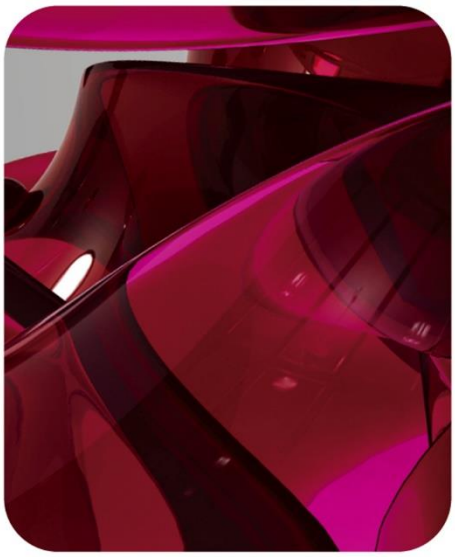
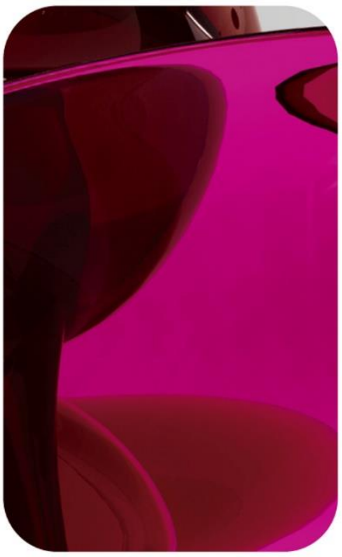
AESKU. DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS


# AESKUBLOTS®

THE DIAGNOSTIC TOOL THAT WORKS

# INSTRUCTION MANUAL

***AESKUBLOTS® Gastro Pro***

***Ref 4005***

DIN EN ISO 13485

# Instruction Manual

## Table of Contents

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# 1 Intended Use

***AESKUBLOTS*® Gastro Pro** is a membrane-based enzyme immunoassay for the qualitative detection of IgG and IgA antibodies against gliadin, tTg neo-epitope, mannan (ASCA), parietal cell antigen and intrinsic factor in human serum. Antigens are located as parallel lines at exactly defined positions on a nitrocellulose membrane.

The assay is a tool in diagnosis of celiac disease, pernicious anaemia and inflammatory bowel diseases.

# 2 Clinical Application and Principle of the Test

Each section of the gastrointestinal tract may be affected by autoimmune gastrointestinal diseases. The diseases are often diagnosed years after the first onset of symptoms and in many cases, they have a severe course.

Celiac patients often have an IgA deficiency. In order to avoid false-negative results, this test detects IgA and IgG antibodies.

**Antibodies against:**

- gliadin are typical for celiac disease. The IgA type is essentially specific for celiac disease. Antibodies of the IgG type occur in 40-50 % of patients with Crohn's disease and also in 10-20 % of patients with ulcerative colitis.
- the neoepitope of tTg (neo-tTg; tissue transglutaminase cross-linked to gliadin specific peptides) constitutes a reliable marker of celiac disease and dermatitis herpetiformis (Duhring’s disease). Due to the structural similarity with the physiological epitopes, antibodies against neo-tTg exhibit greater sensitivity (98-100%) and specificity (93-96%) than anti-tTg antibodies.
- Mannan (ASCA) have a specificity of 97 % for Crohn's disease. They are essential for the differential diagnosis of Crohn's disease and ulcerative colitis. Up to 75 % of Crohn's disease patients show, in contrast to patients with ulcerative colitis, an increased antibody level.
- parietal cells can be detected by immunofluorescence test in 80-90 % of patients with pernicious anaemia but can also be found in 2-5 % of healthy individuals. They also occur in patients with autoimmune endocrinal diseases and chronic atrophic gastritis type A.
- intrinsic factor show for pernicious anaemia a sensitivity of 50-70 % with a specificity of 100 % in a population of healthy blood donors. Also, these antibodies are detected in patients with autoimmune thyroid diseases and chronic atrophic gastritis type A.

