AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS

LOOK CLOSER TO THE TRUTH!

AESKULISA® tTg NEW GENERATION
A NOVEL APPROACH TO CELIAC DISEASE TESTING

AESKULISA® NEW GENERATION tTg: LOOK CLOSER TO THE TRUTH!

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Tissue Transglutaminase (tTg) has been identified as the major target antigen in endomysium in 1997 by Dieterich and Schuppan et.al[1]. Over the last 10 years, gliadin testing has been replaced by tTg testing thus, celiac disease (CD) diagnosis has risen to a sensitivity and specificity level unknown before.
Even after this main serological discovery, a vast majority of celiac patients still remain undetected (ratio of known to unknown CD is 1 to 7)[2] and there's a mean delay on a CD diagnosis of 5 to 11 years.[3]
Several theories about the "iceberg model" were published, and studies have shown that CD also exists in different underestimated forms referred to as potential, latent and silent CD.
Meta-analyses of current studies indicate that celiac disease affects 1% of the population worldwide. Risk groups (like DM type 1 and osteoporosis) have an even higher prevalence (from 1:20 to 1:7).[3]

SO, IS tTg TESTING JUST THE TIP OF THE CD ICEBERG?

In 2002, with this question in mind we started researching on several scientific discoveries. The basic idea behind the research was to observe how tTg and Gliadin, as a component of our diet, could induce toxicity leading to an autoimmune phenomenon.

When looking at the way Gliadin is digested by our enzymes, some peptides of gliadin are resistant to further degradation.[4] These resistant gliadin peptides are an ideal target for tTg due to their high percentage of glutamine residues (35%).

> *"Incubation of tTg … with the known substrate peptide B-α-I* ***resulted in complex formation between the peptide and tTg…*** *These covalent complexes might derive from thioester formation between Cys-277 in the active site of tTg and the side chain of Gln-65 in B-α-I. Alternatively, tTg itself could act as an acyl acceptor molecule, resulting in isopeptide bond formation."*[5]

These residues can be deamidated or crosslinked by tTg on a ratio of 1:4.

> "The binding of the epitope peptide to tTg supports the hypothesis of Sollid et.al (1997 Gut 41) that T cell immune response to gliadin would drive antibody responses towards tTg that is cross-linked to gliadin T cell epitopes. The crosslinking occurs, as demonstrated in this paper and recently by others (Fleckenstein JBC 2004) also outside the active site of tTg."
> ***"…we found a ratio between deamidation and transamidation (cross-linking) of 1:4 for tTg…"*** [6]

After the *"in-vitro"* studies, we looked for an *"in-vivo"* study confirmation, and it was found that all these modifications (crosslinking and deamidation) also occur *"in-vivo"* and complexes of tTg and gliadin can be found in duodenal mucosa.

> *"Taken together, our results suggest that* ***tTg and gliadin form supramolecular complexes*** *directly in normal and diseased duodenal mucosa and that in active CD the levels of both molecules are increased."* [7]

This was the birth of the *AESKULISA®* tTg New Generation!

[1] Dieterich W et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997. 3(7): 797-801
[2] Fasano et.al. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. Gastroenterol. 2001, 163: 636-51
[3] Hopper et.al. Pre-endoscopy serological testing for celiac disease: evaluation of a clinical decision tool. B.M.J. 2007, 334(7596): 729
[4] Shan et.al. Structural basis for gluten intolerance in celiac sprue. Science 2002, 297: 2275-
[5] Fleckenstein B. et.al. Molecular characterization of covalent complexes between tissue transglutaminase and gliadin peptides. J.Biol.Chem. 279 (2004) 17607-16
[6] Skovbjerg H. et.al. Deamidation and crosslinking of gliadin peptides by transglutaminases and the relation to celiac disease. Biochim.Biophys.Acta 1690 (2004) 220-30
[7] R. Ciccocioppo et.al. Gliadin and tissue transglutaminase complexes in normal and celiac duodenal mucosa. Clin.Exp.Immunol. 134 (2003) 516-524

