

Clinical diagnostic of allergy diseases



AllergyScreen® AlleisaScreen®



MEDIWISS
Analytic GmbH

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How allergies work

Allergy is the fifth leading chronic disease in the U.S. and the third most common chronic disease among children under 18 years old.

An allergy is a misguided reaction to foreign substances by the immune system. It is said to be misguided because these foreign substances (called allergens) are generally harmless. A variety of cells play an important role in this type of reaction:

- T-lymphocytes recognize the foreign substances in the body and release chemical cytokines that stimulate the B-lymphocytes
- B-lymphocytes produce immunoglobulin E (IgE) to destroy the substance
- TH1 and TH2 combat the foreign substances
- Mast cells and basophiles (white blood cells) produce mediator chemicals such as histamine and leukotriene

The role of IgE

Class E immunoglobulin (IgE) was first identified in 1964 and plays an important role in Type I allergic reactions. Everyone has IgE antibodies in small amounts to protect the body from parasites. However, allergic persons produce IgE in abnormally elevated quantities.

When stimulated by the appropriate foreign allergen, helper and suppresser cells (sub classes of the T-lymphocytes) stimulate B-lymphocytes to transform into Plasma-cells. Plasma-cells secrete antibodies of various classes which circulate in the blood and are responsible for immunity in the humours. If this regulation fails, a B-lymphocyte can also be converted by a normally harmless antigen. These immunoglobulins migrate via the blood stream to the basophiles and mast cells where they are bound to specific receptors within their Fc region. If the organism has further contact with the specific allergen, it migrates directly to the anchored IgE and links two neighbouring molecules with epitopes via the antigen-binding Fc region. This link formation liberates different vasoactive amines from the mast cells, which together with other highly active mediators can lead to the typical symptoms of a Type I allergic reaction.

The most common allergic conditions include hay fever (allergic rhinitis), asthma, allergic conjunctivitis, allergic eczema, hives (urticaria) and allergic shock (also anaphylaxis and anaphylactic shock).

Common allergy triggers

Airborne allergens: pollen, animal dander, mites and mold

Often pet allergies are triggered by exposure to proteins found in animal skin cells, saliva or urine and are commonly associated with cats, dogs, horses and rodents. Inhaling mold spores can also cause an allergy with incidents typically reported between July and late summer. The most common triggers are *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. A wide variety of tree, grass and weed pollen cause an allergic reaction, the intensity of which is influenced by pollen seasons, location, weather and other environmental factors.

■ Foods

For adults, the most common triggers of food allergies include shellfish, peanuts, tree nuts, fish and eggs, whereas young children tend to experience allergies to eggs, milk and peanuts. Food intolerance is often confused with food allergy as they share similar symptoms.

■ Insect venoms

Insect stings inject chemicals into the body which can cause allergic reactions, in most cases resulting from stings/bites from bees, wasps and fire ants. Mosquitos, flies and fleas can cause milder reactions.

■ Latex

Repeated or prolonged exposure to latex is one of the most common causes of allergic contact dermatitis. Normally harmless, latex is a natural rubber used in a wide range of items: medical supplies (such as gloves and catheters), balloons, children's toys or shoe soles.

■ Medication

Some people develop allergies to certain medications, the most common of which include penicillin and related antibiotics. Reactions range from mild localized rashes or hives to life-threatening severe allergic reaction (anaphylaxis).

Changing the testing paradigm

AllergyScreen® / AlleisaScreen®



Allergy diagnostics and the identification of a sensitization against allergens is an interdisciplinary challenge that incorporates knowledge gained through medical science, biochemistry and biology.

Long years of experience and work done with single test systems in allergological labs have shown that only a few key allergens are responsible for the patient's disorders. And so, a question emerged: Why not collect all these key allergens and combine them in one single reaction trough?

AllergyScreen®: Quick and efficient testing of up to 20 allergens per strip

Mediwiss Analytic has vast experience in the extraction of allergens from raw material and extensive know-how of western blot protein analysis. This led to the idea of binding high quality, self-extracted, lyophilised allergens to a nitrocellulose membrane with the help of a contact plot printer.

The required antibody and substrate for the visualization of the specific IgE/allergen reaction were available and well known from western blot analysis. It was only necessary to develop a plastic reaction trough in which the membrane strips could be glued. This resulted in a ready-to-use test for the determination of specific IgE in human serum.

The original contact plot printer used pencils filled with a special protein solution. New sophisticated equipment was developed featuring up to 21 micro dispensing pumps which can be individually programmed according to variable and individual parameters with minimal divergence.

