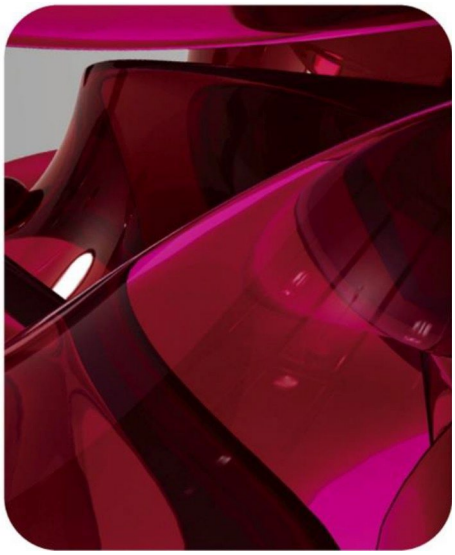
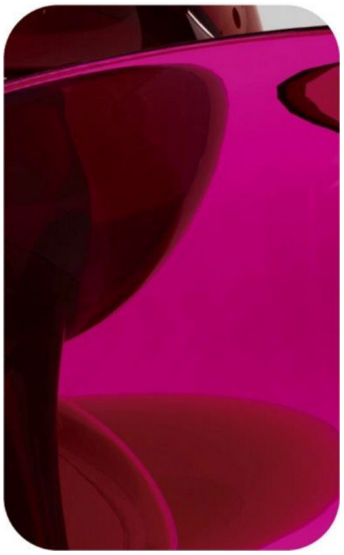
AESKU.DIAGNOSTICS

THE DIAGNOSTIC TOOL THAT WORKS







AESKUBLOTS®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKUBLOTS® Gluten Related Disorders IgA

Ref 401005

CE

Instruction Manual

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1 Intended Use

***AESKUBLOTS*® Gluten Related Disorders IgA** is a membrane-based enzyme immunoassay for quantitative detection of IgA subclass antibodies against gliadin, DGP, tTG, tTG neo, TG3, mTG neo, mTG and Frazer's Fraction in human serum or plasma. Antigens are applied as lines on a nitrocellulose membrane at defined positions.

The test is used as an aid in the diagnosis of gluten-related disorders, which include, for example, celiac disease or non-celiac gluten sensitivity. Samples from patients on a gluten-free diet cannot be detected with this test.

The test-kit is for professional use only in laboratories.

2 Clinical Application and Principle of the Test

Gluten Related Disorders (GRDs) are systemic disorders triggered by gluten, including celiac disease (CD), non-celiac gluten sensitivity (NCGS), dermatitis herpetiformis (DH), and wheat allergy.

Gluten Related Disorders can be classified into several groups:

- Autoimmune disorders: Celiac disease (CD), dermatitis herpetiformis (DH).
- Non-autoimmune, non-allergic: a disorder with unknown cause, probably immunomodulated: non-celiac gluten sensitivity (NCGS).
- Allergic: food allergy (IgE-mediated and non-IgE-mediated), wheat-dependent exercise-induced anaphylaxis (WDEIA), baker's asthma, contact dermatitis

Celiac disease

Celiac disease is a frequently inflammatory disease of the small intestine with the possibility of systemic manifestation, which is triggered by the consumption of foods containing gluten. Gluten is a so-called glue protein, a protein mixture that occurs in various types of cereals (e.g. wheat, barley, rye). The most important protein for the development of celiac disease is gliadin.

Basically, about one 0.5 – 1.0 % of the population is affected by celiac disease, with an upward trend, and it can manifest itself at any age. Thus, nowadays adults and children are affected in approximately equal proportions. In addition to the typical inflammation of the mucosa of the small intestine, those affected also show symptoms such as fatigue, abdominal pain and diarrhea, as well as weight loss, anemia, fertility problems, short stature and osteoporosis as a result of nutrient malabsorption.

Affected patients have HLA-DQ2 or -DQ8 as a genetic predisposition. Another trigger is gliadin ingested through food, which is only partially digested in the intestine. In celiac disease patients, the remaining gliadin peptides can pass through the small intestinal epithelium and reach the underlying connective tissue. Here, deamidation as well as transamidation (complex formation) of the protein fragments occurs by tissue transglutaminase. During deamidation, the amino acid glutamine is converted into glutamic acid. Given a genetic predisposition, these modified peptides are increasingly presented to the immune system by antigen-presenting cells. As a consequence, antibodies are produced against specific deamidated gliadin epitopes and, as a result of so-called epitope spreading, also against the body's own enzyme tTG. This in turn leads to inflammation and

damage of the mucosa of the small intestine, which can also be recognized histologically by an atrophic villous structure and hyperplastic intestinal crypts.

Small bowel biopsy is still considered the gold standard. Celiac disease can be classified into 3 types according to the severity of intestinal histopathology:

Marsh type I: proliferation of intraepithelial lymphocytes (IEL) with normal mucosal architecture.

Marsh type II: additional kypten hyperplasia with still normal villi.

Marsh type III: IEL proliferation, crypt hyperplasia, degeneration of epithelial cells and villous plumping.

When celiac disease is diagnosed in affected individuals, those on a lifelong gluten-free diet show improvement in symptoms. Within 3 - 12 months, celiac serology normalizes (antibodies are no longer detectable) and intestinal inflammation also decreases, albeit slowly.

