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The development and validation of a high-capacity serological assay for celiac disease

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ABSTRACT

Background: The aim of the present study was to develop and clinically validate a high-throughput assay for serum IgA and IgG antibodies against transglutaminase-2 (TG2) and to determine appropriate assay cut-offs for large-scale population screening for celiac disease.

Method: An automated method was developed using dual label time-resolved fluorometry on the AutoDELFIA platform. Individuals (n = 1920) from the general population were screened. Subjects with serum anti-TG2 concentrations above a preliminary cut-off (>0.3 mg*/L anti-TG2 IgA or >0.5 mg*/L anti-TG2 IgG) were offered endoscopic examination and biopsy. A diagnosis of celiac disease was given if villous atrophy (Marsh grade 3) was found.

Results: The assay had a limit of quantification of 0.25 mg*/L (anti-TG2 IgA) and 0.60 mg*/L (anti-TG2 IgG) with imprecision (CV) < 16% and <18% respectively. A total of 66 individuals were above the preliminary cut-off, and 56 underwent endoscopy. Of these, 26 were diagnosed with celiac disease. Sixty-eight percent of subjects with anti-TG2 IgA ≥ 0.7 mg*/L or anti-TG2 IgG ≥ 1.0 mg*/L had biopsy-proven celiac disease, and utilization of these higher cut-offs identified 96% of biopsy-positive patients. At the time of endoscopy, all individuals with anti-TG2 IgA > 2.0 mg*/L had celiac disease, and this cut-off identified 88% of newly diagnosed celiac patients. Eight percent (2/26) of the newly diagnosed patients had primarily anti-TG2 IgG.

Conclusions: In this study we developed and clinically validated a robust and automated assay suitable for celiac disease screening in the general population.

1. Introduction

Celiac disease is a chronic inflammatory disorder of the small intestinal mucosa occurring in genetically predisposed individuals exposed to dietary gluten proteins of wheat (gliadin and glutenin), barley (hordein) or rye (secalin) origin [1]. Peptide fragments of post-translationally modified gluten bind to certain human leukocyte antigens (HLA-DQ2 or HLA-DQ8) and drive an autoimmune response [2].

Currently, in adult patients a diagnosis of celiac disease is usually made through a combination of serological testing and endoscopic biopsy [3]. A common approach is to firstly screen for serum IgA antibodies to transglutaminase 2 (anti-TG2 IgA) and IgG antibodies to deamidated gliadin peptides (anti-DGP IgG). The diagnosis is confirmed

Abbreviations: TG2, Tissue transglutaminase 2; DGP, Deamidated gliadin peptides; rhTG2, Recombinant human transglutaminase-2; ORF, Open reading frame; BAB, Base assay buffer; LoQ, Limit of quantification; CV, Coefficient of variation.

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by histological examination of the small intestinal mucosa, where intraepithelial lymphocytosis (≥25 intraepithelial lymphocytes/100 enterocytes), crypt hyperplasia and villous atrophy (Marsh grades 3a to 3c), are the pathological hallmarks of the disease [4]. Although considered a low risk procedure, endoscopy is resource intensive and uncomfortable for the patient. Cardiopulmonary complications may also occur in 0.6% of esophagogastroduodenoscopies [5].

A recent study has suggested a no-biopsy approach for the diagnosis of adult celiac disease when anti-TG2 IgA titers of ≥ 10 times the upper limit of normal are found [6]. However, this approach has not been tested in an unselected general population. In addition, screening for celiac disease in the population requires a suitable method for the determination of serum anti-TG2 immunoglobulins, including a robust assay with low costs and high degree of automation. Given the increased prevalence of IgA deficiency in the celiac population (2–3%) [7], the assay should also be able to measure both anti-TG2 IgA and IgG, to reduce the number of false negative tests due to IgA deficiency. None of the currently available commercially assays fulfill these criteria.

In this paper, the development and validation of an automated dual-label time-resolved fluorometric assay for the simultaneous measurement of serum anti-TG2 IgA and IgG antibodies is described. In addition, we report the results of a pilot study to identify appropriate assay cut-offs for population screening for celiac disease.

2. Materials and methods

An expanded Materials and Methods section is available in the Supplemental Data.

