RIDA qLine® Allergy

Article no.: A6142 Panel 1 (20 different allergens)

Article no.: A6242 Panel 2 (20 inhalative allergens)

Article no.: A6342 Panel 3 (20 food allergens)

Article no.: A6442 Panel 4 (20 pediatric allergens)

This manual also applies to all other country-specific panels.
See relevant accompanying certificate supplied with each kit for allergenic composition.

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CE

1. Intended use

For *in vitro* diagnostic use**.** This test is an enzyme immunoassay on a nitrocellulose membrane (immunoblot) for the quantitative detection of allergen-specific IgE antibodies in human serum and plasma (citrate).

2. Summary and explanation of the test

The immune system's task is to protect the organism against pathogenic bacteria, viruses and other microorganisms. The defense response protects the organism on first contact with the pathogens, but also provides immunization for recurring exposure. All allergic reactions are preceded by a symptom-free first contact, where specific class E antibodies (IgE antibodies) were already formed. On repeated contact with the triggering allergen, these IgE antibodies react with the allergen and cause the release of mediators (usually from mast cells) like histamine, leukotriens, prostaglandins etc., which lead to the allergy symptoms. By detecting the specific IgE antibodies in the serum the triggering allergens can be identified in the event of allergic reactions. Existing sensitizations without symptoms can also be detected.

3. Test principle

This test is based on the principles of the immunoblot method. Various allergens are attached to the surface of nitrocellulose membranes in separate lines depending on the configuration of the panel. Allergen-specific IgE antibodies react with the appropriate allergens, if they are present in the patients' samples. In a second step, biotin-conjugated anti-human IgE antibodies bind to the attached antibodies. During a third incubation step, the biotin binds to a streptavidin peroxidase conjugate. In a final incubation step, the peroxidase turns the colorless substrate tetramethylbenzidine (TMB) into a bluish purple final product. After each individual incubation, a washing step removes unbound material. The intensity of the blue color is proportional to the amount of allergen-specific antibodies in the patient's serum. The sample is evaluated with RIDA qLine® Scan (IVD) or a common 3D color flatbed scanner (not IVD) validated by R-Biopharm in combination with the software RIDA qLine® Soft. The color intensities of the allergen bands are quantitatively evaluated on the basis of a standard curve on the membrane to determine the corresponding IU/ml or RAST classes.

Standard curve and positive control

RIDA qLine® Allergy is a quantitative test by means of a standard curve calibrated in accordance with the WHO standard. 5 standards are applied to each strip. The 5 standards correspond to the RAST classes 1 - 5, whereby RAST class 6 is extrapolated in the software.

The standard curve is only visible and the validity criteria are only fulfilled if the test has been properly executed and all reagents are functioning properly. The validity criteria of the standard curve / function control are fulfilled if all 5 standards are visible and recognized by the software, the smallest standard reaches at least intensity 10 and the intensity of standard 1 is < standard 2 < standard 3 < standard 4 < standard 5.

The positive control tests the system as a whole. The expected value can only be output if the strips are correctly processed, the measurement system detects the bands correctly and the software performed the calculation correctly. The positive control must reach RAST 4 as a minimum in order to be output as valid.

CCD band

The CCD band is made up of purified carbohydrate side chains which are bound to the nitrocellulose membrane. The CCD band detects specific IgE antibodies against cross-reactive carbohydrate determinants (CCD) in the patient sample.

The immune system forms specific IgE antibodies against genuine allergens, but also the carbohydrate side chains of allergens (anti-CCD-IgE) of vegetable origin, or from insects, molluscs or latex. These anti-CCD-IgEs also lead to cross-reactions with unrelated proteins, and are therefore known as cross-reactive carbohydrate determinants (CCD).

Around 25% of all allergy patients produce anti-CCD-IgEs. However, they are highly unlikely to trigger allergic symptoms, and are therefore not clinically relevant.

These cross-reactions can lead to positive results in in vitro test systems, which must be considered false positives. In order to distinguish correctly between genuine positive results and false positives, the test should be repeated in the event of a positive result for the CCD band (≥ RAST 1), and the serum should be blocked with a CCD inhibitor (ZA0601) before the test in order to prevent binding with CCDs in vitro tests.

