RIDASCREEN® Spec. IgG Foodscreen

Art. Nr.: A8020 RIDASCREEN® Spec. IgG Foodscreen Reagents

Gehört zu:

Art. Nr.: A0301	RIDASCREEN® Foodscreen Allergens Customized Plate
Art. Nr.: A8021	RIDASCREEN® Spec. IgG Foodscreen Starter Kit
Art. Nr.: A8025	RIDASCREEN® Foodscreen Blood Collection Kit
Art. Nr.: A8025-BCC	RIDASCREEN® Foodscreen Blood Collection Card
Art. Nr.: A8025-EB	RIDASCREEN® Foodscreen Elution buffer
Art. Nr.: A8101	RIDASCREEN® Spec. IgG Foodscreen Customized Plate
Art. Nr.: A8111	RIDASCREEN® Spec. IgG Foodscreen Standards and Controls
Art. Nr.: A8121-15	RIDASCREEN® Spec. IgG Foodscreen Plate 1
Art. Nr.: A8121-05	RIDASCREEN® Spec. IgG Foodscreen Plate 1
Art. Nr.: A8221-15	RIDASCREEN® Spec. IgG Foodscreen Plate 2
Art. Nr.: A8221-05	RIDASCREEN® Spec. IgG Foodscreen Plate 2
Art. Nr.: A8321-15	RIDASCREEN® Spec. IgG Foodscreen Plate 3
Art. Nr.: A8321-05	RIDASCREEN® Spec. IgG Foodscreen Plate 3
Art. Nr.: A8621-15	RIDASCREEN® Spec. IgG Foodscreen Plate 6
Art. Nr.: A8821	RIDASCREEN® Spec. IgG Foodscreen Microtiter Plate, Panel 1
Art. Nr.: A8822	RIDASCREEN® Spec. IgG Foodscreen Microtiter Plate, Panel 2
Art. Nr.: A8823	RIDASCREEN® Spec. IgG Foodscreen Plate ES
Art. Nr.: A8921	RIDASCREEN® Spec. IgG Foodscreen Prescreen 1
Art. Nr.: A8931	RIDASCREEN® Spec. IgG Foodscreen Prescreen Customized Plate

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1. Intended use

For *in vitro* diagnostic use. The RIDASCREEN® Spec. IgG Foodscreen test is an enzyme immunoassay (EIA) for the quantitative determination of specific IgG anti-bodies against food allergens in human serum and capillary blood samples. The test should be used in cases of suspected food intolerance.

2. Summary and description of the test

Food intolerance and its triggers can be determined by the detection of specific IgG antibodies. Food intolerances might result from an increased permeability of the intestinal wall to the corresponding food. Immune complexes of IgG antibodies and food can occur due to the penetration of food or food components through the wall of the intestine into the surrounding tissue. These immune complexes might trigger inflammatory reactions which can lead to various clinical pictures.

The test is an enzyme immunoassay (EIA). All reagents listed under Section 4 were validated using the micro titer plates from R-Biopharm AG listed above. The use of RIDASCREEN® Spec. IgG Foodscreen reagents in combination with microtiter plates from other manufacturers respectively the use of RIDASCREEN® Spec. IgG Foodscreen micro titer plates with reagents from other manufacturers is not permitted.

3. Test principle

The food extracts are adsorbed to the inner surfaces of the cavities of a micro titer plate. Patient samples (sera or eluate of capillary blood), standard and control sera are pipetted into the corresponding cavities and incubated at room temperature. During incubation, specific IgG antibodies bind to the corresponding food antigens (adsorbed to the plate). Unbound material will be removed by washing. Subsequently, an anti-human IgG antibody conjugated with alkaline phosphatase is added. During a second incubation, this antibody conjugate binds to the human IgG antibodies of the patient sample. Any unbound conjugate will be removed by washing. Finally, the substrate is added, and will be dephosphorylated by the conjugated enzyme and thus turn into a yellow product. The intensity of the yellow colour is proportional to the quantity of antigen-specific antibodies in the serum and can be measured photometrical at 405 nm after deduction of the reference wavelength of 620/630 nm.