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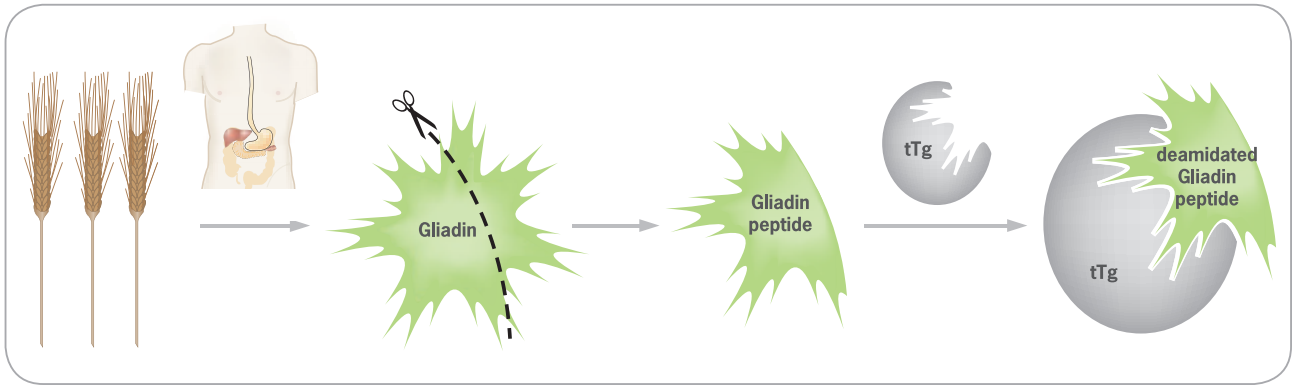


Cereals contain Gliadin

Gliadin digestion 35 % Glutamine* 15 % Proline**

undigested Gliadin peptide

Neo-epitope: deamidated Gliadin peptide crosslinked with tTg

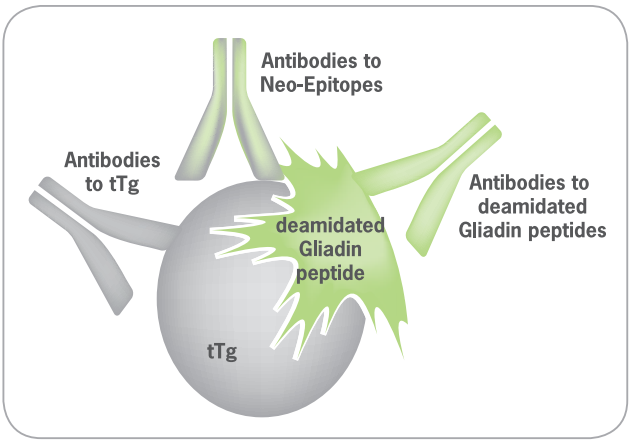
These physiological processes are mimicked by the antigen used for the *AESKULISA*® tTg New Generation:

In our special production process tTg and gliadin peptides are reacting under special conditions comparable to the "*in-vivo*" environment, in order to encourage both reactions: crosslinking and deamidation.

The whole reaction ensures the formation of the neo-epitope as it is encountered by the immune system "*in-vivo*". These crosslinked and deaminated antigens (tTg / deamidated gliadin peptides) are then further purified and coated under special conditions to the microtiter plate.

Due to its special formulation, the *AESKULISA*® tTg New Generation is able to detect 3 different types of antibodies:

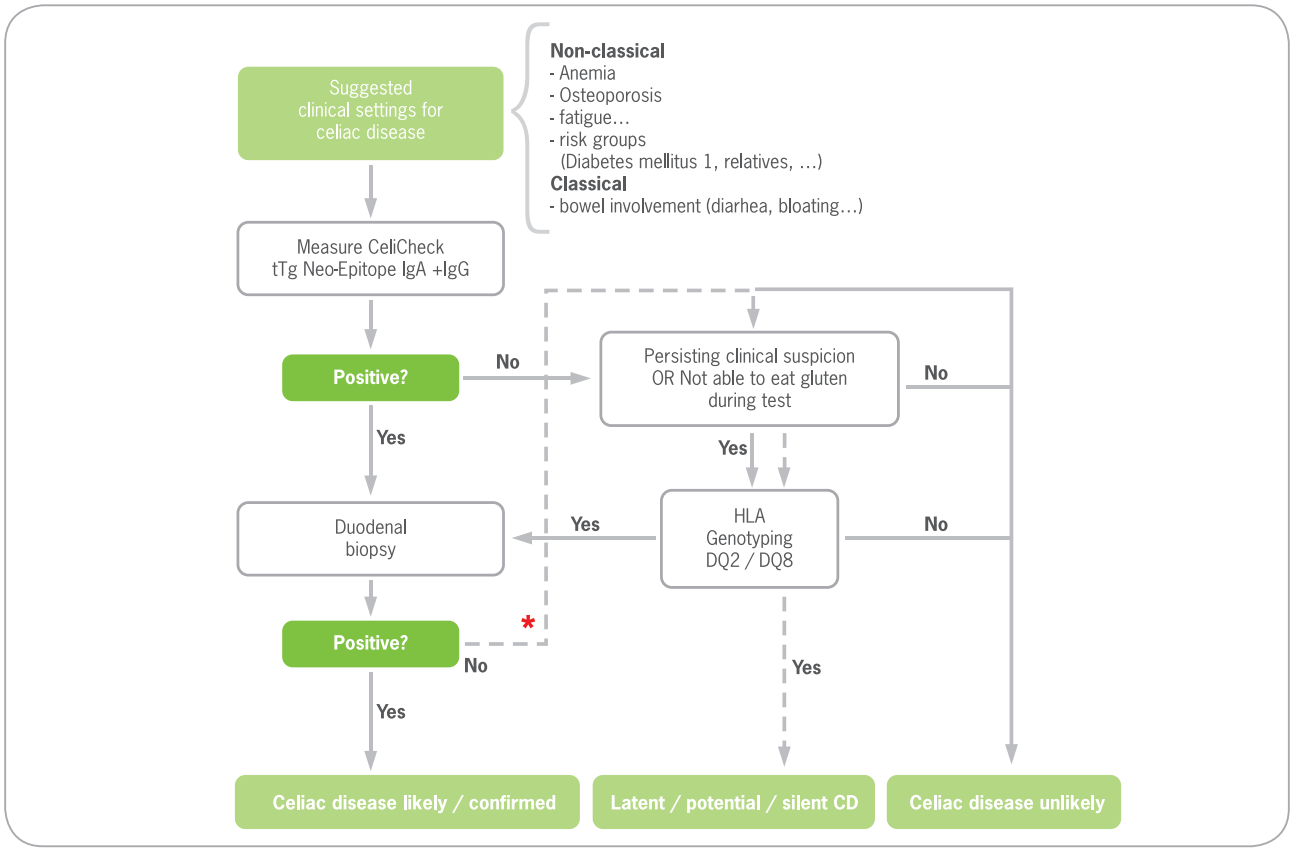
- Antibodies to tTg
- Antibodies to deamidated Gliadin peptides
- Antibodies to Neo-Epitope



* Part of the Glutamine is going to be deamidated or crosslinked
** Proline prevents complete digestion of Gliadin

SUGGESTED APPROACH TO COELIAC DISEASE DIAGNOSIS[8,9]

- Anti-gliadin testing is no longer recommended for routine coeliac screening
- Anti-endomysial antibody testing is no longer recommended for routine coeliac screening
- Celiac HLA Genotype testing is not a good screening test for CD
- Combined IgA and IgG tTg testing offers high sensitivity and specificity for diagnosis of CD where it is clinically suspected, but clinical & histological (gut or skin) parameters remain the final arbiters
- tTg levels correlate with gluten intake, and can be used as a guide to assessment of compliance with a gluten- free diet
- In contrast, Celiac HLA Genotyping displays a high negative predictive value (hence substantially lowering likelihood of CD), and should therefore be considered in any of the settings shown in the following chart. Risks groups has DM1 and osteoporosis should be screened for CD[10]



* SIX CAUSES OF SEROLOGICAL - HISTOLOGICAL DISCREPANCY IN CD[8]

- Self-imposed dietary restriction leading up to time of biopsy (tTg results are falsely negative in the absence of gluten intake)
- Management of associated immune conditions (e.g. corticosteroids can normalize gut architecture)
- Tissue analysis problems (sampling issues; subtle intra-epithelial lymphocytosis)
- Extra-intestinal CD (many dermatitis herpetiformis patients have „normal“ / subtly abnormal small bowel biopsy)
- “Pre-Disease” (Latent CD) - not yet evident at gut biopsy level, but will evolve into CD with time
- True “false-positive” results (unlikely if result > 2 S.D. above mean (> 50 U/L))

[8] G. E. Reeves. Coeliac disease: against the grain. Intern.Med.J. 34 (9-10):521-525, 2004.
[9] personal communication with G.E. Reeves
[10] L. Rodrigo. Celiac disease. World J.Gastroenterol. 12 (41):6585-6593, 2006.