2.1. Calibration, assay protocol and instrumentation

2.1.1. Calibration

A mix comprising two anti-TG2 monoclonal antibodies of either IgA or IgG class (Supplemental Methods) was diluted in normal donor serum to 0, 1.0, 3.0, 10, 30 and 100 mg/L each. Working calibrators were subsequently prepared by dilution (1:100) of the calibrator stock in base assay buffer (BAB: 50 mmol/L Tris-Cl (pH 7.8), 150 mmol/L NaCl, 3.6 mmol/L diazolidinyl urea, 20 μmol/L diethylenetriamine pentaacetic acid, 0.01% (v/v) Tween 20, 20 g/L cold fish gelatin. Aliquots of the working calibrators were stored for up to 2 weeks at 4 °C or long-term at –70 °C.

Results were reported as mg*/L since the calibrators consist of monoclonal reference antibodies and not the true analyte (a polyclonal mixture of anti-TG2 IgA and IgG immunoglobulins that varies within and between individuals). Anti-TG2 IgG antibodies were only reported when anti-TG2 IgA was below 0.9 mg*/L (see 2.2.3 Analytical specificity below).

2.1.2. Assay protocol

Assays were performed in 96-well streptavidin-coated microplates. Before assay, the instrument pre-diluted serum samples 1:100 in assay buffer (BAB containing 1 g/L bovine serum albumin). The solid phase was prepared by dispensing 100 ng/well (150 μL) biotinylated rhTG2 (Supplemental Methods) in assay buffer, followed by 30 min incubation. Wells were washed three times and assay buffer (125 μL/well) was added followed by 25 μL/well of undiluted calibrator, or 25 μL/well of diluted sample or control. The wells were incubated for 60 min to allow anti-TG2 antibodies in the sample to bind to the rhTG2 solid phase. After incubation, wells were washed three times and 150 μL/well tracer reagent mix (0.6 μg/mL europium-labelled mouse anti-human IgA $F(ab)_2$ fragments, 0.2 μg/mL samarium-labelled Protein-A) in assay buffer added. Unbound tracer reagent was removed after 30 min of incubation by washing the wells six times. DELFIA Enhancement Solution (200 μL/well) was then added followed by 10 min of incubation. Eu and Sm signals were measured using time resolved fluorescence (TRF) with excitation/emission filters set to 340/615 nm and 340/642 nm for Eu and Sm, respectively. Signal unit was counts per second (cps). All incubations were performed at ambient temperature with shaking.

The assay was calibrated by correlating the signal intensity and calibrator concentration using the spline smoothed algorithm in the operating software of the instrument (MultiCalc, Wallac Oy, Finland).

The assay principle is shown in Fig. 1.

2.1.3. Instrumentation and automation

The assay protocol was automated on the AutoDELFIA 1235 automated immunoassay platform (PerkinElmer Life Sciences, Oslo, Norway). A total of 192 samples were analyzed per run.

2.2. Assay performance evaluation

2.2.1. Linearity

Linearity of the calibrator curve was assessed by collecting signal intensity and calibrator values from 30 calibrator curves from 10 runs performed on different days. Mean and range of the curves correlation coefficient (R2) were calculated. Mean and SD of the signal strength at each calibrator concentration level were calculated in order to assess day-to-day signal variability. The linearity interval of the assay was assessed in accordance with the CLSI EP06:2020 guidelines [8]. Two separate patient samples with high levels of anti-TG2 IgA (123 mg*/L) and anti-TG2 IgG (22.6 mg*/L) were diluted with blank serum to nine concentrations in the range 0.602 mg*/L-123 mg*/L anti-TG2 IgA and 0.82 mg*/L – 22.6 mg*/L anti-TG2 IgG, respectively. Each dilution was measured in five replicates and deviation from linearity calculated as described in CLSI EP06:2020.

2.2.2. Precision and limit of quantification

Samples (n = 21) from patients with confirmed celiac disease collected in the thematic biobank for celiac patients at Oslo University Hospital, Rikshospitalet were analyzed on three different days and used to identify an assumed limit of quantification (LoQ). The assumed LoQ was defined as the lowest concentration at which the quantity of analyte could be measured with reliable precision (defined as CV < 15%). After identifying a suitable LoQ, new samples were selected for validation of the precision as described in the CLSI EP05 guideline [9]. Patient samples with approximate values around the assumed LoQ, 3x LoQ and 30× LoQ were selected for this experiment. Samples were analyzed in replicates, twice per day, on five days (2 × 2 × 5). Repeatability and within-laboratory precision were calculated as described in the CLSI EP05 guideline. Due to limited sample material available, the samples were run on five different days instead of the recommended twenty. Acceptable assay imprecision was defined as 15%.