Supportive hardware and software systems were also developed for a camera that photographs the membrane and allows a semi-quantitative analysis of the results: Initially, up to 20 allergens could be measured per membrane.

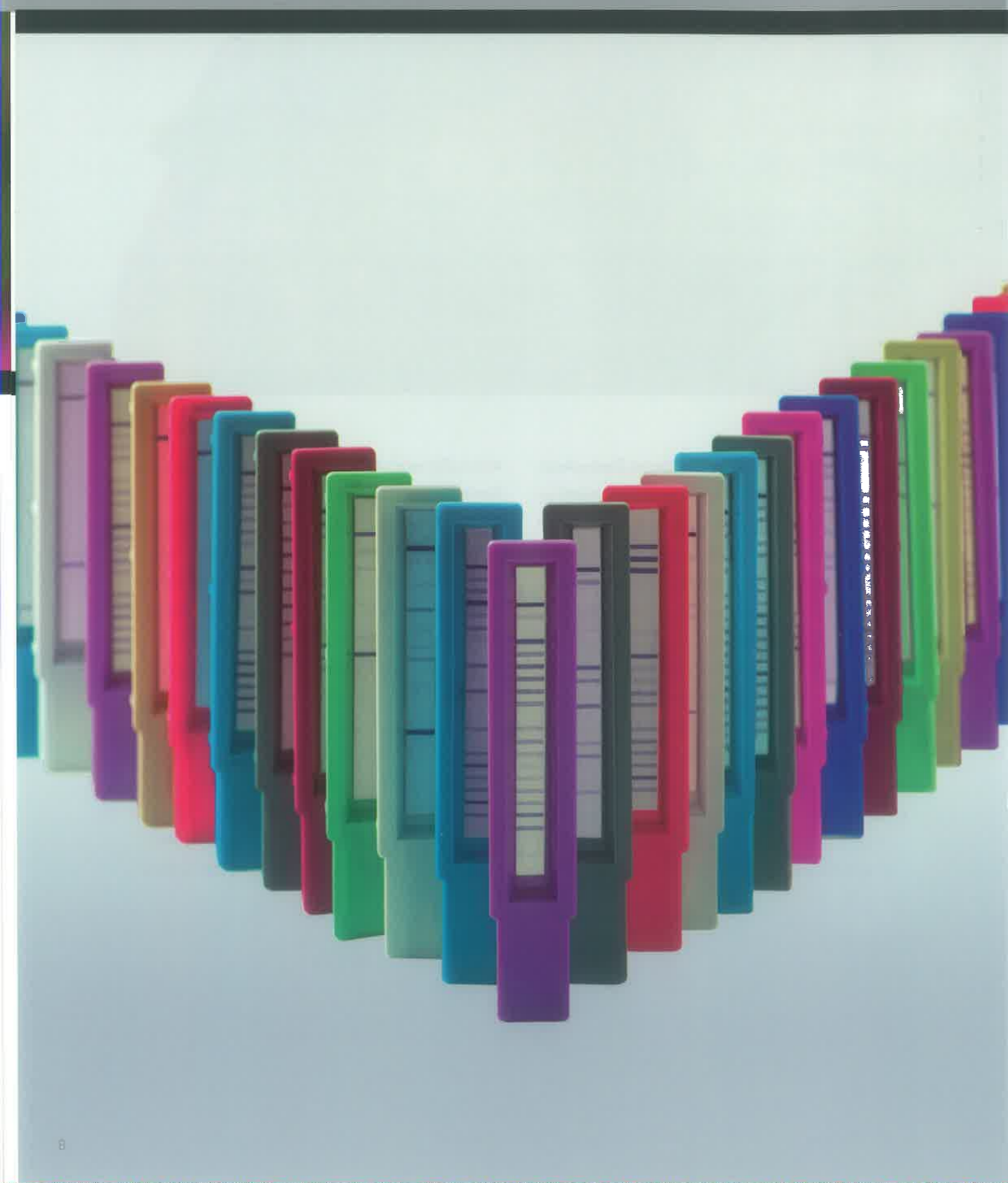
AlleisaScreen®: Precise testing of up to 30 flexible allergens in a single procedure

The wish for more allergen lines on a single membrane surface led to AlleisaScreen®, a bigger biochip with a maximum capability of 30 allergens on one test strip that is glued in a ready-to-use reaction trough. The fact that these new biochips required a higher camera resolution resulted in the development of a new analysis system.