4. Contents

Table 1: Content of RIDASCREEN® Spec. IgG Foodscreen Reagents (A8020)

Sample Buffer IgG	120 ml	Sample dilution buffer, ready to use
	6 x	Wash buffer salt for 1 liter of wash buffer, 10 mM PBS, 0.05% TWEEN20
AllergySub	80 ml	Substrate solution, ready to use; contains pNPP
Standard 1	2 ml	Standard serum 1, diluted human serum; conc.: 2,5 µg IgG/ml; in stabilized protein solution
Standard 2	2 ml	Standard serum 2, diluted human serum; conc.: 10,0 µg IgG/ml; in stabilized protein solution
Standard 3	2 ml	Standard serum 3, diluted human serum; conc.: 40,0 µg IgG/ml; in stabilized protein solution
Standard 4	2 ml	Standard serum 4, diluted human serum; conc.: 200,0 µg IgG/ml; in stabilized protein solution
Conjugate	80 ml	Anti-human IgG-conjugate (sheep), ready to use; alkaline phosphatase conjugated antibodies in stabilized protein solution
Control high	2 ml	Control serum, diluted human serum; valid range stated on the enclosed certificate; in stabilized protein solution
AllergyStop R	80 ml	Stop reagent, ready to use

Table 2: Content of RIDASCREEN® Foodscreen Allergens Customied Plate (A0301)

Plate	15 x 1	Micro titer plate, customized; coated with various food antigens, without standard curve

Table 3: Content of RIDASCREEN® Spec. IgG Foodscreen Starter Kit (A8021)

Plate 1	1 x 5	Micro titer plate 1, coated with various food antigens, standard curve included
Plate 2	1 x 5	Micro titer plate 2, coated with various food antigens, standard curve included
Plate 3	1 x 5	Micro titer plate 3, coated with various food antigens, standard curve included

Table 4: Content of RIDASCREEN® Foodscreen Blood Collection Kit (A8025)

Card	1	Blood Collection Card, for capillary blood
	1 x 2	2 Sterile lancets
	2	Sterile alcohol swabs
	2	Sterile bandages

Table 5: Content of RIDASCREEN® Foodscreen Blood Collection Card (A8025-BCC)

Card	100 x 1	Blood Collection Card, for capillary blood

Table 6: Content of RIDASCREEN® Foodscreen Elution buffer (A8025-EB)

EB	100 ml	Elution buffer, to eluate capillary blood from blood card

Table 7: Content of RIDASCREEN® Spec. IgG Foodscreen Customized Plate (A8101)

Plate	15 x 1	Micro titer plate, customized; coated with various food antigens, standard curve included

Table 8: Content of RIDASCREEN® Spec. IgG Foodscreen Standards and Controls (A8111)

Plate	15 x 1	Micro titer plate, coated with standards and controls

Table 9: Content of RIDASCREEN® Spec. IgG Foodscreen Plate 1 (A8121-15)

Plate 1	3 x 5	Micro titer plate 1, coated with various food antigens, standard curve included

Table 10: Content of RIDASCREEN® Spec. IgG Foodscreen Plate 1 (A8121-05)

Plate 1	1 x 5	Micro titer plate 1, coated with various food antigens, standard curve included

Table 11: Content of RIDASCREEN® Spec. IgG Foodscreen Plate 2 (A8221-15)

Plate 2	3 x 5	Micro titer plate 2, coated with various food antigens, standard curve included

Table 12: Content of RIDASCREEN® Spec. IgG Foodscreen Plate 2 (A8221-05)

Plate 2	1 x 5	Micro titer plate 2, coated with various food antigens, standard curve included

Table 13: Content of RIDASCREEN® Spec. IgG Foodscreen Plate 3 (A8321-15)

Plate 3	3 x 5	Micro titer plate 3, coated with various food antigens, standard curve included

Table 14 Content of RIDASCREEN® Spec. IgG Foodscreen Plate 3 (A8321-05)