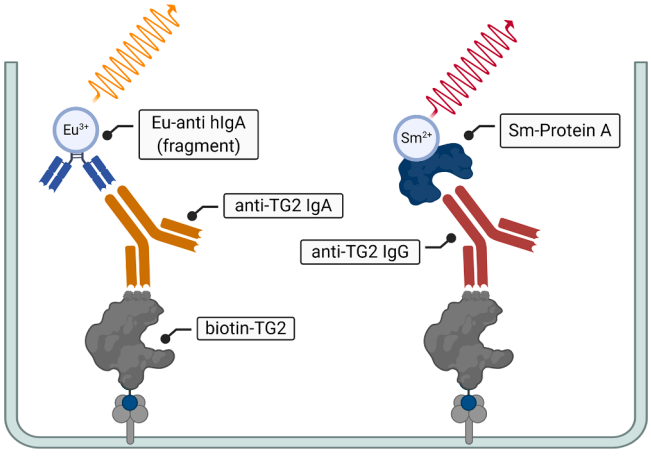

Fig. 1. Assay principle. Dual-label time resolved fluorometry (TRF) using samarium- and europium-labelled tracers to detect anti-transglutaminase 2 (TG2) IgG and IgA, respectively. Figure made in Biorender.com.

Long-term precision was assessed by assaying normal donor serum spiked with reference analyte to a concentration of 1.0 mg/L (level 1) and 10 mg/L (level 2) in 18 different runs over a period of 3 months, with long-term precision defined as coefficient of variation.

2.2.3. Analytical specificity

To assess if unknown components in serum from healthy patients could give falsely elevated signals for either anti-TG2 IgA or anti-TG2 IgG, samples from individuals from the same thematic biobank as outlined above but confirmed negative for celiac disease (n = 14) were analyzed. The mean and standard deviation in signal intensity for anti-TG2 IgA and anti-TG2 IgG were calculated. The number of standard deviations (z-score) between mean signal for negative samples and signal corresponding to LoQ were calculated.

To evaluate potential cross-reactivity of the Eu-labeled anti-human IgA tracer reagent to the anti-TG2 IgG used in calibrators and controls, calibrator solutions containing only anti-TG2 IgG (0–100 mg/L) were analyzed using a tracer comprising only Eu-labeled anti-human IgA F $(ab')_2$ (n = 6 per level).

Since Protein-A has been reported to bind some IgA [10], the binding of Sm-labeled Protein-A to the reference anti-TG2 IgA used in the calibrator and control solutions was also assessed. Calibrator solution containing only anti-TG2 IgA was analyzed using tracer containing Sm-labeled Protein-A only (n = 6 per level).

Since the binding of Protein-A to IgA cannot be excluded in all patient samples, anti-TG2 IgG was not reported in samples with anti-TG2 IgA above 0.9 mg*/L.

2.2.4. Sample stability

To assess sample stability during storage, sera with measurable anti-TG2 IgA (n = 75) or anti-TG2 IgG (n = 14) were analyzed before and after five days storage at 2 – 8°. The percent difference between the runs was calculated.

To assess the effect of freezing and thawing, samples with either measurable anti-TG2 IgA (n = 1011) or measurable anti-TG2 IgG (n = 158) were analyzed before and after a freeze–thaw cycle and the differences between the runs calculated.

2.3. Clinical performance evaluation

Screening samples for clinical evaluation of the assay were acquired from the fourth round of the population-based Trøndelag Health Study (HUNT4) (cohort paper in preparation). In HUNT4, all inhabitants over 20 years of age in Nord-Trøndelag County, Norway, were invited to participate in a health survey running from September 2017 through February 2019. Blood samples from 54,566 participants (53% response rate) were collected and serum aliquots stored at −80 °C at the HUNT Biobank, Levanger, Norway. A random sample of 1,920 serum aliquots from HUNT4 were picked and used in this pilot study.

Screening samples were initially analyzed as singletons and retested in a separate run. Individuals with concordant results above 0.3 mg*/L anti-TG2 IgA or 0.5 mg*/L anti-TG2 IgG were offered follow-up examination for celiac disease by endoscopy and tissue sampling at Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, Norway from June 2019 through November 2019. At endoscopy four biopsies were taken from the second part of the duodenum and two biopsies were taken from the duodenal bulb using the single bite technique by a trained gastroenterologist (ENJ). Biopsies were assessed and graded according to Marsh by expert pathologists (PM and ER) [4] (Supplemental Methods). The celiac disease diagnosis was confirmed in individuals with at least Marsh grade 3a lesions on histology.

