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 Hitachi Chemical Diagnostics, Inc.

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



For *in vitro* diagnostic single use

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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. The dilution method has been developed for use when the patient sample volume available is insufficient to run without dilution. The method incorporates a change in cut-offs as described below. Once the change in cut-offs is implemented, only diluted samples can be run in the instrument. The dilution methods will result in lower sensitivity and/or specificity as defined in Section 13.

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. The sample should be used neat as defined in the package insert for the test doc 0665. An alternative procedure is available in which the patient serum sample can be diluted 1:2, that is, equal amounts of patient sample and the AP1800 sample diluent. This procedure should only be used when the patient sample is not enough to be run without dilution. 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 photo-reagent 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 Sample Diluent Kit:

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

Materials Required:

AP1800 Sample Diluent Kit:

3 bottles, 100 ml

Solution containing:
0.01 M phosphate-buffered saline, 3% Polyethylene glycol, 10% horse serum, surfactant, preservative and yellow dye.

4 Precautions

- The AP 1800 CLA® Allergen-Specific IgE Assay is for *in vitro* diagnostic use.
- 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.
- Mix diluent before use.

5 Storage Instructions

- Store kit components at 2-8°C. When stored as directed, the components can be used until the expiration date printed on the individual component labels.
- Do not freeze kit components.
- Do not use kit components if signs of deterioration are present. Signs of deterioration include unusual odor, and other indications of contamination.

6 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 specimens¹⁻³.

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.

make up the CLA Class Allergy Scoring System of the AP 1800 CLA Allergen-Specific IgE Assay. An alternative scoring system has been developed and validated for the dilution method. The amounts of IgE associated with CLA Class values and instrument readings are listed in the following tables.

Dilution method CLA Class Allergy Scoring System for the AP1800:

CLA Class	Net LUs	Allergen-Specific IgE Concentration
0	0-49	Undetectable
1	50-70	Low
2	71-300	Moderate
3	301-1000	High
4	1001-1400	Very High
5	1401-1650	Very High
6	>1650	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.

7 Serum Sample Dilution

This procedure should only be used when the patient sample volume is not enough to run the standard protocol. A lower sensitivity will be seen with this method, as described in section 13.

1. Determine the serum volume needed as described in section 6.
2. Prepare a 1:2 dilution of the serum by adding equal amounts of the patient serum sample and the AP1800 Sample Diluent in a clean container.
3. Mix and add the diluted sera to the disposable sample cup
4. Follow the standard procedure.
5. Alternatively contact your distributor to implement the changes in the class cut-offs in the AP1800 DMS software.

NOTE: Once the cut-off changes are implemented, non-diluted samples cannot be run.

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.

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. Lab ware that has been decontaminated with bleach solution should be rinsed thoroughly with distilled or deionized water.

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.

13 Performance Characteristics

In-Vitro Allergy Method Comparison for the dilution method with change in class cut-offs.

Expectations for performance are: sensitivity of approximately 80% and specificity of approximately 98% when compared to the non-

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

diluted method. Sensitivity of approximately 85% and specificity of approximately 95% when compared to another allergy system.⁴

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

14 Bibliography

1. 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.
2. Richardson SH, Barkley WE, eds. *Biosafety in microbiological and biomedical laboratories*. 2nd ed. Washington, DC: US Dept of Health and Human Services, 1988.
3. Federal OSHA Standard 1910.1030. *Bloodborne pathogens*. 29 CFR 1910.1030.
4. 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
United States of America
Tel (650) 961-5501
Fax (650) 969-2745

European Office

Hitachi Chemical Diagnostics, Inc.
Hitachi Europe Ltd.
Whitebrook Park
Lower Cookham Road
Maidenhead, Berkshire SL6 8YA
United Kingdom
Tel 44 (0) 1628 585 590
Fax 44 (0) 1628 585 594

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