Dermatitis herpetiformis

Dermatitis herpetiformis is a manifestation of celiac disease on the skin. Affected individuals always exhibit very severe and excruciating itching in the acute stage. The first skin manifestations are small, rarely larger reddish poplars that turn into vesicles.

Diagnosis is made by immunofluorescence optical detection of granular IgA deposits in unaffected skin. Furthermore, a small bowel biopsy should also be performed in this case, as patients show the same changes in the mucosa of the small bowel as those affected by celiac disease, despite having few symptoms. In this special form of celiac disease, autoantibodies against the body's own epidermal tissue transglutaminase (TG3) are formed, among other things; these autoantibodies have established themselves here as serological markers.

Non-celiac gluten sensitivity (NCGS)

A generally accepted definition of NCGS does not yet exist. Nonspecific gastrointestinal, but also extraintestinal symptoms that occur in connection with the intake of gluten-containing food are considered characteristic of NCGS. A prerequisite for the diagnosis of NCGS is a significant improvement of the symptoms under a gluten-free diet on the one hand and a clear exclusion of celiac disease on the other.

The pathophysiology has not yet been adequately elucidated, but studies suggest that not only gluten, but rather the amylase trypsin inhibitors (ATI), which are highly concentrated especially in modern wheat varieties, may play a key role in the pathogenesis.

The clinical presentation of NCGS is characterized by nonspecific gastrointestinal symptoms and thus resembles not only celiac disease, but also irritable bowel syndrome in particular. The following symptoms may be observed: abdominal pain, diarrhea, bloating, nausea, vomiting, headache, muscle discomfort or chronic fatigue.

If the medical history reveals a temporal relationship between gluten intake and symptoms, other gluten-associated diseases such as celiac disease and wheat allergy must be ruled out as the first step in establishing a diagnosis. If neither celiac disease nor wheat allergy can be held responsible for the symptoms, an elimination diet for gluten or wheat products can be carried out in the further course. If the symptoms then clearly and persistently regress, this indicates the presence of NCGS.

Antibodies against:

- **Gliadin:** Antibodies to gliadin are significantly elevated in non-celiac individuals with oral ulceration and are commonly found in CD, subclinical to a small extent in CD, but also in a subset of individuals who do not have the disease.

- **DGP:** (deamidated gliadin-specific peptides) Recent studies have shown that antibodies directed against gliadin from celiac disease patients bind a very limited number of specific epitopes on the gliadin molecule. In addition, selective deamidation of gliadin by tissue transglutaminase enhances binding of anti-gliadin antibodies. Therefore, test systems that use deamidated and defined peptides have higher diagnostic accuracy compared to previous anti-gliadin tests.

- **tTG:** tTG enables a simpler and more reliable diagnosis of celiac disease. tTG is an enzyme that is released during cell damage and is involved in tissue repair. Anti-tTG antibodies show higher sensitivity and specificity for celiac disease than anti-gliadin antibodies and correlate strongly with disease activity.

- **tTG neo:** cross-linking of tTG (transamidation) with gliadin-specific peptides induces the formation of neo-epitopes with tTG. Since these neo-epitopes are structurally more similar to physiological epitopes than previously used antigens, the antigen tTG neo-epitope can achieve a significant increase in the sensitivity and specificity of the test. Studies have also shown that tTG neo-epitope can be used as a marker for dermatitis herpetiformis.

- **TG3:** Transglutaminase 3, or epidermal transglutaminase, is expressed in the epidermis. Mutations in the TGM3 gene lead, among other things, to the syndrome of uncombed hair or dermatitis herpetiformis Duhring. The latter syndrome is a skin disease belonging to the group of blistering autoimmune dermatoses. Besides the conspicuous itchy vesicles and nodules, celiac disease is one of the causes of this disease. For the diagnosis of DH, TGM3 has been shown to be a highly sensitive marker and can further improve the diagnosis of dermatitis herpetiformis.

- **mTG neo:** microbial transglutaminase (mTG) is used as a common additive in the food industry. Like tTG, mTG is able to cross-link with gliadin-specific peptides and is thus able to form so-called mTG neo-epitopes. Anti-mTG neo-epitope antibodies can also be observed in celiac disease patients and correlate with the Marsh index. Furthermore, mTG neo-epitopes can be found in other gluten associated diseases, such as NCGS.

- **mTG:** microbial transglutaminase (mTG) is used as a common additive in the food industry. It is the main cause of the formation of the so-called mTG neo-epitopes, which are suspected to be an additional trigger of celiac disease. However, antibodies against the enzyme itself are very rare.

- **Frazer's Fraction:** This is a gliadin which was digested with pepsin and trypsin. As early as 1959, Frazer et al showed that oral intake of Frazer's Fraction was harmful to celiac patients. The assumption is that a naturally occurring mixture of gliadin peptides significantly increases the sensitivity and specificity of the anti-gliadin antibodies detected in the test. However, this has not yet been proven.

- **IgA-total:** IgA-total is required to detect IgA deficiency. More than 2 % of celiac disease patients have an immunoglobulin A (IgA) deficiency, which leads to a negative result in the IgA autoantibody test (the total IgA signal intensity is below 18 U/ml in ***AESKUBLOTS®*** **Gluten Related Disorders IgA**). If IgA deficiency is proven, additional testing for GRD-relevant IgG antibodies is recommended.

