

AlleisaScreen[®]

Immunoblot for analysing
specific IgE in human serum

Art. No.: A 0297 Panel 30 Food IRAQ (food allergens)
20 test membranes with 30 allergens

Art. No.: A 0298 Panel 30 Resp IRAQ (inhalative allergens)
20 test membranes with 30 allergens

in-vitro test, medical product IVD guideline 98/79/EC

Storage at 2 - 8 °C

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1. Intended use

The AlleisaScreen-Test is an immunoblot assay for the quantitative determination of circulating allergen-specific Immunoglobulin E (IgE) in human serum.

2. General

Class E immunoglobulins were first identified in 1964 and play an important role in triggering Type I allergic reactions. After transforming into the so-called plasma cell, B-lymphocytes excrete antibodies of various classes which circulate in the blood and are responsible for immunity in the humours. The conversion of B-lymphocytes into the antibody-secreting plasma cells is regulated by helper and suppresser cells (a sub-class of the T-lymphocytes). If this regulation fails, a B-lymphocyte can also be converted by a normally harmless antigen. The resulting plasma cell will then produce Immunoglobulin Class E antibodies in preference. These immunoglobulins migrate via the blood stream to the basophilic granulocytes and mast cells where they are bound to specific receptors via their Fc-region. If the organism has further contact with the specific allergen, then this migrates directly to the anchored IgE antibodies and links two neighbouring molecules with its epitopes via the antigen-binding fab region. This link formation liberates different vasoactive amines from the mast cells, histamine in particular, which together with other highly active mediators, can lead to the typical symptoms of a Type I allergic reaction such as urtication (wealing), urticaria (nettle rash), dermatitis, rhinitis (inflammation of the nasal mucosa), hay fever, asthma, and anaphylactic shock.

3. Test principle

Special allergens are bound to the surface of nitrocellulose membranes lying in a reaction trough. The patient's serum is pipetted into the reaction trough and incubated at room temperature. During this time, the allergen-specific IgE-antibodies react with the allergens and bind to the nitrocellulose membranes via the allergen. Non-bound material is removed by washing. After this, an anti-human IgE antibody coupled with biotin is added and incubated at room temperature. This binds to the respective specific IgE in the test fields from the first incubation and to the positive control. Non-bound detector antibodies are removed by washing. Next, a streptavidin is added which is conjugated with alkaline phosphatase and incubated at room temperature. This binds to the biotin from the second incubation in the test fields. Non-bound streptavidin conjugate is removed by washing. After adding the substrate and incubating at room temperature, a specific enzymatic color reaction of the alkaline phosphatase takes place which results in the formation of precipitates on the test strips. The coloration is directly proportional to the specific antibody content of the serum sample. Evaluation is carried out after complete drying of the test strip with the Improvio Scanner, which takes a photo of the membrane.

4. Reagents provided

Each test kit AlleisaScreen contains:

| | | |
|--------|---|---------|
| 2 x 10 | test strips in plastic reaction troughs, nitrocellulose membranes coated with allergen material on 30 test fields | |
| 2 x | washing buffer concentrate (TRIS/NaCl, contains 0.099% NaN ₃), yields 1 x 500 ml washing buffer, pH = 7,5 | (20 ml) |
| 1 x | detector antibody (bottle with white cap), ready for use with biotin conjugated anti-human IgE antibodies, mono/polyclonal contains 0.099% NaN ₃ | (10 ml) |
| 1 x | streptavidin-conjugate (bottle with red cap), ready for use streptavidin conjugated with alkaline phosphatase contains 0.02% methylisothiazolone and 0.02% bromonitrodioxane | (10 ml) |
| 1 x | substrate (black bottle with black cap), ready for use BCIP / NBT (bromochloroindolyl phosphate/ Nitro Blue Tetrazolium), | (10 ml) |
| 1 x | Instruction manual | |

5. Materials required but not provided

5.1. Reagents

Distilled or de-ionised water.

5.2. Accessories

Vortex mixer; measuring cylinder (500 ml), 500 ml laboratory wash bottle, washing retainer to take 10 reaction troughs (optional); incubation box for incubation in the dark (the system consisting of washing retainer and incubation box can be obtained from MEDIWISS Analytic); **ScreenShaker**; hairdryer, conventional, commercially available (optional), measuring device (**Improvio Scanner**) and printer. A **BeeBlot 12** or a **BeeBlot 36 (Bee Robotics)** is also available for an automated procedure.

