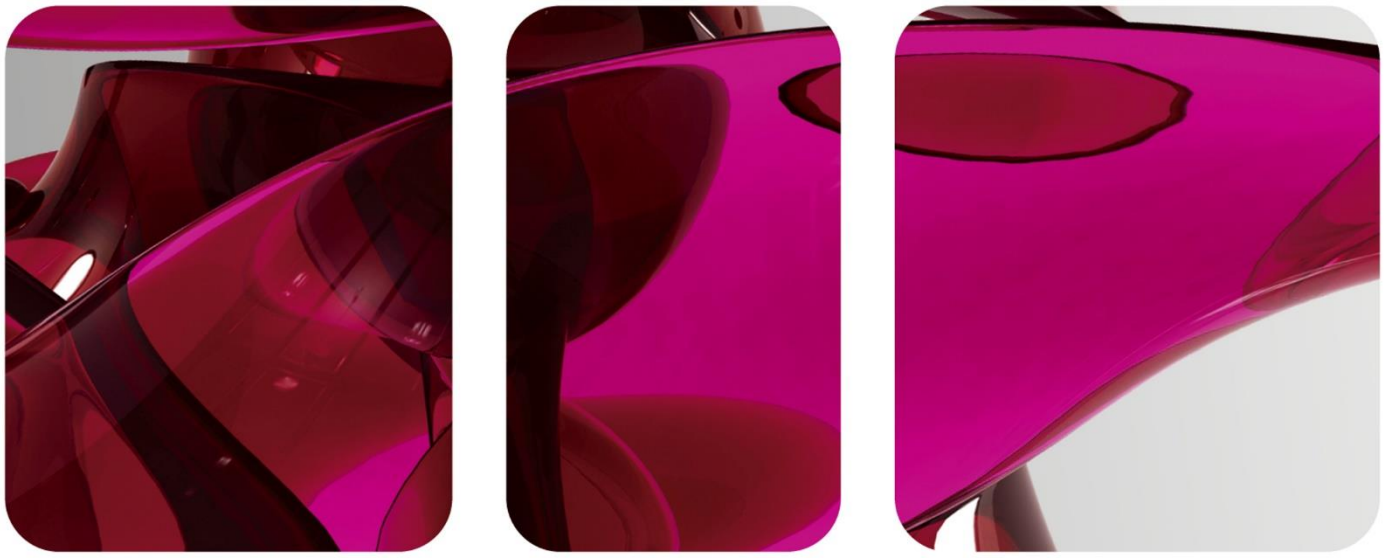
AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS



# AESKUBLOTS®

THE DIAGNOSTIC TOOL THAT WORKS

# INSTRUCTION MANUAL

***AESKUBLOTS® Gluten related disorders IgA***

***Ref 401005***

DIN EN ISO 13485

# Instruction Manual

## Table of Contents



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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-epitope, TG 3, mTG neo-epitope, mTG and PT-Gliadin 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-wheat sensitivity. Samples from patients on a gluten-free diet cannot be detected with this test.

# 2 Clinical Application and Principle of the Test

Gluten-related disorders (GRD) 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 individuals without celiac disease with oral ulceration. Anti-gliadin antibodies are frequently found in CD, to a lesser extent subclinically 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-epitopes:** 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-epitope:** 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.
- **PT-gliadin:** This is a gliadin which was digested with pepsin and trypsin. As early as 1959, Frazer et al showed that oral intake of PT-gliadin 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:** To detect an IgA deficiency. >2% of celiac disease patients have an IgA deficiency and thus receive a negative IgA autoantibody test result (signal intensity of total IgA < 18 U/ml with AESKUBLOT® GRD). The IgA test is evaluated quantitatively or lower than the cut-off calibrator when the test is evaluated qualitatively, even when the disease is active. If IgA deficiency is proven, additional testing for GRD-relevant IgG antibodies is recommended.

**Possible interpretation results total IgA (quantitative evaluation)**

| Results | sIgA concentration arbitary Units (U/ml) |
|---|---|
| Negative | <12,00 |
| Borderline | 12,00 – 17,99 |
| Low positive | 18,00 – 29,99 |
| Positive | 30,00 – 199,99 |
| High positive | ≥ 200,00 |

**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 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 |
| ***MATERIALS REQUIRED, BUT NOT PROVIDED*** | | | | |
| HELIA® from Aesku.Diagnostics, 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 (USP 26 - NF 21) and the European Pharmacopeia (Eur. Ph. 4th ed.). | | | | |

# 4 Storage and Shelf Life

The kit reagents and membrane strips should be stored at 2-8°C/35.6-46.4°F in the original bottles. ***An open vial stability of six weeks can be assumed if the opened and prepared kit components are stored as required at 2-8°C/35.6-46.4°F after the test run. The blocking reagent has a shelf life after preparation at 3 weeks if stored at 2-8°C/35.6-46.4°F as required.*** The expiry dates stated on the packaging and the labels of the individual components must be observed. Kit components for which the expiry date has passed must no longer be used! Avoid exposing the TMB substrate solution to strong 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 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. Do not exchange reagent components between autoimmunity and borrelia diagnostic tests!

