

THE CHAMELEON OF GASTROENTEROLOGY



AESKUBLOTS® • AESKUCARE® • AESKULISA®

GLUTEN RELATED DISORDERS

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WE TAKE CARE OF YOUR HEALTH





GLUTEN RELATED DISORDERS

Gluten related disorders (GRDs) emerging globally, presenting a wide variety of either intra and/or extra-intestinal symptoms. The widespread and rise of gluten consumption as well as processed foods, makes GRDs related to gluten important for different and increasing parts of the world.

The different faces of GRDs

1. Autoimmune diseases like Celiac Disease (CD), Dermatitis herpetiformis (DH), Gluten Ataxia, and further HLA-related autoimmune conditions

CD prevalence ranges from about 1-2% worldwide with a diverse phenotypic spectrum, including classical and non-classical CD. Classical CD show typical signs of malabsorption like diarrhea, weight loss, vitamin deficiencies, and malnutrition. A significant number of patients suffer from non-classical or even asymptomatic CD with non-specific or mild complaints and, therefore, hard to diagnose or even misdiagnose.

2. Non-autoimmune and Non-allergic conditions like non-celiac gluten sensitivity (NCGS)

NCGS is defined by intestinal and/or extraintestinal manifestations related to the digestion of wheat in patients where CD and wheat allergy has been excluded. The prevalence of NCGS ranges from 0.5 to 10%, mainly due to high variations in disease definitions and challenging diagnostic criteria due to the lack of specific biomarkers.

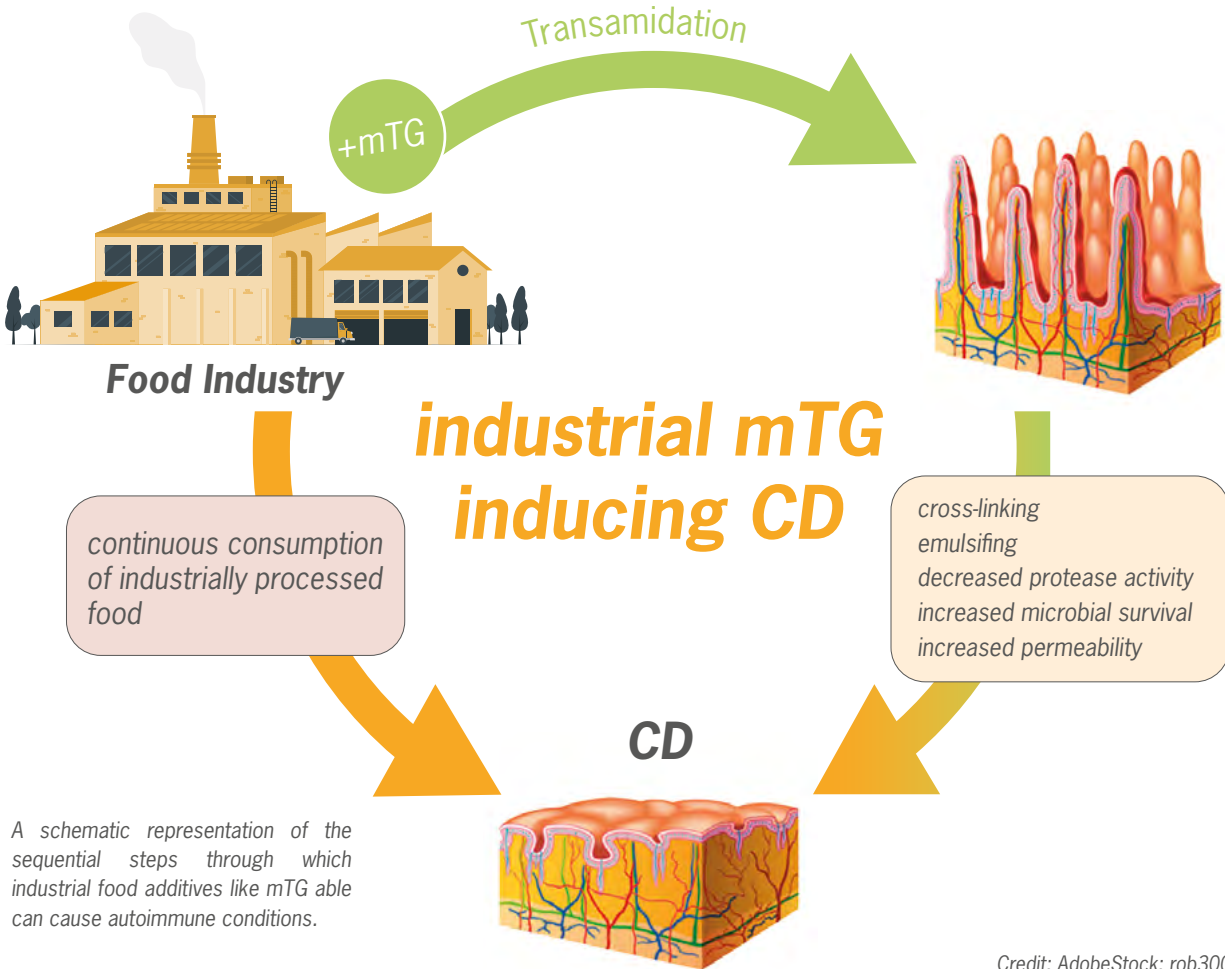
3. Allergic disorders (IgE mediated) like classical wheat allergy and occupational asthma (baker's asthma)