Test Principle

The antigens are applied as lines on a nitrocellulose membrane. The membrane is blocked to prevent unspecific reactions. Membrane-strips with specific antigens at exactly defined positions are incubated in serum/plasma samples diluted 1:101. Patient`s antibodies, if present in specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards, anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples. Unbound conjugate is washed off in the following step. After the addition of the TMB-substrate it is converted by an enzymatic reaction to a blue precipitate. The reaction is stopped by distilled water.

3 Kit Contents

TO BE RECONSTITUTED				
Item	**Quantity**	**Cap color**	**Solution color**	**Description / Contents**
Blocking Reagent	2 x for 10 ml concentrate each	white	N/A	Non-fat dry milk powder for preparation of 2 x 10 ml sample buffer
Wash Buffer (20x)	1 x 50 ml	white	colorless	20x concentrated for preparation of 1 L Tris buffer, pH 6.9 ± 0.2
READY TO USE				
Item	**Quantity**	**Cap color**	**Solution color**	**Description / Contents**
Conjugate IgA	1 x 15 ml	red	colorless	Anti-human Immunoglobulin A (IgA) conjugated to horseradish peroxidase
TMB Substrate	1 x 15 ml	black	colorless	Stabilized TMB/ H_2O_2
Membrane strips	24 strips	color coding: blue/yellow	N/A	Coated antigens see intended use
Tweezers	1 pcs. each	N/A	N/A	N/A
Incubation tray	3 pcs.	N/A	N/A	N/A
Labels for sample buffer	2 pcs.	N/A	N/A	N/A
Set card	1 pcs.	N/A	N/A	N/A
PRODUCT RELATED INFORMATION				
Quality Control Certificate			1 piece	
Instruction for Use			1 piece	
MATERIALS REQUIRED, BUT NOT PROVIDED				
HELIA® Automated Blot System from Aesku.Diagnostics GmbH & Co. KG, cylinder 1000 ml, pipette or cylinder for 10 ml, precision pipettes (15, 1000 µl), absorbent or filter paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia and the European Pharmacopeia.				

4 Storage and Shelf Life

Store all reagents and membrane-strips at 2-8 °C / 35.6-46.4 °F in their original containers. Once prepared, reconstituted wash buffer as well as opened strips, conjugate and TMB are stable at 2-8 °C / 35.6-46.4 °F for six weeks. Reconstituted blocking reagent is stable at 2-8 °C / 35.6-46.4 °F for 3 weeks. Reagents and strips shall be used within the expiry date indicated on each respective component. Don’t use components after the expiry dates. Avoid intense exposure of TMB solution to the light.

5 Precautions of Use and General Introductions

5.1 Health hazard data

This product is for IN VITRO DIAGNOSTIC use only. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit.

All kit components are classified according to Regulation (EC) No. 1272/2008 [CLP]. See the Materials and Safety Document (MSDS) for more information on ingredients.

Substances listed on the so-called "Candidate List of Substances of very High Concern (SVHCV) for authorization" of the European Chemicals Agency (ECHA) are not intentional components of this product. It is therefore not to be expected that these substances are contained in amounts ≥ 0.1 % in the product.

Reagents should be stored safely and be inaccessible to children.

In particular, the mixture does not contain any substances in concentrations ≥ 0.1 % to be classified as PBT or vPvB.

Patient samples should be considered potentially infectious and handled according to national laws. Patient samples and other potentially infectious material should be decontaminated after the test run.

5.2 General directions for use

To differentiate between the various quantitative ***AESKUBLOTS®*** tests available, a color coding is applied above the reference line of the strips:

Color coding	***AESKUBLOTS®***
blue/yellow	Gluten Related Disorders IgA
blue/black	Gluten Related Disorders IgG

In case that the product information, including the labeling, is incorrect please contact the manufacturer or the supplier of the test kit.

Blocking Reagent, TMB Substrate and wash buffer may be interchanged between lots and test kits. All other components are specific for each test kit and are not to be interchanged.

For handling of conjugate do not use polystyrene vessels.

Allow all components to reach room temperature (20-32 °C / 68-89.6 °F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test. The test should be performed in combination with **HELIA® Automated Blot System** from Aesku.Diagnostics GmbH & Co. KG.

Never expose components to higher temperature than 37 °C/ 98.6 °F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips priorly used with other reagents.

The intensity of the band color does not necessarily correlate with antibody titers obtained by other reference methodologies.

Samples from apparent normal blood donors may contain autoantibodies.

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.

Furthermore, it should be noted that under a gluten-free diet, the specific antibodies for celiac disease drop significantly until they can no longer be detected.

6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum/plasma samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolyzed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum/plasma samples should be used during the first 8 h. Alternatively, the samples should be stored in tightly closed vials at 2-8 °C / 35.6-46.4 °F for up to 48 h, or frozen at -20 °C / -4 °F for longer periods. Avoid freezing and thawing samples repeatedly. (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4) Do not use thermally inactivated samples (56 °C / 132.8 °F).

7 Assay Procedure

7.1 Preparations prior to starting

Confirm that no salt crystals have been formed in the concentrate. If this happened, dissolve the crystals by slightly warming the concentrate, room temperature should be enough.

