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See external label



2°C-30°C



Σ=25 or 50 tests



Cat. #15150-1

IgE Serum Test

IgE Test Kit

Cat. No. 15150-1

Summary

Allergic or immediate hypersensitivity reactions are known as Type I immunopathologic reactions that may occur within minutes of an allergic challenge. In 1967 Ishizaka identified the serum factor capable of mediating allergic reactivity as Immunoglobulin E (IgE). The Fc portion of this reaginic antibody attaches to Fc receptors on the surface of target cells, tissue mast cells, and circulating basophils leaving the F(ab) portion of the molecule available to bind with its homologous antigen. Upon contact with a specific allergen, the IgE mediates cell release of pharmacologic substances such as histamine, prostaglandins, and leukotrienes that result in allergic reactions ranging from hay fever, urticaria (hives), and bronchial asthma, to generalized anaphylactic shock.

The discovery of the role of IgE in clinical allergy resulted in a new generation on in-vitro diagnostic assays to test for allergen sensitivity. The first immunoassays were developed to quantitate the serum concentration of total IgE. In normal individuals, IgE is usually present at low levels where 130 ng/mL represents the upper limit of the normal range. However, a significant number of asymptomatic normal individuals, patients with parasitic diseases and patients with depressed cell-mediated immunity exceed this level. Also, some allergic (atopic) persons may exhibit normal total IgE test results in the presence of elevated levels of specific IgE. Therefore, although the total serum IgE level is considered useful in the evaluation of an allergic patient, more important is the demonstration of allergen-specific IgE in patients' serum. RAST was developed for this purpose.

An elevated concentration of Immunoglobulin E (IgE) in serum is a common symptom associated with allergic pathologies. Autoimmune diseases and some infections may cause the elevation of IgE. The One Step IgE test can detect elevated levels of IgE rapidly.

The One Step IgE test is a highly sensitive immunoassay for qualitative determination of human IgE in plasma or serum. This test is intended for professional use as an aid in the diagnosis and treatment of IgE-mediated allergic and autoimmune disorders. One Step IgE test can detect elevated levels of IgE in 5 minutes or less. The sensitivity of the test is 80 IU/ml.

IgE test cassette has a letter of T and C as "Test Line" and "Control" on the surface of the case. Both the "Test Line" and "Control Line" in result window are not visible before applying any samples. The "Control Line" is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. When the specimen flows through a absorbing pad containing purified IgE antibody coupled to purple beads. If the sample contains IgE specific IgG antibody, the antibody will bind to the antigen coupled to the red beads which, in turn, will bind to a monoclonal anti-human IgG antibody spotted on the membrane in the form of a line. As the IgE antigen-antibody complex is captured, a purple Test Line will be visible in the Result Window. If IgE-specific IgG antibody is not present or is present at very low levels in the patient sample, there is no color appears in Test Line.

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A. Background

Allergic or immediate hypersensitivity reactions are known as Type I immunopathologic reactions that may occur within minutes of an allergic challenge. In 1967 Ishizaka identified the serum factor capable of mediating allergic reactivity as Immunoglobulin E (IgE). The Fc portion of this reaginic antibody attaches to Fc receptors on the surface of target cells, tissue mast cells, and circulating basophils leaving the F(ab) portion of the molecule available to bind with its homologous antigen. Upon contact with a specific allergen, the IgE mediates cell release of pharmacologic substances such as histamine, prostaglandins, and leukotrienes that result in allergic reactions ranging from hay fever, urticaria (hives), and bronchial asthma, to generalized anaphylactic shock.

The discovery of the role of IgE in clinical allergy resulted in a new generation on in-vitro diagnostic assays to test for allergen sensitivity. The first immunoassays were developed to quantitate the serum concentration of total IgE. In normal individuals, IgE is usually present at low levels where 130

ng/mL represents the upper limit of the normal range. However, a significant number of asymptomatic normal individuals, patients with parasitic diseases and patients with depressed cell-mediated immunity exceed this level. Also, some allergic (atopic) persons may exhibit normal total IgE test results in the presence of elevated levels of specific IgE. Therefore, although the total serum IgE level is considered useful in the evaluation of an allergic patient, more important is the demonstration of allergen-specific IgE in patients' serum. RAST was developed for this purpose.