What started as a simple qualitative visual test strip has been developed into a highly innovative, semi-quantitative nitrocellulose chip system for the determination of specific IgE, IgG4 and IgG in human serum with customized, high resolution scanner systems.

Clear advantage over single determination tests

AllergyScreen®/AlleisaScreen®



AllergyScreen® and AlleisaScreen® are two test systems to detect allergen-specific immunoglobulins in human serum. Customers can be provided with the necessary test strips and the corresponding hard and software (camera, scanner, testing equipment, robotics).

Testing multiple allergens offers a more detailed and extensive analysis

It is extremely important for allergic persons to identify their sensitizations in order to establish an appropriate life plan – even when, from a clinical point of view, not all of these sensitizations are current. Labs can either use single or multiple allergens for the determination of allergic sensitization. In both cases the allergens are extracted in the same way. Single allergen test systems usually test only allergens based on the patient's complaints and clinical history. By contrast, test membranes with multiple allergens make it possible to test different allergen groups, thereby determining sensitization with no clinical currency at the time of the test. Since most sensitizations are cross-reactive, key allergens in particular are always present. One trough with several key allergens allows for a rapid detection of all possible antigens in a single lab test. This type of approach is naturally cheaper than single tests, uses less lab materials and less patient's serum. The use of allergen specific test strips with multiple food allergens or test panels with multiple respiratory allergens also lead to a more detailed and extensive patient analysis.



AllergyScreen®

This immunoblot test combined with our evaluation software enables:

- Easy test procedure
- Quick testing and evaluation in just 2.5 hours
- Flexible, customized and country-specific panels
- Testing of 20 allergens per strip with only 250 µl serum

AlleisaScreen®

This test offers you all the advantages of our well established AllergyScreen® test, as well as:

- Testing of 30 allergens per strip with only 300 µl serum
- The best price-allergen ratio of all screening systems in allergy diagnostics on the market

Mediwiss Analytic produces its own antigens/allergens and optimizes them for use on the test strips.