SUGGESTED APPROACH TO COELIAC DISEASE DIAGNOSIS

SCREENING FOR CELIAC DISEASE AESKULISA® CELICHECK

Current diagnostics of celiac disease increasingly identifies non-classic, asymptomatic variants. Recent epidemiological studies demonstrate that the prevalence of celiac disease is approximately 1:105, which is significantly higher than previously assumed. Consequently, serological screening methods gain increasing importance, for example in risk groups like diabetes mellitus type I patients.

In a study with 254 individuals[10], tTg tests from various manufacturers have been compared. With a superior sensitivity of 92%, the *AESKULISA®* CeliCheck New Generation turned out to be the ideal screening tool for this purpose - the use of a conjugate mix allows the detection of IgA and IgG tTg antibodies in one working step only.
The *AESKULISA®* CeliCheck provides a sensitivity of 92%, so that it is ideally suited for screening purposes and is also superior to EMA immunofluorescence.

With the *AESKULISA®* tTg New Generation ELISA, a markedly increased sensitivity for IgG has been found as compared to all other manufacturers. Thus, the new generation *AESKULISA®* tTg New Generation ELISA tests are the only one showing comparable high sensitvities for both IgA and IgG. This stresses the significance of IgG tTg testing for all patients, not only for those with a selective IgA deficiency.

Kit	Sensitivity	Specificity
AESKULISA® CeliCheck	92	83
D-Tek Dual	90	81
AESKULISA® IgA	85	86
AESKULISA® IgG	85	89
Bindazyme IgA	81	94
Bindazyme IgG	65	86
Celikey IgA	81	94
Celikey IgG	67	82
D-Tek IgA	17	97
D-Tek IgG	17	90
Euroimmun IgA	81	84
Euroimmun IgG	77	85
Eurospital IgA	92	81
Eurospital IgG	77	86
Fidis IgA	88	85
Genesis IgA	73	95
Genesis IgG	69	83
Inova IgA	77	87
Inova IgG	42	86
Orgentec IgA	88	84
Orgentec IgG	69	82

THE AESKULISA® PRODUCT LINE: INNOVATIVE DIAGNOSTICS EASY TO USE

AESKULISA® tests are already established as an ideal partner for laboratory automation systems as all kits are based on the same workflow:	**The user benefits from:**
• One protocol • One sample dilution • One buffer system • One cut-off • One standard curve	Maximum specificity and sensitivity by • using recombinant antigens • Breakaway microwells • Ready-to-use reagents • Colour-coded vials and reagents • Concentration-dependent colour coding of calibrators • Short incubation times

3503 *AESKULISA®* tTg-A New Generation

Quantitative and qualitative determination of IgA antibodies to neo-epitopes of tissue transglutaminase crosslinked with deamidated gliadin peptides

3504 *AESKULISA®* tTg-G New Generation

Quantitative and qualitative determination of IgG antibodies to neo-epitopes of tissue transglutaminase crosslinked with deamidated gliadin peptides

3510 *AESKULISA®* CeliCheck New Generation

Combined quantitative and qualitative determination of IgA and IgG antibodies to neo-epitopes of tissue transglutaminase crosslinked with deamidated gliadin peptides

3533 *AESKULISA®* tTg-A

Quantitative and qualitative determination of IgA antibodies to tissue transglutaminase

3534 *AESKULISA®* tTg-G

Quantitative and qualitative determination of IgG antibodies to tissue transglutaminase

3501 *AESKULISA®* GLIA-A

Quantitative and qualitative determination of IgA antibodies to alpha gliadin

3502 *AESKULISA®* GLIA-G

Quantitative and qualitative determination of IgG antibodies to alpha gliadin

AESKU.DIAGNOSTICS
MIKROFORUM RING 2 I 55234 WENDELSHEIM I GERMANY
PHONE: +49 (0) 6734 9627-0 I FAX: +49 (0) 6734 9627-27
EMAIL: SALES@AESKU.COM

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