Historically, the diagnosis of allergic disease has been based primarily on two types of in-vivo testing; i.e., provocation (challenge testing) and skin testing. The use of provocation testing involves double-blind challenge that duplicates natural exposure. Responses to challenge other than by natural exposure are dependent on physical characteristics of test material as well as on the dose and duration of exposure to it. Skin testing, either intradermal or prick/puncture (epicutaneous) is a frequently used clinical method of diagnosing allergy. Although these tests do produce false results in some instances, for certain allergens they exhibit greater clinical sensitivity than RAST. However, other recognized clinical modalities may also be used to assess the clinical status of patients. These include nasal or bronchial challenge tests for diagnosing inhalant allergies, and oral challenge testing for diagnosis of food allergies. In-vitro allergen-specific IgE testing is especially useful when skin testing cannot be performed or interpreted at patients with generalized dermatitis, or in those who must continue to take antihistamine medications. In-vitro testing also eliminates the risk of possible systemic reaction. It is essential that allergy test result, regardless of the procedure employed, reflect true clinical sensitivity and specificity in order to prevent mismanagement of patients.

The best correlation between RAST-type tests and skin tests have been with inhalant allergens such as the pollens of trees and grasses. The FDA has noted in data received from various sponsors that concordance with skin tests varies with different allergens to less than 20% for other allergens. Therefore, FDA requests manufacturers to provide allergy specific clinical data for each new allergen they submit for their RAST-type test.

Atopic status of patients may also be defined by physical examination and clinical history and should be included as part of the comparison data. Since January 1990, manufacturers have been required to state in their package inserts the concordance for each allergen between the RAST-type test and the comparative clinical test if the concordance is below 65-70 percent.

In order to alert RAST users to an important potential cause of false negative results for food allergies, the following statement should be incorporated into the Limitations section of package inserts:

"When testing for food allergies, circulating IgE antibodies may not be detected if they are directed towards altered forms of allergens (such as cooked, processed or digested) and the altered forms are not present in the same form as those food allergens which are used in the test."

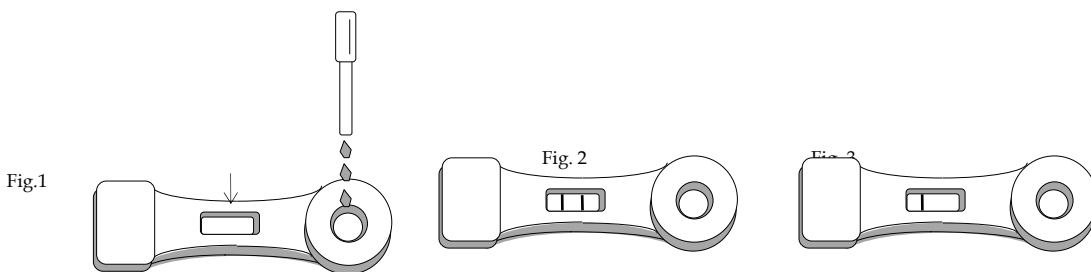
Two other tests for food allergies that are the subjects of scientific concern are cytotoxicity testing, and the measurement of serum levels of IgG and IgG4 antibodies to specific food allergens. Clinical data for these two tests are not conclusive for diagnosis of food allergies and FDA does not recognize these tests to be safe and effective.

The first preamendment (prior to May 28, 1976) test kit intended for measurement of circulating allergen specific IgE in blood specimens used a paper radioimmunosorbent disc (PRIST) which was the forerunner of the current RAST and RAST-type systems. The original RAST methodology was radioimmunoassay. Later modifications included enzymeimmunoassay, luminescence immunoassay and fluorescence immunoassay in fluid phases, as well as a variety of solid phases including dipsticks. Methodologies used to assay Total IgE include radioimmunoassay, enzymeimmunoassay, and laser nephelometry.

In the time span from 1977 to 1981 a total of five 510(k) notifications were submitted by device manufacturers for review. From 1986 to 1992, the total number of RAST submissions received by FDA increased to 167. This increase represents expansion of the numbers of allergens proposed for use with original test systems, modification of existed test kits such as the use of monoclonal antibody reagents, and additional companies manufacturing these tests.