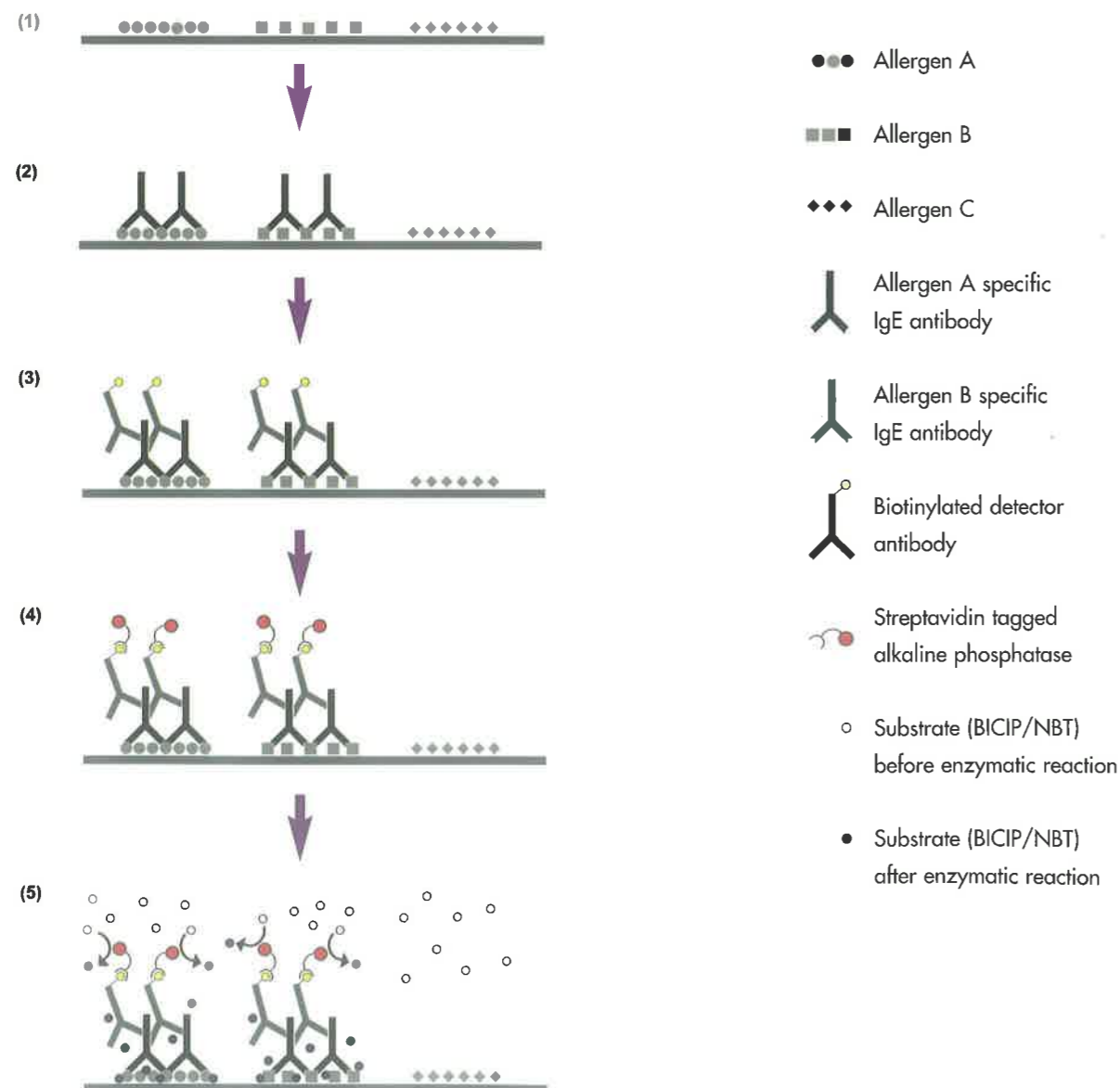


Precise testing methods

AllergyScreen®/AlleisaScreen®

Overview of the AllergyScreen®/AlleisaScreen® processes

- (1) Different allergens are bound to a nitrocellulose membrane as lines.
- (2) Specific IgE antibodies of the patient serum bind to their respective allergen.
- (3) The biotinylated detector antibody, an anti-IgE antibody, binds to the specific IgE antibodies.
- (4) The alkaline phosphatase binds via its streptavidin tag to the biotin group of the detector antibody.
- (5) The colorless substrate (BCIP/NBT) is modified enzymatically, resulting in a blue precipitate.



Key test principles

The AllergyScreen® and the AlleisaScreen® systems are semi-quantitative in vitro systems to determine the presence of allergen-specific IgE antibodies in human serum. A blood sample from the patient is necessary for this test. Liquid allergens, produced for in vitro diagnostic purposes, are passively bound as test lines to a nitrocellulose membrane and the membrane strips are glued into the reaction troughs. Customers can select up to 20 allergens using AllergyScreen® and up to 30 different allergens using AlleisaScreen®.

Test principles

1. Different allergens are bound as test lines to the surface of a nitrocellulose membrane.
2. To determine allergen-specific IgE antibodies, patients sera (250 µl for AllergyScreen® and 300 µl for AlleisaScreen®) is pipetted into the reaction trough and incubated for 45 minutes at room temperature. During this time the allergen-specific IgE antibodies bind to the specific allergens on the membrane. Nonbound IgE antibodies are removed by washing.
3. In a next step, polyclonal anti-human IgE antibody (AllergyScreen®) or a mix of a monoclonal and a polyclonal anti-human IgE antibody (AlleisaScreen®) is pipetted into the reaction trough and incubated for another 45 minutes at room temperature. These biotin coupled detector antibodies bind to the allergen-specific IgE from the patient serum. Nonbound detector antibodies are removed by washing.

4 The membrane is then incubated with a streptavidin tagged alkaline phosphatase (streptavidin-conjugate) for 20 minutes at room temperature. The streptavidin tag binds to the biotin of the detector antibody. Nonbound streptavidin-conjugate is removed by washing.

5. Now the substrate (color solution) is added to the membrane and incubated at room temperature for 20 minutes. An enzymatic reaction of the alkaline phosphatase takes place, resulting in a blue band on the respective allergen line. The reaction is stopped by washing. The coloration is directly proportional to the amount of the specific antibody in the serum sample.

6. After thoroughly drying the membrane, an evaluation of the coloration of the allergen lines is carried out by one of our systems: Improvio scanners, CubeScreen Reader or RapidReader. The concentration of specific IgE in the serum sample is determined and results are given in classes (from 0 to 6) and iU/ml (range 0 to > 100 iU/ml).

Simplicity combined with quick, concise results

AllergyScreen®/AlleisaScreen®



You will need:
 A washing bottle,
 a graduated cylinder,
 a ScreenShaker,
 a comb (trough holder),
 an incubation box,
 a 100 µl – 1000 µl pipette,
 a timer and some gloves.

Precautions:
 Sera and all solutions must be at ambient temperature (20°C-22°C). Dilute the washing buffer concentrate (1:25).