4. Package Contents

Table 1: Package contents sufficient for 10 assays

Membrane	10 pieces	RIDA qLine® Allergy test membranes (nitrocellulose membranes), coated with allergen material on 20 test fields and 5 standards in reaction wells
Wash 25x	5 ml	Wash buffer, in 25-fold concentration, Tris / NaCl
Antibody	5 ml	Detection antibodies; anti-human IgE antibodies (goat) conjugated with biotin, ready to use
Conjugate	5 ml	Streptavidin conjugate, streptavidin conjugated with peroxidase, ready to use
Substrate	5 ml	Substrate; TMB (tetramethylbenzidine), ready to use

5. Storage instructions for reagents

The test membranes have to be stored in a cool, dry and dark place in the aluminium package. The test kit has to be stored at 2-8 °C. The diluted wash buffer has a maximum shelf life of 4 weeks when stored at 2-8 °C. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid.

Contamination of the substrate with the conjugate must be avoided in any case, since this will result in a discoloration of the substrate. At the same time, direct exposure of the substrate to light must be avoided to prevent denaturing or discoloration by autoxidation. When a discoloration has developed, the substrate can no longer be used.

6. Additional necessary reagents and necessary equipment

6.1. Reagents

- Distilled or deionized water

6.2. Accessories

- Vortex mixer
- Measuring cylinder (200 ml)
- Micropipette, 1000 µl
- Strip holder for 10 strips (test membranes)

- Cover box for dark incubation (system of strip holder and cover box (= RIDA qLine® Incubation Set; art. no. ZG2701) can be acquired from R-Biopharm AG)
- Orbital shaker (300 RPM, 3 mm orbital radius; art. no.: ZG2601)
- RIDA qLine® Scan (art. no.: ZG1109) plus personal computer with USB port
- 3D color flatbed scanner validated by R-Biopharm AG (art. no.: ZG1106) plus personal computer with USB port
- RIDA qLine® Soft Software (art. no.: Z9995)

7. Precautionary measures

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. Always strictly adhere to the user instructions for this test.

Samples or reagents must not be pipetted by mouth, avoid contact with injured skin or mucous membranes. Wear disposable gloves when handling samples and wash hands after the test. Do not smoke, eat or drink in areas where samples or test reagents are being used.

Antibodies and wash buffers contain sodium azide as a preservative. This substance must not be allowed to come into contact with skin or mucous membranes. Contact with lead or copper pipes may cause explosive metal azide to develop.

The substrate contains hydrogen peroxide as well as chloromethylisothiazolinone and methylisothiazolinone in subtoxic concentrations.

If the outer package is damaged the individual components must be checked for damage before use. Kit components must not be used, if their individual package is damaged or their containers are leaking.

All components of the kit must be appropriately disposed of by the user after use.

All reagents and materials coming into contact with potentially infectious samples must be treated with suitable disinfectants or autoclaved at 121°C for at least 1 hour.

8. Sample collection and storage

The test was developed to examine human serum and plasma (citrate). After the blood sample is collected, the serum should be separated from the clotted blood as quickly as possible to prevent hemolysis. The samples must be stored in a cool place or frozen until they are tested. Repeated freezing and defrosting as well as bacterial contamination of the serum must be avoided. The use of lipemic, hemolytic, jaundice or opaque serums inactivated by heat may lead to distorted results.

Table 2: Sample storage

2-8 °C	1 week
-20 °C	> 1 week

9. Test procedure

9.1. General information

An exchange or combination of kit components from kits with different batch numbers is allowed.

Reproducible results strongly depend on following the incubation times and temperatures as well as the correct washing of the test membranes.

All test components must be brought to room temperature (20 - 25 °C) before the test is started.

9.2. Preparing the wash buffer

Put 5 ml of wash buffer concentrate Wash 25x in a measuring cylinder and fill up to 125 ml with distilled water (= wash buffer). Dissolve possible crystals in the concentrate by heating (water bath at 37 °C). Keep wash buffer in a container which can be accessed using pipettes.

9.3. First incubation

The test Membrane are removed from the package according to the number of tests to be performed. The wells are put into the test membrane holder to fix the strips when shaking. Each test membrane is coated with 500 µl of wash buffer and shaken on the orbital shaker (300 RPM, 3 mm orbital radius) for 1 minute until the bubbles no longer rise up. The test Membrane are then emptied by tapping them on an absorbent material. The test Membrane are then filled with 400 µl of patient serum and incubated for 30 minutes at room temperature (20-25 °C) on the orbital shaker. It must be ensured that the fluid covers the complete membrane. Should this not be the case, this may be carefully corrected with the tip of a pipette without damaging the membrane.

