MINARIS MEDICAL

INTERNATIONAL PACKAGE INSERT FOR OPTIGEN® Universal Panel 36 (Taiwan Panel) Part Number 85011

"For In Vitro Diagnostic Use" Only - Single Use

CE IVD

Doc.No. 0973
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1 Intended Use

The OPTIGEN Assay is an *in vitro* diagnostic test for use in the semi quantitative determination of circulating allergen-specific IgE concentrations in human serum. It is intended to aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings. The device is designed for use in clinical laboratories.

2 Summary and Explanation of the Test

Immunoglobulin E is a distinct class of serum antibody, which mediates Type 1 hypersensitivity reactions, also known as atopic allergy. When immunocompetent B lymphocyte cells are stimulated by exposure to an antigen (allergen), they may produce allergen-specific IgE antibodies which bind to receptors on mast cells and basophilic leukocytes.

If the same allergen is reintroduced into the system through inhalation, ingestion or dermal contact, the allergen binds with the cell-bound IgE antibodies. This triggers cell degranulation and release of vasoactive amines into the surrounding tissues. Vasoactive amines, such as histamine, are responsible for the bronchial smooth muscle contraction, dermal itch, localized swelling and leakage of extracelluar fluids across mucosal barriers that typify Type 1 hypersensitivity reactions.

The most common clinical manifestations of Type 1 hypersensitivity reactions include sinusitis, asthma, dermatitis, hives and in rare cases, anaphylactic shock.

Assessing the level of allergen-specific IgE in a patient's serum in conjunction with a clinical evaluation based on patient history and subsequent testing can help a physician confirm a diagnosis of atopic allergy and assist in the treatment of the patient.

3 Principle of the Procedure

The OPTIGEN Assay employs a small plastic device known as a pette or test chamber to expose patient serum simultaneously to a number of allergens or allergen mixes. The pette contains a polystyrene solid phase and integrated lenslets, as well as one Negative blanking control and one Positive procedural control.

The OPTIGEN assay can be run manually or with the semi-automated processor AP 720S™.

The assay is run by filling a pette with patient serum after a pre-wash step. As the serum incubates, IgE in the serum binds to the allergen-coated wells. After an incubation period, the pette is washed with buffer solution to remove any unbound serum components.

Next, an enzyme-labeled anti-IgE antibody is introduced into the pette. The antibody couples with the IgE bound to the wells.

After a second washing, the pette is filled a photoreagent mixture which, when combined with the enzyme-labeled anti-IgE antibody gives off a chemically generated light (i.e. chemiluminescence). The amount of light emitted by each well is directly proportional to the amount of allergen-specific IgE in the patient's serum.

4 Reagents/Components

OPTIGEN Assay

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

Component Description	Each 20-Test Kit Includes
Test Chambers Pette test chamber contains a polystyrene solid phase and integrated lenslets, each with an allergen or allergen mix .	20 Pettes
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	One bottle, 50 mL
Antibody Reagent 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, 16 mL
Photoreagent AB Solution containing: 7-15 mM 3-aminophthalhydrazide (luminol), 5-25μM Enhancer and 0.025 M Borate Buffer, pH 9.4	One bottle, 8 mL
Photoreagent CD Solution containing: 0.00125 M Ethyl Orange, 0.002 M Hydrogen Peroxide	One bottle, 8 mL
Pette Plugs (top) Black plugs for the top of the pette	22 plugs
Pette Plugs (bottom) White plugs for the bottom of the pette	22 plugs

5 Precautions

- The OPTIGEN 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.
- Component reagents of the OPTIGEN Assay kits are provided as matched sets (i.e. reagents and pettes). Do not mix with other product lines, as they are not compatible.
- Bleach contamination has been found to interfere with the test.

6 Reagent Preparation

Wash Buffer:

- Allow Wash Buffer Concentrate to reach room temperature, checking to see that any salt crystals that may have formed during refrigeration have dissolved. If crystals persist, place tightly closed buffer bottle into a beaker of warm water until all crystals are 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 a 2 L Wash Buffer Dispenser Bottle.
- Fill Wash Buffer Dispenser Bottle to 1000 mL mark with distilled or deionized water.
- Mix thoroughly.
- 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.
- One bottle of Antibody Reagent is sufficient for twenty (20) OPTIGEN pettes.

Photoreagent Mixture:

Prepare Photoreagent Mixture just before use.