Plate 3	1 x 5	Micro titer plate 3, coated with various food antigens, standard curve included

Table 15: Content of RIDASCREEN® Spec. IgG Foodscreen Plate 6 (A8621-15)

Plate 6	3 x 5	Micro titer plate 6, coated with various food antigens, standard curve included

Table 16: Content of RIDASCREEN® Spec. IgG Foodscreen Microtiter Plate, Panel 1 (A8821)

Plate	15 x 1	Micro titer plate, Panel 1; coated with various food antigens, standard curve included

Table 17: Content of RIDASCREEN® Spec. IgG Foodscreen Microtiter Plate, Panel 2 (A8822)

Plate	15 x 1	Micro titer plate, Panel 2; coated with various food antigens, standard curve included

Table 18: Content of RIDASCREEN® Spec. IgG Foodscreen Plate ES (A8823)

Plate	15 x 1	Micro titer plate ES; coated with various food antigens, standard curve included

Table 19: Content of RIDASCREEN® Spec. IgG Foodscreen Prescreen 1 (A8921)

Plate	15 x 1	Micro titer plate Prescreen 1, coated with various food antigens; Cut-Off included
Cut-Off	10 ml	Cut-off control, dil. human serum; concentration: 10 µg IgG/ml; in stabilized protein solution

Table 20: Content of RIDASCREEN® Spec. IgG Foodscreen Prescreen Customized Plate (A8931)

Plate	15 x 1	Micro titer plate Prescreen customized, coated with various food antigens, Cut-Off included
Cut-Off	10 ml	Cut-off control, dil. human serum; concentration: 10 µg IgG/ml; in stabilized protein solution

5. Storage instructions for reagents

The test kit must be stored at 2 – 8 °C and can be used until the expiry date printed on the label. The diluted washing buffer is stable for up to a maximum of 4 weeks when stored at 2 – 8 °C. Microbial contamination must be prevented. After the expiry date has been reached, the quality guarantee is no longer valid.

It is imperative that the conjugate is prevented from contaminating the ready to use substrate solution because this will discolour the substrate. The substrate solution must also be protected from direct light in order to prevent decomposition or discoloration due to hydrolysis. If the substrate shows a strong yellow colour, it must not be used any longer. See also Section 10.3 Quality control – indications of reagent expiry.

5.1. Storage of the plates

The plates are sensitive to humidity, close and seal the aluminium bags tightly after having removed the required amount of plates. The desiccant bag should remain within the aluminium bag during the entire storage period. The microtiter plates can be stored at 2 - 8 °C in the correctly closed aluminium bag for the indicated shelf life.

6. Additional necessary reagents – and necessary equipment

6.1. Reagents

- Distilled or deionised water

6.2. Equipment

- Photometer for micro titer plates (with 405 and 620/630 nm filters), measurement range 0 to 3.5 OD.
- Micro titer plates washer for 8 – 96 wells
- 12 channel pipette
- Multi step pipette
- Horizontal shaker (for the elution of capillary blood samples)

7. Precautions for users

- For *in vitro* diagnostic use only.
- This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed and the instructions for carrying out the test must be strictly adhered to.

- Samples or reagents must not be pipetted by mouth, and contact with injured skin or mucous membranes must be prevented. When handling the samples, wear disposable gloves and when the test is finished, wash your hands.
- Do not smoke, eat or drink in areas where samples or test reagents are being used.
- The standard sera in the kit have been tested for HIV- and HCV-Ab as well as HbsAg and syphilis CFR 21.640 with negative results. Nevertheless, they must be treated as potentially infectious in the same way as the patient samples and all other materials with which they come into contact and they must be handled in accordance with the relevant national safety regulations.
- Standard sera, conjugate and the washing buffer contains sodium azide as a preservative. This substance must not be allowed to come into contact with the skin or mucous membrane. Explosive metal azides may be produced on contact with lead or copper pipes.
- Avoid contact with the eyes, skin or clothing with stop reagent or substrate solution. Upon skin contact, immediately wash with plenty of soap and water. Upon eye contact, immediately flush under flowing water for 15 minutes with the lids open, immediately consult a doctor. If swallowed, drink plenty of water, avoid vomiting and immediately consult a doctor.
- You are solely responsible for the proper disposal of all components in the kit after use (see safety data sheets of the kit components).
- All reagents or materials that come in touch with potentially infectious samples must be treated with appropriate desinfectants or be autoclaved at 121 °C for at least one hour.