9.4. Washing

The wells are emptied after serum incubation. The test Membrane are now washed in three separate steps with 400 µl of wash buffer each for 1 minute each on the orbital shaker under the same conditions as during incubation. Next, empty the test membranes and tap them on an absorbent material.

9.5. Second incubation

Pipette 400 µl of Antibody onto each test Membrane. It must be ensured again that the fluid covers the complete membrane. The test Membrane are then incubated for 45 minutes at room temperature (20-25 °C) on the orbital shaker.

9.6. Washing

Wash as described in Item 9.4.

9.7. Third incubation

Pipette 400 µl of Conjugate onto each test Membrane. It must be ensured again that the fluid covers the complete membrane. The test Membrane are then incubated for 20 minutes at room temperature (20-25 °C) on the orbital shaker.

9.8. Washing

The quality of the result is highly dependent on this wash step. Rinse each strip three times with 1000 µl of wash buffer each over a sink or suitable receptacle. Hold the strip at an angle and move the pipette from the upper to the lower part of the membrane when pipetting the fluid, without actually touching the membrane. Next, wash the test Membrane in two separate steps with 400 µl of wash buffer each for 1 minute on the orbital shaker under the same conditions as during incubation. Next, empty the test membranes and tap them on an absorbent material.

9.9. Fourth incubation

400 µl of Substrate are applied to each test Membrane, so that the membrane is completely covered. Then the test Membrane are incubated for 15 minutes in the dark at room temperature (20-25 °C) on the orbital shaker.

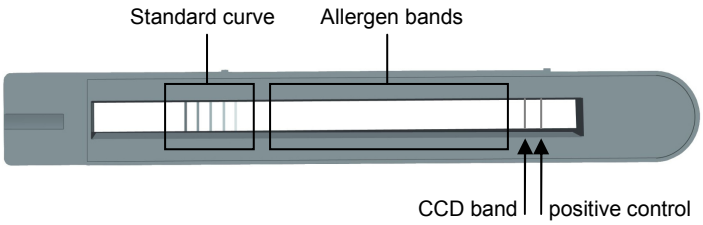
9.10. Washing

The wells are emptied after substrate incubation. Finally, the test Membrane are washed once with 400 µl of wash buffer and once with 400 µl of distilled water for 1 minute each on the orbital shaker (300 RPM, 3 mm orbital radius) under the same conditions as during incubation. Next, empty the test membranes and tap them on an absorbent material.

The test can be evaluated after having air dried for at least 30 minutes or when the membrane is completely dry. We recommend the use of a cold air blow-dryer to shorten this process.

10. Quality control – indications of reagent expiry

The test was performed correctly if the background has completely lost its color, the standard curve of five differentiated bands is visible and the positive control meets specifications.



An opaque reagent or bluish discoloration of the substrate before being added to the test Membrane can be a sign of reagent expiration.

Should the standard curve not be visibly differentiated, the following must be checked before the test is repeated:

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks; any substrate solution which has turned blue must not be used.

If the conditions are not fulfilled again after the test was repeated, please contact the manufacturer or your local R-Biopharm distributor.

11. Evaluation and interpretation

11.1. Membrane configurations of RIDA qLine® Allergy Panel 1, 2, 3 and 4

Panel 1 20 allergens	Panel 2 20 allergens	Panel 3 20 allergens	Panel 4 20 allergens
Standard 5	Standard 5	Standard 5	Standard 5
Standard 4	Standard 4	Standard 4	Standard 4
Standard 3	Standard 3	Standard 3	Standard 3
Standard 2	Standard 2	Standard 2	Standard 2
Standard 1	Standard 1	Standard 1	Standard 1
Derm. pteronyssinus	Derm. pteronyssinus	Hazelnut	Derm. pteronyssinus
Derm. farinae	Derm. farinae	Peanut	Derm. farinae
Alder	Alder	Walnut	Birch
Birch	Birch	Almond	Grass mix
Hazel	Hazel	Milk	Cat
Grass mix	Oak	Egg white	Dog
Rye (pollen)	Grass mix	Egg yolk	Alternaria alternata
Mugwort	Rye (pollen)	Casein	Milk
Plantain	Mugwort	Potato	αlactalbumin
Cat	Plantain	Celery	ß-lactoglobulin
Horse	Cat	Carrot	Casein
Dog	Horse	Tomato	Egg white
Alternaria alternata	Dog	Cod	Egg yolk
Egg white	Guinea pig	Crab	Bovine serum albumin
Milk	Hamster	Orange	Soya bean
Peanut	Rabbit	Apple	Carrot
Hazelnut	Penicillium notatum	Wheat flour	Potato
Carrot	Cladospor. herbarum	Rye flour	Wheat flour
Wheat flour	Aspergillus fumigatus	Sesame	Hazelnut
Soya bean	Alternaria alternata	Soya bean	Peanut
CCD	CCD	CCD	CCD
PosCo	PosCo	PosCo	PosCo