- Allow Photoreagents AB and CD to come to room temperature prior to use.
- Using a micropipette with disposable tips, combine **equal parts of** Photoreagent AB and CD. Draw 250 µL of fluid per pette, from each bottle of Photoreagent AB and CD. Dispense into a disposable container.
  NOTE: Use a new disposable tip for each photoreagent component to avoid contamination of reagents.
- Gently swirl the container to mix.
- Photoreagent mixture should be used within 60 minutes of mixing.

  NOTE: Photoreagent Mixture should be used immediately after preparation for best results.

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 pettes are packaged with desiccant and should be sealed properly after each use. When stored in the sealed bag and refrigerated at 2-8°C, pettes will be stable until the expiration date printed on the labels/kit boxes.
- 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.[2-4]

For the manual method, the minimum volume of human serum required **per individual** pette is as follows:

500 µL for a > 20-allergen pette
300 µL for a ≤ 20-allergen pette

For the semi-automated method (using the AP 720S), the minimum volume of human serum required per individual pette is as follows:

600 µL for a > 20-allergen pette
490 µL for a ≤ 20-allergen pette

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

1. Collect a venous blood sample into a 5 mL serum separator tube or red-top tube. Patient need not be fasting. No special preparations are necessary.
   NOTE: Serum separator tubes (SST) contain an inert material which separates the serum from the cells when centrifuged. Hemolysis can adversely affect the performance of the OPTIGEN allergy assay.
2. Gently invert serum collection tube 3-5 times.
3. Label specimen tube with the patient's name and date of draw.
4. Allow blood to clot in the original stoppered container for up to 2 hours at room temperature or until co-agulation occurs.
5. Centrifuge clotted blood for 10 to 20 minutes at 2000-3000 x g or 2500-3600 rpm in the original stoppered container.
6. Transfer serum from centrifuge tube to an appropriately labeled, clean plastic storage tube.
7. Serum samples may be stored at 2-8°C for up to one week. For longer periods, freeze samples 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. After removal from storage, and immediately prior to performing the assay, serum samples should be re-centrifuged for 10-20 minutes at 2000-3000x g or 2500-3600 RPM.

9 *Assay Procedure*

Refer to the ***OPTIGEN User Guide*** (P/N 60501) and the ***CLA-1 Luminometer Operator Manual*** (Doc. No. 0277) for detailed instructions on the manual test operation. If using the AP 720S Semi-Automated Instrument, please refer to the AP 720S Instruction Manual (Doc. No. 0780) and the AP 720S LCD Panel Guide (Doc. No. 0781).

Materials Provided

- OPTIGEN Assay (see Section 4, REAGENTS/COMPONENTS)

Materials Required But Not Provided

- OPTIGEN Equipment Kit, comprising:
  - Workstation Rack, which holds up to 40 Test Chambers
  - Workstation Reservoir
  - Wash Buffer Dispenser bottle, 2 L graduated.
  - Disposable Reagent Cups, 10ml cups
  - 3 cc Luer lock syringe
  - Electronic or manual fixed volume pipette (optional)
- Graduated cylinder or flask, 1 L, for preparing Wash Buffer
- Deionized or distilled water
- Serum separator tubes or red-top tubes, 10 mL or 5 mL 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 Pettes and Patient Samples

1. Centrifuge serum samples immediately prior to use, if the sample has not been centrifuged on the test day (see Section 8, Specimen Collection and Preparation).
2. Remove pettes (one per patient) from bag.
3. Reseal bag and return kit to the refrigerator.
4. With windows facing down, label each pette with appropriate patient identification.
   NOTE: Keep kit stored at 2-8ºC when not in use.

Procedure

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

B. Re-hydrate Pette

1. Prime the Wash Buffer Dispenser into the sink or reservoir until all air bubbles are removed.
2. Attach the end of the stop cock to the top of the first pette.
3. Wash each pette once with 10 mL of Wash Buffer by depressing the Dispenser pump once with moderate force.
   NOTE: Allow each pette to drain completely before proceeding to the next step.

C. Draw Serum into Pette

1. Tap the pette onto an absorbent paper to remove any residual liquid.
2. Attach the 3 cc syringe to top of the pette.
3. Insert bottom of the pette into vial containing patient serum.
   NOTE: Avoid any precipitate and/or lipid layer.
4. **SLOWLY** withdraw syringe plunger to draw serum into pette until top window is covered. **Check for bubbles.**

   NOTE: Be sure the positive control window is completely covered by serum.