8. Collection and storage of samples (sera and capillary blood)

The RIDASCREEN® Spec. IgG Foodscreen has been developed for testing human serum and capillary blood samples.

After venous blood collection, the serum should be separated from blood clots (after complete clotting) as soon as possible in order to prevent haemolysis. The samples must be stored cold (2 – 8 °C) or frozen (–20 °C) until they are tested. Repetitive freezing and thawing of the serum as well as microbial contamination should be avoided. The usage of heat-inactivated, lipaemic, haemolytic, icteric or turbid sera might lead to false results.

If using capillary blood samples with the RIDASCREEN® Spec. IgG Foodscreen you must use the appropriate accessories (A8025 RIDASCREEN® Foodscreen Blood Collection Kit, A8025-BCC RIDASCREEN® Foodscreen Blood Collection Card and A8025-EB RIDASCREEN® Foodscreen Elution buffer). Only these accessories are validated to this test. The

dried capillary blood samples are stable up to 3 weeks at room temperature (20 – 25 °C) and dry storage. They should not be stored in the fridge.

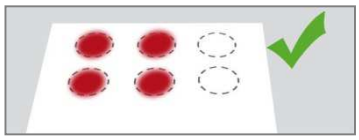
Table 21: Stability of the various sample types

undiluted serum		**diluted serum**	**dried capillary blood sample**	**Eluate of the capillary blood sample**
2 – 8 °C	-20 °C	20 – 25 °C	20 – 25 °C	20 – 25 °C
1 month	>3 months	max. 6 hours	3 weeks	max. 6 hours

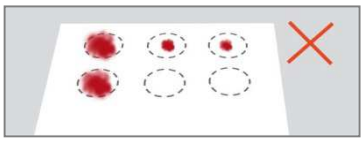
Important information for the processing of capillary blood samples:

The circles must be completely filled and soaked with blood. Also check the backside of the card to make sure that the circles are soaked throughout.

Correctly filled circles



Incorrectly filled circles



9. Test procedure

9.1. General information

All reagents, patients' sera and the micro titer plate must be brought to room temperature before use (important). The reagents must be thoroughly mixed immediately before use. After use, the reagent kit must be stored at 2 – 8 °C.

The coated micro titer plates cannot be used more than once. The reagents and micro titer plates must not be used if the packaging is damaged or the vials are leaking.

All micro titer plates can be used with reagents of different lots!

Using incubation times and temperatures other than those specified will cause the standard curve to be displaced from the standard curve on the certificate. Significant differences in the values on the standard curve could lead to invalid test results.

Any modification of incubation times or temperatures to run the test e. g. fully automatically should be done (validated) by the manufacturer.

The test must not be carried out in direct sunlight. We recommend covering the micro titer plates.

9.2. Preparing the washing buffer

Dissolve one package of washing buffer (salt) in 1 liter of distilled water. Please prepare only the amount of wash buffer needed for the current testing. The diluted washing buffer has a limited shelf life of max. 4 weeks (2 – 8 °C).

9.3. Preparing the micro titer plates

The micro titer plates are supplied pre-coated and can be used immediately. Before pipetting, the plates must be labelled with the name or number of the patient.

9.4. Sample dilution

9.4.1 Sample dilution; serum samples

Dilute each serum-sample 1:100 in sample buffer Sample Buffer IgG

Table 22: Recommended dilutions

Prescreen tests		**up to 50 determinations**		**up to 100 determinations**		**up to 300 determinations**	
Sera	Buffer	Sera	Buffer	Sera	Buffer	Sera	Buffer
20 µl	2 ml	40 µl	4.0 ml	70 µl	7.0 ml	200 µl	20.0 ml

9.4.2. Elution; capillary blood samples

The dried capillary blood sample should be eluted with Elution buffer EB and shaken softly on a horizontal shaker for 1 (-14) hour(s) at 20 – 25 °C.