The membrane configurations for all other country-specific panels for which this package insert is applicable, are available from R-Biopharm AG as a supplement for each panel.

11.2. Evaluation with RIDA qLine® Scan or a 3D color flatbed scanner and RIDA qLine® Soft software.

For this purpose, the test membrane(s) is (are) put into the retainer and measured using one of the mentioned measuring devices and the appropriate software. The IU/ml value is automatically calculated from the measured values and allocated to RAST classes 0-6. The evaluation is based on the standard curve contained on each strip. The intensity of each individual allergen line is related to this standard curve.

Please consult the appropriate manual for information about using the different measuring devices for evaluation.

It must be ensured that the appropriate tests were allocated to the allergy panels to be measured.

Table 3: Connection between the determined class and allergen-specific IgE content of the patient serum

IU / ml	Class	Allergen-specific IgE content
0.00 - 0.34	0 (0.0 - 0.9)	not detectable or trace
0.35 - 0.69	1 (1.0 - 1.9)	low
0.70 - 3.49	2 (2.0 - 2.9)	elevated
3.50 - 17.49	3 (3.0 - 3.9)	significantly elevated
17.50 - 49.99	4 (4.0 - 4.9)	high
50.00 - 99.99	5 (5.0 - 5.9)	very high
≥ 100.00	6	extremely high

11.3. Documentation

The measured data (photo of the test membrane and evaluation) are saved to the hard disk of the PC in a preset directory. This database is used for the management of patient data. A data sheet can be printed out for each tested serum with any standard printer connected to the PC.

12. Limitations of the method

The IgE concentrations determined with this test system provide information about the level of sensitization of the patient regarding the checked individual allergens or allergen mixes.

A correlation between the level of a determined IgE concentration and the occurrence or the severity of clinical symptoms cannot be deduced on that basis. The results obtained must always be interpreted in combination with the entire clinical situation.

Due to the lack of national and international standards and due to the possible differences between prick test solutions and allergen extracts, which are used in in vitro tests, there may be a discrepancy between the results for *in vivo* and *in vitro* tests. Furthermore, IgE titers may result in false negatives or be measured too low directly after the occurrence of anaphylactic reactions. The test should be repeated after 3-4 weeks if there are discrepancies between the *in vivo* and *in vitro* results. If the discrepancies persist, further in vivo tests such as provocation tests should be performed by an allergist. Provocation tests may trigger an anaphylactic shock.

False positive test results may occur due to the cross-reactivity of the tested allergen with other allergens.

13. Performance characteristics

13.1. Intra-assay precision

Intra-assay precision was determined by testing two serums (serum 1 and 2) with 20 test strips each, and one serum (serum 3) with 10 test strips from a single batch.

Mean values (MV), standard deviations (SD) and coefficients of variance (CV) are determined for each allergen individually and summarized in Table 4. CV values of 15% are accepted for the intra-assay precision of all allergens with signals between RAST 1 and RAST 2. CV values of 10% are accepted for allergens with signals **≥** RAST 2. No CV is calculated for any allergen with negative signals (< RAST 1).