How the food industry influences

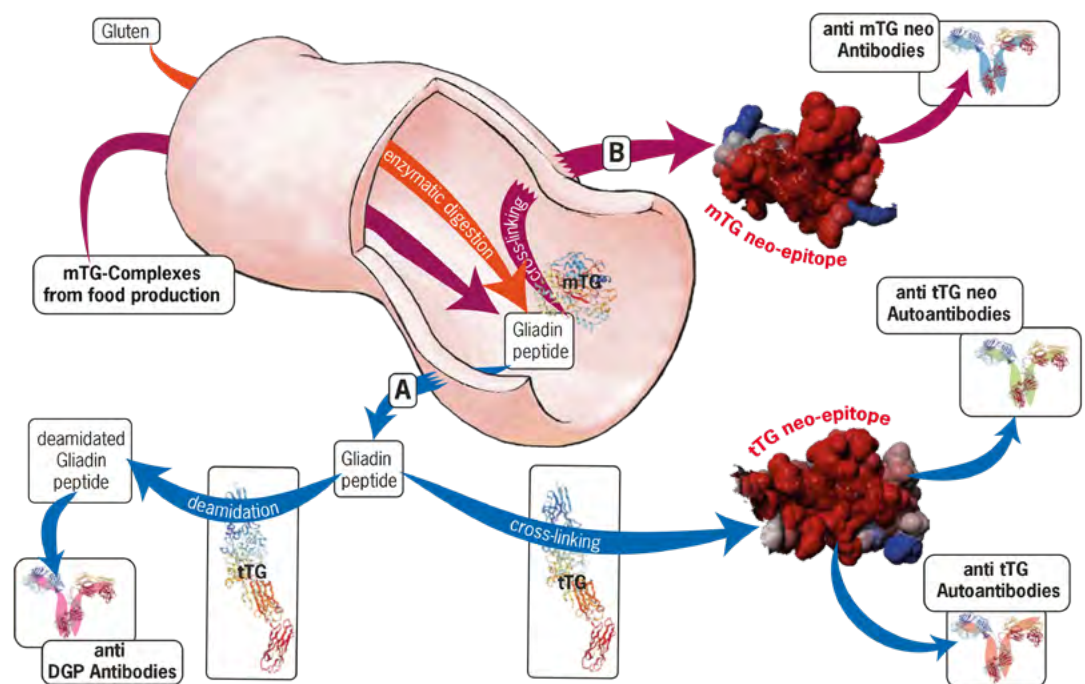
The use of microbial transglutaminase (mTG) creates new immunogenic gluten peptides by cross-linking various macromolecules to gluten, thus establishing new celiacogenic epitopes, resulting in molecular mimicry of immunogenic peptides in patients with Celiac Disease.

Commonly used industrial food additives abrogate human epithelial barrier function, thus increasing intestinal permeability through the opened Tight Junctions (TJ), resulting in foreign immunogenic antigens entering and activating the autoimmune cascade.



What is it – tTG / mTG neo

The schematic hypothesis of the formation of the neo-epitope complexes and their corresponding antibodies in Celiac Disease (CD).



The first part of the figure shows the gluten processing pathway, where cereal products get digested in the intestine by luminal enzymes. During this process, gliadin is cleaved into peptides which then react with tissue transglutaminase (tTG) in the lamina propria and form covalently cross-linked complexes (tTG neo).

Before and after digestion, the protein structures can be further processed by microbial transglutaminase. It thereby imitates the body's own tTG and crosslinks protein structures to form so-called