Dilute concentrated wash buffer 1:20 with distilled water (e.g. 50 ml plus 950 ml).

For preparation of sample buffer: add 10 ml wash buffer to one bottle Blocking Reagent and mix well.

Place test strips in correct orientation (reference line and color coding upwards) into the incubation wells using tweezers (do not touch test strips with your hand). Store unused test strips tightly closed in the strip box with dry bag in a cool place (2-8 °C / 35.6-46.4 °F).

For automated processing with **HELIA® Automated Blot System:** Load the instrument according to the **HELIA® Automated Blot System** specifications. Please use the program: 401005 GRD IgA.

The batch-specific CoA must be used for each batch.

7.2 Manual Test Steps

Important notes:

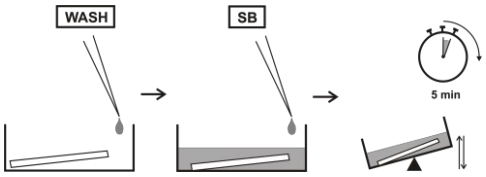
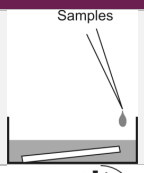
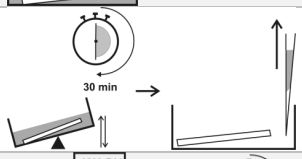
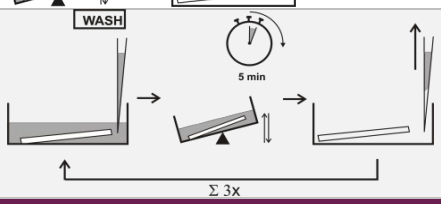
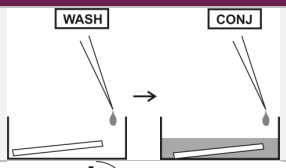
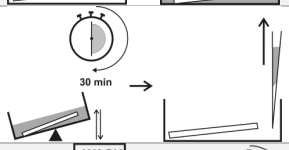
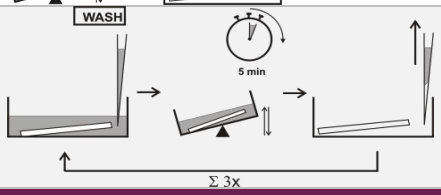
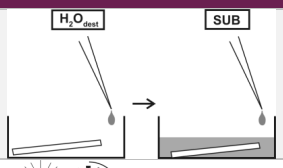
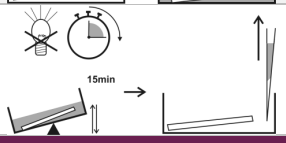
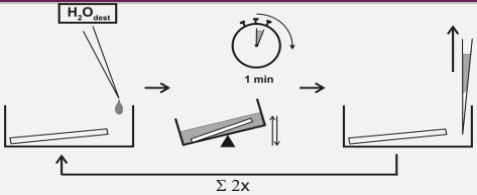
For manual processing please follow the subsequent test procedure. Make sure that the two components mentioned in the protocol are added to the tray in steps 2, 6, 9.

Do not let strips dry out during incubation steps. Do not touch strips with fingers, use tweezers.

Remove diluted samples completely after incubation of strips to avoid carry over.

Continuously shake strips during incubation steps.

Give sample buffer, conjugate and substrate together with the wash buffer to one side of the incubation tray. Do not allow to flow over the strips.

Step	Description
1.	Make sure that the preparations have been made before starting
2.	 Place the strips in the incubation tray in the correct orientation (reference line and color coding facing upwards). Only touch the strips with tweezers. Add 1050 µl wash buffer and 450 µl sample buffer to the incubation tray with strips. Wet the strips completely with the solution and incubate for 5 minutes while shaking.
	Sample
3.	 Place 15 µl of serum/plasma sample in the incubation tray with the strip and sample buffer provided.
4.	 Incubate for 30 minutes at 20-32°C / 68-89.6°F with shaking. Then remove the sample completely.
5.	 Wash 3 times for 5 minutes with 1.8 ml wash solution each time while shaking. Wash solution is removed after each wash step.
	Conjugate
6.	 Add 1050 µl wash solution and 450 µl conjugate to each incubation tray with strips.
7.	 Incubate for 30 minutes at 20-32°C / 68-89.6°F with shaking. Conjugate is removed.
8.	 Wash 3 times for 5 minutes with 1.8 ml wash solution each time while shaking. Wash solution is removed after each wash step.
	Substrate
9.	 Pipette 1050 µl dH_2O and 450 µl substrate into each well.
10.	 Incubate for 15 minutes at 20-32°C / 68-89.6°F with shaking and protect from intense light. Substrate is removed.
	Stop
11.	 Add 2 ml dH_2O and incubate for 1 minute with shaking. Remove dH_2O and repeat this step once.
12.	Put the Strips within the incubation tray into a Reader.
13.	In a time window of 20 minutes, the wet strips can be evaluated with a Reader.

8 Quantitative Evaluation

8.1 Analysis

The evaluation is performed via the software integrated in the **HELIA® Automated Blot System or a Reader**. At the same time, the device checks each strip with regard to the validity criteria.