(1) Wet the membranes with washing buffer. Remove the washing buffer by gently tapping the troughs on a paper towel. The membranes should be wet but with no surplus buffer on its surface.



(2) Pipette 250 µl serum (AllergyScreen®) or 300 µl serum (AlleisaScreen®) into the troughs, taking care that the membranes are completely covered. Carefully slide the comb into the incubation box. Place the incubation box onto the ScreenShaker. Incubate for 45 minutes at ambient temperature.



(3) Carefully take the comb out of the box. Remove the sera by rinsing the troughs with the washing buffer. Rinse each membrane five times, thereby shake the washing buffer in the troughs for some seconds. Remove the surplus by gently tapping the comb on paper towels.



(4) Pipette 250 µl (AllergyScreen®) or 300 µl (AlleisaScreen®) detection antibody in each trough. Slide the comb into the incubation box and incubate for another 45 minutes on the ScreenShaker at ambient temperature.



(5) Wash the membranes as described in step (3).



(6) Pipette 250 µl (AllergyScreen®) or 300 µl (AlleisaScreen®) of the streptavidin conjugate into each reaction trough. Replace the comb into the incubation box and incubate for exactly 20 minutes on the ScreenShaker.



(7) Wash the membranes as described in step (3), but this time rinse each membrane 10 times.



(8) Pipette 250 µl (AllergyScreen®) or 300 µl (AlleisaScreen®) of the substrate into each reaction trough. Slide the comb into the incubation box and replace it on the ScreenShaker. Incubate for exactly 20 minutes at ambient temperature on the incubation shaker.



(9) Rinse each membrane several times under running water. Remove the washing buffer by gently tapping the trough on a paper towel. Leave the membranes to air dry or use a conventional hair dryer. The background of the membranes will turn white, while the control line and positive tested allergens will stay purple.



Standard and customized panels worldwide: Directory of available allergens



Mediwiss Analytics provides our customers worldwide with a wide range of allergens and allergen mixtures for diagnostic testing. In order to obtain exceptional results, our own allergens are manufactured from certificated raw materials to gain the highest quality standards. The allergens are controlled at every stage of production.

Standard and highly customized allergen-specific panels are available for the testing of multiple allergen combinations. Three samples of customized panels are provided at the end of this directory.

If you have any questions about our allergens or about how we support our customers with specific allergen combinations, please contact us.

Sample panels

Multiple combinations of allergens

Worldwide customized panels

Panel CHN Multi (20 allergens)

Control
Derm. pteronyssinus (d1)
House dust (h1)
Mulberry (t70)
Cat (e1)
Dog (e5)
Cockroach (i6)
Pigweed (w14)
Egg white (f1)
Milk (f2)
Shrimp (f24)
Beef (f27)
Mussel (f37)
Crab (f23)
Mango (f91)
Cashew nut (f202)
Pineapple (f210)
Mould fungi mixture (mx3)
Weed mixture CHN (wxCHN)
Tree mixture CHN (txCHN)
Total IgE

Panel 30 Peru PEDI (30 allergens)

Control
Grass mix (gx)
Birch (t3)
Mugwort (w6)
Derm. pteronyssinus (d1)
Derm. farinae (d2)
Cat (e1)
Dog (e5)
Horse (e3)
Cladosporium herbarum (m2)
Aspergillus fumigatus (m3)
Egg white (f1)
Egg yolk (f75)
Cow's milk (f2)
Codfish (f3)
 α -Lactalbumin (f76)
 β -Lactoglobulin (f77)
Casein (f78)
BSA (e204)
Wheat flour (f4)
Rice (f9)
Soy bean (f14)
Peanut (f13)
Hazelnut (f17)
Carrot (f31)
Potato (f35)
Apple (f49)
Bromelain (CCD1)
Horseradish Peroxidase (CCD2)
Ascorbat Oxidase (CCD3)

Panel 12 RUS (12 allergens)

Control
Derm. pteron./Derm. farinae (d1/d2)
House dust/Cockroach (hx/i6)
Birch pollen (t3)
Hazelpollen (t4)
Mixed grasses (gx)
Rye pollen (g12)
Mugwort (w6)
Ambrosia mix (wxrus)
Cat (e1)
Dog (e5)
Penicillium not./Aspergillus fum. (m1/m3)
Cladosporium herb./Alternaria altern. (m2/m6)

Quality controlled from beginning to end

Professional production

Mediwiss Analytic manufactures high quality allergen extracts from certificated raw materials. About 500 different allergens are available for manifold combinations on our AllergyScreen® and AlleisaScreen® test strips.

