

1. Intended use

Allergodip® is an enzyme immuno assay for semi-quantitative determination of allergen-specific IgE in human serum or plasma. The use is limited to professional personnel trained in the use of IVDs.

Allergodip® is used for the diagnosis of allergic type I disease, e.g.

- seasonal and perennial rhinitis and conjunctivitis (hay fever),
- allergic asthma,
- allergic eczema (e. g. against food allergens) and
- gastrointestinal allergies.

Allergodip® is particularly suitable for patients in whom prick testing does not lead to reliable results, e.g. during simultaneous antihistamine medication, persisting dermatitis or unsuitable skin in general (too young/too old patients).

2. Introduction

Immunoglobulin E is a serum protein and major carrier of the reagenic activity of type I reactions (immediate hypersensitivity). IgE is present in the blood circulation system. IgE bound on surfaces of mast cells and basophil granulocytes is responsible for the clinical symptoms of type I reactions. Binding takes place at the Fc receptors of the IgE molecule. If there is a contact between allergen and the corresponding specific IgE on the cell, proinflammatory mediators and enzymes (e.g. histamine) are released. With a test method for the determination of circulating IgE it is not possible to detect stationary specific IgE in the tissue. This is the reason why the results of specific IgE test in serum may only be parts of a diagnostic concept in connection with anamnesis, skin tests and provocation tests.

3. Test Principle

Allergodip® is an enzyme immuno assay for the determination of allergen-specific IgE in serum or plasma and is performed without any laboratory equipment. The solid phase is composed of a chemically activated carrier with covalently bound allergens. In the first step the dipstick is incubated with serum/plasma. This enables the allergen-specific IgE to bind to the different allergens coupled to the test pads. Excess serum/plasma is subsequently removed under flowing cold tap-water.

In the second step the dipstick is incubated with an enzyme anti-human IgE conjugate (Reagent 1). This leads to binding of the labelled anti-IgE to specific IgE linked to the solid phase. Excess anti-human-IgE is removed under flowing cold tap-water.

In a third step the dipstick is incubated with a substrate solution (Reagent 2), which in the case of a positive reaction gives rise to a blue/purple colour on one or more of the allergen coated test pads.

The results of the test are evaluated visually. The intensity of the blue/purple colour of each test pad must be compared with the colour chart and is directly proportional to the concentration of allergen-specific IgE in the serum/plasma. The results are classified into class 0–4.

4. Content of Allergodip®

Pack size: 1 kit with 10 tests incl. reagents and additional materials.

- 1 tube with 10 dipsticks **DIPSTICK | 10**
- 1 dipstick contains 9 allergens plus positive and negative controls. For the respective composition of each panel please see the patient cards in the box.

Reagent 1 (Conjugate): **REAG | 1 | 10 ml**

- 1 bottle with 10.0 ml protein buffered solution of monoclonal anti-human-IgE (mouse) conjugated with alkaline phosphatase; 0.02 % sodium azide (Preservative)

Reagent 2 (Substrate): **REAG | 2 | 10 ml**

- 1 bottle with 10.0 ml 5-Bromo-4-Chloro-3-Indolyl-Phosphate/Nitro Blue Tetrazolium

- 10 Disposable pipettes
- 30 Calibrated test tubes **TEST | TUBES | 30x**
- 1 Test-tube tray
- 10 Patient cards
- 1 Colour chart
- 1 Instruction for use

Legend:

SPE	= Specimen Serum/Plasma)
REAG 1	= Reagent 1 (Conjugate)
REAG 2	= Reagent 2 (Substrate)
DIPSTICK INHA	= Inhalant Allergens
DIPSTICK INHA IND	= Inhalation - India
DIPSTICK INHA AFR	= Inhalation - Africa
DIPSTICK FOOD	= Food Allergens
DIPSTICK FOOD IND	= Food - India
DIPSTICK FOOD AFR	= Food - Africa
DIPSTICK FOOD PL	= Food - Poland
DIPSTICK MITES	= Mites and more
DIPSTICK MEDI	= Mediterranean Allergens
TEST TUBES	= Test tubes

5. Additional materials and devices

- Rubber gloves
- Laboratory coat
- Stop watch
- Flowing cold tap-water
- Absorbent paper towel

6. Limitations of the procedure

- Reliable and reproducible results will be obtained when the assay procedure is carried out by following the instructions (see test procedure chapter 10).
- Negative in vitro results may occur e.g. when:
 - symptoms are not IgE-mediated.
 - samples were taken before the organism was able to produce antibodies against the antigen.