8.1.1 Manual Analysis using BLOTrix Reader with the corresponding software

The analysis of the strips can be carried out manually using the BLOTrix system software version 4.2 or higher. Please refer to the instructions for use of the BLOTrix system for detailed guidance.

Important notes for manual interpretation:

- Visual interpretation of test results is not recommended.
- Test results can be considered valid, if the following criteria are met:
  - Functional controls are clearly visible.
  - Standards 1 to 3 are visible with increasing intensity.
  - Negative control shows a signal less intense than standard 1.

If these criteria are not fulfilled, the test is considered invalid and must be repeated.

Quantitative analysis is based on comparing the colour intensities of the individual antigens with those of the standards. BLOTrix system software calculates signal intensities and performs the evaluation accordingly.

8.1.2 Automated Analysis using HELIA® Automated Blot System

When using the **HELIA® Automated Blot System**, the evaluation is carried out fully automatically. The system's integrated software determines the result in IgA concentration arbitrary (U/ml).

Key features of **HELIA® Automated Blot System** analysis:

- The software automatically checks each strip for validity criteria.
- Signal intensities (bar values) of the test lines are compared to a standard curve created by plotting the grey values of the standards against their known concentrations in U/ml.
- Evaluation is based on a 4-parameter logistic curve fitting (4PL) model.
- The resulting concentration in U/ml for each antigen is calculated and displayed.

8.2 Results Interpretation

8.2.1 Results interpretation of IgA-total

Results	IgA concentration arbitrary (U/ml)
IgA deficiency	< 18 U/ml
Normal IgA level	≥ 18 U/ml

8.2.2 Results interpretation of GRD markers

Results	IgA concentration arbitrary Units (U/ml)
Negative	< 12 U/ml
Equivocal	≥ 12 < 18 U/ml
Weak positive	≥ 18 < 30 U/ml
Positive	≥ 30 < 200 U/ml
High positive	≥ 200 U/ml

8.3 Notes on Validity and Quality

If the values of the controls do not meet the predefined validation criteria, the test is considered invalid and must be repeated. In cases where equivocal or borderline results are obtained, it is recommended to repeat the test using a new sample to ensure reliability.

Check for common sources of error:

- Expiry dates and storage of reagents.
- Calibration and function of pipettes and equipment.
- Accuracy of incubation times and temperatures.

If unusual results or validation issues occur that are not caused by user error, please contact the manufacturer or supplier of the kit.

Medical laboratories must conduct internal quality control procedures using appropriate control sera and follow national laboratory standards.

9 Technical Data

Sample material:	serum / plasma
Sample volume:	15 µl of serum / plasma
Total incubation time:	117 minutes at 20-32 °C / 68-89.6 °F
Measuring range	0-300 U/ml
LoB	Please refer to chapter 10.1
LoD	Please refer to chapter 10.1
Storage:	at 2-8 °C / 35.6-46.4 °F; use original vials only
Number of determinations:	24 tests

10 Performance Data

10.1 Analytical Sensitivity

The Limit of Blank (LoB), which corresponds to the detection limit of the measurement system, was determined by repeating the blocking reagent 20 times for each antigen. The following value is determined for all antigens: 0.20 U/ml.

The Limit of Detection (LoD) corresponds to the detection limit of the measurement system. The LoD is set at 0.58 U/ml for all antigens.

10.2 Normal Range

Sera from healthy donors were tested with the ***AESKUBLOTS*® Gluten Related Disorders IgA** and the following distribution was obtained:

Antigen	Number of samples	Mean Value [U/ml]	Median [U/ml]	Lowest value [U/ml]	Highest value [U/ml]
Gliadin	120	2.31	1.30	0.05	14.56
DGP	120	0.67	0.20	0.03	9.16
tTG	120	1.86	0.86	0.04	15.06
tTG neo	120	0.22	0.11	0.02	1.84
TG3	120	1.08	0.51	0.06	12.18
mTG neo	120	0.71	0.33	0.03	6.06
mTG	120	0.38	0.14	0.03	8.59
Frazer's Fraction	120	0.46	0.25	0.05	4.99
IgA-total	120	29.69	29.73	11.40	47.57

We recommend that each laboratory determine its own normal range value.

10.3 Precision

The precision of the test results obtained with the ***AESKUBLOTS*® Gluten Related Disorders IgA** was investigated by determining the intra- and inter-assay precision as well as the lot variance by analyzing several samples with different concentrations.

Mean values for all samples were calculated and expressed in U/ml. For samples in the positive range, the CV is also listed as a percentage. For samples in the negative range, the indication neg. is used, which confirms the negative results. The indication neg. was introduced, since very small values (with negative samples), even small differences artificially increased, do not result in meaningful deviations.