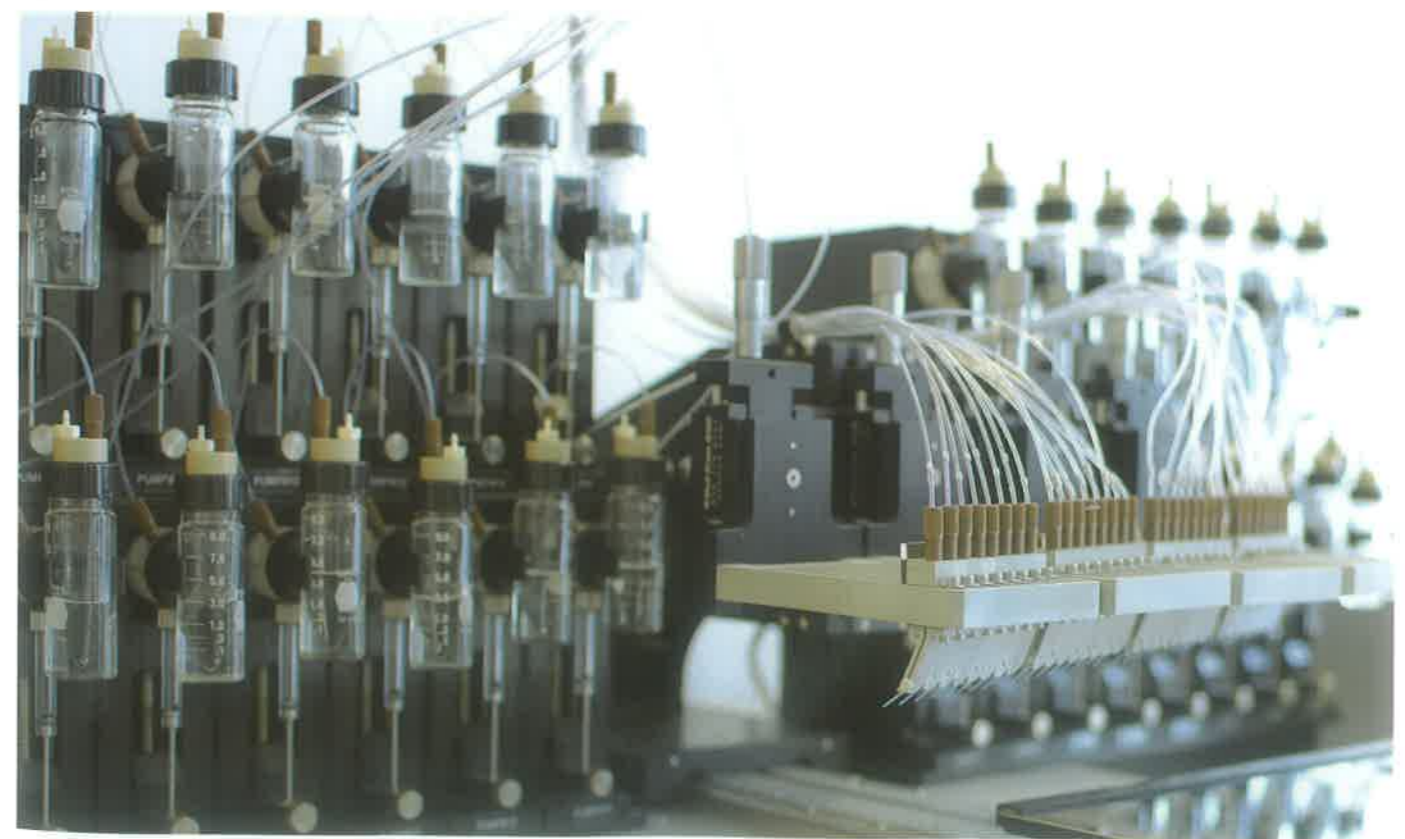
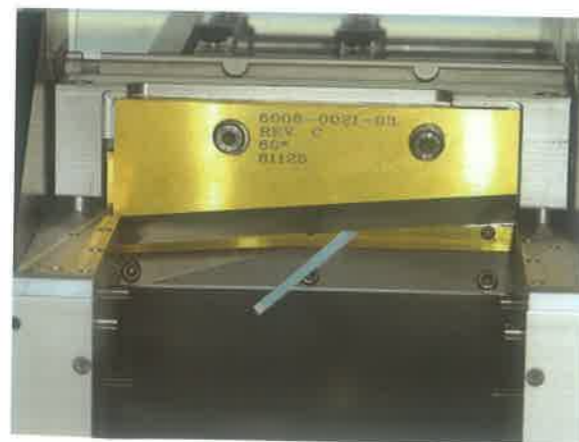
The production of each allergen extract adheres to the following procedures:

- Homogenization of raw material in special buffers
- Purification by dialysation
- Centrifugation and filtration
- Lyophilisation of the extract for long term storage

The potency of each allergen extract is determined by a special in-house reference serum and additional positive/negative patient sera.

