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Hitachi Chemical Diagnostics, Inc.

U.S. PACKAGE INSERT FOR CLA® ALLERGEN-SPECIFIC IgE ASSAY

For *in vitro* diagnostic single use

Doc.No.: 0604
Rev.: 03

1 Reagents/Components

CLA® Allergen-Specific IgE Assay*

Store at 2-8°C until expiration date. Do not freeze.

Component Description	Each 20-Test Kit Includes
Pette Test Chambers Specific allergens or allergen mixes covalently bound to cellulose thread	20 test chambers
Wash Buffer Concentrate Solution that when diluted contains 0.01 M phosphate-buffered saline, 0.1% Tween 20, and 0.001% sodium azide as preservative	Two bottles, 50 mL each
IgE Antibody Solution containing: Blue-colored solution containing Enzyme-labeled goat anti-human IgE, 0.01 M phosphate-buffered saline, pH 7.2, protein stabilizers, 0.1% Proclin® as a preservative.	One bottle, 32 mL
Photoreagent A Solution containing: 14-30 mM 3-aminophthalhydrazide (luminol)	One bottle, 8 mL
Photoreagent B Solution containing: 0.05 M borate buffer, pH 9.4	One bottle, 8 mL
Photoreagent C Red-colored solution containing: 0.0025 M ethyl orange	One bottle, 8 mL
Photoreagent D Solution containing: 0.004 M hydrogen peroxide	One bottle, 8 mL
Test Chamber Rubber Plugs (black) For top of Test Chambers	22 plugs
Test Chamber Rubber Plugs (white) For bottom of Test Chambers	22 plugs

*Available in various kit configurations. Contact your local Hitachi Chemical Diagnostics representative for details.

2 Intended Use

The CLA® Allergen-Specific IgE Assay is a single use *in vitro* test for the semiquantitative determination of circulating allergen-specific IgE concentrations in human serum.

3 Summary and Explanation of the Test

Atopic allergy is a hypersensitive immunologic condition mediated by Immunoglobulin E (IgE).[1, 2] Immunocompetent B lymphocytes, when stimulated by a specific allergen, produce IgE antibodies to the allergen. IgE antibody binds, via its Fc portion, to receptors on the surface of mast cells and basophilic leukocytes.[3] Subsequent binding of allergen to cell-bound specific IgE triggers cell degranulation and the release of vasoactive amines, causing smooth muscle contraction, itching, swelling, and transmucosal leakage of extracellular fluids. The most common clinical manifestations are hay fever, asthma, dermatitis, hives, and anaphylactic shock. The assessment of a patient's IgE level for various allergens is valuable in the diagnosis and treatment of atopic allergy.[4, 5]

The CLA Allergen-Specific IgE Assay permits the simultaneous determination of a patient's IgE level to a multiple number of specific allergens.[6] All CLA allergen panels incorporate internal control features that assess assay performance and compensate for non-specific binding in a patient's serum sample. The CLA Allergen-Specific IgE Assay offers the convenience and simplicity of non-isotopic, simultaneous multiple-allergen testing.[7]

4 Principle of the Procedure

The CLA Allergen-Specific IgE Assay employs a small plastic device known as a Test Chamber to expose patient serum simultaneously to a number of allergens or allergen mixes. The Test Chamber contains discrete segments of cellulose thread, each with an allergen or allergen mix covalently bound to it. Each Test Chamber also contains one Negative Blanking Control and one Positive Procedural Control.

The CLA Allergen-Specific IgE Assay is run by filling a Test Chamber with patient serum. IgE in the serum binds to the allergen-coated cellulose threads during incubation. The Test Chamber is then washed with buffer to remove unbound serum components. Enzyme-labeled anti-IgE is then added to the chamber and couples with the serum IgE bound to the threads. After a second wash, the Test Chamber is filled with a photoreagent mixture that reacts with the labeled antibody to produce chemiluminescence. The amount of light emitted by each thread is directly proportional to the amount of allergen-specific IgE in the patient serum.