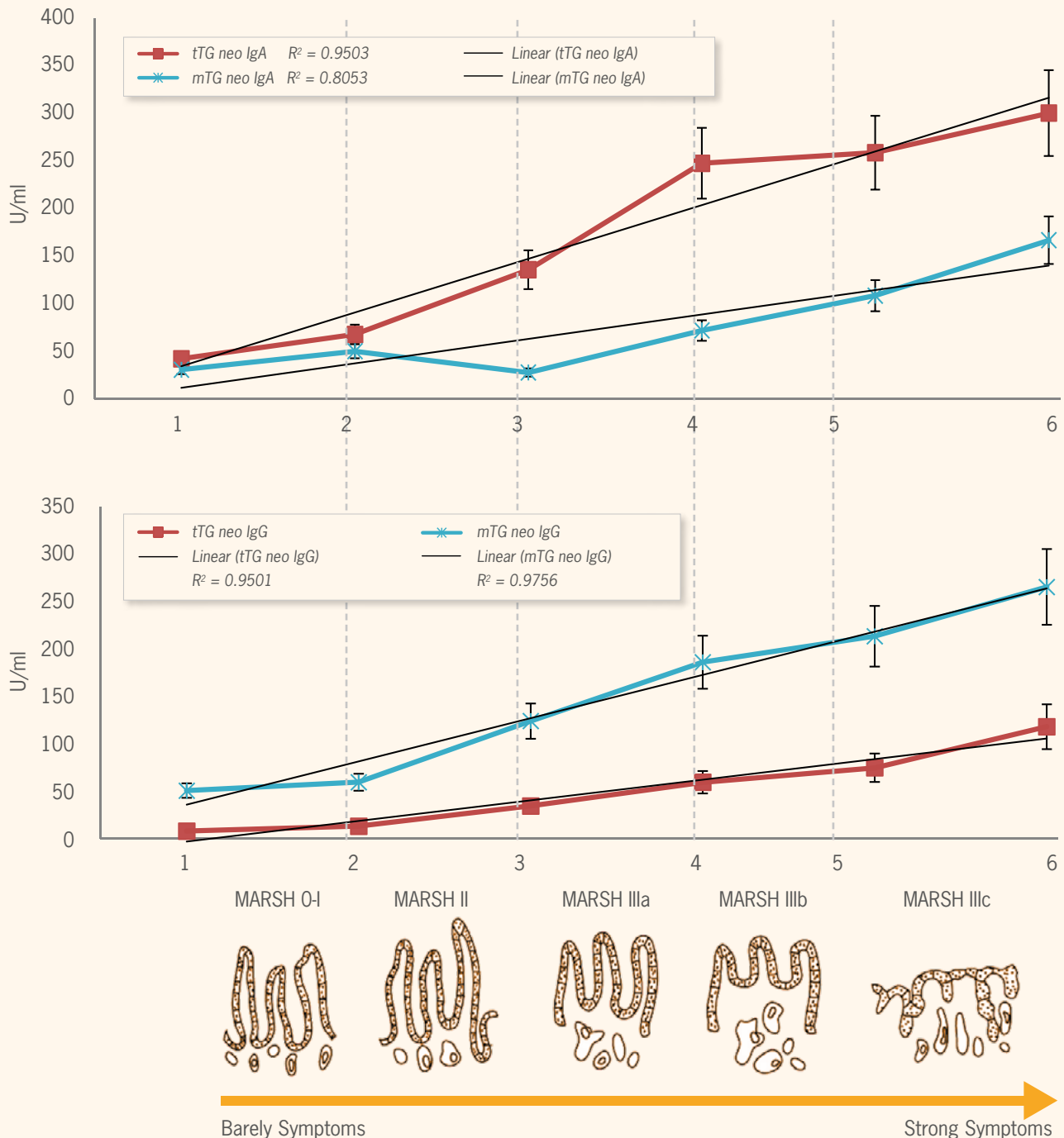
mTG neo. Those tTG and mTG neo-epitopes induce antibody formation, resulting in anti-deamidated gliadin peptide (DGP), tTG, tTG neo, and mTG neo-antibodies.

1. LERNER A, MATTHIAS T. POSSIBLE ASSOCIATION BETWEEN CELIAC DISEASE AND BACTERIAL TRANSGLUTAMINASE IN FOOD PROCESSING: A HYPOTHESIS. *NUTR REV.* 2015; 73: 544-552

2. AGARDH D ET AL., ANTIBODIES AGAINST NEO-EPIPE OF MICROBIAL AND HUMAN TRANSGLUTAMINASE COMPLEXES AS BIOMARKERS OF CHILDHOOD CELIAC DISEASE. *CLIN EXP IMMUNOL.* 2020 MAR;199(3):294-302. doi: 10.1111/CEI.13394. EPUB 2019 Nov 11