New "*peri*-endoscopy" serum samples were also collected at the time of endoscopy and analyzed with the assay described above. In addition, the new serum samples were analyzed using an approved medical device for anti-TG2 IgA quantification (EliA fluoroenzyme immunoassay, Thermo Fisher Diagnostics, Oslo, Norway) for comparison.

Total serum IgA was also measured (Alinity Immunoglobulin A, Abbott Diagnostics, Oslo, Norway) at the time of endoscopy in order to determine if individuals classified as anti-TG2 IgG-responders had lower levels of total IgA when compared to anti-TG2 IgA responders (see definition of these under statistics, software, data handling and data sharing, below).

2.4. Statistics, software, data handling and data sharing

Patients above the preliminary cut-off were stratified into primarily "IgA-responders" or "IgG-responders". IgG-responders were defined as patients with anti-TG2 IgA < 0.5 mg*/L and anti-TG2 IgG > 0.5 mg*/L. All other patients were defined as IgA-responders.

Results from the screening samples and subsequent examination were used to calculate a cut-off value suitable for population screening. A cut-off value applied to the screening sample that identified a large proportion (>90%) of the celiac patients with an acceptable proportion of false positives was favored. In addition, a cut-off value that was associated with high specificity for celiac disease was calculated using data from the *peri*-endoscopy serum samples.

Statistical analysis was performed using R (v 3.5.0, The R Foundation) or Stata/MP (v 16.1, StataCorp, College Station, TX, USA). The data used in this study is available through application to the HUNT Research Centre and approval by a Norwegian Regional Committee for Medical and Health Research Ethics. Researchers from abroad are welcome to apply in cooperation with a Norwegian Principal Investigator.

2.5. Ethics

Samples used for assay development and validation were acquired from a biobank of patients with intestinal disorders at Oslo University Hospital, Rikshospitalet (Regional Committee for Medical and Health Research, South-East ID biobank ID # 20521). All individuals in the biobank have given informed, written consent and use of the material from the biobank is approved by the Regional Committee for Medical and Health Research Ethics, South-East (ID # 6544).

All participants in HUNT4 gave written informed consent when attending the HUNT4 survey and before attending the endoscopy and tissue sampling. The current study is approved by the Regional Committee for Medical and Health Research Ethics, Central (ID # 7943).

2.6. Patient and public involvement

The Norwegian Celiac Society, the patient body for celiac patients in Norway, was involved in the planning of the present study. The study has public funding through the Research Council of Norway and the Central Norway Regional Health Authority.

3. Results

3.1. Assay performance evaluation

During 10 runs, performed over a period of 29 days, signal linearity (R^2) was above 0.970 and 0.998 for anti-TG2 IgA and anti-TG2 IgG, respectively (Fig. 2). Assay signal (cps) was stable during these runs, with a coefficient of variation ranging from 2.8 to 8.1% for anti-TG2 IgA and 3.7 – 5.5% for anti-TG2 IgG.

Assay linearity is summarized in Supplemental table 1. Overall, the assay was linear (deviation from linearity < 11%) in the range 1.24–123 mg*/L anti-TG2 IgA and 1.94–22.6 mg*/L anti-TG2 IgG.

The anti-TG2 IgA and anti-TG2 IgG concentrations in the samples used to determine assay LoQ ranged from 0.246 to 16.7 mg*/L and 0.01 – 62 mg*/L, respectively. No sample had an imprecision > 15% for the anti-TG2 IgA measurements, whilst no sample with an anti-TG2 IgG measurement above 0.613 mg*/L had an imprecision > 15%. The LoQ