**Principle of the test**

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 the 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 | 3 x for 10 ml Concentrate each | white | N/A | Non-fat dry milk powder for preparation of 3 x 10 ml sample buffer |
| Wash Buffer (20x) | 1 x 50 ml | white | colorless | 20x concentrated for preparation of 1 L<br>Tris buffer, pH 6.9 ± 0.2 |
| ***READY TO USE*** | | | | |
| Item | Quantity | Cap color | Solution color | Description / Contents |
| Conjugate, IgG | 1 x 10 ml | blue | colorless | Anti-human immunoglobulin G (IgG) conjugated to horseradish peroxidase |
| Conjugate, IgA | 1 x 10 ml | red | colorless | Anti-human immunoglobulin A (IgA) conjugated to horseradish peroxidase |
| TMB Substrate | 1 x 10 ml | black | colorless | Stabilized TMB/$H_2O_2$ |
| Membrane strips | 24 strips | color coding: black | N/A | Coated antigens see Intended use |
| tweezers, reference template, scoring sheet, adhesive strip (double-sides, white) | 1 pcs. each | N/A | N/A | N/A |
| incubation tray | 3 pcs. | N/A | N/A | N/A |
| Labels for sample buffer | 3 pcs. | N/A | N/A | N/A |
| ***MATERIALS REQUIRED, BUT NOT PROVIDED*** | | | | |
| rocking platform, cylinder 1000 ml, pipette or cylinder for 10 ml, precision pipettes (10, 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

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 at least six weeks. Reconstituted blocking reagent is stable at 2-8°C/35.6-46.4°F for at least 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 ***AESKUBLOTS®***-tests available, a color coding is applied above the reference line of the strips:

| **Color coding** | ***AESKUBLOTS®*** |
|---|---|
| red | ANA-17 comp |
| orange | ANA-17 Pro |
| blue | Myositis Pro |
| brown | Liver Pro |
| purple | Vasculitis Pro |
| black | Gastro Pro |
| green | Borrelia-G and Borrelia-M |

In case 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. Don’t exchange substances 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.

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.**

# 6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed 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 (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4 Vol. 30 No. 10). Avoid repeated thawing and freezing. Do not use heat 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, room temperature should be enough, the concentrate.

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.

## 7.2 Test Steps

**Important notes:**

Follow exactly this protocol. Make sure that the two components mentioned in the protocol are added to the tray in step 2, 6, 9.

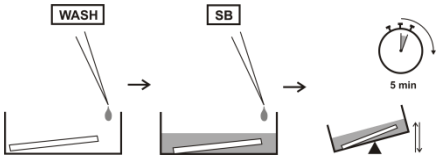
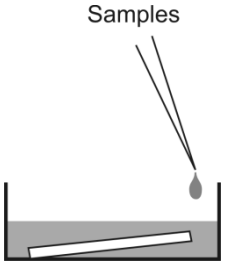
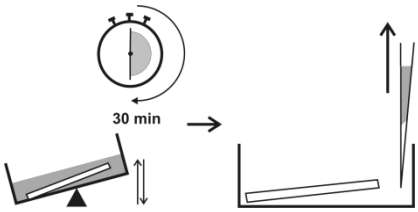
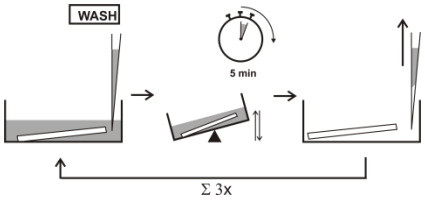
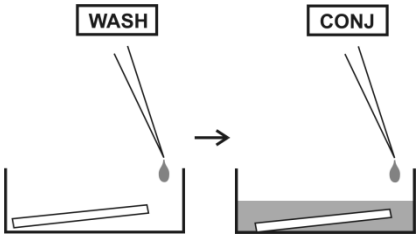
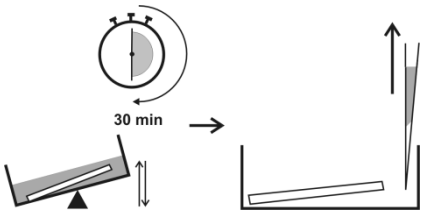
Do not let strip dry out during incubation steps.