tTG / mTG neo performance in disease monitoring

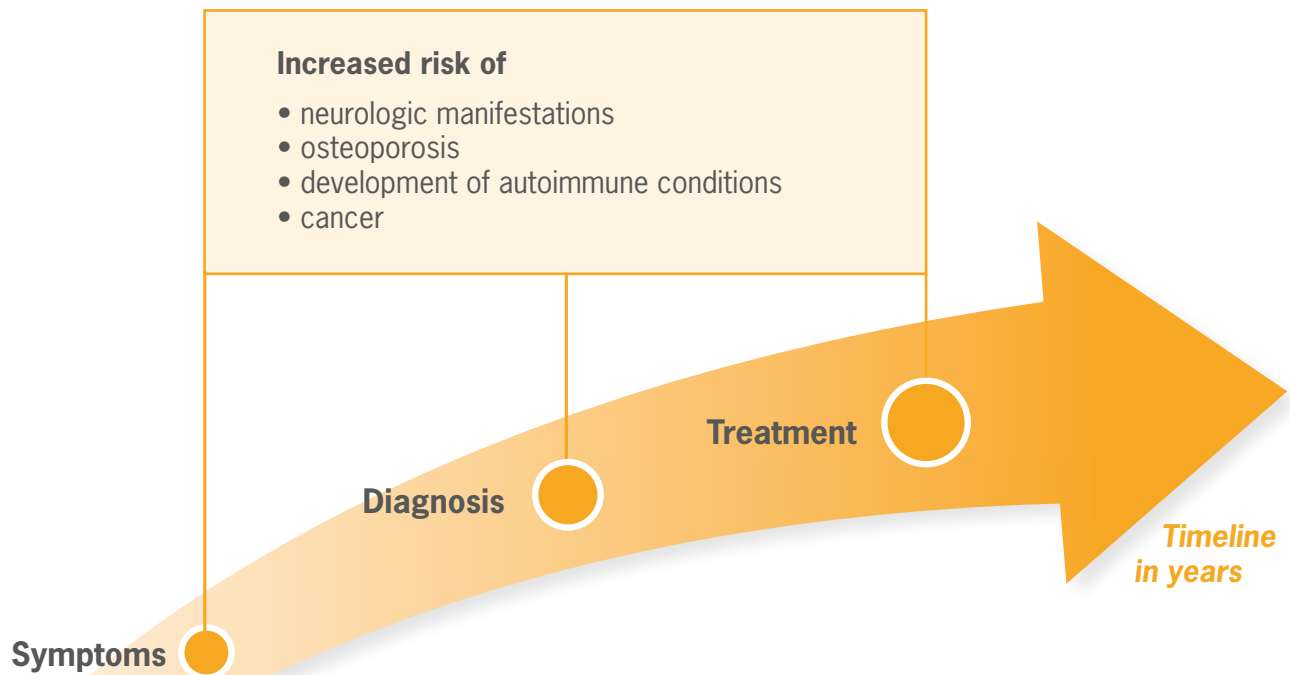
A study performed at the **AESKU.KIPP INSTITUTE** in 2015, with the kind collaboration of Prof. Aaron Lerner, evaluated the reliability of anti-tTG/ mTG neo antibodies to reflect the intestinal damage in children with CD.



95 pediatric CD patients (mean age 8.3 ± 4.4), 45 nonspecific abdominal pain children (AP) (mean age 7.3 ± 5.1), 99 normal children (NC) (mean age 8.5 ± 4.2) and 79 adults (NA) (mean age 28 ± 5.1) were checked for serum IgA and/or IgG antibodies against neo-epitopes. The calculated R squared (R^2) for tTG neo IgA was 0.9503 and 0.8053 for mTG neo IgA.

Looking at the tTG neo IgG isotypes, R^2 was 0.9501, and for the mTG neo IgG resulted in 0.9756 with a statistical significance of $p < 0.0001$, respectively, for all cases. The antibody titer of mTG neos and tTG neos show a strong correlation with the underlying Marsh Index of the patients, indicating the degree of intestinal damage.