Intra-Assay

		Gliadin	DGP	tTG	tTG neo	TG3	mTG neo	mTG	Frazer's Fraction	IgA-total
Sample 1	Mean [U/ml]	1.24	9.96	1.05	97.77	48.21	26.36	0.10	0.49	20.81
	CV [%]	Neg.	Neg.	Neg.	19.40 %	17.66 %	19.25 %	Neg.	Neg.	15.42 %
Sample 2	Mean [U/ml]	151.83	0.14	5.24	1.86	0.59	38.16	0.50	38.41	23.31
	CV [%]	14.66 %	Neg.	Neg.	Neg.	Neg.	17.70 %	Neg.	16.55 %	19.88 %
Sample 3	Mean [U/ml]	4.81	56.15	292.35	300.0	0.77	176.67	0.19	6.86	23.47
	CV [%]	Neg.	14.22 %	9.51 %	0.00 %	Neg.	12.25 %	Neg.	Neg.	14.79 %
Sample 4	Mean [U/ml]	0.48	0.44	0.43	0.36	0.95	0.21	0.13	0.08	19.67
	CV [%]	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	11.24 %
Sample 5	Mean [U/ml]	0.49	0.16	0.91	0.87	1.81	1.00	182.25	0.24	39.22
	CV [%]	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	9.99 %	Neg.	18.93 %

Inter-Assay

		Gliadin	DGP	tTG	tTG neo	TG3	mTG neo	mTG	Frazer's Fraction	IgA-total
Sample 1	Mean [U/ml]	1.41	13.75	1.04	197.85	28.60	28.83	0.28	1.11	22.57
	CV [%]	Neg.	20.70 %	Neg.	21.92 %	24.90 %	22.42 %	Neg.	Neg.	21.71 %
Sample 2	Mean [U/ml]	145.75	0.41	5.07	3.55	0.76	36.42	0.26	36.82	20.75
	CV [%]	16.66 %	Neg.	Neg.	Neg.	Neg.	19.06 %	Neg.	20.49 %	22.05 %
Sample 3	Mean [U/ml]	5.23	59.80	207.97	300.0	0.58	177.42	0.12	8.86	24.24
	CV [%]	Neg.	20.63 %	15.87 %	0,00 %	Neg.	9.40 %	Neg.	Neg.	21.23 %
Sample 4	Mean [U/ml]	1.30	1.05	1.75	4.64	2.54	0.38	0.77	1.31	29.93
	CV [%]	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	21.97 %
Sample 5	Mean [U/ml]	0.76	0.25	3.18	3.53	2.38	1.12	99.68	0.36	41.74
	CV [%]	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	23.12 %	Neg.	20.21 %

Lot variance

		Gliadin	DGP	tTG	tTG neo	TG3	mTG neo	mTG	Frazer's Fraction	IgA-total
Sample 1	Mean [U/ml]	1.30	15.31	3.36	240.09	15.56	32.73	0.30	1.96	28.03
	CV [%]	Neg.	25.59 %	Neg.	23.33 %	27.05 %	23.99 %	Neg.	Neg.	26.21 %
Sample 2	Mean [U/ml]	135.10	0.55	6.96	7.74	1.86	37.35	0.90	40.90	29.04
	CV [%]	14.27 %	Neg.	Neg.	Neg.	Neg.	16.77 %	Neg.	20.60 %	22.84 %
Sample 3	Mean [U/ml]	4.96	67.10	253.69	300.0	2.01	190.09	0.28	9.38	31.18
	CV [%]	Neg.	18.28 %	27.23 %	0.00 %	Neg.	12.61 %	Neg.	Neg.	24.95 %
Sample 4	Mean [U/ml]	2.86	1.65	5.17	7.91	5.15	1.09	1.85	2.06	49.66
	CV [%]	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	20.38 %
Sample 5	Mean [U/ml]	0.39	0.11	3.86	1.97	3.49	3.03	73.06	0.47	35.81
	CV [%]	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	14.91 %	Neg.	17.74 %

10.4 Competitor comparison

Since no clinical data regarding a gluten-free diet were available for the samples measured and this is decisive for the detection of specific antibodies, the samples were pre-characterized with a reference test. Samples pre-assessed as positive by the reference test were assumed to be positive and samples pre-assessed as negative were assumed to be negative. Borderline samples were considered negative. This approach assumes that the reference test correctly identifies the samples, which may not necessarily be the case. Further studies on this have to be done.

10.4.1 DGP and tTG

The DGP and tTG antigens were compared with a commercially available LINE blot (CE-notified reference test) for the determination of celiac disease-specific antibodies.

Disease	measured DGP	measured tTG
Celiac Disease	11	23
Morbus Crohn	31	31
Diabetes mellitus Type 1	23	23
U. colitis	22	22
Gastroenteritis	23	23
Lactose Intolerance	17	17
Healthy Controls	60	60

DGP		Competitor		
		Positive	Negative	Total
AESKUBLOTS® Gluten Related Disorders IgA	Positive	7	1	8
	Negative	7	172	179
	Total	14	173	187

DGP	[%]	95 % CI
Sensitivity	50.0	26.8 - 73.20
Specificity	99.4	96.80 - 99.97
Total-Agreement	95.7	91.8 - 97.8

In the investigated collective, which was pre-characterized using a reference test, a sensitivity of 50.0 % was determined with a specificity of 99.4 % in relation to the reference system. The values obtained are based on the assumption that the reference test correctly classifies all samples. The antigen DGP is most likely 2 different peptide variants.

tTG		Competitor		
		Positive	Negative	Total
AESKUBLOTS® Gluten Related Disorders IgA	Positive	23	2	25
	Negative	1	173	174
	Total	24	175	199

tTG	[%]	95 % CI
Sensitivity	95.8	79.8 - 99.8
Specificity	98.9	95.9 - 99.8
Total-Agreement	98.5	95.7 - 99.5

In the investigated collective, which was pre-characterized using a reference test, a sensitivity of 95.8 % was determined with a specificity of 98.9 % in relation to the reference system. The values obtained are based on the assumption that the reference test correctly classifies all samples.