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 can only performed in combination with HELIA® from Aesku.Diagnostics.

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 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 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 repeated thawing and freezing. Do not use heat-inactivated (56°C/132.8°F) serum/plasma samples.

# 7 Assay Procedure

## 7.1 Preparations prior to starting

**Dilution of concentrated reagents:**

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).

Load the instrument according to the HELIA® specifications.

**Important notes:**

The test can currently only be performed fully automated with the HELIA® from Aesku.Diagnostics. The corresponding test is performed with the program: 401005 GRD IgA.

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

# 8 Quantitative Evaluation

## 8.1 Evaluation

The evaluation is performed via the software integrated in the HELIA®. At the same time, the device checks each strip with regard to the validity criteria.

| Normal Range | Equivocal | Positive Results |
|---|---|---|
| < 12 U/ml | 12 – 18 U/ml | >18 U/ml |

Quantitative evaluation is performed using a standard curve in which the gray values of the standards are plotted against the concentration in U/ml. The evaluation unit of the HELIA® uses a 4-parameter logistic curve fitting (4PL). Using the curve, the concentration in U/ml is determined by the evaluation unit of the HELIA® from the bar value of the sample for each individual antigen.

If the values of the controls do not meet the validation criteria, the test is invalid and must be repeated. In case of borderline results, repetition with a new sample is also recommended.

The following possible sources of error should be checked: Expiration dates of reagents, storage conditions, pipettes, equipment used, and incubation conditions.

If the tested samples show unusual values or deviations, or if the validation criteria are not met for reasons that are not the responsibility of the person performing the test, please contact the manufacturer or supplier of the kit.

Medical laboratories should perform their own quality controls with their own controls and/or pool sera according to national regulations.

# 9 Technical Data

| | |
|---|---|
| Sample material: | Serum |
| Sample volume: | 15 µl of sample |
| 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 |
| TG 3 | 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 |
| PT-Gliadin | 120 | 0.46 | 0.25 | 0.05 | 4.99 |
| IgA-total | 120 | 29.69 | 29.73 | 11.4 | 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 VC 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 | TG 3 | mTG neo | mTG | PT-Gliadin | IgA-total |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample 1 | mean value [U/ml] | 1.24 | 9.96 | 1.05 | 97.77 | 48.21 | 26.36 | 0.10 | 0.49 | 20.81 |
| | VC [%] | neg | neg | neg | 19.40% | 17.66% | 19.25% | neg | neg | 15.42% |
| Sample 2 | mean value [U/ml] | 151.83 | 0.14 | 5.24 | 1.86 | 0.59 | 38.16 | 0.50 | 38.41 | 23.31 |
| | VC [%] | 14.66% | neg | neg | neg | neg | 17.70% | neg | 16.55% | 19.88% |
| Sample 3 | mean value [U/ml] | 4.81 | 56.15 | 292.35 | 300.00 | 0.77 | 176.67 | 0.19 | 6.86 | 23.47 |
| | VC [%] | neg | 14.22% | 9.51% | 0.00% | neg | 12.25% | neg | neg | 14.79% |
| Sample 4 | mean value [U/ml] | 0.48 | 0.44 | 0.43 | 0.36 | 0.95 | 0.21 | 0.13 | 0.08 | 19.67 |
| | VC [%] | neg | neg | neg | neg | neg | neg | neg | neg | 11.24% |
| Sample 5 | mean value [U/ml] | 0.49 | 0.16 | 0.91 | 0.87 | 1.81 | 1.00 | 182.25 | 0.24 | 39.22 |
| | VC [%] | neg | neg | neg | neg | neg | neg | 9.99% | neg | 18.93% |

Inter-Assay

| | | Gliadin | DGP | tTG | tTG neo | TG 3 | mTG neo | mTG | PT-Gliadin | IgA-total |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample 1 | mean value [U/ml] | 1.41 | 13.75 | 1.04 | 197.85 | 28.60 | 28.83 | 0.28 | 1.11 | 22.57 |
| | VC [%] | neg | 20.70% | neg | 21.92% | 24.90% | 22.42% | neg | neg | 21.71% |
| Sample 2 | mean value [U/ml] | 145.75 | 0.41 | 5.07 | 3.55 | 0.76 | 36.42 | 0.26 | 36.82 | 20.75 |
| | VC [%] | 16.66% | neg | neg | neg | neg | 19.06% | neg | 20.49% | 22.05% |
| Sample 3 | mean value [U/ml] | 5.23 | 59.80 | 207.97 | 300.00 | 0.58 | 177.12 | 0.12 | 8.86 | 24.24 |
| | VC [%] | neg | 20.63% | 15.87% | 0.00% | neg | 9.40% | neg | neg | 21.23% |
| Sample 4 | mean value [U/ml] | 1.30 | 1.05 | 1.75 | 4.64 | 2.54 | 0.38 | 0.77 | 1.31 | 29.93 |
| | VC [%] | neg | neg | neg | neg | neg | neg | neg | neg | 21.97% |
| Sample 5 | mean value [U/ml] | 0.76 | 0.25 | 3.18 | 3.53 | 2.38 | 1.12 | 99.68 | 0.36 | 41.74 |
| | VC [%] | neg | neg | neg | neg | neg | neg | 23.12% | neg | 20.21% |

