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Hitachi Chemical Diagnostics, Inc.

INTERNATIONAL PACKAGE INSERT FOR CLA[®] ALLERGEN-SPECIFIC IgE ASSAY



For *in vitro* diagnostic single use

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1 Intended Use

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

2 Summary and Explanation of the Test

Atopic allergy is a hypersensitive immunologic condition mediated by a distinct class of serum antibodies called reagins which were identified in the mid-1960s to be 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 of the biological process 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 is based on a nonisotopic modification of the original RAST method and permits the simultaneous determination of a patient's IgE level to a multiple number of specific allergens.⁶ Semiquantitative results are reported using a classification system similar to that used in RAST testing. 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 combined the specificity and sensitivity of RAST methods with the convenience and simplicity of non-isotopic, simultaneous multiple-allergen testing.⁷

3 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.

4 Reagents/Components

CLA[®] Allergen-Specific IgE Assay*

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

<u>Component Description</u>	<u>Each 20-Test Kit Includes</u>
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, 8mL
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.

5 Precautions

- The CLA Allergen-Specific IgE Assay is for *in vitro* diagnostic 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. If crystals persist, place tightly closed Wash Buffer Concentrate bottle into a beaker of warm water until all crystals have dissolved.
- Rinse Wash Buffer Dispenser and tubing with distilled water.
- Gently invert Wash Buffer Concentrate bottle several times to mix.
- 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).

Antibody Reagent:

- Allow Antibody Reagent to reach room temperature prior to use.
- Gently invert Antibody Reagent Bottle prior to use.
- Antibody Reagent can be used until the expiration date if kept refrigerated (2-8°C) while not in use.

NOTE: One bottle of Antibody Reagent is sufficient for twenty (20) 36-allergen Test Chambers.

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.

NOTE: To avoid contamination of reagents, use a new disposable tip for each photoreagent.

- Gently swirl the container to mix.

NOTE: 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. If the sponge becomes dry, moisten with Wash Buffer and re-seal the bag. When stored in the sealed bag at 2-8°C, Test Chambers can be used until the printed expiration date.
- Do not use kit components if signs of deterioration are present. Signs of deterioration include unusual odor, turbid appearance, and other indications of contamination.

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 **per individual** 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

The following protocol should be used when collecting, preparing, and storing serum for use in CLA allergy testing:

1. 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.

NOTE: Hemolyzed or lipemic serum may adversely affect the performance of the CLA Allergen-Specific IgE Assay.

2. Allow blood to clot in tube for **1 hour** at room temperature.
3. Centrifuge clotted blood for 10 to 20 minutes at 2000-3000 x g or 2500-3600 rpm.
4. Transfer serum to an appropriately labeled clean plastic storage tube.
5. Serum samples may be stored at 2-8°C for up to one week. For longer periods, store samples frozen at -20°C.

NOTE: 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 (see section 4, REAGENTS/COMPONENTS)

Materials Required But Not Provided

- CLA Equipment Kit, comprising:
 - Workstation rack, which holds up to 40 Test Chambers
 - Workstation reservoir
 - Wash Buffer dispenser bottle, 2 L
 - Micropipette and tips
 - Disposable reagent cups, 50 mL
 - 3 cc syringe
- Graduated cylinder or flask, 1 L, for preparing Wash Buffer
- Deionized or distilled water
- Serum separator tubes or red-top tubes, 10 mL, for specimen collection
- Centrifuge capable of 2000-3000 x g or 2500-3600 rpm
- Clean, plastic storage tubes for specimen preparation
- Absorbent paper towels
- Clean, lint-free wipes
- CLA-1 Luminometer System

Preparation of Test Chambers and Patient Samples

1. **Re-centrifuge serum samples** for 10 to 20 minutes at 2000-3000 x g or 2500-3600 rpm immediately prior to use.

NOTE: Use of the Centrifuge Brake may cause the pellet to be dislodged and result in high background values and erroneous results. Turn off the Centrifuge Brake prior to spinning the serum samples.

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

Standard 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. **Be sure top thread is completely covered by serum. This will limit the formation of air bubbles, which may interfere with test results.**

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.

NOTE: Plugs should be pushed in completely to prevent leaking.

3. Place serum-filled Test Chambers upright in workstation rack.
4. Incubate at room temperature for **16 to 24 hours**, noting incubation start time.

C. Prepare Wash Buffer as instructed in Section 6, REAGENT PREPARATION.

D. 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. Note incubation stop time.

E. 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 by depressing dispenser pump once with moderate force.

NOTE: Allow each Test Chamber to drain completely before proceeding to next step.

- Repeat Step 3 two more times for a total of three washes.

NOTE: Test Chambers must be filled with Antibody Reagent immediately after washing to avoid drying of threads.

F. Fill Test Chambers with Antibody Reagent:

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

NOTE: Be sure top thread is completely covered by Antibody Reagent. This will limit the formation of air bubbles, which may interfere with test results.

G. Plug and Incubate Test Chambers:

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

H. Drain Antibody Reagent:

- Remove bottom plug from each Test Chamber and place each Test Chamber back in workstation rack.
- Remove top plug from each Test Chamber, allowing liquid to drain into workstation reservoir. Note incubation stop time.