D. Plug and Incubate Pettes

1. With syringe still attached to top of Test Chamber, insert a white plug into bottom of the pette.
2. Remove the syringe and insert black plug into top of the pette.

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

3. Place serum-filled pettes upright in workstation rack.
4. Incubate at room temperature for **2 hours +/-10 minutes.**

E. Drain Serum

1. Remove bottom plug from each pette and place pette back in workstation rack.
2. Remove top plug from each pette, allowing serum to drain into workstation reservoir.
3. Blot plugs dry and retain for use in subsequent steps.

F. Wash Pettes

1. Prime Wash Buffer Dispenser until all air bubbles are removed.
2. Attach end of stop cock to top of first pette.
3. Wash each pette with 10 mL of Wash Buffer by depressing the Dispenser pump once with moderate force.
   NOTE: Allow each pette to drain completely before proceeding to the next step.

G. Fill Pettes with Antibody Reagent

1. Allow Reagents to come to room temperature prior to use.
2. Gently mix the antibody bottle prior to use.
3. To avoid contamination of Antibody reagent, transfer required amount to a disposable cup or other container.
4. Gently tap bottom of pette tip on absorbent paper to remove any remaining Wash Buffer.
5. Attach the 3 cc syringe to the top of the pette.
6. Place bottom of pette into disposable Antibody Reagent container.
7. **SLOWLY** withdraw syringe plunger to draw Antibody Reagent into the pette, until top window is covered.
   NOTE: Be sure the top window is completely covered by Antibody Reagent. This will limit the formation of air bubbles, which may interfere with test results.

H. Plug and Incubate Pettes

1. Insert white bottom plug into pette with syringe still attached to top of the pette.
2. Remove syringe and insert black top plug.
3. Store reagent-filled pettes upright in workstation rack. Incubate at room temperature for **2 hours +/-10 minutes,** noting incubation start time on the Planner Sheet.

   NOTE: Keep kits stored at 2-8ºC when not in use.

I. Drain Antibody Reagent

1. Remove bottom plug from each pette and place each pette back in the workstation rack.
2. Remove top plug from each pette, allowing liquid to drain into workstation reservoir. Note incubation stop time on the Planner Sheet.
3. Blot plugs dry and retain for use in subsequent steps.

J. Wash Pettes

1. Prime Dispenser into the sink or reservoir until all air bubbles are removed.
2. Attach end of stop cock to the top of the first pette.
3. Wash each pette once with 10 mL of Wash Buffer by depressing the Dispenser pump once with moderate force.

   NOTE: Allow each pette to drain completely before proceeding to the next step.

K. Prepare Photoreagent Mixture

1. Prepare Photoreagent Mixture as instructed in Section 6, Reagent Preparation.
   NOTE: Allow photoreagents to come to room temperature prior to use.
   NOTE: Use photoreagent mixture immediately after preparation for best results.

L. Fill Pette with Photoreagent Mixture

1. Gently tap bottom of pette on an absorbent paper to remove any Wash Buffer remaining in pette.
2. Attach syringe to the top of the pette.
3. Place bottom of pette into container of Photoreagent Mixture.
4. **SLOWLY** withdraw syringe plunger to draw Photoreagent Mixture into the pette, until the pette is completely filled.
   NOTE: Verify the top window is completely covered with the Photoreagent Mixture.

M. Plug Pettes

1. Insert the white bottom plug in pette with the syringe still attached to the top.
2. Remove the syringe and insert the black top plug.
3. Inspect plugged pette for fluid leaks.
4. Wipe away any Photoreagent from the outside of the pette with a clean, damp, lint-free wipe.

N. Allow Filled Pettes to Stand for 10 Minutes

1. Allow all test chambers to incubate for 10 minutes before reading in the Luminometer. All test chambers must be read within 60 minutes after the introduction of photoreagent.
2. Refer to "Reading Test Results" section for Luminometer operation for additional information.

O. Reading Test Results with the CLA-1 Luminometer

NOTE: Do not, under any circumstances, open the CLA-1 Luminometer instrument case. Opening the case will VOID the instrument's warranty, render the CLA-1 Luminometer inoperable and necessitate factory adjustments, as well as exposing the Operator to serious personal injury.

