Review Article

A review of the methods available for the detection of antibodies against transglutaminase and deaminated gliadin in Celiac Disease, traditional and emerging technologies.

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Abstract

Celiac Disease (CD) is an immune-mediated enteropathy where serological testing, centered on IgA anti Tissue Transglutaminase 2 (anti-TG2) and IgA anti Endomysium antibodies (anti-EMA), is the diagnostic cornerstone. Anti-TG2 is recognized as the most sensitive marker, while anti-EMA is the most specific. This review examines the performance characteristics, advantages, and limitations of traditional and emerging technologies for CD serology.

Traditional platforms include ELISA - a cost-effective, open platform used for quantitative anti-TG2 and anti-DGP detection and IIF for anti-EMA, which, despite being labor-intensive and operator-dependent, maintains high specificity by detecting anti-TG2 in its native conformation. Newer technologies prioritize automation in view of clinical laboratory demands.

CLIA and FEIA are fully automated systems that offer enhanced analytical sensitivity and rapid workflow. Meta-analyses confirm that the diagnostic accuracy of IgA anti-TG2 is statistically equivalent across CLIA (Sens. 0.98, Spec. 0.97), FEIA (Sens. 0.97, Spec. 0.99), and ELISA (Sens. 0.96, Spec. 0.97). However, CLIA often requires higher diagnostic thresholds for biopsy-sparing protocols. Emerging solutions include Multiplex Flow Immunoassays (MFI) /Microarrays, which enable simultaneous multi-isotype antibody detection with high concordance to reference assays (PPA 96.0 %, NPA 98.0 % for IgA anti-TG2) while Point-of-Care Tests (POCTs) provide rapid, equipment-free screening for primary care, though they exhibit moderate sensitivity compared to laboratory assays.

The overall trend favors automated and multiplexed methods, improving efficiency and supporting biopsy-sparing strategies based on robust quantitative autoantibody profiling.

Introduction

Celiac Disease is not an "allergy" to gluten, but an autoimmune disease

Celiac disease (CD) is a chronic, immune-mediated enteropathy that predominantly affect the small intestine of genetically predisposed individuals, precipitated by the exposure to food that contains gluten [1]. This disease affects females more frequently than males in a 3:1 ratio, as in most autoimmune diseases (AID), with first-degree relatives being at a higher risk of developing CD than the general population [2,3]. The genetic predisposition depends on the presence of HLA-DQ2/DQ8 (necessary condition, but not sufficient to develop CD on its own). Approximately 95.0 % of people with CD possess the HLA-DQ2 gene, while the remaining patients often carry HLA-DQ8 [4]. The worldwide prevalence of CD varies but is estimated to be approximately 1 % of the population [1].

Transglutaminase 2 (TG2) belongs to a family of enzymes that catalyze post-translational protein modifications through cross-linking or the incorporation of primary amines [5]. These reactions generate products that are highly resistant to mechanical stress and proteolytic degradation, explaining their prevalence in tissues where structural stability is essential [6]. Of the eight transglutaminases identified in the genome, six have been characterized through purification or recombinant methods [7]. Tissue transglutaminase 2 (TG2) participates in processes such as cell death, differentiation, matrix stabilization, and cell adhesion. It is best known for catalyzing covalent cross-links between proteins via transamidation, forming stable bonds between glutamine and lysine residues. This activity enhances extracellular matrix (ECM) stability and promotes cell attachment following enzyme externalization [6]. In addition to cross-linking, TG2 can catalyze deamidation, converting glutamine residues into glutamic acid. Given its broad tissue and cellular distribution, and its multiple enzymatic and non-enzymatic functions, the transglutaminase family is implicated in the pathogenesis of several diseases, including CD. In CD, TG2 is overexpressed and localized to the enterocyte brush border, cytoplasm, and ECM [8]. In patients with HLA predisposition, gluten is recognized as toxic and triggers inflammatory responses involving both the innate and adaptive pathways of immunity. Although the exact mechanism by which different antigens become targets of the immune system is not completely understood, it is thought that in CD the T cells activated by gluten cross-react with TG2 through epitope spreading [2]. The TG2 enzyme deamidates gluten peptides such as gliadin, making them better available to bind to MHC II on the surface of dendritic cells, activating the immune response [1] and increasing gliadin immunogenicity [5]. This process results in the activation of B lymphocytes that differentiate into plasma cells producing autoantibodies against TG2 (among others), leading to a full autoimmune response. Autoantibodies against TG2 (anti-TG2) contribute to CD pathogenesis by interfering with tissue repair, promoting inflammation and increasing permeability through the cells that form the outer lining of the small intestine [6]. The autoantibodies bind to TG2 and inhibit its enzymatic function, which in turn disrupts the tight junctions between the epithelial cells leading toincreased permeability or "leaky gut". In this condition pathogens filter through the cells undetected and undeterred by the immune system towards the lamina propria. Once bound to TG2 they form immune complexes that activate the immune system via the complement cascade and enhance pro-inflammatory cytokine production, mainly through IL-15, driving intraepithelial lymphocyte activation and epithelial damage. These autoantibodies also provoke a dysregulation of the pathways that remodel the extracellular matrix, impairing angiogenesis and contributing to fibrosis by exacerbating mucosal injury and perpetuating inflammation and tissue damage [7].