Corresponding to the amount of determinations, the circles Card filled with blood should be punched out at the perforation line.

You should wear disposable gloves when punching out the dried circles filled with blood with your finger. Alternatively, the circles can be punched out with a suitable sterile object, for example the end of a pipette tip or a laboratory spatula.

Table 23: Recommended elutions

Prescreen tests		**up to 50 determinations**		**up to 100 determinations**		**up to 200 determinations**	
sample	EB	sample	EB	sample	EB	sample	EB
2 circle	4.0 ml	2 circle	4.0 ml	4 circles	8.0 ml	6 circles	12.0 ml

The volume of the eluate correlates with the diluted sample. The eluate is pipetted into the wells according to the pipetting scheme.

9.5. First incubation (sera incubation)

Pipette 50 µl each of the standard sera, Standard 1, Standard 2, Standard 3 and Standard 4, the control Control high OR Cut-Off*and the diluted patient serum or the eluate of the capillary blood sample into the wells of the micro titer plate Plate according to the pipetting scheme. Incubate at 20 – 25 °C for 90 minutes. The plates could be stacked on top of each other; please cover the top plate.

* The Cut-Off is needed only when performing RIDASCREEN® Spec. IgG Foodscreen Prescreen 1 (A8921) and RIDASCREEN® Spec. IgG Foodscreen Prescreen Customized (A8931)

9.6. Washing

Wash each well three times with 350 µl diluted washing buffer. If possible, please use an automated ELISA micro titer plate washer for this step. The last aspiration should take place as quantitatively as possible. To remove the excess washing solution the plates can be knocked out onto an absorbent tissue after washing.

9.7. Second incubation (conjugate incubation)

Pipette 50 µl conjugate Conjugate into **each** of the wells. Incubate the plates Plate at 20 – 25 °C for 90 minutes. The plates Plate could be stacked on top of each other; please cover the top plate Plate.

9.8. Washing

Washing – see Section 9.6.

9.9. Third incubation (Substrate incubation)

Swiftly pipette 50 µl substrate solution (ready to use) AllergySub into **each** of the wells. Subsequently, incubate the plates Plate at 20 – 25 °C for 60 minutes. The plates Plate must not be exposed to direct sunlight during incubation. The plates Plate could be stacked on top of each other; please cover the top plate Plate.

9.10. Stopping the reaction and carrying out the measurement

Stop the reaction by adding 50 µl stop reagent AllergyStop R to each well. Afterwards, knock the plate Plate slightly in order to achieve a thorough mixing within the wells. Measure the extinction at 405 nm against a reference wavelength of 620/630 nm. The plate Plate can be measured again within 24 hours provided that it has been covered and stored cold.

10. Test evaluation and quality control

10.1. Evaluation of RIDASCREEN® Spec. IgG Foodscreen: A8021, A8101, A8111, A8121-15, A8121-05, A8221-15, A8221-05, A8321-15, A8321-05, A8621-15, A8821, A8822, A8823

Standard 1, Standard 2, Standard 3, Standard 4 as well as the control Control high must be always tested on each plate at the same time.

The test has been carried out correctly when the following criteria have been fulfilled

(All figures refer to a difference measurement of 405 nm by 620 nm (or 630 nm) [$OD_{405/620}$]):

- Standard 1 > 1.0.
- Standard 2 minus Standard 1 ≥ 0.08
- Standard 3 minus Standard 2 ≥ 0.30
- Standard 4 minus Standard 3 ≥ 0.70

10.2. Evaluation of RIDASCREEN® Spec. IgG Foodscreen: A8921, A8931

Each time the test is carried out, the Cut-off control Cut-Off has to be tested on each plate. The test has been carried out correctly when the following criteria have been fulfilled (all figures refer to a difference measurement of 405 nm zu 620 nm (or 630 nm) [$OD_{405/620}$]):

- Cut-Off ≥ 0.20

10.3 Indications of reagent expiry

If the values differ from the stipulated values or if the reagent is turbid or the substrate has turned deeply yellow before being added to the wells, it may indicate that the reagents have expired.