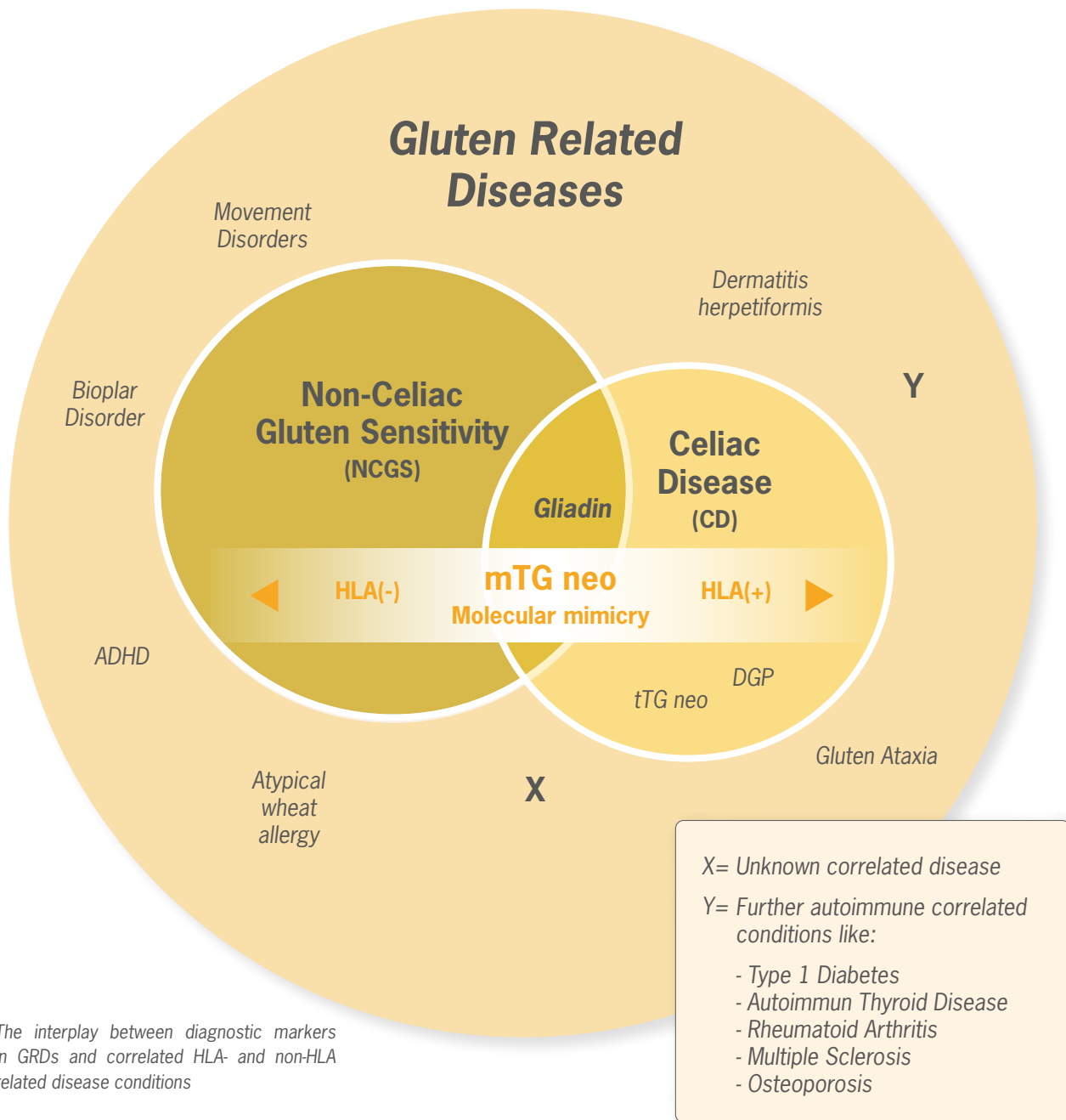
Don't waste time: Early detection, fast reaction



In 2015 the Celiac Disease Center from the University of Chicago outlined a study from Ventura et al. in 1999 that late disease recognition of GRD leads to the appearance or development of further auto- or non-autoimmune co-conditions related to the age of diagnosis. They found that CD patients diagnosed between the ages of 2-4 years had a 10.5% chance of developing an autoimmune disease, but by the age of >20 years at the time of diagnosis, that chance rose to 34 %. [1-3]

1. ALESSANDRO VENTURA, GIUSEPPE MAGAZZÙ, LUIGI GRECO, DURATION OF EXPOSURE TO GLUTEN AND RISK FOR AUTOIMMUNE DISORDERS IN PATIENTS WITH CELIAC DISEASE GASTROENTEROLOGY, VOLUME 117, ISSUE 2, P297-303, AUGUST 01, 1999; DOI: <https://doi.org/10.1053/gast.1999.0029900297> 2. LERNER A, JEREMIAS P, NEIDHÖFER S, MATTHIAS T (2017) COMPARISON OF THE RELIABILITY OF 17 CELIAC DISEASE ASSOCIATED BIO-MARKERS TO REFLECT INTESTINAL DAMAGE. J CLIN CELL IMMUNOL 8: 486. DOI: 10.4172/2155-9899.1000486 (7) (PDF) COMPARISON OF THE RELIABILITY OF 17 CELIAC DISEASE ASSOCIATED BIO-MARKERS TO REFLECT INTESTINAL DAMAGE. 3. STRICKER S ET AL., TISSUE TRANSGLUTAMINASE BUT NOT MICROBIAL TRANSGLUTAMINASE IS INHIBITED BY EXOGENOUS OXIDATIVE SUBSTANCES IN CELIAC DISEASE INT. J. MOL. SCI. 2022, 23, 2248

Why AESKUBLOTS® Gluten Related Disorders and AESKUCARE® Gluten Related Disorders give you the best indication



The interplay between diagnostic markers in GRDs and correlated HLA- and non-HLA related disease conditions

The tests **AESKUBLOTS®** GRD or **AESKUCARE®** GRD are an aid for diagnosis of Gluten related disorders. The presence of anti-mTG neo antibodies can be the first indicator for a underlying HLA-related or HLA-unrelated GRD.