6. Warnings and precautions

The available kit contains in-vitro diagnostic tests for determining specific IgE-antibodies in human serum. For this reason, the patients' samples must be treated as potentially infectious and the appropriate safety precautions must be observed.

AlleisaScreen detector antibody and AlleisaScreen washing buffer contain sodium azide as a preservative (0.0995). Contact with the skin or mucous membrane must be avoided. Explosive metal azides may be produced on contact with lead or copper pipes.

AlleisaScreen streptavidin conjugate contains methylisothiazolone and bromonitrodioxane in subtoxic concentrations as preservatives. Methylisothiazolone may cause an allergic skin reaction.

After use, the user is personally responsible for disposing of all the components of the kit in the proper manner.

All reagents and materials which come into contact with potentially infectious samples must be treated with suitable disinfectants or autoclaved at 121°C for at least one hour.

Exchanging or combining kit components with those from a kit of another batch number is not possible. In case of destroyed outer wrapping, the single components have to be tested for integrity. Kit components must not be used in case of destroyed individual wrappings or leaky vials.

7. Storage instructions

Test strips in the reaction troughs must be stored in plastic packaging under cool, dry and dark conditions. All reagents must be stored at 2-8°C and can be used anytime before the expiry date printed on the label. Microbial contamination must be avoided. The quality is no longer guaranteed after the expiry date has passed. The diluted washing buffer can be stored at 2-8°C for a maximum of 8 weeks before use.

Contamination of the substrate solution (AlleisaScreen substrate) with the streptavidin conjugate solution (AlleisaScreen streptavidin conjugate) must be avoided at all costs as this would lead to coloration of the substrate. Likewise, the substrate must be kept away from direct sunlight in order to avoid decomposition or coloration due to auto-oxidation. If the substrate should become colored, it will no longer be suitable for use.

8. Indication of instability or deterioration of reagents

Turbidity or purple coloration of the substrate before addition to the reaction trough indicates that the reagent has become degraded.

9. Specimen collection and storage

AlleisaScreen was developed for the analysis of human serum samples. The blood samples should be acquired using venipuncture and the serum separated out after coagulation (30–40 min) by centrifugation for 10 min at 4000g. Repeated freezing and thawing of the samples and microbial contamination must be avoided at all costs. Using heat-inactivated, lipaemic, haemolytic, icteric, or turbid samples may lead to erroneous results.

If the analysis is not carried out immediately, the sample material may be stored at 2-8 C for up to a week. The samples may be stored for longer periods at -20 C or lower.

10. Test procedure

10.1. General information

Before use, all reagents and reaction troughs must be warmed to room (20 – 22°C) temperature. The reagents must be thoroughly mixed before use. The reproducibility of the results is strongly dependent on the accuracy of pipetting, maintenance of the incubation times and temperature as well as the uniformity of washing of the test strips. If higher temperatures are given the developing time of the substrate (last step) should be reduced ½ minute per °C (> 25°C) (general rule).

Direct sunlight must be avoided while carrying out the test. It is recommended that the reaction troughs be covered during incubation in order to avoid losses due to evaporation. Reaction troughs must only be grasped by the handle. Contact with the reaction surface must be avoided. The patient data (such as the laboratory number) can be written on the handle of the trough using a permanent felt-tip marker

Deviation from the stipulated incubation times and temperature will lead to erroneous results for the patients' sera.

The substrate incubation (fourth incubation see Section 10.9.) must be carried out in the dark to avoid auto-coloration of the substrate.

The test has to be used only by experienced laboratory personal. Please refer to guidelines for safety regulations in medical laboratories. The test protocol must be followed strictly.

10.2. Preparation of the washing buffer

The contents of each bottle of AlleisaScreen washing-buffer concentrate is placed in a 500 ml measuring cylinder, dissolved and made up to 500 ml with distilled water and then transferred to a laboratory wash bottle. If necessary, dissolve any crystals found in the concentrate beforehand by heating in a water bath at 37 C.

10.3. First incubation

The corresponding number of reaction troughs required for the tests to be carried out is removed from the packaging, briefly rinsed for about several seconds over the sink with the diluted washing buffer in the wash bottle and filled with 300 µl patient's serum using a pipette. A new pipette tip must be used for each serum! The reaction troughs are then incubated at room temperature (20 – 22°C) on the **ScreenShaker** for 45 minutes.

10.4. Washing

The reaction troughs are rinsed over the sink for 5 seconds using the prepared washing buffer (wash bottle). The troughs are held diagonally downwards. The stream of washing solution should be made to pass over the test strips several times.