Lot variance

| | | Gliadin | DGP | tTG | tTG neo | TG 3 | mTG neo | mTG | PT-Gliadin | IgA-total |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample 1 | mean value [U/ml] | 1.30 | 15.31 | 3.36 | 240.09 | 15.56 | 32.73 | 0.30 | 1.96 | 28.03 |
| | VC [%] | neg | 25.59% | neg | 23.33% | 27.05% | 23.99% | neg | neg | 26.21% |
| Sample 2 | mean value [U/ml] | 135.10 | 0.55 | 6.96 | 7.74 | 1.86 | 37.35 | 0.90 | 40.90 | 29.04 |
| | VC [%] | 14.27% | neg | neg | neg | neg | 16.77% | neg | 20.60% | 22.84% |
| Sample 3 | mean value [U/ml] | 4.96 | 67.10 | 253.69 | 300.00 | 2.01 | 190.09 | 0.28 | 9.38 | 31.18 |
| | VC [%] | neg | 18.28% | 27.23% | 0.00% | neg | 12.61% | neg | neg | 24.95% |
| Sample 4 | mean value [U/ml] | 2.86 | 1.65 | 5.17 | 7.91 | 5.15 | 1.09 | 1.85 | 2.06 | 49.66 |
| | VC [%] | neg | neg | neg | neg | neg | neg | neg | neg | 20.38% |
| Sample 5 | mean value [U/ml] | 0.39 | 0.11 | 3.86 | 1.97 | 3.49 | 3.03 | 73.06 | 0.47 | 35.81 |
| | VC [%] | 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 | | reference test | | |
|---|---|---|---|---|
| | | 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 to 73.20 |
| Specificity | 99.4 | 96.80 to 99.97 |
| Total-Agreement | 95.7 | 91.8 to 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 | | reference test | | |
|---|---|---|---|---|
| | | 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 | 79.76 to 99.79 |
| Specificity | 98.9 | 95.93 to 99.80 |
| Total-Agreement | 98.5 | 95.7 to 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-epitope

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

| 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** | | **reference test** | | |
|---|---|---|---|---|
| | | 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 to 92.4 |
| Specificity | 97.2 | 94.0 to 98.7 |
| Total-Agreement | 96.1 | 92.7 to 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-epitope** | | **reference test** | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| AESKUBLOTS Gluten related disorders IgA | Positive | 27 | 0 | 27 |
| | Negative | 15 | 216 | 231 |
| | Total | 42 | 216 | 258 |

| **tTG neo-pitope** | [%] | 95% CI |
|---|---|---|
| Sensitivity | 64.3 | 49.2 to 77.0 |
| Specificity | 100 | 98.3 to 100.0 |
| Total-Agreement | 94.2 | 90.6 to 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. If the antigen tTG is included in the evaluation of the discrepant samples, it can be seen that the *AESKUBLOTS*® Gluten related disorders IgA achieves a sensitivity of 90.0% with a specificity of 100.0% due to the addition of the antigen tTG. The values obtained are based on the assumption that the reference test correctly classifies all samples.

### 10.4.3 TG 3

The TG 3 antigen was compared with a commercially available ELISA for the determination of IgA antibodies against TG 3. 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).

| TG 3 | | reference test | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| AESKUBLOTS Gluten related disorders IgA | Positive | 8 | 1 | 9 |
| | Negative | 3 | 15 | 18 |
| | Total | 11 | 16 | 27 |

| TG 3 | [%] | 95% CI |
|---|---|---|
| Sensitivity | 72.7 | 0.0 to 94.87 |
| Specificty | 93.8 | 97.27 to 99.74 |
| Total-Agreement | 85.2 | 96.8 to 99.5 |

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-epitope and mTG

For the antigens mTG neo-epitope 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) for the determination of mTG neo-epitope 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-epitope | | reference test | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| AESKUBLOTS Gluten related disorders IgA | Positive | 41 | 1 | 42 |
| | Negative | 9 | 200 | 209 |
| | Total | 50 | 201 | 251 |

| mTG neo-epitope | [%] | 95% CI |
|---|---|---|
| Sensitivity | 82.0 | 69.2 to 90.2 |
| Specificity | 99.5 | 97.2 to 99.9 |
| Total-Agreement | 96.0 | 92.8 to 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 | | reference test | | |
|---|---|---|---|---|
| | | 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 to 94.87 |
| Specificity | 99.05 | 97.27 to 99.74 |
| Total-Agreement | 98.75 | 96.8 to 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 | - |
| TG 3 | 0.976 | 0.958 | - | - |
| mTG neo | 0.998 | 0.960 | 0.965 | - |
| mTG | 0.956 | 0.984 | 0.974 | - |
| PT-Gliadin | 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 Literature

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

| | | |
|---|---|---|
| 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 | |
| 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 | |
| +8°C +2°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 | ¨ |
| 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 | |