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

J. Prepare Photoreagent Mixture as instructed in Section 6, REAGENT PREPARATION.

NOTE: Test Chambers must be filled with Photoreagent mixture immediately after the last wash to avoid drying of threads.

K. Fill Test Chambers with Photoreagent Mixture:

- Gently tap bottom of Test Chamber tip on absorbent paper towel to remove any remaining Wash Buffer.
- Attach syringe to top of Test Chamber.
- Place bottom of Test Chamber into disposable cup containing Photoreagent Mixture.
- Slowly withdraw syringe plunger to draw Photoreagent Mixture into Test Chamber until chamber is completely filled to the top. This will limit creation of air bubbles, which may interfere with test results.

L. Plug Test Chambers:

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

M. Allow all Test Chambers to incubate for 10 minutes before reading in the Luminometer. All Test Chambers must be read within 60 minutes of introduction of photoreagent.

N. To read results, refer to the *User Guide & Procedural Manual*.

Alternative (Same-Day) Procedure

NOTE: In house studies have shown significant reductions in sensitivity with same day procedure. Each laboratory must determine the suitability of the Alternative Procedure for their specific use.¹²

Requires CLA Rotator Kit, comprising:

- Main assembly with power cord
- Test Chamber rack
- Operating instructions
- Statpet, 750 µL

A. Fill Test Chamber with Serum:

- Attach Statpet (from Rotator Kit) to top of test chamber.

- Insert bottom of Test Chamber into tube containing patient serum. **Avoid any precipitate and/or lipid layer.**
- Depress and slowly release Statpet plunger to draw serum into Test Chamber. Test Chamber should be only half-filled with serum.

NOTE: Depress Statpet plunger before inserting Test Chamber tip into serum samples. Injecting air into serum sample may disrupt precipitate in bottom of sample tube.

B. Plug Test Chambers, Load them into Rotator, and Rotate:

- With Statpet still attached to top of Test Chamber, insert white bottom plug into bottom of Test Chamber.
- Remove Statpet and insert black plug into top of Test Chamber.

NOTE: Plugs should be pushed in completely to prevent leaking.

- To coat all threads with sera, invert to a 45° angle and gently tap one end of the plugged Test Chamber several times until serum flows to lower end. Then invert and repeat tapping until the serum flows to the other end.
- Load Test Chambers into the CLA Rotator Rack.
- Rotate Test Chambers as follows:
 - Set Rotator timer for **3 hours** and begin rotation. Note start and stop times for rotated serum incubation.
 - Observe each Test Chamber for serum flow while Rotator is in motion.
 - If serum is not flowing smoothly from one end of the Test Chamber to the other:
 - Stop rotator, remove Test Chamber, and tap it against counter surface.
 - Return Test Chamber to Rotator rack.
 - Resume rotation, observe serum flow.
 - Repeat if necessary.

C Through G. Perform as described for Standard Procedure.

H. Plug and Incubate Test Chambers:

- Insert white bottom plug into Test Chamber with syringe still attached to top.
- Remove syringe and insert black top plug.
- Store reagent-filled Test Chamber upright in workstation rack. Incubate at room temperature for **3 hours ± 15 minutes**, noting incubation start time.

Note: Do not rotate Test Chamber during Antibody Reagent incubation stage.

I through N. Perform as described for Standard Procedure.

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 33 LUs in the CLA-1 Luminometer.

Unacceptable Internal Control Outcomes: If a result for either internal control is not within acceptable limits as defined above, the following actions should be taken:

- Re-position Test Chamber in Pette Cassette (ensuring Test Chamber is fully inserted) and reread.
- If results are still unacceptable, refer to Sections 6 and 7 of the *User Guide & Procedural Manual*.

B. IgE Positive and Negative Control Sera

Hitachi Chemical Diagnostics recommends that each new kit lot of CLA Allergen-Specific IgE Assay reagents and Test Chambers be tested 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. Regulatory agencies may re-

quire more frequent use of Positive and Negative Control Sera. Check with your regulatory agency for specific details.

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. CLA 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 of the CLA Allergen-Specific IgE Assay. The amounts of IgE associated with CLA Class values and instrument readings are listed in the following table.

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

CLA Class values of 1/0 or above represent progressively increasing concentrations of allergen-specific antibodies. CLA 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.

NOTE: 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 for Standard Procedure

A. Precision¹²

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. Sensitivity¹²

The detection limit of the assay is 10 LUs.

C. Specificity¹²

There is no detectable cross-reactivity with human serum immunoglobulins IgA, IgM, IgG, or IgD at normal physiological levels.

D. In-Vitro Allergy Method Comparison¹²

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%.

Note: 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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12. Data available upon request.

For technical assistance, please contact Hitachi Chemical Diagnostics. Outside the United States, please contact your local Hitachi Chemical Diagnostics representative.

United States Office

Hitachi Chemical Diagnostics, Inc.
630 Clyde Court
Mountain View, California 94043
Tel. (650) 961-5501
Fax (650) 969-2745

European Office

Hitachi Europe Ltd.
Whitebrook Park
Lower Cookham Road
Maidenhead, Berkshire SL6 8YA
United Kingdom
44 (0) 1628 585 590

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