# Value and Use of Serologic Markers of Celiac Disease


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**Abbreviations**

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|---|---|
| AGA | Antigliadin antibodies |
| ARA | Antireticulin antibodies |
| TGA-IgA | IgA antibodies against type-2 (tissue) transglutaminase |
| TGA-IgG | IgG antibodies against type-2 (tissue) transglutaminase |
| EMA-IgA | IgA endomysial antibodies |
| DGP | Deamidated gliadin peptides |
| ELISA | Enzyme Immuno assay |
| IF | Immunofluorescence |
| DGPA-IgG | IgG Deamidated gliadin peptides antibodies |
| DGPA-IgA | IgA Deamidated gliadin peptides antibodies |
| SM | Serological marker |
| SBB | Small bowel biopsy |
| TG2 | Tissue Transglutaminase |

## 1 Introduction

Identification of SM of CD has no doubt modified our perception of the disease compelling to revisiting both disease definition and diagnostic approach, mainly over the last twenty years principally [1–6].

Antigliadin antibodies (AGA) both IgG and IgA [7, 8] and antireticulin antibodies (ARA) [9] were the first SM available and this since the early 1980s. Measurement of AGA by ELISA methods consolidated its widespread use while

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ARA decayed overtime partially due to the need of immunofluorescence (IF) methods for detection [9]. In the late eighties a new marker was identified, the anti-endomysium antibodies (EMA), which turned out to display a higher diagnostic performance than the previous AGA and ARA [10–12]. Its widespread use in the nineties firmly demonstrated that initial EMA positivity in patients with small intestinal villous atrophy was, in children, as predictive of CD diagnosis as traditional gluten challenge and full Interlaken criteria [1, 13]. Thanks to availability of TGA and EMA the number of SBB required for diagnosis were reduced in the new 1990 ESPGHAN CD diagnostic criteria for the paediatric population [2].

In 1997 the identification of tissue transglutaminase (TG2) as the autoantigen in CD led to IgA and IgG antibodies detection specific for TG2 (TGA) [14]. The production of TGA was shown to be related to dietary exposure to gluten and to small bowel mucosal atrophy. Moreover easy to use automated methods helped promoting their wide use as SM for CD diagnosis after the year 2000 [15, 16].

Subsequent studies on the immunopathogenesis of CD demonstrated that selective deamination of gliadin by TG2 helps the gliadin fragments bind to the antigen-presenting cells which is a fundamental step in the immunological response that leads to CD [17]. Serologic assays based on deamidated gliadin peptides (DGP) were then developed detecting antibodies against DGP (DGPA); when comparing the DGPA tests performance with the previous SM, DGPA display a higher diagnostic accuracy than the traditional AGA test [18], but lower than TGA and EMA [19, 20].

Lately easy to run rapid visual tests, based on various SM and different SM combinations have been developed, which can be run at the patients bed side and display a high efficiency [21]. Results need however to be confirmed by conventional laboratorial based tests but can be useful as a preliminary diagnostic screening tool [22].

Availability of these high efficient SM from the 80´s onwards have completely modified the approach to CD diagnosis, specially but not exclusively in the paediatric population [2–4, 6].

In this chapter we perform a thorough revision of the definition of the different SM, the methods used for detection and their accuracy for CD diagnosis.

## 2 Anti-endomysium Antibodies

In 1983, Chorzelsky et al. [23] detected for the first time IgA class EMA using sections of monkey oesophagus by indirect IF, in the serum of patients with dermatitis herpetiformis (DH) and CD. Ever since, EMA have been used for the diagnosis of CD due to their high sensitivity and specificity, replacing other less reliable tests such as AGA or ARA.

Enzyme tissue transglutaminase (TG2) was identified as the target antigen of EMA in 1997 [14], therefore EMA recognize the same antigen as TGA antibodies and they only differ in terms of detection method. Immunoenzyme assays (ELISA)

used to detect TGA-IgA showed high sensitivity, so have gradually replaced the EMA test in the serological diagnosis of CD.