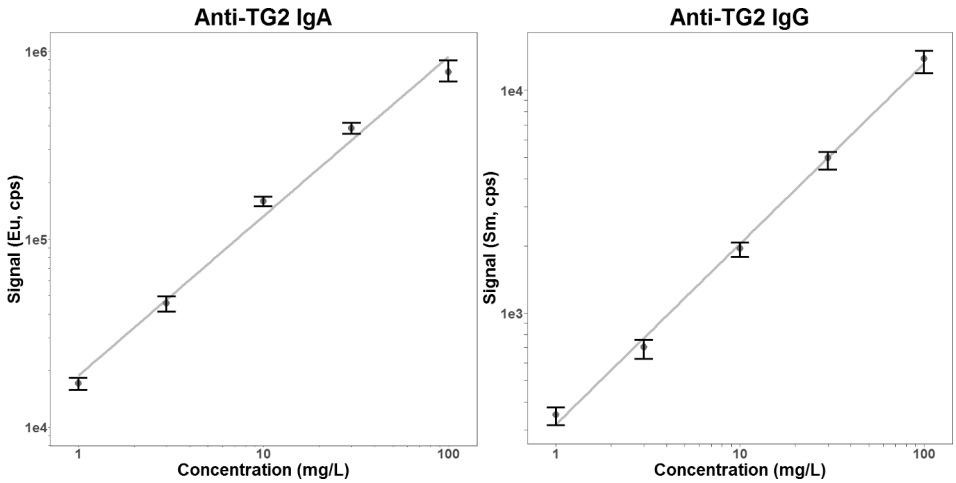

Fig. 2. Signal-concentration relationship for anti-TG2 IgA and anti-TG2 IgG. Points indicate mean (± range) in signal from 10 calibrator curves from different days. Cps; counts per second, TG2; transglutaminase 2.

was therefore set to 0.25 mg*/L for IgA and 0.60 mg*/L for IgG. The assay imprecision was < 15.6% for anti-TG2 IgA and < 17.8% for anti-TG2 IgG (Table 1).

When the method was run with calibrators containing only anti-TG2 IgG and with only Eu-labeled anti-human IgA F(ab')$_2$ present in the tracer solution the signal ranged from 236 to 1158 cps (n = 36). Conversely, when calibrators with just anti-TG2 IgA were analyzed using tracer solution containing only Sm-Protein-A, the signal ranged from 74 to 114 cps (n = 36). These signals were below or comparable to blank (mean, SD) for anti-TG2 IgA (1012 ± 326) and anti-TG2 IgG (165 ± 10), respectively. This indicated that the Eu-labeled anti-human IgA F(ab')$_2$ and Sm-labeled Protein-A are specific for the reference anti-TG2 IgA and anti-TG2 IgG, respectively, used in the calibrators and controls.

Mean (± standard deviation, SD) signal strength in samples from patients without celiac disease was 1179 cps (± 485) for IgA and 145 cps (± 20) for IgG. Signal strength for the corresponding LoQ was 4002 cps and 281 cps, respectively, resulting in a z-score of 5.8 IgA and 6.7 for IgG.

Sample stability is shown in Supplemental Fig. 1. After five days of storage, the mean difference in measured anti-TG2 IgA was −5.9% and difference in measured anti-TG2 IgG was 2.5%. After one freeze and thaw cycle, a mean difference of 5.1% (anti-TG2 IgA) and 7.5% (anti-TG2 IgG) was observed.

Table 1
Assay imprecision.

Repeatability and within-laboratory precision					
		Repeatability		Within-laboratory precision	
Anti-TG2	Mean, mg*/L	SD, mg/L	CV, %	SD, mg/L	CV, %
IgA, low	0.38	0.007	1.8	0.034	8.9
IgA, medium	1.41	0.054	3.8	0.208	14.7
IgA, high	13.9	0.553	4.0	2.164	15.6
IgG, low	0.77	0.056	7.3	0.102	13.2
IgG, medium	1.40	0.08	5.7	0.215	15.3
IgG, high	17.0	1.061	6.2	3.031	17.8
Long-term precision					
Anti-TG2	Mean, mg*/L	SD, mg/L	CV, %		
IgA, level 1	0.97	0.03	3.1		
IgA, level 2	8.71	0.31	3.6		
IgG, level 1	0.85	0.12	14.1		
IgG, level 2	8.46	0.46	5.4		

Note: Repeatability and within-laboratory precision; data from 10 runs, performed over 5 days, with duplicate per run. Long-term imprecision; data from 18 runs over a period of 3 months.

All samples for the clinical performance evaluation were measured in singletons in two different runs (18 runs in total) over a one month period with a runtime between 4 and 5 h resulting in a total runtime of 89 h. Of this, 77 h (86%) was performed on the instrument without operator involvement.