A repeated effect of the washing buffer in the reaction troughs over some seconds by shaking the buffer in the troughs increases the washing effect

This process can be simplified by using the washing retainer in which 10 troughs can be inserted or an automated western-blot analyzer is to be used (**BeeBlot 12 or BeeBlot 36, Bee Robotics**).

10.5. Second incubation

Next, place 300 µl AlleisaScreen detection antibody in each reaction trough. Incubate the troughs on the ScreenShaker at room temperature for 45 minutes.

10.6. Washing

Wash as described under Section 10.4.

10.7. Third incubation

After this, place 300 µl AlleisaScreen streptavidin conjugate in each reaction trough. Incubate the troughs on the ScreenShaker at room temperature for 20 minutes.

10.8. Washing

Wash as described under Section 10.4.

10.9. Fourth incubation

Next, place 300 µl AlleisaScreen substrate in each reaction trough. Incubate the reaction troughs on the ScreenShaker at room temperature for 20 minutes **in the dark**. After incubation is complete, the color reaction is terminated by briefly rinsing the test strips under flowing water. The strips are then dried in the air or by using a conventional hair dryer which will speed up the drying process. The blue-purple color of the background disappears as the test strip dries. The evaluation must not be carried out until the test strips in the trough are thoroughly dry.

Summary of the test procedure

- A. Warm up the reagents to room temperature (20 – 22°C).
- B. Dilute the AlleisaScreen washing buffer concentrate 1:25.
- C. Moisten the membranes in the reaction trough by means of the washing buffer. Pipette 300 µl of each of the serum samples into the corresponding reaction troughs charged with the allergen strips; incubate on the ScreenShaker at room temperature for 45 minutes.
- D. To wash, guide the stream of diluted wash solution from the wash bottle over the test strips several times while holding the reaction trough diagonally. Fill the trough several times with buffer und shake the solution in the trough manually for several seconds
- E. Add 300 µl AlleisaScreen detector antibody; incubate on the ScreenShaker at room temperature for 45 minutes.
- F. Wash (see point D.)
- G. Add 300 µl AlleisaScreen streptavidin conjugate; incubate on the ScreenShaker at room temperature for 20 minutes.
- H. Wash (see point D.)
- I. Add 300 µl AlleisaScreen substrate; incubate on the ScreenShaker at room temperature for 20 minutes in the dark.
- J. Terminate the substrate reaction by briefly rinsing under flowing water.
- K. Dry the membranes completely and evaluate in the Improvio Scanner.

11. Analysis

11.1. Strip configuration

The strip **AlleisaScreen Panel 30 Food IRAQ (Art. No. A 0297)** contains following 30 allergens:

Positive control,

Milk, Egg white, Sunflower seed, Egg yolk, Peach, Soybean, Banana, Olive, Strawberry, Tomato, Carrot, Cacao, Kiwi, Fig, Broad bean, Orange, Hazelnut, Pistachio nut, Cod fish, Apricot, Meat mix (Chicken, Lamb, Beef), Gluten, Sesame seed, Potato, Garlic, Pepper, Aubergine, Walnut, Raspberry, Almond

The strip **AlleisaScreen Panel 30 Resp IRAQ (Art. No. A 0298)** contains following 30 allergens:

Positive control,

Dermatophagoides pteronyssinus, Dermatophagoides farinae, Cockroach, Camel hair, Absinthe, Oak, Olive (Pollen), Cypress, Mulberry, Timothy grass, Mixed grasses (Sweet vernal grass, Bermuda grass, Orchard grass, Meadow fescue, Ryegrass, Timothy grass, Smooth brome grass, Velvet, Meadow foxtail, Quitch, Oat grass), Grain pollen mix (Oat, Rye, Wheat, Maize), Ragweed, Mugwort, Parietaria, Sweet vernal grass, Nettle, Chenopodium album, Camomile, Cat epithel, Candida albicans, Dog epithel, Feather mix (Pigeon, Goose, Chicken, Duck), Cage bird mix (Parrot/Budgerigar/Hollandicus), Cow/Sheep, Chicken feather, Penicillium notatum, Aspergillus fumigatus, Cladosporium herbarum, Alternaria alternata

The surface of the negative control test strip has not been coated with allergen (area below the relevant last allergen). Anti-goat IgG (rabbit) has been applied as a positive control (top end of each test strip).