*Methods for EMA Detection*

EMA described in CD are IgA antibodies directed against the intermyofibril substance of the smooth muscle [23]. They are detected by indirect IF method on sections of monkey oesophagus or human umbilical cord as substrate. In short, a tissue section is incubated with serum from the patient under study. After washing to remove unbound Immunoglobulin to the tissue, an anti-human Immunoglobulin antibody labelled with a fluorochrome, usually fluorescein isothiocyanate (FITC), is added. Mounting medium is used before carefully covering with a coverslip. Slides should be blindly examined to identify the cellular or tissue location by means of the observation under the fluorescence or confocal microscope of antibodies recognized by the patient. These substrates allow the identification of the classic honeycomb pattern identifiable in *muscularis mucosae* (Fig. 1).

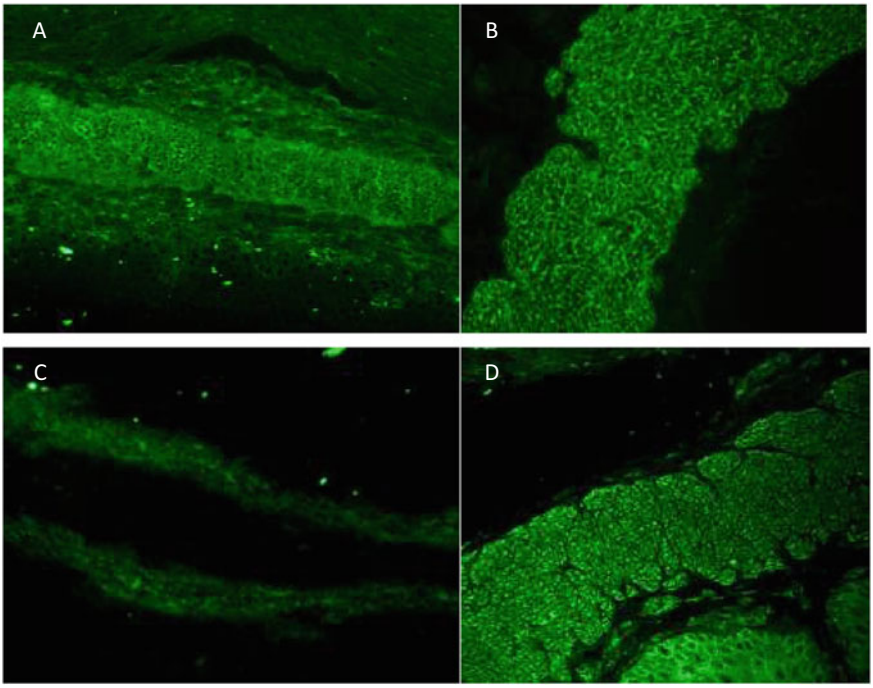


**Fig. 1** IgA anti-endomysial antibodies by indirect immunofluorescence (IF) on section of monkey oesophagus. **a** Positive IF pattern of IgA-EMA staining the connective tissue structures that surround individual muscle fibrils (20x). **b** Positive classic honeycomb pattern (40x). **c** Negative pattern, the lack of fluorescence is remarkable (20x). **d** False positive pattern, fluorescence is found inside cells (20x)

Routine serial dilutions of patient's serum are not necessary for clinical purposes due to cost, time, and sacrifice of laboratory animals. Analysis of the sera at an initial dilution of 1:5 is recommended, but in case of doubtful positivity, the serum can be diluted or concentrated further, depending on whether there is an overlap with other autoantibody stains or weak stains, in order to draw conclusions. Regarding the optimal dilution of the fluorochrome-labelled antibody, the concentration should be adjusted for each antibody, according to the instructions of the manufacturer.

In patients with IgA deficiency, EMA-IgG can be performed, although according to the few studies published, it seems that the sensitivity of EMA-IgG is lower than of EMA-IgA [24]. However, a study in patients with IgA deficiency showed that EMA-IgG was very sensitive and specific [25]. CD is 5–20 times more common in patients with IgA deficiency compared to the general population, in these cases, EMA-IgG autoantibody tests are highly efficient in detecting celiac disease in IgA deficient patients.

To obtain accurate results of the fluorescence pattern, since there is a certain degree of subjectivity in interpreting the images, high-quality biological materials as well as expertise in result interpretation of the assay are required; therefore interpretation errors and added costs are found. Moreover, the IF can only be partially automated, so it should only be used in settings with appropriate expertise. Limitations would be, the fact that it cannot be completely automatized, and so are the workload and subjective reading of the results.