The One Step IgE test is a highly sensitive immunoassay for qualitative determination of human IgE in plasma or serum. This test is intended for professional use as an aid in the diagnosis and treatment of IgE-mediated allergic and autoimmune disorders. One Step IgE test can detect elevated levels or IgE in 5 minutes or less. The sensitivity of the test is 80 IU/ml.

IgE test cassette has a letter of T and C as "Test Line" and "Control" on the surface of the case. Both the "Test Line" and "Control Line" in result window are not visible before applying any samples (Fig. 1). The "Control Line" is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working (Fig. 2). When the specimen flows through an absorbing pad containing purified IgE antibody coupled to purple beads. If the sample contains IgE specific IgG antibody, the antibody will bind to the antigen coupled to the red beads which, in turn, will bind to a monoclonal anti-human IgG antibody spotted on the membrane in the form of a line. As the IgE antigen-antibody complex is captured, a purple Test Line will be visible in the Result Window (Fig. 3). If IgE-specific IgG antibody is not present or is present at very low levels in the patient sample, there is no color appears in Test Line.



Cortez Diagnostics IgE test kit contains following items to perform the assay; **Cortez Diagnostics** IgE test device, disposable sample dropper and instructions for use. However, the test kit do not contains specimen collection container and clock or timer. The test kit should be stored at room temperature. The expiration date was determined under normal laboratory conditions.

B. Summary of Safety and Effectiveness Studies

1. Name of Product:

One Step Antigen of the Immunoglobuline E (Ig E)Test.

2. Indication:

The One Step Ig E test is designed for the qualitative determination of human surface antigen of the immunoglobuline E in serum.

3. Standard Substance:

Antibody to Antigen of the Ig E.

4. Component Composition:

Each test unit contains:

Major Ingredients	Quantity/Test
Goat Anti-(Mouse IgG) Polyclonal Antibody	62 ug/test
Goat anti-Ig E antibody	44 ug/test
anti-Ig E antibody-Colloidal Gold Conjugate	34 ug/test

5. Production Process:

- a). Nitrocellulose membrane is coated with a Goat Anti – (Mouse IgG) Polyclonal Antibody solution in the Control Reaction Zone, and coated with anti-Ig E antibody conjugate solution in the Test Reaction Zone. The coated membrane is dried overnight, blocked with a PBS/Triton X-100 buffer for 5 minutes, and then dried overnight again.
- b). A buffered solution of anti-Ig E. antibody–Colloidal Gold Conjugate is sprayed on fiberglass and then lyophilized in a 48-hour cycle.
- c). The blocked and dried membrane is then applied to an adhesive-backed vinyl strip. An absorbent material is applied to the vinyl strip so that it is in contact with the membrane close to the Control Reaction Zone. The lyophilized conjugate – coated fiberglass is applied to the vinyl strip so that it is in contact with the membrane close to the Test Reaction Zone. The completed strips are then die-cut at a 7 mm width or 5 mm.
- d). The die-cut strips are assembled in plastic housings and sealed in moisture-proof foil pouches along with a desiccant packet and a disposable plastic pipette.

6. Analytical Sensitivity:

An in-house study was conducted with three separate lots of the One Step Ig E.Serum Test to determine the analytical sensitivity of the test. Each lot was tested three times at each control level, with the comparison results shown in the following table.

Table I: Analytical sensitivity testing.

Test Lot	# of times	0	20	40	80	160	320
A	1	(-)	(-)	(-)	(+)	(+)	(+)
	2	(-)	(-)	(-)	(+)	(+)	(+)
	3	(-)	(-)	(-)	(+)	(+)	(+)
B	1	(-)	(-)	(-)	(+)	(+)	(+)
	2	(-)	(-)	(-)	(+)	(+)	(+)
	3	(-)	(-)	(-)	(+)	(+)	(+)
C	1	(-)	(-)	(-)	(+)	(+)	(+)
	2	(-)	(-)	(-)	(+)	(+)	(+)
	3	(-)	(-)	(-)	(+)	(+)	(+)

7. Specificity:

An in-house study is conducted with 3 separated lots of the One Step Ig E Serum Test to determine the Specificity of One Step Ig E. test.