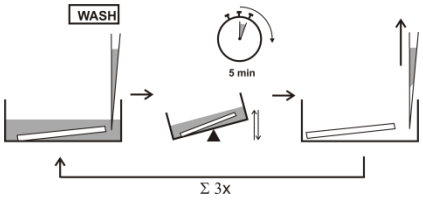
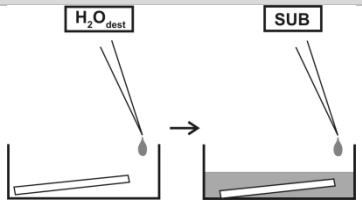
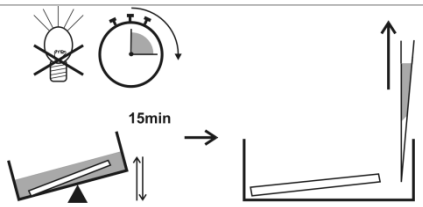
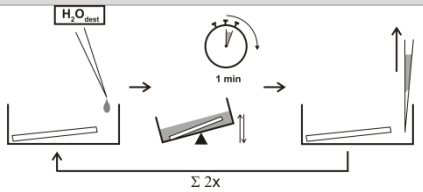
Do not touch strip with fingers, use tweezers.

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

Continuously shake strip 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 strip.

| *Step* | *Description* |
|---|---|
| 1. | Ensure the preparations, from step 7.1 above, have been carried out prior to test begin. |
| 2. |  Put strip in correct orientation into incubation tray (reference line and color coding upwards). Put 700 µl wash buffer and 300 µl sample buffer in the incubation tray. Moisten strip with the solution and incubate for 5 minutes with agitation. |
| CONTROLS & SAMPLES | |
| 3. |  Pipette 10 µl serum sample into the designated incubation trays with sample buffer. |
| 4. |  Incubate for 30 minutes at 20-32°C/68-89.6°F with agitation. After that remove sample completely. |
| 5. |  Wash 3 times for 5 minutes with 1.5 ml wash buffer by agitation. Remove wash buffer after every washing step. |
| CONJUGATE | |
| 6. |  Pipette 700 µl wash buffer and 300 µl conjugate into each incubation tray with strip. |
| 7. |  Incubate for 30 minutes at 20-32°C/68-89.6°F with agitation. Remove conjugate. |

| | | |
|---|---|---|
| 8. |  | Wash 3 times for 5 minutes with 1.5 ml wash buffer by agitation. Remove wash buffer after every washing step. |
| SUBSTRATE | | |
| 9. |  | Pipette 700 µl $dH_2O$ and 300 µl substrate into each incubation tray with strip. |
| 10. |  | Incubate for 15 minutes at 20-32°C/68-89.6°F with agitation, protected from intense light. Remove substrate. |
| STOP | | |
| 11. |  | Pipette 2 ml $dH_2O$ into each incubation tray with strip. Incubate 1 minute with agitation. Remove $dH_2O$. Repeat this step one time. |
| 12. | Remove strip of the incubation tray. Dry strip between filter paper | |
| 13. | Analyze results within 24 h. | |

*AESKUBLOTS*® Gastro Pro is also intended to be automatically processed and evaluated on the HELIA® Automated blot system.
Reagent preparation for HELIA®: Dilute 1 part wash buffer concentrate (WASH) with 19 parts ultrapure water (e.g. 50 ml wash buffer concentrate and 950 ml ultrapure water) to obtain a ready-to-use wash buffer. All other reagents are ready to use when processed in HELIA®. For detailed handling of the test on HELIA® refer to the instruction manual of the HELIA®.

# 8 Qualitative Interpretation

## 8.1 Manual Analysis

Test results can be considered valid, if:

- Functional control is visible
- Cut-off control is visible
- Color intensity of cut-off control is weaker than color intensity of functional control

Fix dried strip onto scoring sheet aligned with reference line. Align reference template with the strip reference line. Interpret results only in reference to cut-off control of each strip.

Each test kit contains a color copy with all bands provable in the test.

The analysis is carried out by means of comparing the color intensities of the bands with color intensity of the cut-off control. The test is equivocal if the intensities do not significantly differ. If the color is more intense, the test result is positive, if the color intensity is weaker, the test is negative.

The results can be recorded on the scoring sheet.

In case that the values of the controls do not meet the criteria, the test is invalid and has to be repeated. We recommend retesting samples that are borderline.

The following technical issues should as well be checked: expiry date of (prepared) reagents, storage conditions, pipettes, equipment, incubation conditions and washing methods.

If the samples tested show aberrant values or any kind of deviation or if the validation criteria are not met because of reasons outside the operator's responsibility, please contact the manufacturer or the supplier of the test kit.