If typical pattern high positive results are found, the reading is easy, but in case of a low EMA-IgA level or atypical patterns, it can be more difficult. Despite the human umbilical cord is a good alternative to monkey oesophagus, the staining intensity is considerably weaker and the interpretation can be a real challenge.

*Efficiency of EMA for CD Diagnosis*

In mixed populations of children and adults, EMA-IgA tests have shown a very high specificity ($\sim$100%) in studies using monkey oesophagus. However, those studies had some variation in sensitivities; one study reported a very low sensitivity (75%), while in others, the sensitivity ranged from 86 to 98% [26]. In studies assessed EMA-IgA using human umbilical cord in a mixed-age population, the pooled sensitivity was 93% (95% CI, 88.1–95.4%), while the specificity was 100% (95% CI, 97.5–100%).

In adult patients, in different studies the sensitivity reported of EMA test is slightly above 86% and the specificity close to 100% [27–30]. The diagnostic performance of the EMA-IgA using monkey oesophagus as substrate in adults showed a pooled sensitivity of 97.4% (95% CI, 95.7–98.5), and a pooled specificity of 99.6% (95% CI, 98.8–99.9). The specificity of the EMA-IgA using human umbilical cord in adults was reported as 100% [26, 31]; however, there was greater variability in the sensitivity, ranging from 87 to 100%. The pooled sensitivity and specificity of this test were 90.2% (95% CI, 86.3–92.5) and 99.6% (95% CI, 98.4–99.9), respectively [26].

In children, IgA EMA using monkey oesophagus as substrate showed a pooled sensitivity and specificity of 96.1% (95% CI, 94.5–97.3) and 97.4% (95% CI, 96.3–98.2), respectively [26]. Studies that assessed EMA-IgA using human umbilical cord performance in children reported some variability in specificity [26] close to 100%. The pooled sensitivity in children was 96.9% (95% CI, 93.5–98.6).

In paediatric patients, a systematic meta-analysis of the diagnostic accuracy of CD antibody tests, the EMA-IgA sensitivity was $\geq$ 90%, pooled specificity 98.2% (ranged 94.7–100%), and the positive likelihood ratio is 31.8 [19]. This evidence report on CD serology estimates that EMA has a higher reliability for the diagnosis of CD that reveals almost an absolute specificity.

An international prospective study concluded that children can be accurately diagnosed with celiac disease without biopsy analysis, based on level of TGA-IgA tenfold or more the ULN, positive results from the EMA tests of 2 blood samples, and the presence of one symptom with a PPV of 99.75 (95% CI, 98.61–99.99). The inclusion of HLA analyses did not increase accuracy [32].

In a recent retrospective study in children, EMA-IgA and TG2-IgA, reached similar sensitivities (98% and 99%), while EMA had a higher specificity (99%) than anti-TG2 (93%). The results support the use of EMA to increase CD diagnostic accuracy in a non-biopsy approach, especially in asymptomatic children [33].

In a study including children and adults, EMA positivity has been observed as a very strong predictor of subsequent CD diagnosis irrespective of the initial titres or initial clinical presentation and it is a very strong predictor of forthcoming CD also in subjects with initially normal villi [34]. In multicentre studies, it has been found that inexperienced personnel in reading IF preparations can incorrectly evaluate EMA serological markers. Additionally, it has been found that EMA test specificity, detected through routine diagnostic analysis in a study performed over a long period, was considerably lower than expected, due to the degree of subjectivity in interpreting the results [35]. These statements support the importance of the evaluation in an expert laboratory by skilled technicians.

In general, EMA tests are more specific and TG2 more sensitive. Specificity is greater in EMA-IgA than in TG2-IgA, since EMA only recognize the TG2 epitopes related to CD, usually extracellular TG2 combined with fibronectin.

EMA is currently considered the most specific laboratory test for the diagnosis of CD due to its high sensitivity and specificity, even though there is no general agreement regarding its use. Furthermore, the EMA-IgA test is highly specific, but less sensitive than TG2-IgA, and should therefore preferably be used as a confirmation test.