- IgE level has reached a minimum a long time after sensitisation.

- Identical results with different patients do not suggest identical reaction situations since the reaction situations may vary.
- Positive results with spec. IgE in vitro tests do not have to correlate with clinical manifestations.
- Many IgE antibodies show cross reactivity with various allergens e.g. birch pollens/apple, mugwort/celery, latex/banana. The diagnostic identification must consider this circumstance.
- Recommended times must be followed closely if multiple tests are performed simultaneously.
- There is no reuse protocol for this product.
- The use of samples other than serum or plasma has not been validated in this test.
- Clinical diagnosis should not be made on the basis of a single determination only, but should include all clinical and laboratory findings. The determination of IgE should never be the sole decision basis for commencing a hyposensitisation therapy. In addition skin tests – and if possible – provocation tests for the determination of clinical relevance must be performed (see 17, point 1).
- Especially with food allergens it is common to find a negative in vitro result although marked clinical symptoms may be present. This explains itself through the effect of maturing, industrial processing, cooking, frying etc. but also through the digestive process which leads to the problem that the protein structure in vivo might be different from the protein structure on the solid phase of the testkit. Furthermore some food is likely to be very sensitive so that not all allergens which are present in the native form may be coupled to a solid phase.

7. Specific performance data

- Analytical specificity
 - Cross reactivity with other specific IgE entities is not expected
- Diagnostic specificity 100 %
- Diagnostic sensitivity 97.5 %
- Accuracy
 - the expected values were found in all cases
- Reproducibility 6 CV (%) (Inter-assay)
- Repeatability 0 CV (%) (Intra-assay)
- Lower detection limit < Class 1
- Measurement range Class 0–4

The results are semi-quantitative and show a very good - concordance with EAST/CAP (86–97 %) and also with skin test (87–98 %). See literature 2, 3.

8. Relevant interferences

Icterus	0–0.1 mg/ml bilirubin – no interference
Haemolysis	0–8 mg/ml haemoglobin – no interference
Lipaemia	0–5 mg/ml triglyceride – no interference

9. Preparation and storage of specimen

The serum or plasma sample (0.9 ml/test) to be tested can be stored for 5 days at 2 to 8°C. If the test cannot be performed in this time, the serum/plasma has to be kept at -20°C (Stable at -20°C at least 2 years). Do not refreeze serum/plasma.

When using serum samples, it must be ensured that the blood was completely clotted before serum separation.

10. Test procedure**Test preparation:**

All assay reagents, test components and test specimens must be at room temperature (RT 18 to 22°C) prior to use. Wear rubber gloves and a laboratory coat. Don't touch the test pads.

Straighten the tray and add three empty test tubes for each serum/plasma to be tested. Complete the patient card and mark the dipstick with the respective patient's initials.

Fill patient's serum/plasma into the first test tube up to the blue mark using one of the enclosed disposable pipettes. Then pour Reagent 1 directly (without pipette) into the second test tube up to the blue mark.

On the next day pour Reagent 2 directly (without pipette) into the third test tube up to the mark. Avoid air bubbles while filling liquids into the tubes.