Table 4: Intra-assay precision of all 43 allergens in standard panels 1-4

	Serum 1			**Serum 2**			**Serum 3**		
Allergen	**MV (RAST)**	**SD**	**CV (%)**	**MV (RAST)**	**SD**	**CV (%)**	**MV (RAST)**	**SD**	**CV (%)**
Alder	4.2	0.1	**2.9**	5.4	0.1	**2.6**	5.9	0.1	**2.1**
Almond	2.8	0.1	**3.4**	4.2	0.2	**4.4**	1.7	0.1	**5.1**
Alternaria alternata	0.3	0.0	**-**	0.4	0.1	**-**	3.5	0.1	**2.7**
Apple	2.8	0.1	**5.0**	2.8	0.2	**5.3**	2.2	0.1	**5.2**
Aspergillus fumigatus	0.3	0.0	**-**	0.4	0.1	**-**	1.9	0.1	**7.8**
Birch	5.5	0.1	**2.0**	6.0	0.0	**0.0**	1.7	0.1	**4.9**
Bovine serum albumin	0.2	0.1	**-**	0.2	0.1	**-**	5.2	0.1	**2.2**
Carrot	3.2	0.1	**2.3**	1.9	0.1	**7.8**	0.6	0.0	**-**
Casein	0.1	0.1	**-**	0.4	0.1	**-**	5.2	0.1	**2.2**
Cat	2.5	0.1	**4.3**	4.4	0.1	**2.7**	6.0	0.1	**1.2**
Celery	3.4	0.1	**3.7**	2.4	0.2	**6.5**	3.4	0.1	**3.5**
Cladosporium herbarum	0.3	0.0	**-**	0.4	0.1	**-**	3.7	0.1	**2.6**
Cod	0.1	0.0	**-**	0.3	0.1	**-**	5.2	0.1	**2.1**
Crab	2.4	0.1	**4.1**	4.0	0.1	**3.3**	2.2	0.1	**4.8**
D. farinae	2.1	0.1	**2.6**	6.0	0.0	**0.0**	2.9	0.1	**3.8**
D. pteronyssinus	1.4	0.1	**7.1**	6.0	0.0	**0.0**	2.2	0.1	**2.8**
Dog	0.3	0.0	**-**	6.0	0.0	**0.8**	4.1	0.1	**3.1**
Egg white	0.4	0.1	**-**	1.0	0.1	**-**	3.5	0.1	**2.8**
Egg yolk	0.1	0.1	**-**	0.5	0.1	**-**	3.2	0.1	**2.9**
Grass mix	5.9	0.1	**2.0**	5.5	0.2	**3.4**	4.1	0.3	**8.3**
Guinea pig	0.3	0.0	**-**	1.3	0.1	**8.8**	0.7	0.1	**-**
Hamster	0.6	0.1	**-**	1.8	0.1	**6.9**	0.7	0.1	**-**
Hazel	4.5	0.2	**4.4**	5.7	0.1	**2.2**	5.7	0.2	**2.7**
Hazelnut	3.0	0.1	**2.6**	5.1	0.1	**2.7**	3.8	0.1	**1.9**
Horse	0.3	0.1	**-**	0.3	0.1	**-**	0.3	0.1	**-**
Milk	0.5	0.1	**-**	0.5	0.1	**-**	5.2	0.1	**2.7**
Mugwort	2.5	0.1	**3.9**	4.3	0.1	**3.3**	1.4	0.2	**13.8**
Oak	4.7	0.1	**2.8**	5.9	0.1	**1.4**	5.1	0.2	**3.7**

Orange	2.3	0.1	**5.1**	1.2	0.1	**11.2**	1.2	0.1	**10.7**
Peanut	1.3	0.1	**9.4**	1.5	0.2	**13.3**	3.4	0.1	**2.7**
Penicillium notatum	1.1	0.1	**6.7**	1.4	0.1	**7.9**	4.0	0.1	**2.4**
Ribwort plantain	2.8	0.1	**3.7**	4.5	0.1	**3.0**	2.0	0.1	**5.1**
Potato	3.4	0.2	**4.6**	2.2	0.2	**8.5**	1.7	0.2	**9.1**
Rabbit	1.1	0.1	**6.6**	1.9	0.1	**3.5**	1.5	0.2	**11.0**
Rye (pollen)	5.5	0.2	**3.9**	4.4	0.2	**4.5**	5.2	0.2	**3.9**
Rye flour	2.7	0.1	**4.1**	2.8	0.2	**6.8**	2.9	0.1	**4.7**
Sesame	3.0	0.1	**4.0**	2.9	0.2	**6.1**	2.7	0.1	**5.5**
Soya bean	0.2	0.1	**-**	0.4	0.1	**-**	4.7	0.1	**1.6**
Tomato	3.7	0.1	**2.5**	2.3	0.1	**6.0**	2.7	0.1	**4.3**
Walnut	0.9	0.1	**-**	3.7	0.2	**4.5**	0.2	0.0	**-**
Wheat flour	2.8	0.1	**3.9**	2.5	0.1	**4.5**	5.5	0.1	**2.5**
α lactalbumin	0.2	0.1	**-**	0.4	0.1	**-**	2.7	0.2	**7.0**
ß-lactoglobulin	0.3	0.1	**-**	0.8	0.1	**-**	4.5	0.2	**4.0**
CCD	2.2	0.1	**4.1**	0.3	0.1	**-**	3.1	0.1	**4.3**