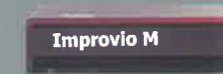
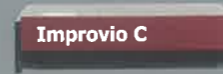
High qualitative allergens are dispensed as lines on a nitrocellulose membrane by a Biodot printing device with high resolution syringe pumps.

After drying and blocking, a Biodot cutting device precisely cuts the nitrocellulose membranes into test strips. The final test strips are glued into reaction troughs and sent to our customers worldwide.



A new generation of Allergy Screening

The Improvio Family



- Scans 1–36 test strips at a time
- Print out and program in all languages available
- Data export via Windows-Excel™ or CSV
- Data import via CSV
- Data transfer to LIMS possible
- Integrated self-control of the system by Fuji™ gray scale



	Improvio C	Improvio M	Improvio L
Strips scanned at a time	1–2 strips	1–10 strips	1–36 strips
Dimensions (h x l x w)	7.5 x 29.5 x 23 cm	7.7 x 29.5 x 43 cm	10.5 x 60 x 35
Weight	2.9 kg	5.1 kg	25 kg
Plastic contents	2 housing parts	2 housing parts	3 housing parts
Drawer	mechanical drawer	mechanical drawer	mechanical drawer
AllergyScreen®/AlleisaScreen®	yes	yes	yes
Minimized shadowing	yes	yes	yes
Free position of test strips	yes	yes	yes
Easy to clean	yes	yes	yes
Contamination prevention	yes	yes	yes
Integrated image acquisition and analyzation	no	yes	no
Footprint	small	medium	large
Simplified setup	yes	completely installed	yes
Calibration card	yes	yes	yes
Computer connection	USB 2.0	2 x USB 2.0, 1 x HDMI, 1 x DVI RJ45 LAN	USB 2.0
Keyboard	no	yes	no
Mouse	no	yes	no
Power supply	1 x external power supply, CE certified	2 x external power supply, CE certified	1 x external power supply, CE certified
Input	220 V-240 V 50 Hz, 0.4 A	220 V-240 V 50 Hz, 0.4 A	220 V-240 V 50 Hz, 0.4 A
Output	10V- 1A	10V- 1A	10V- 1A

A new generation of Allergy Screening

The CubeScreen Reader



Scans one test strip at a time

Print out and program in all languages available

Data export via Windows-Excel™ or TXT

Data transfer to LIMS possible

Micro-stepping software-controlled drawer

Sony sensor: 600 dpi

Quiet operation

CubeScreen Reader

Strips scanned at a time	1 strips
Dimensions (h x l x w)	17 x 15 x 15 cm
Weight	2.5 kg
Plastic contents	2 parts of acetal body
Drawer	automatic drawer
AllergyScreen®/AlleisaScreen®	yes
Minimized shadowing	yes
Free position of teststrips	no
Easy to clean	yes
Contamination prevention	yes
Integrated image acquisition and analyzation	no
Footprint	small
Simplified setup	yes
Calibration card	no
Computer connection	USB 2.0
Keyboard	no
Mouse	no
Power supply	1 x external power supply, CE certified
Input	100-240 V, 50-60 Hz, 0,7 A
Output	16-21 V DC, 1,56-1,19A



Bring the future to your lab

Beeblot 12 and Beeblot 36

The AllergyScreen® and AlleisaScreen® systems can be used in combination with an optional automated processor in two different model sizes: Beeblot 12 and Beeblot 36.

Bee Robotics Ltd., located in the UK, designed and manufactured the 'BeeBlot' system especially to support blot strip-based assays like AllergyScreen®. The technology of scientific blot analysis integrated into the routine of automated in-vitro laboratories results in enhanced efficacy and eases the operating process.

The Bee36 automated processor makes it possible to automatically process up to 36 diagnostic membranes within 2.5 hours, whereas the smaller Bee12 model can process up to 12 membranes. The only manual step is the pipetting of the serum (depending on the test system used, this requires only 250 or 300 µl of the patient's sera). The

rest is completed by the processor. The results can be directly evaluated with the Improvio L Scanner by transferring the tray with 36 test strips from the Bee36 to the scanner. We recommend our Improvio M Scanner for evaluating the results of the Bee12 process as it lets you measure 10 membranes simultaneously.