Serum with trygliceride concentration up to 500 mg/ml,

Serum with Dilirbin concentration up to 10 mg/100ml,

Hemolized specimens with hemoglobin concentration up to 10 mg/ml

Prostatic acid phosphatase with concentration up to 1000 ng/ml

albumin with concentration up to 20 mg/ml.

All of the above were analyzed and did not show interference or cross reactivity with the test.

8. Reproducibility & Stability:

Method

Reproducibility and stability studies were carried out on three different lots of the One Step Ig E. Serum Test. The lots, upon approval (t=0) were stored at 15 – 38 degrees C, and tested over an eighteen month time period from date of manufacture. In-house controls were tested according to the Instructions for use with the three lots at regular intervals (0,3,6,9,12,15, and 18 months from date of approval).

Acceptance Criteria

Each stability test is passed if:

- a. The test gave a negative result when tested with a Ig E-negative serum sample.
- b. The test gave a positive result when tested with a 80 IU/ml Ig E-positive serum sample.

Results are shown in the following table.

Table II: Stability testing.

Month	Number	Ig E.	negative	sample	Ig E.	positive	sample
		Lot A	Lot B	Lot C	Lot A	Lot B	Lot C
0	1	(-)	(-)	(-)	(+)	(+)	(+)
	2	(-)	(-)	(-)	(+)	(+)	(+)
	3	(-)	(-)	(-)	(+)	(+)	(+)
3	1	(-)	(-)	(-)	(+)	(+)	(+)
	2	(-)	(-)	(-)	(+)	(+)	(+)
	3	(-)	(-)	(-)	(+)	(+)	(+)
6	1	(-)	(-)	(-)	(+)	(+)	(+)
	2	(-)	(-)	(-)	(+)	(+)	(+)
	3	(-)	(-)	(-)	(+)	(+)	(+)
9	1	(-)	(-)	(-)	(+)	(+)	(+)
	2	(-)	(-)	(-)	(+)	(+)	(+)
	3	(-)	(-)	(-)	(+)	(+)	(+)
12	1	(-)	(-)	(-)	(+)	(+)	(+)
	2	(-)	(-)	(-)	(+)	(+)	(+)
	3	(-)	(-)	(-)	(+)	(+)	(+)
	1	(-)	(-)	(-)	(+)	(+)	(+)

15	2	(-)	(-)	(-)	(+)	(+)	(+)
	3	(-)	(-)	(-)	(+)	(+)	(+)
	1	(-)	(-)	(-)	(+)	(+)	(+)
18	2	(-)	(-)	(-)	(+)	(+)	(+)
	3	(-)	(-)	(-)	(+)	(+)	(+)

Comparison Study:

(1) Clinical Symptoms Criteria:

The bronchial asthma is only allergic disorder selected for this study. Bronchial asthma may be defined as episodic expiratory dyspnea associated with wheezing. Such patients are apt to show temporary relief after the administration of epinephrine. The cough in asthma is dry, tight, wheezy and usually occurs in paroxysms. Chest examination during an attack reveals the suppressed breath sounds of small airway obstruction and musical rales or rhonchi. Postmortem examination reveals extensive blocking of the finer airways with inspissated mucus plugs. Hay fever and fatigue may occur.

(2) Population Tested:

The population tested at three sites as following:

Population tested;

Table 3

Population tested

	Site A	Site B	Site C
Male	10	10	4
Female	10	10	6
Age: <20	5	6	2
20-30	4	6	2
31-50	6	5	3
51-70	4	3	1
>70	1	0	1

(3) Criteria for diagnosis:

Patient suffered with episodic expiratory dyspnea associated with wheezing has been diagnosis with bronchial asthma that is only allergic disorder selected for this study.

(4) Inclusion / Exclusion Criteria:

If patient has above symptoms that has been release by medication and also were willing tested by laboratory IgE test and following confirmed tests were selected in study group.

(5) Test procedure:

An independent comparison study was performed at hospital laboratory on 50 symptomatic patients. Each specimen was tested with the One Step IgE Serum Test and a commercially available test (ELISA). The results are summarized in the following tables.

