

HITACHI

Hitachi Chemical Diagnostics, Inc.

INTERNATIONAL PACKAGE INSERT FOR AP 1800 CLA® ALLERGEN-SPECIFIC IgE ASSAY



For *in vitro* diagnostic single use

Doc.No. 0665
Rev.: 03
Rev. Date: 08/06

1 Intended Use

The AP 1800 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, utilizing the AP 1800 automated instrument.

2 Principle of the Procedure

The AP 1800 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 Positive Procedural Control, one Negative Blanking Control, and a Serum Indicator Control.

The CLA® Allergen-Specific IgE Assay is run on the AP 1800, which fills a Test Chamber with patient serum. IgE in the serum binds to the allergen-coated cellulose threads during incubation. The Test Chamber is then automatically 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.

3 Reagents/Components

AP 1800 CLA® Allergen-Specific IgE Assay
Store at 2-8°C until expiration date. Do not freeze.

NOTE: Each kit of AP 1800 Reagents is sufficient for a single run of fifty (50) Test Chambers.

Follow the instructions in the Data Management System (AP DMS) to set up the volume of reagents and Wash Buffer required for the number of Test Chambers in the run.

Materials Required:

Pette Kit:

Test Chambers*
Specific allergens or allergen mixes covalently bound to cellulose thread

Each Test Kit Includes:

50 test chambers

Reagent Kit:

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

Four 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, 100 mL

Photoreagent I
Solution containing:
15 mM 3-aminophthalhydrazide (luminol)
11.5 µM 4-[2'-(4'-methyl)thiazolyl]phenol
0.025 M borate buffer, pH 9.4

One bottle, 50 mL

Photoreagent II
Red-colored solution containing:
0.00125 M ethyl orange
0.0035 M hydrogen peroxide

One bottle, 50 mL

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

AP 1800 Instrument:

AP 1800 Consumables

Disposable Sample Cups
Disposable Pette Tips

Miscellaneous Items:

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

4 Precautions

- The AP 1800 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.¹
- Do not use kit components after the expiration date. The expiration date is printed on each component.
The reagent kit of the AP 1800 CLA® Allergen-Specific IgE Assay is universal and can be used with various pette kits.
- Bleach contamination has been found to interfere with the test.

5 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 Bottle 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 Bottle.
- 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).

6 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.

7 Specimen and Test Chamber 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^{2,4}.

The minimum volume of human serum required per individual Test Chamber with a disposable tip is as follows:

- One 33 allergen Test Chamber requires 1.5 mL of serum
- One 28 allergen Test Chamber requires 1.4 mL of serum
- One 18 allergen Test Chamber requires 1.1 mL of serum

The following protocol should be used when collecting, preparing, and storing serum for use in AP 1800 CLA allergy testing:

1. Collect a venous blood sample into a 10mL 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 AP 1800 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.

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.

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.

6. Remove Test Chambers from plastic bag. Reseal plastic bag and return unused portion to refrigerator.
7. Wipe moisture from outside of each Test Chamber. Gently tap Test Chamber tip onto an absorbent paper towel to remove any residual liquid from inside the Test Chamber.

8 Assay Procedure

Refer to the AP 1800 User Guide for detailed instructions on the AP 1800 CLA® Allergen-Specific IgE Assay and the AP 1800 Instrument.

9 Quality Control

A. Internal Control Threads

Each Test Chamber contains a Positive Procedural Control, a Negative Blanking Control, and a Serum Indicator 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 AP 1800.

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 9 LUs in the AP 1800.

Serum Indicator Control: The Serum Indicator Control is used to ensure that patient serum was aspirated to the top of the Test Chamber. As a precautionary measure, if serum does not reach the top, the AP 1800 will not report the assay results.

