DRG® Allerquant IgG Food Additives Elisa (EIA-4725)

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Intended Use

The *Allerquant IgG Food Additives Screening Elisa Test* is for measuring the relative amount of food additive-specific IgG antibody in human serum. Since the elevation of IgG to certain food additives may not express clinical symptoms all the time, the values must be correlated with clinical manifestation.

Background

The topic of food allergy and the role of food and food additives as causative factors in food hypersensitivity diseases have prompted a considerable interest for many years. It is also suspected that food additives contribute to the role of causative factors in food intolerance. Recent studies have linked dietary factors as a significant contributor to the pathophysiology of irritable bowel syndrome.
Most common food and food additive allergy symptoms are gastrointestinal-related and may include nausea, diarrhea, and abdominal pain. The clinical manifestations of food allergy also include classic allergic symptoms such as anaphylaxis, allergic rhinitis, atopic dermatitis, and urticaria. The role of food allergy in conditions such as migraine headaches and allergic tension-fatigue syndrome is controversial. It is important to remember that the symptoms of food allergy, especially gastrointestinal symptoms, can be mimicked by a variety of other conditions.

Principal of the Test

Specific food additives are immobilized separately onto microtiter wells. These allergens are allowed to react with specific antibodies present in the patient's serum. Excess serum proteins are removed by the wash step.
Enzyme labeled antibody conjugate is allowed to react with allergen-antibody complex. A color is developed by the addition of a substrate that reacts with the coupled enzyme. The color intensity is measured and is directly proportional to the concentration of IgG antibody specific to a particular allergen.

Reagents and Materials

This test kit contains sufficient wells and reagents to assay 18 patient sera for antibodies to 14 different food additives.

PLA FOOD = 14 Food Additives Coated Microwells ..... 3 plates
DIL SPE 1X = Serum Diluent (Green)..... 1 x 56 ml
BUF WASH 66.67X = Wash Buffer (concentrate)..... 1 x 30 ml
CAL FOOD IgG = Food Additive IgG Calibrator.....1 x 1.0 ml
CTRL + IgG = Food Additive IgG Positive Control ..... 1 x 1.0 ml
CONJ ENZ IgG-HRP = Food Additive IgG-HRP Conjugate..... 1 x 33 ml
SUBS A TMB = Substrate Solution A (TMB)..... 2 x 12 ml
SUBS B H2O2 = Substrate Solution B (hydrogen peroxide)..... 2 x 12 ml
SOLN STOPPING = Stopping Solution (1N H2SO4)..... 1 x 20 ml

Warnings and Precautions

Potential Biohazardous Material The matrix of the Calibrators and Controls is human serum. The human serum used has been found nonreactive to HbsAg, anti-HIV 1/2 and anti-HCV when tested with FDA licensed reagents. Because there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled as if potentially infectious

Sodium Azide Some reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper or brass to form explosive metal azides. When disposing of these materials, always flush with large volumes of water to prevent azide buildup.

Stopping Solution Stopping Solution consists of 1N H2SO4. This is a strong acid and should be handled with caution. It can cause burns and should be handled with gloves. Wear eye protection and appropriate protective clothing. Avoid inhalation. Dilute a spill with water before absorbing the spill with paper towels.

Preperation of Patient Sample

Dilute patient's serum 1:100 in Serum Diluent. Take
0.025 ml (25µl) of patient serum and add it to 2.5 ml of Serum Diluent.

Reagent Preperation and Storage

Wash Buffer: Wash the contents of the vial into a 2000 ml flask with distilled water and Q.S. to 2000 ml mark with distilled water. Label it as Working Wash Buffer and store refrigerated at 2-8°C. The Working Wash Buffer is stable for 6 months at 28°C.

Substrate Solution: Mix Substrate Solution A and B in equal proportions 30 minutes before use. (*For example mix 5 ml each of A and B for each microwell plate to be used).* Discard the unused substrate mix solution. Do not interchange the caps on these solutions. If the mixed substrate solution looks blue in color before use, it should be discarded. Mixed substrate solution is stable for 60 minutes at room temperature.

Assay Procedure

Bring all the test kit reagents to room temperature before use.

1. ***PREPARATION OF CALIBRATORS (it is important to accurately pipette in making different calibrators):*** Label four 12 x 75 mm glass tubes as 50, 100, 200 & 400 U/ml. Dispense 150 µl of Serum Diluent into these four tubes. Add 150 µl of Food Additive IgG Calibrator to the tube labeled 400 U/ml. Mix and transfer 150 µl into tube labeled 200 U/ml. Mix and transfer 150 µl into the tube labeled 100 U/ml. Again mix and transfer 150 µl into the tube labeled 50 U/ml. At this point you should have 150 µl in tubes 100, 200 & 400 U/ml, and 300 µl in tube 50 U/ml. This is the calibration curve to be used in the assay. Transfer 100 µl from each of these tubes to the microplate wells as follows. **Double these quantities to run the calibration curve in duplicate.**

The microtiter plate is designed with two sets of calibrators sufficient to test 6 patient samples.

1) Place 100 µl of the diluted patient serum (**See Preparation of the Patient Sample – Section VI above)** into all the other wells.
2) Cover the plates with parafilm or plastic wrap and incubate at room temperature (22-25°C) for 1 hour.
3) After one-hour incubation, wash all the microwells three times with 300 µl of working wash buffer each time. **(See Reagent Preparation – Section VII).** If you use an automated washer, check the manufacturer's instructions for a three cycle wash procedure with 300 µl wash volume.

    1. Add 100 µl of Foods IgG-HRP Conjugate to all the wells.
    2. Incubate the plates for 30 minutes at room temperature (22-25°C).
    3. Wash the plate again as in step #4.
    4. Add 100 µl of Working Substrate mix to all the wells **(See Reagent Preparation – Section VII)**.
    5. Cover the plates and Incubate for 10 minutes at room temperature (22-25°C).Add 50 µl of Stopping Solution to all the wells. (Blue color in the wells will change toyellow).
    6. Set the microplate reader at 450 nm and read absorbance in all the wells.
    7. Prepare DRC (Dose Response Curve),using specific absorbance vs. concentration of the calibrators (as identified in the chart), on linear-linear graph sheet. Read the absorbance of the sample wells on DRC and obtain the sample values to each food additive.

Quality Control

For the test to pass, it must meet the following Q.C. specifications for O.D. (Optical Density) at 450 nm.

O.D. BLANK < 0.2
O.D. 50 CAL > 1.2 x OD BLANK
O.D. 100 CAL > 1.2 x OD 50 CAL
O.D. 200 CAL > 1.2 x OD 100 CAL
O.D. 400 CAL > 1.2 x OD 200 CAL Concentration Positive > 100 U/ml

Set 1		Set 2	
Microwell	Calibrator	Microwell	Calibrator
A1	Blank	A7	Blank
A2	50	A8	50
A3	100	A9	100
A4	200	A10	200
A5	400	A11	400
A6	Positive Control	A12	Positive Control

2. Add 100 µl of serum diluent (blank) to well A1 and 100 µl of calibrators of 50, 100, 200, and 400 to wells A2, A3, A4, and A5 respectively. Add 100 µl of Positive Control to well A6.

Interpretation of Results
The absorbance readings, after extrapolation as DRG U/ml, should be interpreted as follows for each allergen.

READING INTERPRETATION

< 50 U/ml Negative **0** 50 - 100 U/ml Mildly Positive **+1** 100 - 200 U/ml Moderately Positive **+2** > 200 U/ml Strongly Positive **+3**

References

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