	Ig E one step Test Positive	Ig E one step Test Negative
ELISA Positive (31)	30	1
ELISA Negative (19)	0	19

For symptomatic patients, the relative sensitivity is 96.77% (30/31), and the relative specificity is 100% (19/19).

The data demonstrates that the One Step Ig E. Test is substantially equivalent to the commercially available test. The clinical significance of the two tests is comparable.

Near Patient Test Result

a, Clinical Specificity

The clinical specificity is defined as the probability to have a negative result in the absence of the particular condition.

The clinical specificity assessed by studying expected negative subjects.

We classified negative a sample that, tested by IgE test, showed below 80 IU/ml.

Sample was tested in replicates of 20 in a single run.

The results are shown in the following table.

	number of replic	Results	remark
Control conc. 0 IU/ml	20	(-)	

b, Clinical Sensitivity

The clinical sensitivity is defined as the probability to have a positive result when the particular condition is present.

The clinical sensitivity was evaluated testing samples of positive control that resulted reactive for Ig E.

We classified as positive a sample that, tested by Ig E., showed a level 80 IU/ml, 160 IU/ml.

The results are reported in the following table:

Sample conc.	number of replica	results	Remark
80 IU/ml	10	(+)	
160 IU/ml	10	(+)	

The reproducibility is evaluated on 2 controls containing different levels of Ig E.

Each sample was tested in replicates of 10 in a single run.

The results are shown in the following table:

Sample conc.	number of replic	results	Remark
80 IU/ml	10	(+)	
0 IU/ml	10	(-)	

C. QUALITY CONTROL OF IgE TEST

Trial site

The final QC certificate is issued by Cortez Diagnostics, Inc.

Trial Protocols

Different protocols were used in the performance evaluation of Ig E., as listed below.

Precision
 intra – assay
 inter – lot
 analytical sensitivity

Detection limits

Precision

Intra – Assay Precision

The within assay precision was evaluated on 5 samples containing different levels of Ig E.

Each sample was tested in replicates of 10 in single run.

The results are shown in the following table:

sample of Ig E. Conc.	# of replic	Results	Remark
80IUng/ml	10	(+)	
160IU/ml	10	(+)	
320IU/ml	10	(+)	
640IU/ml	10	(+)	
1000IU/ml	10	(+)	

Inter – lot Precision

The between assay precision was evaluated on 3 samples containing different levels of Ig E.

Each sample was tested in replicates of four using three different lots.

The results are shown in the following table.

Lot #	# of test per Lot	Ig E.	Antibody Conc.	320 IU/ml
		80 IU/ml	160 IU/ml	
A	4	(+)	(+)	(+)
B	4	(+)	(+)	(+)
C	4	(+)	(+)	(+)

Detection limits

Analytical Sensitivity

An in-house study was conducted with three separate lots of the One step Ig E. Test to determine the analytical sensitivity of the test. Each lot was tested five times at each control level, with the results shown in the following table:

Analytical sensitivity testing

Test Lot	# of times	Ig E.	Antibody	Conc.	(IU/ml)	
		0	80	160	320	640
	1	(-)	(+)	(+)	(+)	(+)
	2	(-)	(+)	(+)	(+)	(+)
A	3	(-)	(+)	(+)	(+)	(+)
	4	(-)	(+)	(+)	(+)	(+)
	5	(-)	(+)	(+)	(+)	(+)
B	1	(-)	(+)	(+)	(+)	(+)
	2	(-)	(+)	(+)	(+)	(+)
	3	(-)	(+)	(+)	(+)	(+)
	4	(-)	(+)	(+)	(+)	(+)
	5	(-)	(+)	(+)	(+)	(+)
	1	(-)	(+)	(+)	(+)	(+)
	2	(-)	(+)	(+)	(+)	(+)
C	3	(-)	(+)	(+)	(+)	(+)
	4	(-)	(+)	(+)	(+)	(+)
	5	(-)	(+)	(+)	(+)	(+)

Results:

The analytical sensitivity is 80 IU/ml.

C. Bibliography

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- 5, Wall R and Kuehl M, "Biosynthesis and Regulation of Immunoglobulins," *Annu Rev Immunol*, 1983, 1:393-422.



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