Medical laboratories might perform an in-house quality control by using their own controls and/or internal pooled sera, as stated in national regulations.

## 8.2 Software-supported evaluation

The analysis of the strips can be carried out by means of using AESKU.SCAN Software. Please refer to the instructions for use of AESKU.SCAN.

Test results can be considered valid, if:

- Functional control is visible
- Cut-off control is visible
- Color intensity of cut-off control is weaker than color intensity of functional control

<u>AESKU.SCAN 2.0:</u>

Fix dried strip onto scoring sheet (printable) aligned with reference line. Align reference template with the strip reference line.

Evaluate strips according to the instructions for use of AESKU.SCAN 2.0 software.

Qualitative result analysis is carried out by means of comparing the color intensities of the individual antigens with the color intensity of the cut-off control.

AESKU.SCAN 3.0:

Put strips within the incubation tray into the reader.

Evaluate strips according to the instructions for use of AESKU.SCAN 3.0 software.

Qualitative result analysis is carried out by means of comparing the color intensities of the individual antigens with the color intensity of the cut-off control.

HELIA®:

Using a HELIA® Automated blot system, the results are analyzed automatically. The results can be determined in Index-values.

The following interpretation according to the signal intensity is suggested:

| Result Interpretation | Symbol | Index | Color |
|---|---|---|---|
| Negative | - | 0.0 - <0.8 | Colorless |
| Equivocal | +/- | ≥0.8 - <1.15 | Blue |
| Weak positive | + | ≥1.15 - <2.5 | Yellow |
| Positive | ++ | ≥2.5 - <4.0 | Red |
| Strong positive | +++ | ≥ 4.0 | Dark red |

In case the values of the controls do not meet the criteria, the test is invalid and has to be repeated. We recommend retesting samples that are borderline. The following technical issues should as well be checked: expiry date of (prepared) reagents, storage conditions, pipettes, equipment, incubation conditions and washing methods.

If the samples tested show aberrant values or any kind of deviation or if the validation criteria are not met because of reasons outside the operator's responsibility, please contact the manufacturer or the supplier of the test kit.

Medical laboratories might perform an in-house quality control by using their own controls and/or internal pooled sera, as stated in national regulations.

# 9 Technical Data

| | |
|---|---|
| Sample material: | serum |
| Sample volume: | 10 µl of sample |
| Total incubation time: | 112 minutes at 20-32°C/68-89.6°F |
| Storage: | at 2-8°C/35.6-46.4°F; use original vials only. |
| Number of determinations: | 24 tests |

# 10 Performance Data

## Normal Range Study

Expected values for AESKUBLOTS® Gastro Pro were analyzed with a panel of 120 sera from healthy donors.

All assays were performed fully automated according to the current IFUs.

*AESKUBLOTS®* Gastro IgA

| AESKUBLOTS Gastro Pro, REF 4005 - Normal Range - Report | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Antigen | Number of samples | positive samples | | equivocal samples | | negative samples | | Min [Index] | Max [Index] | mean [Index] |
| | | n | [%] | n | [%] | n | [%] | | | |
| Gliadin | 120 | 0 | 0.00 | 9 | 7.50 | 111 | 92.50 | 0.00 | 1.08 | 0.27 |
| Neo-tTG | 120 | 1 | 0.83 | 0 | 0.00 | 119 | 99.17 | 0.00 | 1.30 | 0.03 |
| ASCA | 120 | 0 | 0.00 | 11 | 9.17 | 109 | 90.83 | 0.00 | 1.10 | 0.28 |
| PCA | 120 | 0 | 0.00 | 10 | 8.33 | 110 | 91.67 | 0.00 | 1.08 | 0.24 |
| IF | 120 | 0 | 0.00 | 0 | 0.00 | 120 | 100.00 | 0.00 | 0.70 | 0.04 |