If the stipulated values are not met, the following points must be checked before repeating the test:

Expiry date of the reagents used.

Functionality of the equipment being used (e.g. calibration).

Correct test procedure.

A substrate solution which has turned a deep yellow must not be used any longer.

If the stipulated values are still not fulfilled after repeating the test, please contact your local R-Biopharm distributor.

11. Evaluation and interpretation

11.1 Findings for the sera

11.1.1 Basis for the calculations

A valid standard curve must be generated in order to evaluate the test, a calculation on the basis of the standard curve is a prerequisite. This is why the standards have to on each plate. In order to set up the standard curve, the extinction values for the standards are plotted semi-logarithmically (y-lin/x-log presentation) as a function of the associated concentrations (µg/ml) in a point-to-point presentation. If a standard is present twice on the micro titer plate (A8621, plate 6), the average extinctions from the double determinations are used to prepare the standard curve. The standard curve can be used to determine the concentrations (µg/ml) of the specific IgG antibodies. These can then be converted into IgG classes (see Tab. 24 and 25). The evaluation can also be carried out using an appropriate software.

The standard curve for RIDASCREEN® Spec. IgG Foodscreen is calibrated against an international reference preparation “1st WHO IRP 67/86 for human IgG”.

11.1.2. Concentrations, IgG classes and calculations for RIDASCREEN® Spec. IgG Foodscreen

Table 24: Relationship between the determined µg/ml, IgG classes and antigen-specific IgG content of the patient out of serum samples

µg / ml	IgG class	Allergen-specific IgG content	Alternative IgG class	Allergen-specific IgG content
< 7.5	0 (0.0 – 0.9)	negative	0 (0,0 – 0,9)	negative
7.5 – 12.49	1 (1.0 – 1.9)	weak (standard titer)	1 (1,0 – 1,9)	elevated
12.5 – 19.99	2 (2.0 – 2.9)	increased		
20.0 – 49.99	3 (3.0 – 3.9)	high	2 (2,0 – 2,9)	highly elevated
≥ 50	4 (4.0 – 4.9)	very high		

These values should only be understood as rough guidelines. International standards for spec. IgG against foods do not exist.

Tab. 25: Relationship between the determined µg/ml, IgG classes and antigen-specific IgG content of the patient out of capillary blood samples

µg / ml	IgG class	Allergen-specific IgG content	Alternative IgG class	Allergen-specific IgG content
< 8.0	0 (0.0 – 0.9)	negative	0 (0,0 – 0,9)	negative
8.0 – 17.99	1 (1.0 – 1.9)	weak (standard titer)	1 (1,0 – 1,9)	elevated
18.0 – 39.99	2 (2.0 – 2.9)	increased	2 (2,0 – 2,9)	highly elevated
≥ 40	3 (3.0 – 3.9)	high		

These values should only be understood as rough guidelines. International standards for spec. IgG against foods do not exist.

Table 26: Calculation of the sample value and its interpretation for RIDASCREEN® Spec. IgG Foodscreen Prescreen 1 and Customized (A8921, A8931) from serum or capillary blood samples

Q	Evaluation	Interpretation
≤ 1.0	–	Negative
> 1.0	+	Weak
≥ 2.5	++	Increased
≥ 5.0	+++	High

Q = $OD_{405/620}$ (sample) / $D_{405/620}$ (Cut-off), The test is valid, if $OD_{405/620}$ Cut-off ≥ 0.20

12. Limitations of the method

The IgG concentrations determined by using this test system allow a statement regarding the degree of sensitisation of the patient to the food antigens or mixtures tested.

They cannot be used to derive a relationship between the determined IgG concentration and occurrence or severity of serious clinical symptoms. The results obtained must always be interpreted in combination with the complete clinical picture.

False positive test results may be produced by cross reactivity of the antigens being tested with the epitopes of other antigens.