Product highlights

- Up to 12 or 36 membranes can be worked off per process
- Required assay protocol pre-installed
- Application support
- User-friendly Windows-based software
- Easy maintenance
- Test duration of only 2.5 h
- Only 250 µl/ 300 µl serum needed
- Usable for AllergyScreen® and AlleisaScreen® test strips



Beeblot 12 – Specifications

- Capacity: minimum 2, maximum 12 samples per run
- Dimensions: 400mm(w) x 480mm(d) x 500mm(h)
- Weight: 23 kg
- Dispensing accuracy: less than or equal to 10%
- Display unit: LCD with 2 rows of 16 digits
- Reagent save feature
- Easy walk away system
- Acoustical signals
- Color coding
- Quiet-running

Beeblot 36 – Specifications

- Capacity: minimum 2, maximum 36 samples per run
- Dimensions: 640 mm(w) x 620mm (d) x 450mm(h)
- Weight: 45 kg
- Dispensing accuracy: less than or equal to 10%
- Display unit: LCD with 2 rows of 16 digits
- Reagent save feature
- Easy walk away system
- Acoustical signals
- Color coding
- Quiet-running

Excellent results at a greatly reduced cost

Comparative Studies

AllergyScreen® in comparison with a single allergen determination system (internal studies)

Latex

Sensitivity:	92.9 %
Specificity:	100.0 %
Accuracy:	92.9 %

Bee

Sensitivity:	93.8 %
Specificity:	100.0 %
Accuracy:	97.2 %

Wasp

Sensitivity:	95.7 %
Specificity:	78.6 %
Accuracy:	89.2 %

Food

Sensitivity:	76.8 %
Specificity:	77.5 %
Accuracy:	77.1 %

IgG4

Sensitivity:	79.6 %
Specificity:	75.0 %
Accuracy:	79.1 %

Comparison of a single allergen determination system (SADS), skin-prick test and AllergyScreen® (Inhalative allergens 142 sera tested) (Kersten, 2002)

Sensitivity

Prick-test/AllergyScreen®:	95.1 %
Prick-test/SADS:	95.8 %
SADS /AllergyScreen®:	84.3 %

Specificity

Prick-test/AllergyScreen®:	80.2 %
Prick-test/SADS:	76.1 %
SADS/AllergyScreen®:	95.0 %

Accuracy

Prick-test/AllergyScreen®:	88.3%
Prick-test/SADS:	87.5%
SADS/AllergyScreen®:	90.6%

Inter-assay and Intra-assay variations - IgE and IgG4 (n=10)

Inter-assay variation IgG4	4.0 %
Intra-assay variation IgG4	1.7 %
Inter-assay variation IgE	3.9 %
Intra-assay variation IgE	4.5 %
Inter-batch variation IgE	8,8 %

A comparison with an established single allergen determination system (SADS) and skin test has shown that the Allergy-Screen® system offers a highly effective method for determining a patient's comprehensive specific sensitisation pattern. The sensitivity and specificity of the system is very similar to skin testing, and corresponds to a conventional single allergen system.

What's more, the Allergy-/Alleisa-Screen® systems achieve precise results at a low cost and with a minimum material expenditure.

A complete study overview is available on our website: www.mediwiss-analytic.de. Simply register and you will be provided with a login-password. You are also welcome to contact us directly for further information.

Kersten, W. (2002). Allergologie, Band 25(4): 203-208.

MEDIWISS Analytic GmbH

MEDIWISS Analytic GmbH has been developing, manufacturing and selling their own medical products since the foundation in 1999 in Moers, Germany. Our experience in allergologic in vitro diagnostics, developments and technologies have transformed us into a worldwide company that plays an important role in allergy screening diagnostics.

Our guiding objective is to provide a complete system for the allergy screening of specific IgE antibodies and further developed areas such as IgG4 and IgG antibody detection with test panels, hardware, software and customer support. Team members are certified biologists and are regularly trained in the latest medical requirements in the field of allergy and immunology.

Our aim is to simplify professional allergy diagnostics with concomitant sensitivity and specificity. To this end, our products are developed and manufactured to meet the highest standards. Customer suggestions and requirements are taken into consideration since their input ensures the optimal support of customers and distributors around the world.

Our pursuit of quality is demonstrated by our quality control system ISO 9001 from 2008 and ISO 13485 from 2003 as well as a certification for in vitro test systems in allergology from March 2011.



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