



**A novel quantitative ELISA as accurate and reproducible tool to detect epidermal transglutaminase antibodies in patients with Dermatitis Herpetiformis**

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**TITLE**

A novel quantitative ELISA as accurate and reproducible tool to detect epidermal transglutaminase antibodies in patients with Dermatitis Herpetiformis

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3 Serology for anti-epidermal transglutaminase antibodies (Anti-TG3 Abs) has attracted interest,  
4 being inexpensive and non-invasive and could ideally replace skin biopsy for the diagnosis of  
5 Dermatitis Herpetiformis (DH)<sup>1</sup>. The current commercially available ELISA (Elisa Kit) for serum  
6 Anti-TG3 Abs overall has a good specificity (84-100%)<sup>2-4</sup> however, its clinical use is limited due to  
7 variable sensitivity (45-100%) and low specificity in CD patients (47-85%) in whom Anti-TG3 Abs  
8 may be present in the absence of DH<sup>2,3,5-7</sup>. Here, we developed and evaluated the diagnostic  
9 performance of a novel ELISA to quantitatively measure Anti-TG3 Abs.  
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18 The novel ELISA was set-up using a human activated recombinant TG3<sup>8</sup> (Zedira, Darmstadt,  
19 Germany). To quantify serum Anti-TG3 Abs we produced a human gut-derived monoclonal IgA  
20 Anti-TG3 Ab (hrm-IgA anti-TG3 Ab) and built a dose-response curve of the absorbance vs  
21 concentration correlating the optical density values of different known amounts of the hrm-IgA anti-  
22 TG3. The curve had high affinity and was highly specific and not cross-reactive with TG2. The  
23 intra- and inter-assay CV values for each concentration of the hrm-IgA anti-TG3 Ab and for each  
24 sample of our novel ELISA were good being less than 10% and 15% respectively.  
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33 Stored serum samples of 46 DH patients, 212 CD patients, 120 patients with non-gluten related  
34 gastrointestinal diseases (CTRL) and 168 healthy controls (HC), collected at four University  
35 Hospitals, in Italy and Finland, between 2013 and 2017 were analysed at IRCCS Burlo Garofolo,  
36 Trieste, Italy, using the novel ELISA and the ELISA kit (Immundiagnostik, Bensheim, Germany).  
37 Both assays were run in duplicate for all sera.  
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43 Using the novel ELISA we found that DH patients had significantly higher concentrations of Anti-  
44 TG3 Abs compared to all the other groups ( $p < 0.0001$ ) (Fig. 1a) and that Anti-TG3 Abs  
45 concentrations significantly decreased with gluten free diet ( $p < 0.0001$ ) (Fig. 2). In DH patients  
46 there was a direct correlation between Anti-TG3 and Anti-TG2 Abs concentrations ( $R^2 = 0.3$ )  
47 whereas no statistically significant relationship was found in CD patients ( $R^2 = 0.01$ ).  
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53 For the novel ELISA, the ROC curve analysis identified a cut-off value of IgA Anti-TG3 Abs  
54 concentration of 1.4  $\mu\text{g/ml}$  able to produce a sensitivity of 93% and a specificity of 99% (AUC 0.98)  
55 to distinguish DH patients from HC. This cut-off was used to define a positive test across all  
56 groups. Applying the manufacturer cut-off value (positive value  $> 22$  AU/ml) the ELISA kit gave a  
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3 sensitivity of 83% and a specificity of 90.5% in the same cohort (Fig. 1b). In CD patients, the novel  
4 ELISA gave a specificity of 85% whereas the ELISA kit gave a specificity of 48%. Interestingly, the  
5 specificity of the novel ELISA varied when considering CD patients by age and was higher in  
6 patients  $\leq 25$  years (specificity 97%) versus patients  $> 25$  years (specificity 55%). This difference  
7 was not observed using the ELISA kit.  
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11 To conclude, in this multicentre study, we developed an accurate and reproducible quantitative  
12 novel ELISA assay for the measurement of Anti-TG3 Abs. One reason to explain the good  
13 reproducibility of the test could be the fact that to create the dose-response curve, we used a  
14 monoclonal antibody isolated from the intestinal mucosa, which is likely the primary site of Anti-  
15 TG3 Abs synthesis in DH patients<sup>9</sup>. The availability of a specific standard curve will improve the  
16 comparison of the results obtained from different laboratories and allow a shared diagnostic cut-off  
17 value for the diagnosis of DH. Given its high diagnostic performance, this novel ELISA is suitable  
18 as a screening tool in patients in whom DH is clinically suspected and may serve as the sole  
19 confirmatory test in younger patients. Further studies in larger cohorts are needed to confirm these  
20 results.  
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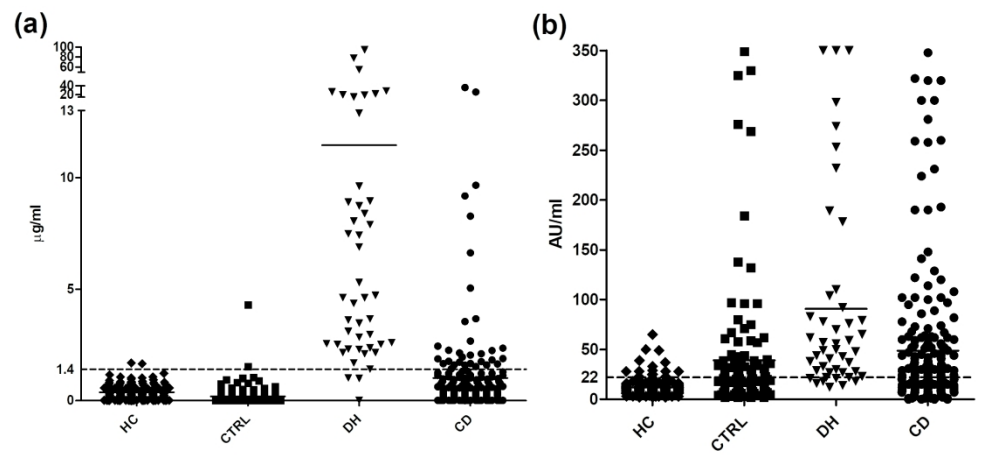