5 Precautions

- The CLA Allergen-Specific IgE Assay is for *in vitro* diagnostic single use.
- The Wash Buffer Concentrate contains sodium azide as a preservative. Sodium azide has been reported to react with lead or copper plumbing to form potentially explosive metal azides. Therefore, use caution when disposing of this reagent, and always flush with an adequate volume of water to prevent metal azide buildup in plumbing systems.[8]
- Do not use kit components after the expiration date. The expiration date is printed on each component.

- Component reagents of the CLA Allergen-Specific IgE Assay kits are provided as matched sets. Do not mix with other kit lot reagent sets.
- Bleach contamination has been found to interfere with the test.

6 Reagent Preparation

Wash Buffer:

- Allow Wash Buffer Concentrate to reach room temperature. Check to see that all salt crystals have dissolved.
- Add contents of Wash Buffer Concentrate bottle (50 mL) to 950 mL of distilled or deionized water in a clean 1 L graduated cylinder or flask. Mix thoroughly.
- Transfer solution to Wash Buffer Dispenser.
- Once prepared, the Wash Buffer solution can be used for up to 1 month when stored at room temperature (20-25°C) or refrigerated (2-8°C).

IgE Antibody:

- Allow IgE Antibody to reach room temperature before use.

Photoreagent Mixture:

Prepare Photoreagent Mixture just before use.

- Allow Photoreagents A, B, C, and D to reach room temperature.
- Using a micropipette with disposable tips, combine **equal parts of** Photoreagent A, B, C, and D in a container. A minimum of **350 µL** of **each** Photoreagent is required per Test Chamber being assayed, i.e., **1.4 mL** of Photoreagent Mixture per Test Chamber.
- Gently swirl the container to mix.
- Photoreagent Mixture should be used within 60 minutes of mixing.

7 Storage Instructions

- Store kit components at 2-8°C. When stored as directed, the components can be used until the expiration dates printed on the individual component labels.
- Do not freeze kit components.
- The Test Chambers are packaged in a plastic bag with a moist sponge. Be sure the plastic bag is sealed properly before and after use. When stored in the sealed bag at 2-8°C, Test Chambers can be used until the printed expiration date.

8 Specimen Collection and Preparation

Handle all patient samples and used kit components as recommended for any potentially infectious human serum or blood specimen. Follow Universal Precautions or other guidelines as established by your institution when handling patient specimens.[9-11]

The minimum volume of human serum required for each Test Chamber is as follows:

One 36-allergen Test Chamber, 1.4 mL of serum
One 16-or-fewer-allergen Test Chamber, 0.8 mL of serum

Collect a venous blood sample into a 10 mL serum separator tube or red-top tube. Patient need not be fasting. No special preparations are necessary. Hemolyzed or lipemic serum may adversely affect the performance of the CLA Allergen-Specific IgE Assay. Serum samples may be stored at 2-8°C for up to one week. For longer periods, store samples frozen at -20°C. Repeated freezing and thawing of serum samples should be avoided. Frozen samples that have been thawed should be thoroughly mixed before centrifugation.

9 Assay Procedure

Refer to the *User Guide & Procedural Manual* for detailed instructions on test operation.

Materials Provided

- CLA Allergen-Specific IgE Assay
- CLA Equipment Kit,
- CLA-1 Luminometer System

Materials Required But Not Provided

- Graduated cylinder or flask, 1 L, for preparing Wash Buffer
- Deionized or distilled water

Preparation of Test Chambers and Patient Samples

Re-centrifuge serum samples for 10 to 20 minutes at 2000-3000 x g immediately prior to use. Turn off the centrifuge brake prior to spinning the serum samples.

1. Remove Test Chambers (one per specimen) from plastic bag. Reseal plastic bag and return unused Test Chambers to refrigerator.
2. With colored grid facing down, label each Test Chamber with appropriate patient identification.
3. Record kit lot numbers, panel lot numbers, and patient identifications.
4. Gently tap Test Chamber tip onto an absorbent paper towel to remove any residual liquid from inside the test chamber.