3.2. Clinical performance evaluation

The mean age of the 1,920 random participants from HUNT4 used in the clinical evaluation was 54.8 (SD 17.5, range 19.2–98.3) years and 53.3% were women. A total of 66 individuals had serum anti-TG2 IgA and/or IgG above the preliminary cut-off and were invited to endoscopy with biopsy. The mean age of these 66 individuals was 55.4 (SD 16.7, range 19.5–84.7) years and 53.0% were women. Twenty one percent (14/66) were defined as IgG-responders according to the predefined definition. In addition, two patients had 0.5 and 0.7 mg*/L anti-TG2 IgA, but considerable levels of anti-TG2 IgG (3.1 and 5.7 mg*/L, respectively). These were subsequently defined as IgG-responders, resulting in 50 IgA-responders and sixteen IgG responders in total. In IgA-responders, the mean (range) anti-TG2 IgA was 4.23 mg*/L (0.31 – 34.5 mg*/L), whilst mean (range) anti-TG2 IgG was 1.51 mg*/L (0.59 –5.67 mg*/L) in IgG-responders. One IgG-responder had inconclusive anti-TG2 IgG value due to large difference in the two parallels (anti-TG2 IgG 0.59 and 0.10 mg*/L). Among 66 individuals, nine either declined participation, did not respond or had deceased, leaving 57 individuals for further evaluation. Of these, two IgA-responders had known celiac disease and were not examined with endoscopy. One IgG-responder did not manage to complete the endoscopy, but celiac disease was ruled out due to negative HLA-DQ2 and HLA-DQ8 genotyping. Marsh grades and anti-TG2 measurements of the remaining 54 individuals are presented in Table 2. A total of 59% (24/41) of IgA-responders and 15% (2/13) of

Table 2
Anti-TG2 IgA and IgG vs. Marshgrade - Screening sample.

		Marsh grade					
		0	*1/2*	*3a*	*3b*	*3c*	*Missing*
IgA-Responders	*0.30 – 0.69*	7	2	1			6[a,b,c]
Anti-TG2 IgA (mg/L)*	*>0.70*	1	7	6	10	7	3[c,d]
IgG-Responders	*0.50 – 0.99*	3	3*				2[c]
Anti-TG2 IgG (mg/L)*	*> 1.00*	2	2	1	1		1[c]
	Total	**13**	**14**	**8**	**11**	**7**	**12**

Note: Missing due to (a) deceased, (b) known celiac disease, (c) declined examination or did not respond and/or (d) endoscopy not completed. *In addition; one patient with uncertain anti-TG2 IgG between 0.1 and 0.5 mg*/L.

IgG-responders had Marsh grade 3 and were diagnosed with celiac disease. In IgA-responders, a cut-off at ≥ 0.7 mg*/L anti-TG2 IgA identified 96% (23/24) of individuals with celiac disease and 74% (23/31) of IgA-responders above this cut-off had celiac disease (Fig. 3A). Correspondingly, in IgG-responders (n = 13) a cut-off at ≥ 1.0 mg*/L identified both individuals with celiac disease, whilst 33% (2/6) above this cut-off were positive (Supplemental Fig. 2A). Setting a combined cut-off of either > 0.7 mg*/L anti-TG2 IgA or > 1.0 mg*/L anti-TG2 IgG therefore identified 96% (25/26) of celiac patients, whilst 68% (25/37) of individuals above this cut-off had celiac disease. Based on these results, seropositivity for population screening purposes was defined as ≥ 0.7 mg*/L for anti-TG2 IgA or ≥ 1.0 mg*/L for anti-TG2 IgG.

The time delay between collection of the screening serum sample and endoscopy was mean 1.34 (SD 0.41, range 0.34 to 2.05) years.

New serum samples collected at the time of endoscopy were available for 98% of the examined patients (40/41 IgA-responders and 13/13 IgG-responders). The correlation (R^2) between the screening sample and the sample collected at the time of endoscopy was 0.688 for IgA-responders and 0.893 for IgG-responders (Supplemental Fig. 3).

All IgA-responders with anti-TG2 IgA > 2.0 mg*/L at the time of endoscopy had a Marsh grade ≥ 3a (n = 21), whilst three patients with Marsh grades ≥ 3a had anti-TG2 IgA levels below 2.0 mg*/L (Fig. 3**B**). Thus 88% of celiac patients with a primarily IgA response were identified using this cut-off. Similar results were seen using the EliA Celikey assay (Supplemental Fig. 4). Anti-TG2 IgA measured using our assay correlated well with the EliA Celikey assay (R^2 = 0.758) and the EliA Celikey cut-off (10 U/mL) corresponded to approximately 2.0 mg*/L (Supplemental Fig. 5). In IgA-responders (n = 40), all 21 individuals with measurements > 10 U/mL had celiac disease, whilst three celiac patients had values below the stated cut-off. All IgG-responders in our assay, including the two with celiac disease, had values < 2 U/mL in the EliA Celikey method.