In combination with specific markers for CD, you get the direct determination of an underlying autoimmune condition or a non-autoimmune gluten-related condition. Therefore, fast nutritional recommendations like gluten-free diet compliance and further treatments are possible using the **AESKUBLOTS®** GRD or **AESKUCARE®** GRD.

AESKU PRODUCTS FOR DIAGNOSIS OF GRD

AESKULISA® tTg New Generation

AESKU leads by example

In 2002 **Aesku**.Diagnostics introduced the tTG neo as a diagnostic marker for CD. The **AESKULISA®** tTg New Generation ELISA is a diagnostic tool for evaluating antibody levels against tTG neo in CD patients, which is still unique in the market [1].

Study results of measurements of numerous samples from 2002 until today show that the (tissue transglutaminase) tTG neo ELISA has superior performance as a screening assay, high sensitivity, and specificity value even in a pediatric population, has an excellent correlation with MARSH criteria, and can be an early marker for silent/latent CD. Further study results recommend the use of this ELISA as an aid for diagnosis of other Gluten related disorders like Dermatitis herpetiformis or Ataxia [2, 4].

Furthermore, in 2012 **AESKU** presented the (microbial transglutaminase) mTG neo, which show comparable diagnostic power to the tTG neos, even without any structural similarity of these transglutaminases from different species. mTG can cross-link numerous molecules, revolutionizing industrial food product qualities. Despite declarations of the safety of mTG, it can produce cross-linked complexes that, due to molecular mimicry, can be immunogenic in CD. Until now, direct evidence for its pathogenicity in celiac patients is lacking. To further investigate the role of the mTG concerning GRDs induction and to test our hypotheses, the immunogenicity of the enzyme was investigated in CD and NCGS patients' sera, as well as their



degree of intestinal damage compared to controls [1-5].

Less is known about the underlying pathomechanisms of GRDs. Still, the increasing awareness and introduction of new markers in diagnostics like mTG neo could lead to early detection, early treatment, and the avoidance of further autoimmune or non-autoimmune co-conditions.

In 2019, the Mayo Clinic in Rochester, Minnesota, stated that neo-epitopes are a promising tool to predict persistent mucosal injury in treated CD patients.[8]

Antigen	Sensitivity % (95% CI)	Specificity % (95% CI)	Overall Accuracy % (95% CI)
tTG neo	84 (75-90)	95 (88-98)	90 (84-94)
tTG ^a	27 (19-38)	93 (85-97)	61 (53-68)
DGP ^a	48 (38-59)	91 (83-95)	70 (63-76)

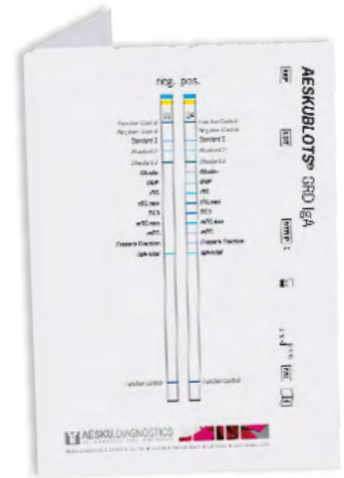
Table: Sensitivity, Specificity and Overall Accuracy of using tTG neo, anti-tTG IgA and anti-DGP IgA to predict healing status in treated CD patients.

^a Determined using tTG-IgA or DGP-IgA ELISA (competitor kit)