## 3 Anti-tissue Transglutaminase Antibodies

In 1997 Dieterich and colleagues [14] identified the enzyme TG2 as the target antigen of EMA. They demonstrated that CD patient's serum with high EMA-IgA levels tested negative when preadsorbed with TG2, showing that TGA recognize the same antigen as EMA.

TG2 is a ubiquitous calcium-dependent enzyme. Eight different isoenzyme forms have been described, depending to their location in the tissues, like type TG2 of intestinal origin, type 3 (TG3 present) in the skin, which is the target of autoantibodies in dermatitis herpetiformis or type 6 (TG6), which targets the central nervous system and have been identified in patients with ataxia. TG2 plays a significant biological role, catalyzing the connection between glutamine and lysine in different proteins as well as in the conversion of glutamine into glutamic acid. It is involved in tissue repair and also in the removal of cell detritus after cell death and apoptosis [36]. In normal subjects, TG2 has been detected in all layers of the small intestinal wall.

TGA are present in different organs and can be detected not only in the blood, saliva or intestinal mucosa (TGA-IgA deposits) but also in liver and other tissues.

In CD patients an inappropriate immune response to gluten ingestion leads to mucosal damage and the release and activation of TG. Gluten and glutamine may be the target of the enzyme, which can bind it to other proteins including transglutaminase itself. TG deaminates gliadin peptides increases their affinity for HLA-DQ8 and DQ2 receptors [37] and activate lymphocytes T CD4 that afterwards stimulate B lymphocytes for the production of TGA antibodies of IgA and IgG class.

*Methods for TGA Measurement*

The first commercial immunoenzymatic (ELISA) assay were based on guinea pig liver TG and showed a very good diagnostic accuracy. However, after the later introduction of extractive or human recombinant TG2 (rhTG) as the antigen, higher sensitivity and specificity for CD diagnosis was obtained. Human erythrocytes are one of the most widely used sources of TG2, and human recombinant TG2 is obtained with eukaryotic expression systems or baculoviruses. The antigens obtained by these procedures show high stability and maintain the conformational epitopes of the protein unchanged, thus providing excellent analytical performance.

The use of TGA monoclonal antibodies demonstrates that the target region of the TGA antibodies in CD patients is located in the core of the molecule corresponding to a peptide not exceeding 237 aminoacids and that the epitopes are conformational, as they require the presence of the C and N terminal domains to maintain stability and immunogenicity [38].

The TGA can be detected by different methods: ELISA, Fluorometric Enzyme Immunoassay (FEIA) or Chemiluminescence Immunoassay (CLIA) and by radio binding assay (RBA) [39]

The tests are generally quantitative; as there are no international standards the values are expressed using different units based on calibration curves from each manufacturer. A standardization, harmonizing the results obtained in different laboratories with the various testing methods, is still needed. Thus cut-off level for each method and commercial kit needs to be identified on the basis of receiver operating characteristic (ROC) curve analysis.

Considering the recent guidelines, which attach considerable importance to the results obtained from the assay of TGA-IgA in diagnosing CD without biopsy, the importance of standardizing the results obtainable with the various commercial kits is all the more obvious. Although the measurement TGA-IgA antibodies is not standardized, most commercially available tests are highly accurate, especially at high values [40]. However, there is evidence of variability between different tests or different laboratories using the same test when it comes to moderate TGA levels.

A study comparing five commercial kits and RBA performed on the sera of children at risk for CD highlighted significant differences in the responses, affecting the interpretation of the results and the diagnosis of CD [41]. An European Workshop stressed the lack of reference materials and procedures [42] and also the American Gastroenterological Association Institute underlined the need for significant international collaboration to improve and harmonize the results of TGA testing [43].

Laboratories must be extremely rigorous in their internal quality control measures, accurately calculating the calibration curve, which should include the value of 10 times the ULN.

