New serological biomarkers of celiac disease: The neo-epitope tTg/mTg story

Prof. Aaron Lerner¹,², Dr. Torsten Matthias²
5th European Immunology Conference, July 21-23, Berlin, Germany

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Human Transglutaminase Family

**The TG family**

- TG1: keratinocyte transglutaminase
- **TG2: tissue transglutaminase**
- TG3: epidermal transglutaminase
- TG4: prostate transglutaminase
- TG5: transglutaminase 5
- **TG6: neuronal transglutaminase**
- TG7: transglutaminase 7
- FXIII: blood coagulation factor XIII
- B4.2: inactive structural protein

**Functions**

- Post translational modifier of proteins:
  - Cross-linking
  - Deamidation

**Autoimmune Diseases**

- Celiac Disease
- Dermatitis Herpetiformis
- Gluten Ataxia

[adapted and modified from www.zedira.de]
Tissue Transglutaminase (TG2): central role in biological processes and diseases

Compact (inactive) and extended (active) conformations of tTG
Schematic representation of the proposed model for CD pathogenesis

Adapted and modified from: Sanz Y. Microbiome and Gluten. Ann Nutr Metab. 2015;67 Suppl 2:28-41
Antibodies in Celiac Disease

Anti-tTg antibodies

Anti-DGP antibodies

Gliadin [PDB ID 2Q3Z]

Gliadin peptide

Glutamine rich

Deamidation

(glutamic acid ← glutamine)

25%

Crosslinking

25%

75%

Crosslinking by tTg

Anti-tTg neo-epitope antibodies

Courtesy of Dr. Christian Meesters, AESKU.KIPP Institute 2012//
A. Lerner et al., Antibodies against neo-epitope tTg complexed to gliadin are different and more reliable then anti-tTg for the diagnosis of pediatric celiac disese (Journal of immunological Methods 429 (2016) 15-20)
Overall immunoreactivity in PCD patients

A significantly higher OD activity was detected for tTg neo-epitope IgA, IgG and IgA+ IgG than for tTg.

A. Lerner et al., Antibodies against neo-epitope tTg complexed to gliadin are different and more reliable than anti-tTg for the diagnosis of pediatric celiac disease (Journal of immunological Methods 429 (2016) 15-20)
# Diagnostic power of neo-epitopes

**n=198 pediatric samples of an actual study**

<table>
<thead>
<tr>
<th>ELISA</th>
<th>sensitivity</th>
<th>specificity</th>
<th>AUC</th>
<th>Correlation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>hTG2 IgG</td>
<td>12.12</td>
<td>98.99</td>
<td>0.56</td>
<td>0.2601</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hTG2 IgA</td>
<td>58.59</td>
<td>98.99</td>
<td>0.79</td>
<td>0.4692</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tTg neo IgG</td>
<td><strong>77.78</strong></td>
<td>97.98</td>
<td>0.88</td>
<td>0.5334</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hTG2 Check</td>
<td>79.8</td>
<td>98.99</td>
<td>0.89</td>
<td>0.6093</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tTg neo IgA</td>
<td><strong>86.87</strong></td>
<td>98.99</td>
<td>0.93</td>
<td>0.6165</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tTg neo check</td>
<td><strong>97.98</strong></td>
<td>100</td>
<td>0.99</td>
<td><strong>0.6492</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EMA IgA</td>
<td>90.2</td>
<td>94.1</td>
<td>0.94</td>
<td><strong>0.8098</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EMA IgG</td>
<td>55.7</td>
<td>99.3</td>
<td>0.78</td>
<td>0.5996</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

> **tTg showed the highest sensitivity in its combined version IgA + IgG (Check)**

A. Lerner et al., Antibodies against neo-epitope tTg complexed to gliadin are different and more reliable then anti-tTg for the diagnosis of pediatric celiac disease (Journal of immunological Methods 429 (2016) 15-20)
The genetic environmental balance in autoimmunity
The human microbiome project says the human body has 100 trillion microscopic life forms living in it.

You call this living?
Microbiological transglutaminase (mTg)

- Molecular weight: 38 kDa
- Most of the identified mTgs derived from *Streptomyces* (ubiquitary living soil microorganisms)
- Comparable enzymatic activity as tTg
- Difference to tTg:
  - Ca^{2+} independent protein
  - Lower substrate specificity

Increase of publications about "microbial transglutaminase" after discovery in 1989.