| | Panel 30 Food IRAQ | Panel 30 Resp IRAQ |
|--|---------------------------|-------------------------------------|
| | Control | Control |
| | Milk (f2) | Derm. pteronyssinus (d1) |
| | Egg white (f1) | Derm. farinae (d2) |
| | Sunflower seed (f114) | Cockroach (i6) |
| | Egg yolk (f75) | Camel hair (e17) |
| | Peach (f95) | Absinthe (w5) |
| | Soybean (f14) | Oak, white (t7) |
| | Banana (f92) | Olive (t9) |
| | Olive (f458) | Cypress (t23) |
| | Strawberry (f44) | Mulberry (t70) |
| | Tomato (f25) | Timothy grass (g6) |
| | Carrot (f31) | Mixed grasses (gx) |
| | Cacao (f93) | Grain pollen mix (gx12) |
| | Kiwi (f84) | Ragweed (w1) |
| | Fig (f328) | Mugwort (w6) |
| | Broad Bean (f500) | Parietaria (w21) |
| | Orange (f33) | Sweet vernal grass (g1) |
| | Hazelnut (f17) | Nettle (w20) |
| | Pistachio nut (f203) | Chenopodium album (w10) |
| | Cod fish (f3) | Camomile (w206) |
| | Apricot (f237) | Cat epithel (e1) |
| | Meat mix (fx23) | Candida albicans (m5) |
| | Gluten (f79) | Dog epithel (e5) |
| | Sesame seed (f10) | Feather mix (ex70) |
| | Potato (f35) | Parrot/Budgerigar/Hollandicus (ex8) |
| | Garlic (f47) | Cow/Sheep (ex9) |
| | Pepper (263) | Chicken feather (e85) |
| | Aubergine (f262) | Penicillium notatum (m1) |
| | Walnut (f256) | Aspergillus fumigatus (m3) |
| | Raspberry (f343) | Cladosporium herbarum (m2) |
| | Almond (f20) | Alternaria alternata (m6) |

The sequence of allergens is given in **Fig. 1**

11.2. Sera findings

The basis of development of the software is the digital photographic evaluation of Western-Blot lines. The system takes photographs of the test strips and a software programme evaluates the colouration of the allergen lines. This is achieved by comparing the calculated surface integral of each line of the membrane with an internal standard curve, based on a grass pollen standard, and grouping these into classes. These classes are directly related to the specific IgE content in iU/ml. Interferences on the strips are taken into account mathematically via a rolling disc and a "cut-off" value.

After measurement a printout provides the user with a photo of the strip, the densitometer curve of the membrane, the classes and the concentration data for each allergen band in iU/ml. The data are stored and documented in the system, specifically for each patient, and are retrievable. In the case of each allergen, the color intensity on the test fields is directly proportional to the amount of specific IgE antibodies in the serum of the patient.

The quantitative evaluation is carried out using the Scanner by inserting the reaction trough with the membrane into the Improvio Scanner followed by the measurement. The results of the analysis were printed out and assigned to the test Classes 0 – 6. The classes which have been read off can be converted into the IgE content of each allergen using Table 1.

Care must be taken to use the correct pre-defined test depending on the allergen panel when starting the measurement.

Fig. 2: Optical density correlated with the grass antibody concentration.

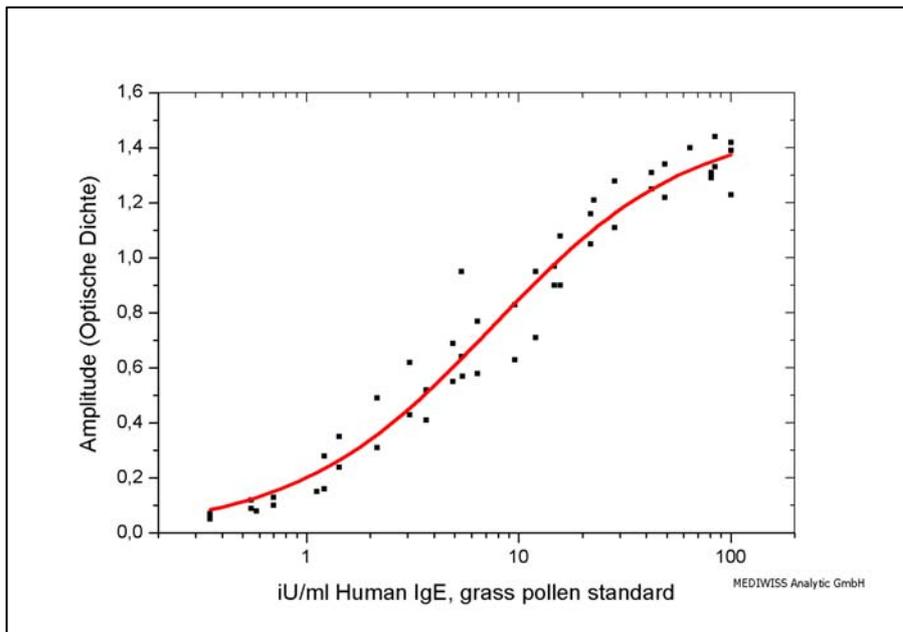


Table1: Relationship between the classes found and the IgE content of specific allergens of the patient's serum