Test procedure:

1. Put the dipstick into the first test tube filled with serum/plasma. All pads have to be covered with liquid. This applies for all test steps. Incubate for 3 hours at room temperature (RT).
2. Remove the dipstick from the first test tube. Rinse the dipstick under flowing cold tap-water for 1-2 minutes. All test pads have to be rinsed evenly.
3. Put the rinsed dipstick into the second test tube filled with Reagent 1 (Conjugate). Incubate for 18 to 20 hours (overnight) at room temperature (RT).
4. Important: Now prepare the third test tube: Pour Reagent 2 (substrate) directly into the third test tube up to the mark. Then remove the dipstick from the second test tube. Rinse the dipstick under flowing cold tap-water for 1-2 minutes. All test pads have to be rinsed evenly.
5. Now insert the rinsed dipstick into the third test tube filled with Reagent 2 (substrate). Incubate for 45 minutes at room temperature (RT).
6. Remove the dipstick from the third test tube. Rinse the dipstick under flowing cold tap-water for 10 seconds. Dry the dipstick lightly with paper tissue and let it dry for half an hour.
7. Compare the colour of the test result with the colour chart. Read the classes from 0 to 4 for each test pad (see 11). Note the results on the patient card. Fix the dipstick onto the patient card for the purpose of documentation (see 13).

Please note: Positive and negative controls prove a correctly performed test. The positive control must show a clear blue/purple colour, the negative control has to stay white. In individual cases a faint blue/purple colour of the negative control is also possible. If controls do not give the expected results, the test results must be considered invalid.

8. Once the testing has been started, all steps, temperature settings and reaction times must be performed without any interruption.

Attention! If alterations are made concerning the test run (e.g. time, succession, temperature etc.) or if interferences concerning the analytical quality are evident (e.g. control serum values outside of the permitted range or marked variation of duplicates etc.) the values shall not be used. An examination of the test and/or the test run is necessary before continuation of work. If in doubt please consult a specialist of Omega Diagnostics.

Alternative test procedure:

- Put the dipstick into the first test tube filled with serum/plasma. All pads have to be covered with liquid. This applies for all test steps. Incubate for 60 minutes at 37°C.
- Remove the dipstick from the first test tube. Rinse the dipstick under flowing cold tap-water for 1-2 minutes. All test pads have to be rinsed evenly.
- Put the rinsed dipstick into the second test tube filled with Reagent 1 (Conjugate). Incubate for 60 minutes at 37°C.
- Important: Now prepare the third test tube: Pour Reagent 2 (substrate) directly into the third test tube up to the mark. Then remove the dipstick from the second test tube. Rinse the dipstick under flowing cold tap-water for 1-2 minutes. All test pads have to be rinsed evenly.
- Now insert the rinsed dipstick into the third test tube filled with Reagent 2 (substrate). Incubate for 45 minutes at 37°C.
- Remove the dipstick from the third test tube. Rinse the dipstick under flowing cold tap-water for 10 seconds. Dry the dipstick lightly with paper tissue and let it dry for half an hour.
- Compare the colour of the test result with the colour chart. Read the classes from 0 to 4 for each test pad (see 11). Note the results on the patient card. Fix the dipstick onto the patient card for the purpose of documentation (see 13).
Please note: Positive and negative controls prove a correctly performed test. The positive control must show a clear blue/purple colour, the negative control has to stay white. In individual cases a faint blue/purple colour of the negative control is also possible. If controls do not give the expected results, the test results must be considered invalid.
- Once the testing has been started, all steps, temperature settings and reaction times must be performed without any interruption.

11. Calculation

The results of the tests are evaluated visually. The intensity of the blue/purple colour of each test pad is compared with a colour chart. The intensity of the blue/purple colour is directly proportional to the concentration of allergen-specific IgE in the serum/plasma. The results are classified into class 0–4.

A positive result of an allergen pad is indicated if the pad colour compared with the colour chart shows a class ≥ 1 . A negative result of an allergen pad is indicated if the pad colour compared with the colour chart shows a class 0.