Procedure

A. Fill Test Chambers with Serum:
1. Attach the 3 cc syringe to top of Test Chamber.
2. Insert bottom of Test Chamber into tube containing patient serum. Avoid any precipitate and/or lipid layer.
3. Slowly withdraw syringe plunger to draw serum into Test Chamber until top thread is covered.

B. Plug and Incubate Test Chambers:
1. With syringe still attached to top of Test Chamber, insert a white plug into bottom of Test Chamber.
2. Remove the syringe and insert black plug into top of Test Chamber.
3. Incubate upright in workstation rack at room temperature for **16 to 24 hours.**

C. Drain Serum:
1. Remove bottom plug from each Test Chamber and place Test Chamber back in workstation rack.
2. Remove top plug from each Test Chamber, allowing serum to drain into workstation reservoir.

D. Wash Test Chambers:
1. Prime Wash Buffer dispenser until all air bubbles are removed.
2. Attach end of opened dispenser tubing to top of first Test Chamber.
3. Sequentially wash each Test Chamber once with 10 mL of Wash Buffer. Allow each Test Chamber to drain completely before proceeding to next step.
4. Repeat Step 3 two more times for a total of three washes. Test Chambers must be filled with Antibody Reagent immediately after washing to avoid drying of threads.

E. Fill Test Chambers with Antibody Reagent:

1. Gently tap bottom of Test Chamber tip on absorbent paper towel to remove any remaining Wash Buffer.
2. Attach the 3 cc syringe to top of the Test Chamber.
3. Place bottom of Test Chamber into Antibody Reagent container. Use disposable reagent cup to hold Antibody Reagent.
4. Slowly withdraw syringe plunger to draw Antibody Reagent into Test Chamber until top thread is covered.

F. Plug and Incubate Test Chambers:

1. Insert white bottom plug into Test Chamber with syringe still attached to top of the Test Chamber.
2. Remove syringe and insert black top plug.
3. Store reagent-filled Test Chambers upright in workstation rack. Incubate at room temperature for **4 hours ± 15 minutes.**

G. Drain Antibody Reagent:

1. Remove bottom plug from each Test Chamber and place each Test Chamber back in workstation rack.
2. Remove top plug from each Test Chamber, allowing liquid to drain into workstation reservoir.

H. Wash Test Chambers three (3) times as described in Steps E1 through E4.

I. Prepare Photoreagent Mixture as instructed in Section 6, **REAGENT PREPARATION.** Immediately after the last wash to avoid drying of threads.

J. Fill Test Chambers with Photoreagent Mixture:

1. Gently tap bottom of Test Chamber tip on absorbent paper towel to remove any remaining Wash Buffer.
2. Attach syringe to top of Test Chamber.
3. Place bottom of Test Chamber into disposable cup containing Photoreagent Mixture.
4. Slowly withdraw syringe plunger to draw Photoreagent Mixture into Test Chamber until chamber is completely filled to the top.

K. Plug Test Chambers:

1. Insert white bottom plug into Test Chamber with syringe still attached to top of the Test Chamber.
2. Remove syringe and insert black top plug.
3. Wipe away any Photoreagent mixture from outside of Test Chambers with a clean, damp, lint-free wipe.

L. Allow all Test Chambers to incubate for 20 minutes before reading in the Luminometer. All Test Chambers must be read within 60 minutes of introduction of photoreagent.

M. To read results, refer to the *User Guide and Procedural Manual.*

10 *Quality Control*

A. Internal Control Threads

Each Test Chamber contains a Positive Procedural Control and a Negative Blanking Control. These threads function as internal indicators for each Test Chamber.

Positive Procedural Control: The Positive Procedural Control checks the performance of kit reagents. The Positive Procedural Control must generate a reading greater than or equal to 243 LUs in the CLA-1 Luminometer.

Negative Blanking Control: The Negative Blanking Control compensates for any nonspecific IgE binding that may occur. The Negative Blanking Control must generate a reading of less than or equal to 33 LUs in the CLA-1 Luminometer.