13.2. Inter-assay precision

In order to determine inter-assay precision, 3 serums were assayed in duplicate on 10 consecutive days. The experiment was performed by two different individuals.

Mean values (MV), standard deviations (SD) and coefficients of variance (CV) are determined for each allergen individually and summarized in Table 5. CV values of 20% are accepted for the inter-assay precision of all allergens with signals between RAST 1 and RAST 2. CV values of 15% are accepted for allergens with signals **≥** RAST 2. No CV is calculated for any allergen with negative signals (< RAST 1).

Table 5: Inter-assay precision

	Serum 1			**Serum 2**			**Serum 3**		
Allergen	**MV (RAST)**	**SD**	**CV (%)**	**MV (RAST)**	**SD**	**CV (%)**	**MV (RAST)**	**SD**	**CV (%)**
Alder	5.4	0.2	**3.6**	2.2	0.2	**9.1**	5.9	0.1	**2.0**
Almond	1.6	0.2	**13.5**	1.1	0.2	**16.1**	1.4	0.2	**15.2**
Alternaria alternata	2.4	0.2	**8.4**	1.7	0.2	**13.5**	3.5	0.2	**6.3**
Apple	2.2	0.1	**6.4**	0.2	0.1	**-**	3.3	0.3	**7.7**
Aspergillus fumigatus	0.2	0.1	**-**	0.2	0.1	**-**	2.0	0.2	**10.4**
Birch	1.0	0.2	**-**	2.7	0.3	**9.8**	5.9	0.1	**1.4**
Bovine serum albumin	4.9	0.2	**3.7**	2.0	0.3	**14.4**	0.1	0.1	**-**
Carrot	2.4	0.2	**7.7**	0.4	0.1	**-**	0.9	0.1	**-**
Casein	5.1	0.2	**3.6**	0.1	0.1	**-**	0.3	0.1	**-**
Cat	4.4	0.2	**3.7**	2.9	0.2	**7.4**	5.9	0.2	**2.7**
Celery	3.5	0.2	**5.0**	0.8	0.1	**-**	2.2	0.2	**7.1**
Cladosporium herbarum	0.5	0.1	**-**	0.4	0.1	**-**	3.8	0.2	**4.7**
Cod	5.2	0.2	**3.1**	1.3	0.3	**26.0**	0.4	0.1	**-**
Crab	2.9	0.2	**6.6**	2.6	0.2	**6.4**	1.4	0.3	**18.3**
D. farinae	4.6	0.2	**4.9**	5.1	0.3	**5.5**	2.3	0.2	**7.1**
D. pteronyssinus	1.6	0.2	**14.5**	5.4	0.2	**3.2**	1.5	0.2	**14.1**
Dog	0.7	0.1	**-**	0.3	0.1	**-**	3.8	0.2	**4.8**
Egg white	3.5	0.1	**4.2**	0.2	0.0	**-**	0.5	0.1	**-**
Egg yolk	3.3	0.2	**4.8**	0.1	0.0	**-**	0.2	0.1	**-**
Grass mix	4.9	0.3	**5.3**	3.1	0.2	**5.3**	5.9	0.1	**1.6**
Guinea pig	2.3*	0.3	**10.8**	0.4	0.1	**-**	0.5	0.1	**-**
Hamster	1.8	0.2	**11.2**	0.4	0.1	**-**	0.6	0.1	**-**
Hazel	5.0	0.2	**4.5**	2.0	0.2	**11.8**	5.6	0.2	**3.8**
Hazelnut	3.8	0.2	**5.1**	3.2	0.2	**7.2**	3.7	0.2	**5.4**
Horse	1.4	0.2	**15.2**	0.2	0.1	**-**	0.3	0.1	**-**
Milk	5.3	0.2	**4.3**	0.1	0.1	**-**	0.3	0.1	**-**
Mugwort	2.7	0.4	**13.6**	0.3	0.1	**-**	1.1	0.3	**22.9**
Oak	3.9	0.2	**5.1**	0.9	0.1	**-**	4.4	0.3	**6.4**
Orange	1.2	0.1	**10.2**	0.2	0.1	**-**	0.8	0.1	**-**
Peanut	3.7	0.2	**4.9**	0.3	0.1	**-**	1.8	0.2	**11.8**
Penicillium notatum	1.0	0.2	**-**	1.5	0.2	**10.5**	4.0	0.2	**5.4**