B. IgE Positive and Negative Control Sera

Hitachi Chemical Diagnostics recommends that each new kit lot of AP 1800 CLA® Allergen-Specific IgE Assay reagents and Test Chambers be tested with two levels of controls: AP 1800 Positive Control Reagent and AP 1800 Negative Control Reagent. For instructions on their use and acceptability of results, refer to the AP 1800 Positive and Negative Control Reagent Package Insert. Regulatory agencies may require more frequent use of Positive and Negative Control. Check with your regulatory agency for specific details.

10 Results

The AP 1800 measures the amount of light emitted by the threads in the Test Chambers. The AP 1800 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 6, 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 AP 1800 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
0	0-40	Undetectable
1	41-93	Low
2	94-389	Moderate
3	390-1237	High
4	1238-1674	Very High
5	1675-1868	Very High
6	>1868	Very High

CLA Class values of 1 or above represent progressively increasing concentrations of allergen-specific antibodies. CLA Class 0 represents an absence of or undetectable level of allergen-specific antibodies.

11 Limitations of the Procedure

- Hemolyzed or lipemic serum may adversely affect the performance of the AP 1800 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 AP 1800 CLA® Allergen-Specific IgE Assay provides semi-quantitative 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.
- The Serum Indicator Control ensures the patient serum was aspirated to the top of the Test Chamber. It does not ensure that the Test Chamber was completely filled with the patient serum.
- If total IgE values are greater than 1,000 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.

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

©2004, Hitachi Chemical Diagnostics, Inc.
CLA is a registered trademark of Hitachi Chemical Diagnostics, Inc.

Manufactured under one or more of the following United States Patent Nos.: 3,941,876, 4,031,197, 4,459,360 (and corresponding patents issued in Canada, Australia, Japan, Spain, France, Germany, Italy, Sweden, and Great Britain), 4,510,393, 4,558,013, 5,567,149 (and corresponding patents issued in Canada, Australia, Japan, Spain, France, Germany, Italy, Sweden, Switzerland, Austria, Belgium, the Netherlands, Luxembourg, and Great Britain), 4,568,184, 285,485, 4,743,541 (and corresponding patents issued in Canada, Australia, Japan, France, Germany, Sweden, Switzerland, and Great Britain), and 5,082,768 (and corresponding patent issued in Japan).

12 Expected Values

The AP 1800 CLA® Classes were originally determined via scientific studies to establish calibration curves using serum containing specific IgE antibodies to multiple allergens. The cutoff threshold between positive and negative results was statistically established as three standard deviations above the mean value of the normal population.

13 Performance Characteristics for Standard Procedure

A. Precision

Within-Assay: Five replicates of three serum samples were run on four days. The average of the mean coefficient of variation from all of the allergens tested, when calculated as net LUs, was 11.7%.

Between-Assay: Three serum samples and one test per serum were run on four days. The average of the mean coefficient of variation from all of the allergens tested, when calculated as net LUs, was 13.7%.

B. Sensitivity

The detection limit of the assay is 41 LUs.

C. Specificity

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

D. In-Vitro Allergy Method Comparison

Expectations for performance are sensitivity ³ 85% and specificity ³ 80% when compared to another allergy system. In a large clinical study (2700 data points), AP 1800 CLA Allergen-Specific IgE assay demonstrated 88% sensitivity, 89% specificity and 88% concordance.⁵

Note: There are no standardized reference allergens available for comparison between methods, nor for the great majority of clinically relevant allergens.

14 Bibliography

1. Safety Management No. CDC-22, *Decontamination of laboratory sink drains to remove azide salts*. Atlanta, GA: Centers for Disease Control, April 30, 1976.
2. U.S. Dept. of Health and Human Services. Centers for Disease Control. *Guidelines For Prevention of Transmission of Human Immunodeficiency Virus and Hepatitis B Virus to Health-Care and Public-Safety Workers*. February 1989.
3. Richardson SH, Barkley WE, eds. *Biosafety in microbiological and biomedical laboratories*. 2nd ed. Washington, DC: US Dept of Health and Human Services, 1988.
4. Federal OSHA Standard 1910.1030. *Bloodborne pathogens*. 29 CFR 1910.1030.
5. Data available upon request.