Due to increasing quality of current available assays, which are well-suited for automation and high-throughput testing, as well as to lower price of the assays, methods based on recombinant human enzyme has consolidated TGA in the last years almost a standard in CD diagnosis

*Efficiency of TGA for CD Diagnosis*

A meta-analysis of studies investigating the diagnostic accuracy of ELISA tests showed that the SROC curve indicated the absence of heterogeneity, and the superiority of recombinant human TGA (rh-TGA) and purified human TGA (ph-TGA) compared to guinea pig-TGA (gp-TGA). The sensitivities (all individual assays) for rh-TGA, ph-TGA, and gp-TGA were 94%, 94%, and 91%, respectively, and the specificities 95%, 94%, and 89%, respectively [44].

The sensitivity in adults of the TGA- IgA assays, using rh-TG, was above 95% and specificity in the range of 92–100% [4, 45]. The higher the value of the test, the greater the likelihood of a true positive result.

In paediatric patients a systematic meta-analysis of the diagnostic accuracy of CD antibody tests, covering the years 2004 to 2009, showed that the sensitivity was around 90% and specificity around 95% in most studies [19].

A recent systematic review with meta-analysis [46] in asymptomatic patients (children and adults) found a sensitivity of 92.8% (95% confidence interval [CI], 90.3–94.8%) and a specificity of 97.9% (95% CI, 96.4–98.8%).

In a study comparing 10 commercial ELISA kits a high level of accuracy was reported for all the methods examined. The sensitivity of TGA-IgA ranged from 91

to 97% and the specificity between 93 and 100%, using the producer's cut-off. The diagnostic accuracy of all the kits can be improved further by adjusting the cut-off through ROC-curve analysis [47].

On suspicion of CD the initial test in the diagnosis evaluation (in the diagnostic approach) should be the TGA-IgA test on account of its high sensitivity and specificity as well as its wide availability and the use of an automated and objective method. In previous years TGA-IgA was consider to have lower sensitivity in infants under 2 years of age but latter studies did not confirmed this [48].

In patients with IgA deficiency, whose risk of developing CD is higher, TGA-IgG is used. TGA-IgG achieved a performance inferior to TGA-IgA assays. Studies investigating the diagnostic accuracy of TGA-IgG have mainly been conducted using ELISA methods, in a mixed population which also included patients with selective IgA deficiency. The sensitivity of the test as reported in the various studies and comparing different commercial kits, ranges from 67.6 to 100%, and the specificity from 80 to 100% [19, 49, 50].

The relationship between TGA-IgA and the degree of the histological lesion has been evaluated, this showing sensitivity is significantly lower in cases with milder histological damage as for the EMA test; it drops to 67% in patients with partial mucosal atrophy and fall to only 7.69% in patients with Marsh 1 lesions, both in adults and in children [51]. Different groups of researchers [15, 52–54] have attempted to determine whether high TGA-IgA levels can justify avoiding diagnostic biopsies, especially in paediatric patients. Considering the differences between the various commercial methods, Hill et al. [55] report that a TGA-IgA equal or above 10 times the cut-off level for the specific test can detect 100% of patients with intestinal atrophy.

In contrast no studies have been conducted to establish the levels of TGA-IgG that can reliably predict the presence of enteropathy. There is also not enough evidence in children with type1 diabetes in whom a spontaneous normalization of CD serology at moderate titres [56] has been described.

False positive results for TGA have been occasionally reported, as in some diseases positive TGA-IgA can occur in the absence of CD and this, usually at low values and in a limited percentage (2–3%) of patients. This might be the case in autoimmune diseases, liver diseases, other foods sensitizations or infections [57–59] like giardiasis. On the other hand, false negative results can be expected in patients on immunosuppressive therapy and in dermatitis herpetiformis. Measurement in haemolyzed samples may also yield a false decrease in antibody levels [60]. Serological testing must be performed while the patient is consuming gluten regularly, as antibody levels decrease after initiation of a low-gluten or a gluten-free diet.

## 4 Anti-deamidated Gliadin Peptides Antibodies

For the traditional AGA test based on native gliadin, a wide range of sensitivity and specificity has been reported related mainly to the detection kit used and largely to the quality (specificity) of the gliadin employed in the ELISA test. Sensitivity for AGA-IgA ranges from 60.9 to 96.0% and specificity from 79.4 to 93.8%; worse performance, mainly for specificity, is reported for AGA-IgG in paediatric patients [19, 24]. Overall results were better for children than for adults [24]. In fact for many years AGA-IgA were considered extremely useful in the diagnostic approach in children as they were the only available marker and after the discovery of EMA, combined testing for AGA plus EMA was the most widely used laboratory approach, thus amending the lack of specificity of AGA [2]. After the year 2000, a higher sensitivity and specificity for TGA than for AGA was definitely confirmed and the use of the latter was no longer endorsed [3, 6, 61].