In the IgG responder group, both individuals with celiac disease had anti-TG2 IgG values above 1.0 mg*/L at the time of examination. Three additional individuals had anti-TG2 IgG above 1.0 mg*/L, but did not have the disease. The remaining nine individuals all had < 1.0 mg*/L anti-TG2 IgG (Supplemental Fig. 2B). Anti-TG2 IgA was < 0.3 mg*/L in all IgG-responders at examination.

Total IgA measurements were available for 14 (86%) of the IgG responders and 42 (84%) of the IgA responders with a mean total IgA of 2.19 g/L in both groups. None were considered IgA deficient (<0.7 g/L total IgA).

4. Discussion

This paper describes the development and validation of a high capacity assay for the simultaneous determination of serum anti-TG2 IgA and IgG antibodies. The method has performance characteristics that make it suitable for screening for celiac disease in the general population. The assay utilizes a recombinant TG2 solid phase to capture anti-TG2 antibody and a tracer mix comprising Eu-labeled anti-IgA monoclonal antibody and Sm-tagged Protein-A to quantify IgA and IgG classes, respectively. The assay is automated and able to perform complete analysis of 192 samples in<5 h with minimal operator involvement.

During methods development samarium-labeled Protein-A was selected to detect IgG-class anti-TG2 antibodies, rather than an anti-human IgG immunoglobulin reagent, because it has a remarkably low background binding to the assay solid phase [11]. This is important since samarium displays a 50-fold less fluorescent yield when compared to europium. However, the Protein-A tracer can also bind a subset of IgA molecules via a non-Fc mediated mechanism [12]. High levels of anti-TG2 IgA could therefore result in falsely elevated results for anti-TG2 IgG. However, this was found not to be problematic since the samarium channel is used solely to identify patients with low or no IgA (patients where such interference is not expected). In this regard, anti-TG2 IgG values were not reported when anti-TG2 IgA values were above 0.9 mg*/L.

In our new method, calibrators are prepared using two recombinant human anti-TG2 monoclonal antibodies reactive to a dominant N-terminal epitope expressed with either human IgA1 or IgG1 constant regions. Using this approach, a potentially unlimited supply of reference material was obtained together with significant reductions in the variability of individual calibrator batches. This is in marked contrast to the common practice of using pooled patient sera or similar material. Furthermore, by using anti-TG2 IgA and IgG with the same epitope binding site as reference material, a direct comparison between the immunoglobulin classes can be made.

Assay imprecision (Table 1) was above the target imprecision of 15%, but only to a lesser degree. The imprecision around limits for clinical decision were below 15% and we therefore consider the assay precision overall to be acceptable.