It cannot be ruled out that the epitopes which trigger the antigens are missing when manufacturing the food extracts and coating the micro titerl plates. This can also lead to false negative results. IgG antibodies against food antigens which first appear during industrial

preparation, food preparation or during digestion may not be detectable because they are not present in the original food for which the patient was tested.

13. Performance characteristics

13.1 Precision

The precision of the RIDASCREEN® Spec. IgG Foodscreen assay was tested with reference samples which cover the full measuring range. For the Inter-lot precision three reagent lots and two micro titer plate lots were tested. In the case of the Inter-assay precision the samples were tested over five days in two runs each.

Tab. 27: Intra-assay precision

Samples	2
Replicates (per sample)	30 (15)
Average CV	**8.1 %**

Tab. 28: Inter-assay precision

Samples	2
Replicates (per sample)	60 (30)
Average CV	**11.1 %**

Tab. 29: Inter-lot precision

No. of lots	3 x reagents, 2 x micro titer plates
Samples / combinations	3 / 18
Replicates (per comb.)	54 (3)
Average CV	**19.1 %**

Based on Intra-Assay, Inter-Assay and Inter-Lot precision measurements, it can be concluded that the RIDASCREEN® Spec. IgG Foodscreen assay is precise and reproducible under repeatability and changed conditions as well as over several different reagent and micro titer plate lots in the whole intended assay range.

13.2 Stability

13.2.1 Stability after transport

The stability during transportation was investigated for the RIDASCREEN® Spec. IgG Foodscreen reagents and the micro titer plates. Two transport simulations as well as one real transport was investigated. The temperatures during shipping and during the simulations were monitored with a data logger. Temperatures between 4.5 °C and 45.0 °C were recorded.

None of the test results was influenced by the transport conditions.

13.2.2 Stability of the opened kit components

The kit was analyzed for its stability after opening of each individual kit component over a period of 6 weeks.

Both, the reagents as well as the micro titer plates did not show any perturbed results over the period of 6 weeks.

13.3 LOD

The limit of detection (LOD) is a measure for the sensitivity of the test at very low concentrations of the analyte.

An average LOD of ~ 4 µg/ml could be calculated from the analyses.

13.4 Interference

The following putative interfering substances were tested: Hemoglobin, Bilirubin, triglycerides, HSA.

None of the listed substances did show any significant perturbation of the test results.

Importantly, it is crucial to mention, that combinations of these substances were not tested. Thus, it is always important to evaluate the full clinical picture of the patient, especially if implausible results were obtained from hemolytic, hemorrhagic or lipemic samples.

13.5. Method comparison

Tab. 30: Comparison to the RIDASCREEN® Spec. IgG Allergen Disc (A0629)

Allergens tested	88
Samples	5
Overall agreement	**95,45%**

14 Literature

Bentz S et al.(2010) Clinical relevance of IgG antibodies against food antigens in Crohn's disease: a double-blind cross-over diet intervention study. Digestion 81(4):252-64.

Alpay K et. al (2010) Diet restriction in migraine, based on IgG against foods: a clinical double-blind, randomised, cross-over trial. Cephalalgia. 30(7):829-37.

Aydinlar EI et. al (2013) IgG-based elimination diet in migraine plus irritable bowel syndrome. Headache. 53(3):514-25.

Uzunısmaıl H et. al (2012) The effects of provocation by foods with raised IgG antibodies and additives on the course of Crohn's disease: a pilot study. Turk J Gastroenterol. 23:19-27.

G. E. Mullin et al. (2010) Testing for Food Reactions: The Good, the Bad, and the Ugly. Nutr Clin Pract 25(2):192-198.

A. Aljada et al. (2004) Increase in intranuclear nuclear factor B and decrease in inhibitor B in mononuclear cells after a mixed meal: evidence for a proinflammatory effect. Am J Clin Nutr 79:682–90.

A. R. Gaby. (1998) The Role of Hidden Food Allergy/Intolerance in Chronic Disease. Alternative Medicine Review, 3(2):90-100.