*AESKUBLOTS®* Gastro IgG

| AESKUBLOTS Gastro Pro, REF 4005 - Normal Range - Report | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Antigen | Number of samples | positive samples | | equivocal samples | | negative samples | | Min [Index] | Max [Index] | mean [Index] |
| | | n | [%] | n | [%] | n | [%] | | | |
| Gliadin | 120 | 0 | 0.00 | 8 | 6.67 | 112 | 93.33 | 0.00 | 1.10 | 0.21 |
| Neo-tTG | 120 | 0 | 0.00 | 0 | 0.00 | 120 | 100.00 | 0.00 | 0.21 | 0.00 |
| ASCA | 120 | 0 | 0.00 | 5 | 4.17 | 115 | 95.83 | 0.00 | 0.98 | 0.30 |
| PCA | 120 | 0 | 0.00 | 4 | 3.33 | 116 | 96.67 | 0.00 | 1.03 | 0.14 |
| IF | 120 | 0 | 0.00 | 0 | 0.00 | 120 | 100.00 | 0.00 | 0.27 | 0.01 |

With *AESKUBLOTS®* Gastro Pro IgA there was one positive result for anti-Neo-tTG. The most equivocal results were detected for anti-Gliadin in both variants. The lowest Index-value measured was 0.0, the highest Index-value measured was 1.3 (Neo-tTG) for *AESKUBLOTS®* Gastro Pro IgA.

The low number of positive samples we found with AESKUBLOTS® Gastro Pro in the tested healthy population correlates well with the numbers reported in the literature.

We also recommend in the IFU in chapter 8: “Quantitative and qualitative interpretation” that each laboratory should establish its own normal range.

## Precision

The precision of the test results obtained with the AESKUBLOTS® Gastro Pro was investigated by determining the intra- and inter-assay precision as well as the lot variance by analyzing several samples. The number of positive and negative samples (considering each individual antigen) is compared and related to the previously determined reference. In this way, the positive and negative agreement, as well as the overall agreement, can be calculated.

### Intra-Assay

To determine the within-lab / intra-assay variances five different sera (S1-S4) have been tested for 20 times on AESKUBLOTS® Gastro Pro. The positive- and negative agreement were calculated from these test values (n=20).

*AESKUBLOTS®* Gastro IgA

| Calculation of agreements | |
|---|---|
| Percent positive-agreement [%] | 100.0 |
| Percent negative-agreement [%] | 100.0 |
| Percent overall-agreement [%] | 100.0 |

*AESKUBLOTS*® Gastro IgG

| Calculation of agreements | |
|---|---|
| Percent positive-agreement [%] | 100.0 |
| Percent negative-agreement [%] | 100.0 |
| Percent overall-agreement [%] | 100.0 |

**Inter-Assay**

To determine the inter-assay / day to day variances four different sera (S1-S4) over the whole range have been tested 8 times per run, in a total of 5 runs on 5 different days (n=40). For day 1 the results from the within-lab / intra-assay variances are used (n=20), the positive- and negative agreement were calculated from these test values.

*AESKUBLOTS*® Gastro IgA

| Calculation of agreements | |
|---|---|
| Percent positive-agreement [%] | 100.0 |
| Percent negative-agreement [%] | 100.0 |
| Percent overall-agreement [%] | 100.0 |

*AESKUBLOTS*® Gastro IgG

| Calculation of agreements | |
|---|---|
| Percent positive-agreement [%] | 100.0 |
| Percent negative-agreement [%] | 100.0 |
| Percent overall-agreement [%] | 100.0 |

**Lot to Lot Variation**

To determine the Lot to Lot variation four different sera (S1-S4) over the whole range have been tested 8 times on 3 Lots (n=24). Lot 1 is taken from Run 1 of the Intra Assay data. For each sample the variation coefficients and the mean values have been calculated across the test values of the three different lots, positive- and negative agreement were calculated from these test values.

*AESKUBLOTS*® Gastro IgA

| Calculation of agreements | |
|---|---|
| Percent positive-agreement [%] | 100.0 |
| Percent negative-agreement [%] | 100.0 |
| Percent overall-agreement [%] | 100.0 |

*AESKUBLOTS*® Gastro IgG

| Calculation of agreements | |
|---|---|
| Percent positive-agreement [%] | 100.0 |
| Percent negative-agreement [%] | 100.0 |
| Percent overall-agreement [%] | 100.0 |

## Diagnostic Sensitivity and Specificity

A clinical study with different serum samples from patients with celiac disease, Dermatitis herpetiformis, Morbus Crohn, Ulcerative Colitis, SLE, RA, Diabetes type 1 and healthy controls were analyzed with the AESKUBLOTS® Gastro Pro. Furthermore, these samples were measured with a competitor Western blot. Also, a pediatric sample cohort with clear diagnosis and medical history of 87 samples was included.