| iU/ml | Class | Allergen-specific IgE content |
|-------------------|-------|-------------------------------|
| < 0.35 iU/ml | 0 | None or hardly any found |
| 0,35 – 0.69 iU/ml | 1 | Low |
| 0.7 – 3,4 iU/ml | 2 | Increased |
| 3,5 – 17,4 iU/ml | 3 | Significantly increased |
| 17,5 – 49.9 iU/ml | 4 | High |
| 50 – 100 iU/ml | 5 | Very high |
| > 100 iU/ml | 6 | Extremely high |

11.3. Quality control

A positive control is provided on each test strip. It is used to check that the test has been carried out properly. If there is a class value < 2.5 for the positive control, the test is not valid.

In this case, please check the following before repeating the test:

- Expiration date of the reagents.
- Calibration of the used instrument with the correct calibration file.
- Exact test procedure.
- Exact placing of the reaction trough in the Improvio device.
- Visual examination of kit components for signs of contamination, deterioration or leakage; the substrate must not be used if turned to dark.

If the control data are not fulfilled after repeating analysis, please contact your local distributor.

11.4. Documentation

After drying the test strips and evaluation in the Scanner has been completed, they can be removed from the reaction trough with a pair of tweezers and documented in a work log. The measurement data (photo of the test strip and evaluation) are saved to the PC hard disk in a preselected directory. A data printing of each serum tested can be done using a graphical standard printer connected to the PC.

The analysis results obtained with Improvio Scanner depend from the quality of the test procedure in the lab. The software may interpret drags, points or fluff on the membranes as false positive results, due to the fact that these stains – as an allergen line – has a contrast to the background. The user must verify the conformity of the measurement.

12. Statistical data: variations, sensitivity, specificity and accuracy

| | |
|------------------------|------------------|
| Intra-assay variation: | mean value 4,5 % |
| Inter-assay variation: | mean value 3,9 % |
| Inter-batch variation: | mean value 8,8 % |

In order to determine the sensitivity and specificity of the method 737 comparisons between the skin-prick test and the AllergyScreen were done in a framework of a clinical study (1) with 142 sera.

| | |
|--------------|--------|
| Sensitivity | 95.1 % |
| Specificity: | 80.2 % |
| Accuracy: | 88.3 % |

Also 881 comparisons were done in this study between the AllergyScreen and an ELISA - single test system.

| | |
|--------------|--------|
| Sensitivity: | 84.3 % |
| Specificity: | 95.0 % |
| Accuracy: | 90.6 % |

13. Remarks about the clinical significance of specific IgE determinations.

Allergy diagnosis is not only based on the results of a laboratory test. It is not possible to see the whole picture until the anamnesis (clinical history) is combined with the clinical profile (results from different in-vivo and in-vitro tests).

The classes determined using this test system make it possible to make a statement about the degree of sensitivity of the patients in regard to the individual allergens examined. Here, the determination of the presence of specific antibodies represents an important and practical extension of the in-vivo methods such as the skin test.

Furthermore, in-vitro testing is frequently the only possible way of identifying Type 1 allergen-specific sensitivity.

The AlleisaScreen is constructed in this way that a significant positive colouring to a specific allergen line results in a specific immunological reaction of the IgE-antibodies in the serum of the patient. However this must not be necessarily demonstrated in an actual clinical picture of the patient to the appropriate allergen, if there is only a sensitization without clinical relevance.

14. Literature:

Kersten, W. (2002) : Vergleich des AllergyScreen (MEDIWISS Analytic, Moers) mit dem Hauttest (HAL, Düsseldorf -in-vivo) und dem CAP-System (Pharmacia, Freiburg -in-vitro). – Allergologie 25/4:203-208

Herzum et al. (2005) : Diagnostic and analytical performance of a screening panel for allergy. – Clin Chem Lab Med 43(9): 963-966

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