Peptides crosslinking,
de/amination/deamidation,
de/phosphorylation,
a/deacetylation,
de/tyrosination,
de/glutamylation,
de/glycylation,
ubiquitination,
palmitoylation,
glycosylation,
galactosylation,
arginylation,
methylation,
citrullination,
sumoylation
carbamylation
Ect................
The reactions catalyzed by transglutaminase include:

**A. acyl-transfer reaction;**

**B. cross-linking** reaction between Gln and Lys residues of proteins or peptides.

**C. deamidation**
A schematic presentation of the interplay between gliadin, tTg and mTg enzymes and their corresponding complexes and specific antibodies.

A Lerner, P Jeremias, S Neidhöfer, T Matthias. Antibodies against neo-epitope tTg complexed to gliadin are different and more reliable then anti-tTg for the diagnosis of pediatric celiac disease. J Immunol Methods. 2016;429:15-20.

Deamidation
(glutamic acid ← glutamine)

Gliadin
Gliadin peptide
Glutamine rich

Anti-DGP antibodies

Anti-tTg antibodies

Anti-tTg neo-epitope antibodies

Deamidated Gliadin peptide

25%

75%

Crosslinking by tTg

Crosslinking by mTg

Courtesy of Dr. Christian Meesters, AESKU.KIPP Institut 2012
The different conformational states of tTg and mTg and their effects on cells

**tTg**
- **Closed Conformation** (catalytically inactive)
- **Open Conformation** (catalytically active)
- **Ca^{2+}** regulated
- **GTP or GDP** regulated
- **α-hel**
- **β-st**
- **PDB ID:** 4PYG
- **PDB ID:** 2Q3Z

**mTg**
- **α-hel**
- **β-st**
- **PDB ID:** 1IU4

- **Crosslinking**
- **Ca^{2+} independent**
- **Nucleotide independent**

**tTg**
- **Nucleotide-bond**
- **Crosslinking deficient**
- **PROMOTES CELL GROWTH AND SURVIVAL**

**mTg**
- **Nucleotide-free**
- **Crosslinking active**
- **INDUCES CELL DEATH**

Singh, Jingwen Zhang, Yilun Ma, Richard A. Cerione and Marc A. Antonyak; The Different Conformational States of Tissue Transglutaminase Have Opposing Affects on Cell Viability Garima, JBC Papers in Press on February 18, 2016
## Application of mTgase on food processing

<table>
<thead>
<tr>
<th>Source</th>
<th>Product</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>Restructured meat</td>
<td>Restructured meat texture and appearance, increased hardness</td>
<td>Kuraishi et al. (1997); Motoki and Seguro (1998); Trespalacios and Pla (2007)</td>
</tr>
<tr>
<td>Fish</td>
<td>Fish paste, restructured product</td>
<td>Increased hardness</td>
<td>Téllez-Luis et al. (2002)</td>
</tr>
<tr>
<td>Milk</td>
<td>Cream, deserts, milk drinks, dressings</td>
<td>Improved quality and texture</td>
<td>Lauber et al. (2000); Şanlı et al. (2011)</td>
</tr>
<tr>
<td>Casein</td>
<td>Cross linked protein</td>
<td>Allergenicity reduction</td>
<td>Lauber et al. (2000); Ozer et al. (2007)</td>
</tr>
<tr>
<td>Wheat</td>
<td>Baked foods</td>
<td>Improved texture and high volume</td>
<td>Gerrard et al. (2001)</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Sweet foods</td>
<td>Low calorie foods with good texture and elasticity</td>
<td>Giosafatto et al. (2012)</td>
</tr>
</tbody>
</table>


Mahta Mirzaei et al. 2011 // M. Griffin et al. 2002
Examples of mTg application versatility

<table>
<thead>
<tr>
<th>Food applications</th>
<th>Protein-DNA</th>
<th>Protein-Polymer</th>
<th>Protein-Protein</th>
<th>Antibody radioimmunoconjugate</th>
<th>Antibody Drug conjugate</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Food applications" /></td>
<td><img src="image2" alt="Protein-DNA" /></td>
<td><img src="image3" alt="Protein-Polymer" /></td>
<td><img src="image4" alt="Protein-Protein" /></td>
<td><img src="image5" alt="Antibody radioimmunoconjugate" /></td>
<td><img src="image6" alt="Antibody Drug conjugate" /></td>
</tr>
</tbody>
</table>

The change in modern nutrition!

www.onegreenplanet.org
Change in nutrition responsible for increasing CD numbers?