10.4.2 Gliadin and tTG neo

The antigens gliadin and tTG neo were compared with commercially available ELISA (CE-notified products of Aesku.Diagnostics GmbH & Co. KG) for the determination of antibodies against gliadin and tTG neo.

Disease	measured Gliadin	measured tTG neo
Celiac Disease	10	40
Morbus Crohn	31	31
Diabetes mellitus Type 1	23	23
U. colitis	24	24
Gastroenteritis	23	23
Lactose Intolerance	17	17
Healthy Controls	100	100

Gliadin		Competitor		
		Positive	Negative	Total
AESKUBLOTS® Gluten Related Disorders IgA	Positive	11	6	17
	Negative	3	208	211
	Total	14	214	228

Gliadin	[%]	95 % CI
Sensitivity	78.6	52.4 - 92.4
Specificity	97.2	94.0 - 98.7
Total-Agreement	96.1	92.7 - 97.9

In the investigated collective, a sensitivity of 78.6 % was determined with a specificity of 97.2 % in relation to the reference system. The values determined are based on the assumption that the reference test correctly classifies all samples.

tTG neo		Competitor		
		Positive	Negative	Total
AESKUBLOTS® Gluten Related Disorders IgA	Positive	27	0	27
	Negative	15	216	231
	Total	42	216	258

tTG neo	[%]	95 % CI
Sensitivity	64.3	49.2 - 77.0
Specificity	100	93.3 - 100.0
Total-Agreement	94.2	90.6 - 96.4

In the investigated collective, a sensitivity of 64.3 % was determined with a specificity of 100.0% in relation to the reference system. The values obtained are based on the assumption that the reference test correctly classifies all samples.

10.4.3 TG3

The TG3 antigen was compared with a commercially available ELISA for the determination of IgA antibodies against TG3. For this purpose, 27 samples from patients with dermatitis herpetiformis were precharacterized with a CE-notified reference test and compared with the ***AESKUBLOTS***® **Gluten Related Disorders IgA** (borderline results were considered negative).

TG3		Competitor		
		Positive	Negative	Total
AESKUBLOTS® Gluten Related Disorders IgA	Positive	8	1	9
	Negative	3	15	18
	Total	11	16	27

TG3	[%]	95 % CI
Sensitivity	72.7	43.4 - 90.3
Specificity	93.8	71.7 - 99.7
Total-Agreement	85.2	67.5 - 94.1

In the investigated collective, a sensitivity of 72.7 % and a specificity of 93.8 % were determined in relation to the reference system. The values determined are based on the assumption that the reference test correctly classifies all samples.

10.4.4 mTG neo and mTG

For the antigens mTG neo and mTG there are currently no comparative products on the market. Nevertheless, it was possible to perform a comparative test using ELISA RUO kits (company Aesku.Diagnostics GmbH & Co. KG) for the determination of mTG neo and mTG antibodies.

Disease	measured mTG neo	measured mTG
Celiac Disease	33	102
Morbus Crohn	31	31
Diabetes mellitus Type 1	23	23
U. colitis	24	24
Gastroenteritis	23	23
Lactose Intolerance	17	17
Healthy Controls	100	100

mTG neo		Competitor		
		Positive	Negative	Total
AESKUBLOTS® Gluten Related Disorders IgA	Positive	41	1	42
	Negative	9	200	209
	Total	50	201	251

mTG neo	[%]	95 % CI
Sensitivity	82.0	69.2 - 90.2
Specificity	99.5	97.2 - 99.9
Total-Agreement	96.0	92.8 - 97.8

In the investigated collective, a sensitivity of 82.0 % was determined with a specificity of 99.5 % in relation to the reference system. The values determined are based on the assumption that the reference test correctly classifies all samples.

mTG		Competitor		
		Positive	Negative	Total
AESKUBLOTS® Gluten Related Disorders IgA	Positive	0	3	3
	Negative	1	316	317
	Total	1	319	320

mTG	[%]	95 % CI
Sensitivity	0.0	0.0 - 94.87
Specificity	99.1	97.27 - 99.74
Total-Agreement	98.8	96.8 - 99.5

Previous studies have shown that antibodies against the mTG itself are very rare. This could be confirmed for the ***AESKUBLOTS*®** **Gluten Related Disorders IgA**. A specificity of 99.1 % was determined in relation to the reference system. The values determined are based on the assumption that the reference test correctly classifies all samples.

10.5 Linearity

For selected sera a linear relationship between dilution and antibody concentration in this assay could be determined. All samples listed show an $R^2 > 0.95$. However, due to the heterogeneity of human antibodies, it cannot be excluded that individual sera show a non-linear behavior.

Antigen	Sample 1	Sample 2	Sample 3	Sample 4
	R^2	R^2	R^2	R^2
Gliadin	0.965	0.953	0.959	-
DGP	0.963	0.969	0.971	0.956
tTG	0.996	0.951	0.970	-
tTG neo	0.997	0.961	0.959	-
TG3	0.976	0.958	-	-
mTG neo	0.998	0.960	0.965	-
mTG	0.956	0.984	0.974	-
Frazer's Fraction	0.995	0.960	0.955	0.952

10.6 Calibration

The quantitative measurement system is calibrated in provisional units due to the lack of an international reference standard. The results are expressed in U/ml.