However new knowledge on the immunopathogenesis of CD, reported the observation that selective deamination of gliadin by TG2 is a crucial step in the immunologic pathway. This event changes glutamine into glutamic acid, thus enhancing the affinity of gliadin fragments for the antigen-presenting cells [17]. The HLA-DQ/gliadin/tTG complex induces a response by the immunocompetent cells, with production of TGA and also antibodies against DGP (DGPA), both IgA and IgG class; these can be detected circulating in serum of patients with active CD on a gluten containing diet [62, 63].

Thereafter several studies promptly showed that comparing the traditional AGA test with DGPA this latter has a higher diagnostic accuracy than AGA, both in terms of sensitivity and of specificity [18, 64].

*Methods for DGPA Detection*

DGPA were first determined by immune assay methods (ELISA methods) and promptly easy to run automated serologic assays using a pool of deamidated gliadin peptides as the antigen became commercially available for their detection.

*Accuracy for CD Diagnosis*

Contrary to AGA and EMA or TGA, preliminary studies found that accuracy of DGPA- IgG to be superior to DGPA-IgA for CD diagnosis, and this on account of a lower specificity for the latter. So, sensitivity of DGPA IgA in adults ranges from 83.6 to 98.3% with a specificity between 90.3 and 99.1% [65]. For DGPA- IgG the sensitivity is between 84.4 and 96.7% with a specificity of 98.5–100% [65]

Data reported in paediatric patients showed a sensitivity for DGPA-IgA in the range of 80.7–95.1% and a specificity between 86.3 and 93.1%; the positive likelihood ratios (LR+) and negative likelihood ratios (LR-) were from 6.9 to 12.7 and from 0.06 to 0.21 respectively. The diagnostic odds ratios (DOR) were between

56 and 93 which is lower than for TGA and EMA. The sensitivity in DGPA-IgG tests ranged between 80.1 and 98.6%, the specificity between 86.0 and 96.9%, the LR+ between 6.8 and 25.8, the LR_ between 0.02 and 0.21 and the DOR between 115 and 948 [19, 65]. In early studies patients with more severe mucosal damage had higher DGPA levels, but other studies have not confirmed a strict relationship. Also, although in general the levels of DGPA (IgG and IgA) increase in proportion to the degree of mucosal damage, a cut-off point to differentiate between patients with and without atrophy has not been identified [65, 66].

Although it is overall considered that performance of DGP-IgG is inferior to TGA, nevertheless there are still discrepancies on their use. Some authors affirm that as TGA tends to appear later in life than AGA, normally after the age of 1–2.5 years, DGPA should be used mainly in children under 2 years of age [67]. However this has not been confirmed in more recent studies [68] and it has even been shown that AGA can appear early in life and disappear without CD developing [48]. Thus, the role of DGPA in the diagnosis in children younger than 2–3 years still requires further assessment in large prospective studies, especially in comparison with TGA or EMA detection [62, 69].

There is also some debate on DGP being an earlier marker of mild histological lesion than TGA. Kurppa et al. reported that the sensitivity of DGPA was superior to TGA and comparable to EMA in patients having early-stage celiac disease with normal villous morphology. The authors conclude that DGPA seems to offer a promising tool for case-finding and follow-up in this entity [70].

Other authors however consider DGPA to have a diagnostic accuracy comparable to, or slightly lower than TGA-IgA [18]. So, it has been shown that DGPA-IgG are positive in the majority of patients negative for TGA, such as young children and patients of any age with selective IgA deficiency [50, 71].

Further studies confirmed these data as well as the fact that in this population DGPA-IgG maintained a high specificity and nowadays DGPA-IgG is considered to add real value in the diagnostic approach of CD in IgA deficiency [3].