U.S. FOOD CONSUMPTION
AS A % OF CALORIES

PLANT FOOD:
Vegetables, Fruits, Legumes, Nuts & Seeds, Whole Grains
Fiber is only found in plant foods.

NOTE: Up to half of this category may be processed, for example almonds in candy bars, apples in apple pies or spinach in frozen spinach soufflé, and of course these would not be healthy choices. The focus should be on whole unprocessed vegetables, fruits, legumes, nuts and seeds and whole grains.

12%

ANIMAL FOOD:
Meat, Dairy, Eggs, Fish, Seafood
Cholesterol is only found in animal foods. Animal foods are the PRIMARY source of saturated fat.

25%

63%

PROCESSED FOOD:
Added Fats & Oils, Sugars, Refined Grains

New York Coalition for Healthy School Food * www.healthychoicesfood.org
Special thanks to Joel Fuhrman, MD, author of Disease Proof Your Child: Feeding Kids Right * Graphics by MichelleBando.com
© 2009, New York Coalition for Healthy School Food

http://draxe.com/charts-american-diet/
Change in nutrition responsible for the increasing numbers of ADs?

Lerner A, Matthias T. Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease. Autoimmun Rev. 2015;14:479-89
### Mechanisms of food additives to increase intestinal permeability

<table>
<thead>
<tr>
<th>Food additives</th>
<th>Net % increase/year</th>
<th>Mechanism of TJ integrity breaching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>2.9±2.8</td>
<td>Change in distribution of ZO-1, claudin-1, E cadherin. Perijunctional cytoskeleton condensation.</td>
</tr>
<tr>
<td>Salt</td>
<td>7.1±5.3</td>
<td>Increased phosphorylation of myosin light chain, contraction of the perijunctional actomyosin ring, loss of function of claudin 2 and 15.</td>
</tr>
<tr>
<td>Organic solvents</td>
<td>-2.9±9.3</td>
<td>Alterations in TJ proteins, dissociates the PTP1B-E-cadherin-beta-catenin complex</td>
</tr>
<tr>
<td>Emulsifiers</td>
<td>6±8.4</td>
<td>P-glycoprotein inhibition, decrease the hydrophobicity of the mucus layer, actine disbandment and structural separation of TJ, change the distribution of ZO-1 and actine.</td>
</tr>
<tr>
<td>Gluten</td>
<td>1.8±0.4</td>
<td>Rearrangement of the cytoskeleton through the zonulin pathway, reduces F-actin content, interaction between occludin and Zo-1 is compromised, zonulin release is leading to PKC-mediated cytoskeleton reorganization, zonulin release by binding to the CXCR3 receptor in intestinal cells, in a MYD88-dependent pathway and subsequent transactivation of EGFR by PAR2.</td>
</tr>
<tr>
<td>Microbial transglutaminase</td>
<td>21.9±7.4</td>
<td>Increases luminal microbial load, <strong>Hypothesis</strong>: cross-linking TJ proteins, imitating emulsifiers and nanoparticles functions.</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>25.8±21.5</td>
<td>Redistribution of ZO-1 TJ proteins, clustering of integrin α(V)β(3) along the cell border, F-actin reorganization and claudin 4 down regulation, open epithelial TJ via C-Jun Nh2-terminal kinase-dependent pathway, TJ electrostatic interactions</td>
</tr>
</tbody>
</table>
Microbial transglutaminase & Celiac Disease

<table>
<thead>
<tr>
<th>Processed/raw</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doughnut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>processed</td>
<td>118</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>Raw</td>
<td>130</td>
<td>85</td>
<td>32</td>
</tr>
<tr>
<td>wheat</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>barley</td>
<td>10</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>yeast</td>
<td>14</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>soy</td>
<td>18</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Cake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>processed</td>
<td>138</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>Raw</td>
<td>134</td>
<td>84</td>
<td>32</td>
</tr>
<tr>
<td>wheat</td>
<td>66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>egg</td>
<td>12</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>corn</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>milk</td>
<td>34</td>
<td>54</td>
<td>18</td>
</tr>
<tr>
<td>Cereal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>processed</td>
<td>124</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>Raw</td>
<td>138</td>
<td>84</td>
<td>30</td>
</tr>
<tr>
<td>wheat</td>
<td>64</td>
<td>42</td>
<td>20</td>
</tr>
<tr>
<td>oat</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>corn</td>
<td>10</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>rice</td>
<td>8</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>barley</td>
<td>9</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>milk</td>
<td>34</td>
<td>52</td>
<td>12</td>
</tr>
</tbody>
</table>
Similarity between mTg and tTg?