Ribwort plantain	2.6	0.3	**10.2**	0.7	0.1	**-**	1.7	0.3	**15.1**
Potato	1.6	0.3	**18.9**	0.7	0.1	**-**	1.5	0.2	**12.9**
Rabbit	1.3	0.2	**12.2**	0.5	0.1	**-**	0.6	0.1	**-**
Rye (pollen)	5.8	0.2	**4.2**	2.0	0.3	**13.7**	4.8	0.4	**7.6**
Rye flour	2.9	0.2	**6.3**	1.0	0.2	**14.8**	3.3	0.1	**3.9**
Sesame	2.7	0.2	**8.5**	1.1	0.2	**16.9**	3.0	0.2	**5.9**
Soya bean	4.9	0.2	**3.8**	0.3	0.1	**-**	2.5	0.3	**12.5**
Tomato	2.7	0.2	**6.9**	0.8	0.1	**-**	1.3	0.3	**19.6**
Walnut	0.3	0.1	**-**	1.0	0.2	**19.1**	0.2	0.1	**-**
Wheat flour	5.6	0.2	**3.3**	0.7	0.1	**-**	2.1	0.1	**5.2**
α lactalbumin	2.6	0.4	**15.7**	0.0	0.0	**-**	0.2	0.1	**-**
ß-lactoglobulin	4.7	0.2	**4.7**	0.1	0.1	**-**	0.2	0.1	**-**
CCD	0.4	0.1	**-**	0.6	0.1	**-**	3.2	0.2	**5.5**

13.3. Comparison with a quantitative in vitro IgE reference system

In order to determine the concordance between RIDA qLine® Allergy and a quantitative IgE reference system (Phadia ImmunoCap, Thermo Scientific, USA), both test systems were used to test 50 serums for 42 allergens in RIDA qLine® Allergy Standard Panel 1-4. The resulting RAST classes of both test systems were compared. (See table 6). A difference between the test systems of ΔRAST ≤ 1 is regarded as concordant.

Table 6: Comparison to the IgE reference system

	Δ qLine / IgE reference system
Concordance (Δ ≤ 1 RAST)	1904
Discrepancy (Δ > 1 RAST)	196
Samples in total	2100

% Concordance	90.7%
% Discrepancy	9.3%

13.4. Interfering substances

The serum of patients suspected to have an allergic condition was tested for interfering substances in order to identify interfering substances in human serum samples. Potentially interfering substances were found at concentrations exceeding the physiological level in two serum samples. The RIDA qLine® Allergy test was performed for these samples.

The results indicate that Omalizumab treatment can lead to values being reduced or false negatives.

Because it is impossible to exclude the possibility of interference from triglycerides in serum, lipermic serums may not be used for testing (see also Section 8).

Table 7: Substances with the potential to interfere with RIDA qLine® Allergy

		Serum 1		Serum 2	
Substance	**Final serum concentration**	**Number of allergens**	**Δ RAST ≥ 1**	**Number of allergens**	**Δ RAST ≥ 1**
IgG (human non-specific)	20 mg/ml	43	1	43	1
Ceterizine	7.7 µmol/L	43	0	43	0
Loratadine	0.78 µmol/L	43	0	43	0
Desloratadine	0.97 µmol/L	43	0	43	0
Omalizumab	75 µg/ml	43	10	43	7
Hemoglobin	2 mg/ml	43	0	43	0
Triglycerides	32.75 mg/ml	43	4	43	0
Bilirubin	200 µg/ml	43	0	43	0
Cholesterol	5 mg/ml	43	1	43	0

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