All assays were performed according to the current IFUs. After that the results were compared to determine the diagnostic sensitivity* and specificity* of the assay.

### Anti-Gliadin

Gliadin are typical for celiac disease. The IgA type is essentially specific for celiac disease. Antibodies of the IgG type occur in 40-50 % of patients with Crohn's disease and also in 10-20 % of patients with ulcerative colitis.

*AESKUBLOTS*® Gastro IgA and *AESKUBLOTS*® Gastro IgG

Celiac samples were analyzed, however, no sensitivity for gliadin can be calculated as the reference kit does not have gliadin for IgA and IgG variant. The prevalence of celiac disease-specific autoantibodies has been studied in patients with SLE, RA, and diabetes type 1.

| | n (65) | AESKUBLOTS Gastro IgA anti-Gliadin positive | AESKUBLOTS Gastro IgG anti-Gliadin positive |
|---|---|---|---|
| SLE | 28 | 7.1% | 10.7% |
| RA | 11 | 0.0% | 0.0% |
| Diabetes type 1 | 26 | 0.0% | 34.6% |

| Gliadin antibodies: analysis of healthy controls | AESKUBLOTS Gastro Pro IgA | | |
|---|---|---|---|
| | positive | equivocal | negative |
| | 0 | 9 | 111 |

| Gliadin antibodies: analysis of healthy controls | AESKUBLOTS Gastro Pro IgG | | |
|---|---|---|---|
| | positive | equivocal | negative |
| | 0 | 8 | 112 |

In the analysis of 120 antibody-negative healthy patient samples, no positive samples were measured, and only 9 and 8 samples, respectively, showed borderline results. It is known from the literature that gliadin antibodies can also occur in healthy patients or in patients with other diseases.

**Anti-tTG neo**

*AESKUBLOTS*® Gastro Pro IgA

| Neo-tTG 2x2 | Diagnose | |
|---|---|---|
| | Celiac Diseases | Controls |
| positive | 28 | 1 |
| negative | 18 | 40 |

| Diagnostic performance tTG-neo IgA | | CI |
|---|---|---|
| Sensitivity | 60.9 | 45.4% to 74.9% |
| Specificity | 97.6 | 87.1% to 99.9% |
| AUC | 0.792 | 0.692 to 0.872 |

In a pediatric sample cohort of 87 samples including Celiac Disease, Morbus Chron, Ulcerative Colitis and healthy blood donors a diagnostic sensitivity for tTG-neo IgA of 60.9% and a diagnostic specificity of 97.6% could be calculated.

*AESKUBLOTS*® Gastro Pro IgG

| Neo-tTG 2x2 | Diagnose | |
|---|---|---|
| | Celiac Diseases | Controls |
| positive | 38 | 3 |
| negative | 10 | 33 |

| Diagnostic performance tTG-neo IgG | | CI |
|---|---|---|
| Sensitivity | 79.2 | 65.1% to 89.5% |
| Specificity | 91.7 | 77.5% to 98.2% |
| AUC | 0.854 | 0.760 to 0.922 |

In a pediatric sample cohort of 84 samples including Celiac Disease, Morbus Chron, Ulcerative Colitis and healthy blood donors a diagnostic sensitivity for tTG-neo IgG of 79.2% and a diagnostic specificity of 91.7% could be calculated.

**Anti-ASCA**

*AESKUBLOTS*® Gastro Pro IgA

The analysis of 24 sera from patients with Crohn`s disease (anti-ASCA positive n = 15) showed a sensitivity of 100% for the detection of autoantibodies against ASCA with respect to the reference method Western blot of a competitor. In comparison to the competitor assay a specificity of 100% could be measured.

| ASCA antibodies: analysis of anti-ASCA in Crohn`s disease patients | | competitor assay | | |
|---|---|---|---|---|
| | | positive | equivocal | negative |
| AESKUBLOTS Gastro Pro IgA | positive | 15 | 1 | 0 |
| | equivocal | 2 | 1 | 0 |
| | negative | 0 | 1 | 4 |

The prevalence of ASCA autoantibodies has been studied in patients with Crohn`s disease, SLE, RA, diabetes type 1 and healthy controls.