## 5 POC Tests

In the last decade POC tests for CD have been developed and are commercially available worldwide. These are immunochromatographic rapid visual methods which are performed with whole blood/serum and use a strip coated with the antigen [21, 22, 72]. After the blood/serum diffuses down the strip, if antibodies are present in the patient's sample, antigen–antibody complexes are detected by labelled anti-human IgA and/or IgG antibodies, thus showing a series of coloured lines after a few minutes [21, 22, 72].

The first POC tests developed were based on AGA-IgA, but the number of published studies is too low to draw sounded conclusions about sensitivity and specificity, although both might be slightly above 95% [19, 21, 22, 72].

TGA-IgA based POC tests were developed later on with overall reported pooled sensitivities of 96.4% and pooled specificities of 97.7%; pooled LR+ was 40.6, LR- was 0.04, and DOR was 1343 [19, 22, 73], however, TGA-IgA or EMA perform better [3, 19, 22].

The main advantage of POCs is that they are easy to perform, do not require a laboratory or experienced laboratory staff, and results are rapidly available. Also because of stability for most of these methods, strips can be sent to a central lab for a centralized lecture or checking of the results.

Therefore, POCTs have the potential to increase CD diagnosis rates worldwide, facilitate early diagnosis, at a reduced cost. They are especially useful in primary care or in settings with inadequate infrastructure. Although they have also been used for screening in the general population, it is a matter of debate whether they could be performed by lay people if properly instructed [3, 19, 22]. Anyhow, results of POCs need to be confirmed by conventional tests performed in skilled laboratories by expert professionals, before a CD diagnosis can be established(Table 1).

## 6 Conclusions

Serological markers, specifically EMA-IgA, TGA-IgA and DGPA/ IgG have a high accuracy for CD diagnosis, particularly in the paediatric population, provided that IgA deficiency has been ruled out for IgA class SM and the patient is consuming a gluten-containing diet. The best performance for sensitivity is reported for TGA-IgA, which together with availability of automated methods, makes it the most popular SM used worldwide. EMA is superior in terms of specificity but detection by non-automated IF method requires a well-equipped laboratory and, above all, skilled personnel. One of the limitations of ELISA methods is the need for standardization, thus high quality commercial kits and quality controls of the laboratories are mandatory. POC tests, being rapid, cheap and easy to use, could be used in the initial approach of CD diagnosis but results needs to be confirmed by conventional assays.

**Table 1** Summary of performance and characteristics of the CD serological markers

| | Adults | | Children | | Detection methods | Pros | Cons | Use |
|---|---|---|---|---|---|---|---|---|
| | Specificity (range) | Sensitivity (range) | Specificity (range) | Sensitivity (range) | | | | |
| EMA-IgA | > 90% (86-100) | > 99% (90-100) | ≥ 90% (82-100) | 98.2 % (94-100) | Manual IF methods | The most specific test | Time consuming and high cost plus subjective interpretation | Confirmatory test |
| TGA-IgA | ≥ 95% (90-100) | ≥ 97% (92-100) | ≥ 95% (73.9-100) | ≥ 95% (77.8-100) | Automatized ELISA detection method | The most sensitive test | No standardization | As initial screening test and follow-up |
| TGA-IgG | ≥ 70% (67.6-100) | ≥ 90% (80-100) | (12.6-99.3) | ≥ 94%( 86.3-100) | Automatized ELISA detection method | Usually positive in IgA deficient CD patients | Variability of commercial detection kits' precision | For IgA deficient patients |
| DPGA-IgG | (84.4-96) | (98.5-100) | ≥ 95% (80.1-98.6) | ≥ 90% (86.0–96.9) | Automatized ELISA detection method | Usually positive in TGA-IgA negative CD children | Lower accuracy than TGA-IgA | For children below 3 years of age and IgA deficient patients |
| DPGA-IgA | (83.6-98.3) | (90.3-99.1) | ≥ 90% (80.7 - 95.1) | ≥ 90% (86.3-93.1) | Automatized ELISA detection method | No benefit | Lower accuracy than TGA-IgA and DGP-IgG | Not recommended |

EMA, anti-endomysial antibodies; TGA, antibodies against type-2 (tissue) transglutaminase; DPGA, deamidated gliadin peptides antibodies; IF, immunofluorescence staining; ELISA, enzyme linked immunosorbent assay; CD, celiac disease

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