3D structure alignment of mTg and tTg

Yellow = conserved segments.

Structural alignment of mTg and tTg on their catalytical triade.

Closer look to the active center

mTg (magenta) Cys 64 – Asp 255 – His 274

tTg, active (green) Cys 277 – His 335 – Asp 358

tTg, inactive (blue)
Docking of gliadin peptide (PDB-ID: 1NNA) with mTg (left side) and tTg (right side). The arisen complexes got stained among their electrostatic profile; red = negative charges.

[Pictures produced with YASARA, Courtesy of Dr. Christian Meesters, AESKU.KIPP Institute 2012]
Sera immunoreactivity of the neo-epitopes

Immunoreactivity of the mTg/tTg neo-epitope anti IgA and IgG and the single compounds mTg, tTg and gliadin (not complexed to gliadin complexes) anti IgA and IgG.
Antibodies’ activity in relation to the degree of intestinal damage in CD

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Correlation coefficient r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>tTg neo check</td>
<td>0.6492</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>tTg neo IgA</td>
<td>0.6165</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>mTg neo IgG</td>
<td>0.5633</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>tTg neo IgG</td>
<td>0.5334</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>mTg neo check</td>
<td>0.5127</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>mTg neo IgA</td>
<td>0.3018</td>
<td>0.003</td>
</tr>
</tbody>
</table>
Competition ELISA (human IgA)

Competition to the “IgA neo-epitope” showed a decrease in response if transglutaminases complexed with gliadin peptides were added.

* p<0.05 // ** p< 0.005
## Diagnostic power of neo-epitopes

n=198 pediatric samples (CD=99, N=99)

<table>
<thead>
<tr>
<th>ELISA</th>
<th>sensitivity</th>
<th>specificity</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>mTg neo IgA</td>
<td>64.65</td>
<td>98.99</td>
<td>0.82</td>
</tr>
<tr>
<td>tTg neo IgG</td>
<td>77.78</td>
<td>98.0</td>
<td>0.88</td>
</tr>
<tr>
<td>mTg neo Check</td>
<td>90.91</td>
<td>87.88</td>
<td>0.89</td>
</tr>
<tr>
<td>hTG2 Check</td>
<td>79.8</td>
<td>98.99</td>
<td>0.89</td>
</tr>
<tr>
<td>tTg neo IgA</td>
<td>88.89</td>
<td>98.99</td>
<td>0.94</td>
</tr>
<tr>
<td>mTg neo IgG</td>
<td>94.96</td>
<td>93.94</td>
<td>0.95</td>
</tr>
<tr>
<td>tTg neo check</td>
<td>97.98</td>
<td>98.99</td>
<td>0.98</td>
</tr>
</tbody>
</table>

- mTg neo-epitope IgG had higher sensitivity than tTg neo-epitope IgG
- tTg neo-epitope showed the highest sensitivity in its combined version IgA + IgG (Check)
Schematic representation of the proposed model for CD pathogenesis

Adapted and modified from: Sanz Y. Microbiome and Gluten. Ann Nutr Metab. 2015;67 Suppl 2:28-41
Effects of mTg that might drive autoimmunity

Luminal (green) and systemic (red) effects of the mTg that might drive autoimmunity

Intestinal dysbiotic transglutaminases are potential environmental drivers of systemic autoimmunogenesis
Aaron Lerner, Rustam Aminov, Torsten Matthias. Frontiers in Microbiology, 2016
Effects of mTg that might drive autoimmunity

It is tempting to estimate the amount and activity of mTg that reside in the human gut during daily life. Three sources can be envision: from the industrial processed food, from the ingestion of probiotic and from the micro/dysbiota luminal residents. Based on the following observations, a hypothetical estimate can be suggested:

1. Each kilogram of food treated with microbial transglutaminase contains about 50–100mg of microbial transglutaminase.
2. Examples of the mean yield of bacteria used by the industries for mTg production is Streptoverticillium sp 1.43 unit/ml, Streptoverticillium mobaraense (ajinomoto “wild type”) 1.25 unit/ml or 22 unit/mg and Escherichia coli 19.55 unit/mg.
3. No information to my knowledge, is available, on the amount of tTg secreted in the human gut lumen, however Tgase activity is depicted in the human jejunum.
4. As a result of the increasing use of microbial transglutaminase in the food industry, the common Western diet now contains large amounts of microbial transglutaminase, with the maximum daily intake ranging up to 15 mg.
5. Assuming additive effect of a daily intake of 10⁸ viable human originated probiota (Bifidobacterium bifidum/longum/adolescentis/infantis) , as probiotics, that secret mTg to the 100 trillion bacteria constituting the human microbiome, with their mTg load, one can reach a substantial continuous mTg activity residing constantly in the human intestinal lumen.

- Interestingly enough, mTg crosslinking can be used as a new probiotic carrier for enhanced gastrointestinal transit and storage.

Conclusions

- **tTg is the autoantigen of CD** and plays a **pivotal role in PTM** of the gliadin and CD autoimmunogenesis
- **tTg is essential for human** and **mTg for bacterial survival**
- **mTg & tTg are capable to deamidate and crosslink proteins, including gliadin**
- **mTg** is **Ca^{+2} independent**, less substrate specific, secreted by the luminal microbes and is a heavily used industrial food additive.
- **CD patients** are **immunoreactive to the neo-epitopes mTg and tTg**. Both antibodies represents **new bio-markers** for CD diagnosis.
<table>
<thead>
<tr>
<th>mTg parameters</th>
<th>Industrial producers</th>
<th>Processed food products</th>
<th>Human effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td>None</td>
<td>Yes</td>
<td>Intestinal luman</td>
</tr>
<tr>
<td>Toxic</td>
<td>No</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Immunogenic</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Allergic</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>pathogenic</td>
<td>no</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
Environmental and pathophysiological aspects involving tTg & mTg in CD

Gut-Systemic autoimmunity Axis


Intestinal permeability and TJ functions

Small bowel organoid
CD animal model
Subepithelial mTg deposits
Reactive, innate immunity

Immunogenicity of mTg products
HLA presentation of mTg treated peptides

ADs serum biobanks
Intestinal permeability and TJ functions

Looking for scientific cooperation

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Dr. T. Matthias
Dr. S. Neidhöfer
P. Jeremias
T. Barth
Dr. S. Reuter
Dr. P. Trinder
Dr. K. Prager
A. Neu

Carmel Medical Center,
Technion-Israel Institute of Technology
B. Rappaport School of Medicine
Haifa, Israel
**mTg-neo-epitope check diagnostic performance in CD and other ADs, compare to controls**

<table>
<thead>
<tr>
<th>Diseases</th>
<th># Tested</th>
<th># positive AESKULISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celiac Disease</td>
<td>99</td>
<td>97 (97.9%)</td>
</tr>
<tr>
<td>Celiac Disease (GFD)</td>
<td>5</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Disease control (total)</td>
<td>1037</td>
<td>87 (8.4%)</td>
</tr>
<tr>
<td>IBD</td>
<td>52</td>
<td>7 (13.5%)</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>575</td>
<td>54 (9.4%)</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>58</td>
<td>5 (8.6%)</td>
</tr>
<tr>
<td>Adult Healthy donors</td>
<td>264</td>
<td>7 (2.7%)</td>
</tr>
<tr>
<td>Pediatric Healthy Donors</td>
<td>88</td>
<td>14 (15.9%)</td>
</tr>
</tbody>
</table>

**mTg-neo-epitope check diagnostic performance**

<table>
<thead>
<tr>
<th>AESKULISA mTg-neo-epitope check</th>
<th>Pos</th>
<th>Neg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>97</td>
<td>2</td>
<td>99</td>
</tr>
<tr>
<td>Neg</td>
<td>87</td>
<td>950</td>
<td>1037</td>
</tr>
<tr>
<td>Total</td>
<td>184</td>
<td>952</td>
<td>1136</td>
</tr>
</tbody>
</table>

Area under the Curve: 0.95

Sensitivity: 97.98 %
Specificity: 91.61 %

- The mTg-gliadin complexes were separated by size fractionation.
- Defolding and digesting experiments were performed to prove the complex formation as well as the stability of the immunopotent epitopes after treatment of the complexes.
- The ELISA antigenic complexes are not linear and the 3D confirmation carry the antigenic epitopes.