1. Load Pette into Pette Cassette Tray
   a. Insert the pette into the Pette Cassette in the order indicated on the OPTIGEN Planner Sheet.
   b. Slide the pette, black plug first with windows facing up, all the way to the end of the Pette Cassette Tray.
   c. Inspect loaded pette for fluid leakage. Wipe with a clean, damp, lint-free wipe.
2. Load Pette Cassette into the CLA-1 Luminometer
   a. Press the "OPEN/CLOSE" key on the CLA-1 Luminometer once to open the Transport Door.
   b. Grasp the handle of the Pette Cassette and insert the loaded tray into the Cassette Transport slot, until it clicks.
   c. Press the "OPEN/CLOSE" key again. At this point, the Pette Cassette will automatically be transported inside the CLA-1 Luminometer and the Transport Door will close.
3. Program the Load List into the CLA-1 Luminometer
   a. Identify the panel loaded into each of the 5 positions in the pette cassette, using the Luminometer Planner Sheet as a guide.
   b. Press the "UP" or "DOWN" keys on the CLA-1 Luminometer to scroll through the panel selections.
   c. Press the "ENTER" key when the appropriate selection is displayed with the listed Cassette position.
   d. Repeat the above steps until all of the pettes in the Pette Cassette have been properly programmed into the CLA-1 Luminometer.
4. Read and Print Results
   a. After all 5 Pette Cassette positions have been programmed, the CLA-1 Luminometer screen will display a corresponding "LOAD LIST". If it correctly matches the pettes in the Pette Cassette, press the "ENTER" key to begin analysis.
   b. The CLA-1 Luminometer will print out the test results in approximately 1 minute.
   c. Write the patient's name on the printed test results and attach results to the Test Record for the CLA-1 Luminometer.

10 *Quality Control*

A. Internal Control Wells

Each pette contains a Positive Procedural Control and a Negative Blanking Control. These controls function as internal indicators for each pette.

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 LU 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 equal to or less than 69 LU 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 pette in Pette Cassette (ensuring that the pette is fully inserted) and reread.
- If results are still unacceptable, refer to Sections 6 and 7.

B. IgE Positive and Negative Control Sera

Minaris Medical America recommends that each new kit lot of reagents and pettes used in performing the OPTIGEN Allergen-Specific IgE Assay be tested with two levels of serum controls: IgE Positive Control Serum and IgE Negative Control Serum.

Regulatory agencies may require more frequent use of Positive and Negative control sera. Check with your regulatory agency for specific details.

OPTIGEN IgE Positive and Negative Control Sera are available for purchase from Minaris Medical America, Inc. and are shipped with a printout of expected values. Control sera are shipped frozen and must remain frozen until used.

Internal controls and Serum control need to pass specifications in order for the results to be reportable.

11 Results

The CLA-1 Luminometer measures the amount of light emitted by the allergens in the pettes. 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 Control from the emission level of each allergen. CLA Class Values are assigned from 0 to 4 based on the amount of light emitted by the individual allergens in the pette. These values make up the CLA Class Allergy Scoring System of the OPTIGEN 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	Levels of antibodies detected Allergen-Specific IgE Concentration
4	>242	Very high levels of antibodies
3	143-242	High levels of antibodies
2	66-142	Moderate levels of antibodies
1	27-65	Low levels of antibodies
0	0-26	No antibodies detected

CLA Class values of 1 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

- Measured results could vary within +/- 1 class. Low positive results should be interpreted in conjunction with clinical findings.
- Hemolyzed or lipemic serum may adversely affect the performance of the OPTIGEN 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 OPTIGEN 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.
- If total IgE values are greater or equal to 2500 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

It is recommended that each laboratory establish its own expected range of values for the population of interest. The cut-off threshold between positive and negative results was established as three standard deviations above the mean value of the normal population.

14 Performance Characteristics for Standard Procedure

A. Precision [5]

Within-Assay: Ten serum replicates were run in one batch. The average mean coefficient of variation of the responses was calculated per class:

Class	% CV
1	31
2	16
3	16
4	5

Between-Assay: Ten replicates of a serum sample were run on five different days. The mean coefficient of variation of the responses of all allergens tested was calculated per class:

Class	% CV
1	25
2	15
3	9
4	1

B. Detection limit [5]

The detection limit of the assay ranges from 12- 26 LUs and is allergen dependent.

C. Analytical Specificity [5]

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[5]

On average, concordance (calculated as efficiency) between each CLA allergen and alternate in-vitro assay is approximately 90%; the range of concordances is 83% to 98%.

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

15 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.

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