Figure 1 Serum IgA Anti-TG3 antibody concentrations in the four study groups were measured by TG3 ELISA (a) and commercial ELISA kit (b). Dashed lines mark the cut-off value for test positivity. Solid line in each study group is the mean value. DH, Dermatitis Herpetiformis (closed inverted triangles); CD, Coeliac Disease (closed circles); CTRL, non-gluten gastrointestinal disease (closed squares); HC, Healthy Controls (open circles).

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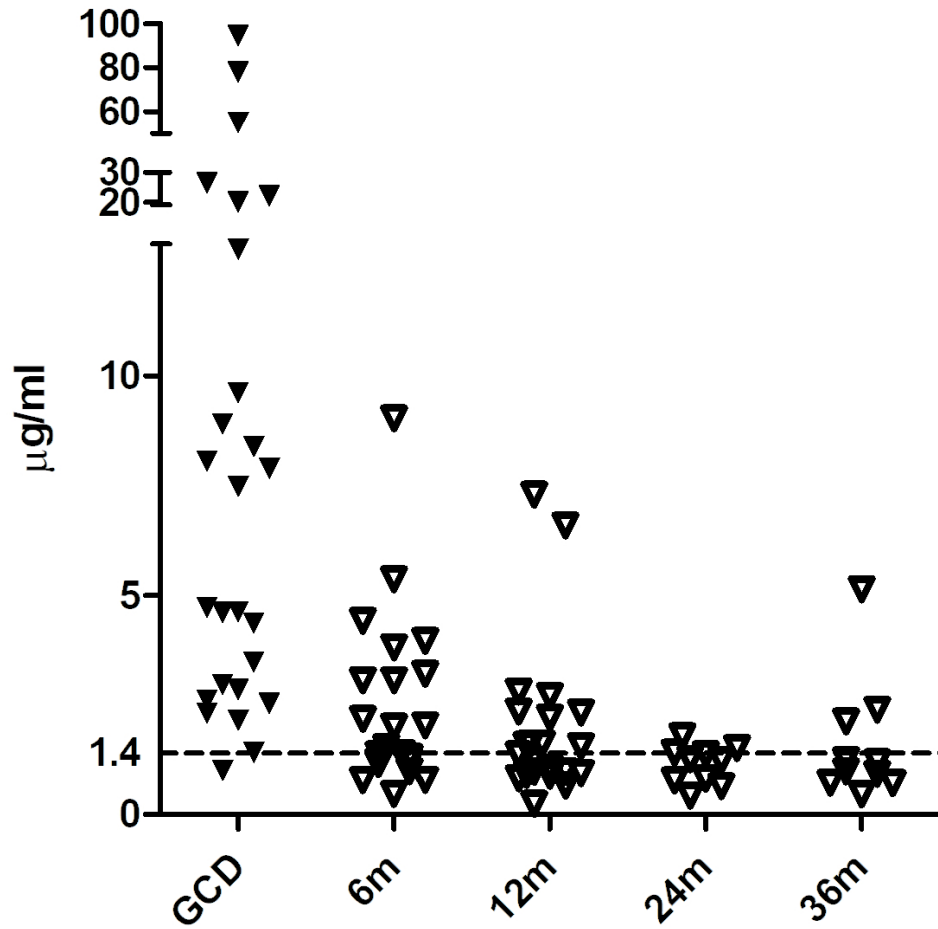


Figure 2. Serum IgA Anti-TG3 antibody concentrations were measured in 26 patients with Dermatitis Herpetiformis on gluten-containing diet (GCD) (closed inverted triangles) and after 6, 12, 24 and 36 months on gluten-free diet (open inverted triangles) using the TG3 ELISA. The dashed line marks the cut-off value for positivity (1.4 µg/ml).

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