11 Disposal

Please observe the relevant statutory requirements!

12 Note to the User (European Union)

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or patient is established.

13 Literature

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CLSI Guideline GP44-A4: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

14 Regulatory Symbols

IVD	¨ Diagnosi in vitro	¨ For in vitro diagnostic use
	¨ Pour diagnostic in vitro	¨ Para uso diagnóstico in vitro
	¨ In Vitro Diagnostikum	¨ In Vitro Διαγνωστικό μέσο
	¨ Para uso Diagnóstico in vitro	
REF	¨ Numero d'ordine	¨ Cataloge number
	¨ Référence Catalogue	¨ Numéro de catálogo
	¨ Bestellnummer	¨ Αριθμός παραγγελίας
	¨ Número de catálogo	
LOT	¨ Descrizione lotto	¨ Lot
	¨ Lot	¨ Lote
	¨ Chargen Bezeichnung	¨ Χαρακτηρισμός παρτίδας
	¨ Lote	
UDI	¨ Identificatore univoco del dispositivo	¨ Unique Device Identifier
	¨ Identifiant unique de l'appareil	¨ Identificador único del dispositivo
	¨ eindeutige Produktidentifizierung	¨ Μοναδικό αναγνωριστικό συσκευής
	¨ Identificador único do dispositivo	
CE	¨ Conformità europea	¨ EC Declaration of Conformity
	¨ Déclaration CE de Conformité	¨ Declaración CE de Conformidad
	¨ Europäische Konformität	¨ Ευρωπαϊκή συμφωνία
	¨ Déclaracão CE de Conformidade	
Σ 24	¨ 24 determinazioni	¨ 24 tests
	¨ 24 tests	¨ 24 pruebas
	¨ 24 Bestimmungen	¨ 24 προσδιορισμοί
	¨ 24 Testes	
	¨ Rispettare le istruzioni per l'uso	¨ See instructions for use
	¨ Voir les instructions d'utilisation	¨ Ver las instrucciones de uso
	¨ Gebrauchsanweisung beachten	¨ Λάβετε υπόψη τις οδηγίες χρήσης
	¨ Ver as instrucões de uso	
	¨ Da utilizzarsi entro	¨ Use by
	¨ Utilise avant le	¨ Utilizar antes de
	¨ Verwendbar bis	¨ Χρήση μέχρι
	¨ Utilizar antes de	
+2°C +8°C	¨ Conservare a 2-8°C (35.6-46.4°F)	¨ Store at 2-8°C (35.6-46.4°F)
	¨ Conserver à 2-8°C (35.6-46.4°F)	¨ Conservar a 2-8°C (35.6-46.4°F)
	¨ Lagerung bei 2-8°C (35.6-46.4°F)	¨ Φυλάσσεται στους 2-8°C (35.6-46.4°F)
	¨ Conservar entre 2-8°C (35.6-46.4°F)	
	¨ Prodotto da	¨ Manufactured by
	¨ Fabriqué par	¨ Fabricado por
	¨ Hergestellt von	¨ Κατασκευάζεται από
	¨ Fabricado por	
STRIP	¨ Strip di nitrocelluslosa rivestita	¨ Coated nitrocellulose strip
	¨ Strip de nitrocellulose couché	¨ Tira de nitrocelulosa recubierta
	¨ Nitrozellulosemembran-Streifen mit aufgebrachten Antigenen	¨ Επίστρωση λωρίδα νιτροκυτταρίνης
	¨ Tira de nitrocelulose revestido	
WASH 20x	¨ Tampone di lavaggio	¨ Wash buffer
	¨ Tampon de Lavage	¨ Solución de lavado
	¨ Waschpuffer	¨ Ρυθμιστικό διάλυμα πλύσης
	¨ Solucão de lavagem	
BLOCK-REAG	¨ Reagente bloccante	¨ Blocking Reagent
	¨ réactif de blocage	¨ Reactivo bloqueante
	¨ Blockier-Reagenz	¨ Αντιδραστήριο αποκλεισμού
	¨ Bloqueio de reagente	
RCNS 10ml	¨ Ricostituire con 10 mL	¨ Reconstitute with 10 mL
	¨ reconstituer avec 10 mL	¨ reconstituir con 10 mL
	¨ rekonstituiren mit 10 mL	¨ Ανασύσταση με 10 mL
	¨ reconstituir com 10 mL	
SB	¨ Tampone campione	¨ Sample buffer
	¨ Tampon Echantillons	¨ Tampón Muestras
	¨ Probenpuffer	¨ Ρυθμιστικό διάλυμα δειγμάτων
	¨ Diluente de amostra	
CONJ	¨ Coniugato	¨ Conjugate
	¨ Conjugé	¨ Conjugado
	¨ Konjugat	¨ Σύζευγμα
	¨ Conjugado	
SUB	¨ Tampone substrato	¨ Substrate buffer
	¨ Substrat	¨ Tampón sustrato
	¨ Substratpuffer	¨ Ρυθμιστικό διάλυμα υποστρώματος
	¨ Substrato	