;88:827-34.
6. Duran-Tauleria E, et al. The utility of specific immunoglobulin E measurements in primary care. Allergy 2004;59 (Suppl.78):35-41.
7. Matsson, P; Analytical performance characteristics and clinical utility of immunological assay for human immunoglobulin E (IgE) antibodies and defined allergen specificities: approved guideLINE. Clinical and Laboratory Standards Institute. 2nd ed. Wayne, PA : Clinical and Laboratory Standards Institute, 2009.
8. Chang ML, Cui C, Liu YH, Pei LC, Shao B. Analysis of total immunoglobulin E and specific immunoglobulin E of 3721 patients with allergic disease. Biomed Rep 2015;3:573-577.
9. Sanz ML, et al. Diagnostic reliability considerations of specific IgE determination. J Investig Allergol Clin Immunol. 1996 May-Jun;6(3):152-61)