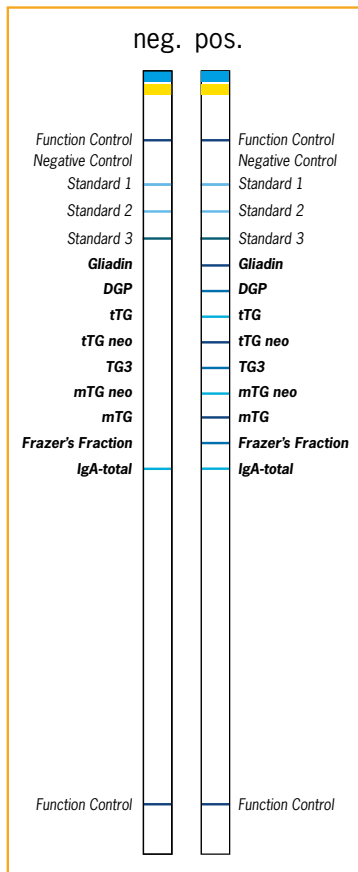
1. SRIKANT MOHTA, MAHENDRA S RAJPUT, VINEET AHUJA, AND GOVIND K MAKHARIA ET AL., EMERGENCE OF CELIAC DISEASE AND GLUTEN RELATED DISORDERS IN ASIA, J NEUROGASTROENTEROL MOTIL, VOL. 27 No. 3 JULY, 2021 <https://doi.org/10.5056/jnm20140> 2. LERNER A, MATTHIAS T. POSSIBLE ASSOCIATION BETWEEN CELIAC DISEASE AND BACTERIAL TRANSGLUTAMINASE IN FOOD PROCESSING: A HYPOTHESIS. NUTR REV. 2015; 73: 544-552] 3. AARON LERNER, AND TORSTEN MATTHIAS, "FOOD INDUSTRIAL MICROBIAL TRANSGLUTAMINASE IN CELIAC DISEASE: TREAT OR TRICK." INTERNATIONAL JOURNAL OF CELIAC DISEASE, VOL. 3, NO. 1 (2015): 1-6. doi: 10.12691/ijcd-3-1-10 4. DANIEL AGARDH, TORSTEN MATTHIAS, PATRICIA WUSTERHAUSEN, SANDRA NEIDHÖFER, ANETTE HELLER, AARON LERNER, ANTIBODIES AGAINST NEO-EPITOPES OF MICROBIAL AND HUMAN TRANSGLUTAMINASE COMPLEXES AS BIOMARKERS OF CHILDHOOD CELIAC DISEASE. CLINICAL & EXPERIMENTAL IMMUNOLOGY, 2019, 199: 294-302. doi: 10.1111/cei.13394 5. CURRENT GUIDELINES FOR THE MANAGEMENT OF CELIAC DISEASE: A SYSTEMATIC REVIEW WITH COMPARATIVE ANALYSIS. WORLD J GASTROENTEROL. 2022 JAN 7; 28(1): 154-175. PUBLISHED ONLINE 2022 JAN 7. doi: 10.3748/wjg.v28.i1.154 8. CHOUNG RS, KHALEGHI ROSTAMKOLAEI S, JU JM, MARIETTA EV, VAN DYKE CT, RAJASEKARAN JJ, JAYARAMAN V, WANG T, BEI K, RAJASEKARAN KE, KRISHNA K, KRISHNAMURTHY HK, MURRAY JA. SYNTHETIC NEOEPITOPES OF THE TRANSGLUTAMINASE-DEAMIDATED GLIADIN COMPLEX AS BIOMARKERS FOR DIAGNOSING AND MONITORING CELIAC DISEASE. GASTROENTEROLOGY. 2019 FEB;156(3):582-591.e1. doi: 10.1053/j.gastro.2018.10.025. EPUB 2018 OCT 17. PMID: 30342033.

AESKUBLOTS® Gluten Related Disorders

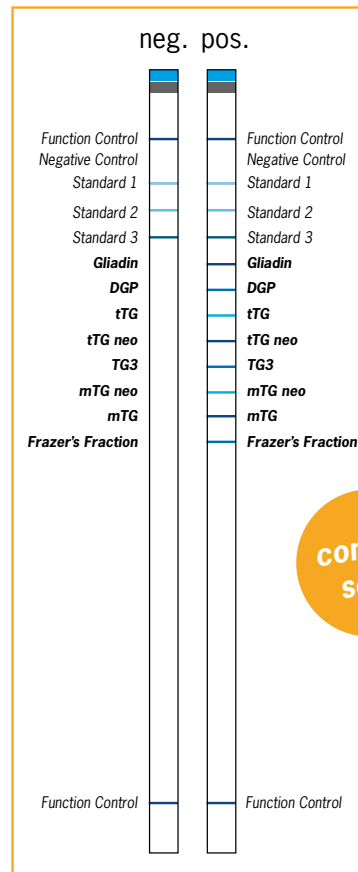
The **AESKUBLOTS®** Gluten Related Disorders is a membrane-bound enzyme immunoassay for the quantitative determination of IgA and/or IgG antibodies against gliadin, DGP, tTG, tTG neo, TG 3, mTG neo, mTG, and Frazer's Fraction (digested gliadin by Frazer et al. 1959) in human serum or plasma.



AESKUBLOTS® Gluten Related Disorders IgA



AESKUBLOTS® Gluten Related Disorders IgG



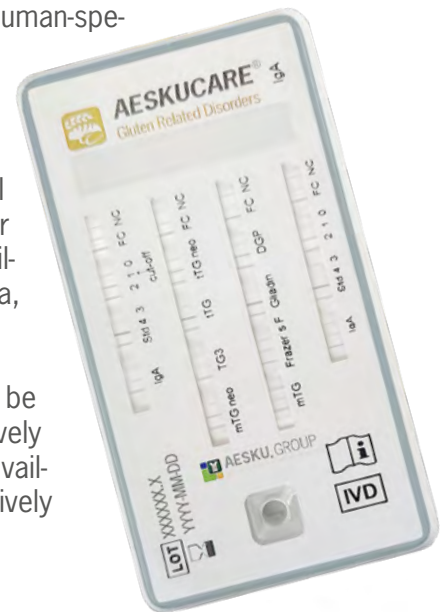
coming soon

AESKUCARE® Gluten Related Disorders IgA



AESKUCARE® Gluten Related Disorders IgA is a near-patient (point of care) in-vitro immunoassay intended for the qualitative and quantitative simultaneous measurement of human-specific IgA antibodies against tTG, tTG neo, mTG, mTG neo, DGP, gliadin, Frazer's Fraction, TG3, and total IgA in heparinized or Na-EDTA venous or capillary whole blood, plasma, or serum.