B. IgE Positive and Negative Control Sera

Hitachi Chemical Diagnostics recommends testing with two levels of serum controls: CLA IgE Positive Control Serum and CLA IgE Negative Control Serum. For instructions on their use and acceptability of results, refer to the CLA IgE Positive and Negative Control Sera Package Insert. Frequency of testing with control should be decided by each laboratory according to regulatory agencies' requirements.

11 *Results*

The CLA-1 Luminometer measures the amount of light emitted by the threads in the Test Chambers. The luminometer measures light emission in luminescence units (LUs). To calculate the patient's IgE response, the instrument automatically subtracts the emission level of the Negative Blanking Control Thread from the emission level of each specific IgE thread. Class values are assigned from 0 to 4 based on the amount of light emitted by the individual threads in the Test Chamber. These values make up the CLA Class Allergy Scoring System as seen in the table below.

CLA Class	Net LUs	Allergen-Specific IgE Concentration
4	>242	Very High
3	143-242	High
2	66-142	Moderate
1	27-65	Low
1/0	12-26	Very Low
0	0-11	Nondetectable

Class values of 1/0 or above represent progressively increasing concentrations of allergen-specific antibodies. Class 0 represents an absence of or nondetectable levels of allergen-specific antibodies.

12 *Limitations of the Procedure*

- Hemolyzed or lipemic serum may adversely affect the performance of the CLA Allergen-Specific IgE Assay.
- Definitive clinical diagnosis and/or dosage regimens for immunotherapy should not be based solely on the results of any single diagnostic test, but should be made by the physician after all clinical and laboratory findings are evaluated.
- The CLA Allergen-Specific IgE Assay provides semiquantitative results. The method has no absolute standard and has been arbitrarily assigned levels of classification.
- Since the binding capacity for specific IgE antibody may vary from allergen to allergen, similar classifications of different allergens do not necessarily imply clinical equivalence.
- 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 that are used in this test. False-positive test results in persons who are tested for food allergies may lead to inappropriate dietary restriction, while false-negative results in food-sensitive

persons may result in anaphylactic reactions of varying severity.

- When testing for inhalant allergies, false-positive results may lead to improper medication of those persons. False-negative test results may lead to lack of proper medical treatment.
- If total IgE values are greater or equal to 500 IU/mL, low-level allergen-specific IgE response should be interpreted with caution.
- Reliable and reproducible results will be obtained when the assay procedure is carried out in complete accordance with the product's instructions for use and adherence to good quality control procedures.
- Bleach contamination has been found to interfere with the test.
  Labware that has been decontaminated with bleach solution should be rinsed thoroughly with distilled or deionized water.
- The use of alcohol-based solutions to disinfect the workstation will result in cracking of the plastic and premature failure of the workstation.

13 *Expected Values*

The CLA Classes were originally determined via scientific studies to establish calibration curves using serum containing specific IgE antibodies to White Birch. The cutoff threshold between positive and negative results was statistically established as two standard deviations above the mean value of the normal population.[6]

It is recommended that each laboratory establish its own expected reference range for the population of interest.

14 *Performance Characteristics*

A. Precision[12]

Within-Assay: Five replicates of four serum samples were run in one batch. The average mean coefficient of variation of the responses of all the allergens tested, when calculated as net LUs, was 11.7%.

Between-Assay: Five replicates of four serum samples were run on four different days. The mean coefficient of variation of the responses of all allergens tested, when calculated as net LUs, was 11.6%.

B. Sensitivity[12]

The detection limit of the assay is 10 LUs.

C. Specificity[12]

There is <1% cross-reactivity with human serum immunoglobulins IgA, IgM, IgG, or IgD at normal physiological levels.

D. In-Vitro Allergy Method Comparison[12]

On average, concordance (calculated as efficiency) between each CLA allergen and alternative in-vitro assay is approximately 95%; the range of concordances is 86% to 100%. There are no standardized reference allergens available for comparison between methods, nor for the great majority of clinically relevant allergens

15 *Bibliography*

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