Class	Specific IgE concentration
0	Not detectable
1	Low
2	Medium
3	High
4	Very high

12. Normal values

- Class Allergodip® 0 is negative
- Class Allergodip® ≥ 1 is positive

13. Warnings and precautions

These rules must be followed:

- Handling of test components must be performed according to corresponding safety regulations.
- Do not use damaged or contaminated kit components.
- Serum/plasma samples may be potentially infectious. Contaminated regions must be disinfected by appropriate means/procedures.
- Attention: A developed dipstick may be potentially infectious.
- Reagent 1/Reagent 2: Avoid contact with skin and eyes. Do not inhale the reagents. Do not ingest.
- Do not smoke, eat or drink in the laboratory.
- Do not pipette by mouth.
- Reclose all vials immediately after use. Do not interchange vial stoppers.
- Avoid cross-contamination while pipetting.
- Do not use or mix components from different batches.
- Reagents must not be used after expiry date.
- Handling and disposal of reagents and chemical products must be done according to all applicable laws. List of materials, which must be treated with extra care:
 - Reagent 1 (Conjugate)
Sodium azide < 0.1 % w/w CAS 26628-22-8
Bovine serum albumin CAS 90604-29-8
 - The disposal of all other test components must be done according to the current applicable regulations of the respective region.

14. Quality control

- Internal quality control
Each dipstick contains a positive and a negative control

15. Storage of the test kit

2 to 8°C

16. Expiry date

The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Expiry date is the last day of the month on the bottle and the kit label. Do not use reagents after the expiry date.

17. References

- In-vitro Tests: Immunglobuline E und G; Debelic M., Wahl, R., in Fuchs/Schulz, Manuale allergologicum IV.9, 1996, Düstri-Verlag, Deisenhofen
- Allergodip – a new dipstick test for detection of specific IgE antibodies in sera from allergic patients in comparison with CAP, skin prick test and clinical history; Schlenvoigt, G.; Wahl, R.; Cromwell, O.; Fiebig, H.; Jäger, L.; Allergologie 10, 512–518 (1997)
- Straumann F., Wüthrich B.; Food allergies associated with birch pollen: Comparison of Allergodip and Pharmacia CAP for detection of specific IgE antibodies to birch pollen related foods.; J Invest Allergol Clin Immunol 2000; 10:135-141

18. Date of information

This instruction for use is valid starting from January, 1st, 2015.

19. Ordering information

Allergodip® Inhalant Allergens	Article number REF 38006000
Allergodip® Inhalation - India	REF 38006001
Allergodip® Inhalation - Africa	REF 38006010
Allergodip® Food Allergens	REF 38007000
Allergodip® Food - India	REF 38007001
Allergodip® Food - Africa	REF 38007010
Allergodip® Food - Poland	REF 38002060
Allergodip® Mediterranean Allergens	REF 38009000
Allergodip® Mites and more	REF 38010006

20. Composition of the Mixes

Allergodip® Inhalant Allergens

- 6-Grass Mix:
 - Velvet grass
 - Orchard grass
 - Perennial ryegrass
 - Timothy grass
 - Blue grass
 - Meadow fescue

Allergodip® Inhalation - India

- Mould Mix
 - Cladosporium herbarum
 - Rhizopus nigricans
 - Curvularia lunata
 - Alternaria tenuis

- Grass Mix
 - Rye
 - Bermuda grass
 - Sweet vernal grass
 - Timothy grass
 - Sorghum vulgare

- Weed Mix
 - Xanthium strumarium
 - Amaranth
 - Mugwort
 - Lambs quarter
 - P. hysterophorus

- Epithelia Mix
 - Cat epithelia
 - Dog epithelia

- Animal Mix
 - Pigeon droppings
 - Rat droppings

Allergodip® Food - India

- Fish/Crustacean Mix
 - Crab
 - Lobster
 - Shrimp
 - Kingfish
 - Mackerel

- Legume Mix
 - Lentil
 - Pea
 - White bean

- Cereal/Lentil Mix
 - Rice
 - Corn flour
 - Semolina
 - Yellow pigeon pea
 - Brown chick pea
 - Mung bean

- Onion/Garlic Mix
 - Onion
 - Garlic

Allergodip® Inhalation - Africa

- 6-Grass Mix:
 - Velvet grass
 - Orchard grass
 - Perennial ryegrass
 - Timothy grass
 - Blue grass
 - Meadow fescue

21. Manufacturer

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