IgA concentrations can be determined quantitatively by using a separately available reader or qualitatively by the naked eye.



AESKU GRD antigens – Description

Gliadin

Anti-gliadin antibodies are significantly elevated in non-celiac individuals with oral ulcers 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

Anti-gliadin antibodies from CD patients bind to a very limited number of specific epitopes on the gliadin molecule. Binding of the anti-gliadin antibodies is strengthened by the selective deamidation of gliadin by the tTG. Therefore, test systems using DGPs show a higher diagnostic accuracy compared to previous anti-gliadin tests.

tTG

The enzyme tTG 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

Formation of neo-epitopes with tTG is induced by cross-linking of tTG with gliadin-specific peptides. These neo-epitopes are structurally more similar to physiological epitopes than previously used antigens and can significantly increase the sensitivity and specificity of the test. Studies have also shown that tTG neo can be used as a marker for dermatitis herpetiformis (DH).

TG 3

Transglutaminase 3, or epidermal transglutaminase, is expressed in the epidermis. Mutations in the TGM3 gene lead, among other things, to the syndrome of uncombable hair or dermatitis herpetiformis Duhring. The latter syndrome is a skin disease from the group of blistering autoimmune dermatoses. In addition to the noticeable itchy blisters and lumps, CD is one of the causes of this disease. For the diagnosis of DH, it was

shown that TGM3 is a highly sensitive marker and can further improve the diagnosis of DH.

mTG neo

The microbial transglutaminase (mTG) is a common additive used in the food industry. Like tTG, mTG is able to crosslink with gliadin-specific peptides and is, therefore, able to form so-called mTG-epitopes. Anti-mTG-epitopes antibodies can also be observed in Celiac Disease patients and correlate with the Marsh index. Furthermore, mTG-epitopes can be found in other GRDs like Gluten Sensitivity.

mTG

is the main cause of the formation of the so-called mTG-epitopes, which are suspected of being an additional trigger of GRD. However, antibodies against the enzyme itself are very rare.

Frazer's Fraction

Gliadin and glutenin partially peptic-tryptic digested.^[1] As early as 1959, Frazer et al. showed that oral intake of PT gliadin is harmful to CD 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.

IgA

To detect an IgA deficiency. 2-2.5% of Celiac Disease patients have an IgA deficiency and thus negative IgA autoantibody tests even with active disease. Testing for IgG antibodies is recommended if there is a proven IgA deficiency.

1. REINKE, Y., ZIMMER, K.-P., & NAIM, H. Y. (2009). TOXIC PEPTIDES IN FRAZER'S FRACTION INTERACT WITH THE ACTIN CYTOSKELETON AND AFFECT THE TARGETING AND FUNCTION OF INTESTINAL PROTEINS. EXPERIMENTAL CELL RESEARCH, 315(19), 3442-3452

HIGHLIGHTS OF NEO-EPITOPE TESTING

- ▶▶▶ Superior performance as a screening assay
- ▶▶▶ High sensitivity and specificity in the pediatric population
- ▶▶▶ Excellent correlation with Marsh criteria
- ▶▶▶ Patient benefits due to early disease recognition



ORDERING INFORMATION

AESKUCARE® Point-Of-Care Test Kits

REF 860001 AESKUCARE® Gluten Related Disorders IgA

AESKUBLOTS® BLOT Test Kits

REF 401005 AESKUBLOTS® Gluten Related Disorders IgA (24 Tests)

REF 401006 AESKUBLOTS® Gluten Related Disorders IgG (24 Tests)

coming soon

AESKULISA® ELISA Test Kits

REF 3503 AESKULISA® tTg-A New Generation

REF 3504 AESKULISA® tTg-G New Generation

REF 3516 AESKULISA® tTg-GA New Generation

REF 3510 AESKULISA® CeliCheck New Generation

REF 3501 AESKULISA® GliA-A

REF 3502 AESKULISA® GliA-G

REF 3500 AESKULISA® Gliadin-Check

REF 3513 AESKULISA® DGP-A

REF 3514 AESKULISA® DGP-G

REF 3517 AESKULISA® DGP-GA

REF 3515 AESKULISA® DGP-Check



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