Antibodies against DGP may present an alternative or supplementary

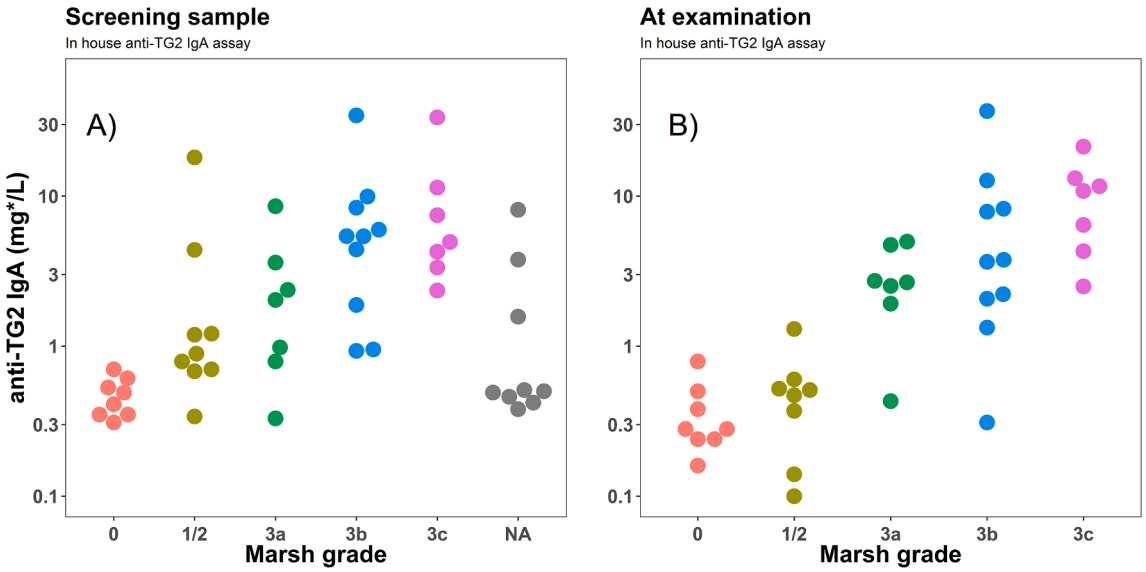

Fig. 3. Anti-TG2 IgA vs. Marsh grade. Anti-TG2 IgA in screening sample (A), sample collected at endoscopic examination (B) and subsequent Marsh grade. Patients without score (NA); Missing due to being deceased, having known celiac disease, declined examination/did not respond or endoscopy not completed. Time from screening sample to exam was median (range) 1.34 (0.34 to 2.05) years.

assay for serological assessment for celiac disease. Studies suggest that assays measuring anti-TG2 perform better [13], but that a combination could help to also identify celiac disease in IgA-deficient individuals [3].

Of the 66 individuals undergoing endoscopy and tissue sampling, 13 had primarily an anti-TG2 IgG response and two were diagnosed with celiac disease. These would not have been identified without the inclusion of anti-TG2 IgG measurements using the suggested anti-TG2 IgA screening cut-off of 0.7 mg*/L. Surprisingly, none of the IgG-responders were considered to be IgA-deficient and would therefore not have been tested for anti-TG2 IgG (alternatively anti-DGP IgG) as this is usually reserved for IgA deficient individuals. These results could suggest that simultaneous measurement of both isotypes has merit, but more data should be available to conclude. However, the data accumulated from our on-going main study with 54,566 participants should resolve this question.

Another reason for the primary IgG-response in individuals without IgA-deficiency could be a self-imposed reduced exposure to dietary gluten. The half-life of circulating IgA in serum is less than one week, whilst IgG has a half-life closer to one month. After reducing the exposure to gluten, there could have been a substantial reduction of circulating anti-TG2 IgA, whilst anti-TG2 IgG remained elevated. These subjects would display an IgG dominating phenotype at the time of sampling and be classified as “IgG responder” according to our criteria. We are unable to substantiate this possible explanation as information in this cohort regarding changes in gluten consumption prior to blood sampling is not available.

Strengths of this study include the clinical performance evaluation that was done in an unselected general population with a wide age span of both sexes, arguing for high validity. As a weakness, none of the individuals with measurement below the cut-off were examined and the assays diagnostic sensitivity could therefore not be determined. Determining the diagnostic sensitivity would have required endoscopic examination of patients with a low probability of having celiac disease (3) and could not be performed due to both ethical and logistical reasons. However, the assay clearly separated negative and positive patients (both biopsy-confirmed) from the thematic biobank used in the validation of the assay, even if the number of negative samples were limited ($n = 14$).

Based on the assumption that no individuals with negative serology (<0.3 mg*/L anti-TG2 IgA and < 0.5 mg*/L anti-TG2 IgG) did have celiac disease, a cut-off of > 0.7 mg*/L anti-TG2 IgA alone identified 88% (23/26) of celiac patients and correctly classified 99.5% as not having the disease. An anti-TG2 IgG cut-off of > 1.0 mg*/L alone (regardless of anti-TG2 IgA value) identified 77% (20/26) of celiac patients and correctly identified 99.7% of the healthy individuals. Combining the cut-offs (either anti-TG2 IgA > 0.7 mg*/L or anti-TG2 IgG > 1.0) correctly identified 96% (25/26) of the celiac patients and 99.4% of those not having the disease. Eight of the IgA-responders with anti-TG2 IgA > 0.7 mg*/L were negative for the disease. Including anti-TG2 IgG with the suggested cut-off measurement increased the number of false negatives to twelve, or 32% (12/37). This could still be acceptable for screening purposes, and it illustrates the utility of including anti-TG2 IgG in the assay.

In conclusion, this study describes a robust, high-capacity and fully automated assay with excellent performance suitable for celiac disease screening in the general population.

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2022.05.010.

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