| | n (124) | AESKUBLOTS Gastro IgA anti-ASCA positive |
|---|---|---|
| Crohn`s disease | 24 | 70.0% |
| SLE | 28 | 0.0% |
| RA | 11 | 0.0% |
| Diabetes type 1 | 26 | 25.0% |
| healthy controls | 35 | 0.0% |

*AESKUBLOTS*® Gastro IgG

The analysis of 20 sera from patients with Crohn`s disease (anti-ASCA positive n = 9) showed a sensitivity of 100% for the detection of autoantibodies against ASCA with respect to the reference method Western blot of a competitor. In comparison to the competitor assay a specificity of 100% could be measured.

| ASCA antibodies: analysis of anti-ASCA in Crohn`s disease patients | | competitor assay | | |
|---|---|---|---|---|
| | | positive | equivocal | negative |
| AESKUBLOTS Gastro Pro IgG | positive | 9 | 1 | 0 |
| | equivocal | 0 | 3 | 3 |
| | negative | 0 | 1 | 3 |

The prevalence of ASCA autoantibodies has been studied in patients with Crohn`s disease, SLE, RA, diabetes type 1 and healthy controls.

| | n (136) | AESKUBLOTS Gastro IgG anti-ASCA positive |
|---|---|---|
| Crohn`s disease | 20 | 50.0% |
| SLE | 28 | 7.1% |
| RA | 11 | 9.1% |
| Diabetes type 1 | 18 | 5.6% |
| healthy controls | 59 | 0.0% |

**Anti-PCA**

Parietal cells can be detected by immunofluorescence test in 80-90 % of patients with pernicious anaemia but can also be found in 2-5 % of healthy individuals. They also occur in patients with autoimmune endocrinal diseases and chronic atrophic gastritis type A.

In the sample panel used, there were no samples of pernicious anaemia, in which antibodies against PCA typically occur. Therefore, no sensitivity can be given here for *AESKUBLOTS*®

Gastro IgA and IgG. In sera from patients with SLE, RA, diabetes type 1 and healthy controls the prevalence for anti-PCA was studied.

| | n (185) | AESKUBLOTS Gastro IgA anti-PCA positive | AESKUBLOTS Gastro IgG anti-PCA positive |
|---|---|---|---|
| SLE | 28 | 10.7% | 10.7% |
| RA | 11 | 9.1% | 18.2% |
| Diabetes type 1 | 26 | 26.9% | 23.0% |
| healthy controls | 120 | 0.0% | 0.0% |

**Anti-Intrinsic Factor**

Intrinsic factor show for pernicious anaemia a sensitivity of 50-70 % with a specificity of 100 % in a population of healthy blood donors. Also, these antibodies are detected in patients with autoimmune thyroid diseases and chronic atrophic gastritis type A.

In the sample panel used, there were no samples of pernicious anaemia , in which antibodies against Intrinsic Factor typically occur. Therefore, no sensitivity can be given here for *AESKUBLOTS*® Gastro IgA and IgG. In sera from patients with SLE, RA, diabetes type 1 and healthy controls the prevalence for anti-PCA was studied.

| | n (185) | AESKUBLOTS Gastro IgA anti-IF positive | AESKUBLOTS Gastro IgG anti-IF positive |
|---|---|---|---|
| SLE | 28 | 0.0% | 3.5% |
| RA | 11 | 0.0% | 0.0% |
| Diabetes type 1 | 26 | 0.0% | 0.0% |
| healthy controls | 120 | 0.0% | 0.0% |

The sensitivities and specificities as well as the competitor agreements show very good results. In some cases, the sensitivity could not be calculated for individual parameters due to the lack of positives samples in the cohorts. Here further samples are to be investigated in the future.

## Relative Sensitivity and Specificity

In order to determine the positive agreement (relative sensitivity), 30 sera from IIF or ELISA antibody-positive patients were tested in ***AESKUBLOTS*® Gastro Pro.** For determination of the negative agreement (relative specificity), 100 sera from blood donors were analyzed.

| | positive agreement (relative sensitivity) | negative agreement (relative specificity) |
|---|---|---|
| gliadin | 100 % | 100 % |
| Neo-tTg | 100 % | 100 % |
| mannan (ASCA) | 100 % | 100 % |
| parietal cell antigen | 100 % | 100 % |
| intrinsic factor | 100 % | 100 % |

# 11 Literature

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| | | |
|---|---|---|
| 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 | |
| +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 | ¨ |
| 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 | |