Gliadin is the main protein contained in wheat and due to its aminoacidic sequence and structure it is difficult to digest by human endopeptidases [8]. The proline/glutamine-rich fragment (peptide p31-43) is resistant to digestion by gastric, pancreatic, and intestinal proteases and it plays a crucial role in epithelial stress, increased permeability, and innate immune activation. The undigested peptide residues can have different biological activities such as inducing an innate or adaptive immune response related to their ability to bind to HLA-DQ 2.5, DQ 8 or DQ 2 [9,10].

Antibodies against deamidated gliadin are specific to modified gliadin peptides that are deamidated by TG2 as discussed above. These posttranslational modifications are believed to contribute to CD by augmenting gliadin immunogenicity [11]. Beyond the increased immunogenicity of TG2-mediated deaminated peptides, gliadin itself can trigger inflammation in a context of increased intestinal permeability. Gliadin is a plant component, thus acts as a foreign antigen and can directly stimulate innate immune cells, even in individuals without CD. Once in contact with gluten the intestinal epithelium releases zonulin which increases permeability and the translocation of gliadin to the lamina propria [2]. Overall, CD pathogenesis involves gluten-induced activation of adaptive immunity driven by TG2-deamidated peptides that stimulate Th1 $CD4^+$ T cells, IFN-γ production, and a specific autoantibody response leading to intestinal damage [12].

The cornerstone of CD diagnosis is the laboratory testing of autoantibodies.

In addition to anti-TG2, in CD one can find IgA and IgG -antibodies against gliadin (anti-gliadin antibodies - AGA) as well as against deamidated gliadin peptides (anti-DGP) [13], (Box 1). AGA can be found in other enteropathies or even in healthy individuals, which lowers its specificity and may lead to false positive results. For this reason, other autoantibodies are preferred, such as anti-TG2, anti-DGP or anti-EMA [1]. The endomysium is a layer of connective tissue that surrounds muscle fibers and thus is filled with TG2 enzymes either in the ECM or on the cell surface, allowing for the autoantibodies to be able to detect the enzyme in its natural conformational structure within the tissue. Essentially, the anti-EMA assay detects the presence of anti-TG2 in the sample, although other autoantibodies could be present - such as antibodies against other transglutaminases TG3 and TG6 [14]. These antibodies have been related to extraintestinal manifestations of CD [7] such as dermatitis herpetiformis (DH), and neurological complications like ataxia [15].

Many AIDs are linked, meaning the presence of one disorder seems to increase the risk of developing others. CD is no exception and is linked to selective IgA deficiency (sIgAD), type 1 diabetes, autoimmune thyroid disease and some chromosomal abnormalities [16].

The case of sIgAD is especially relevant, since IgA plays a homeostatic role regulating the intestinal mucosa. Patients that lack the proper concentrations of this antibody are more at risk of developing an immune reaction to certain foreign antigens, in the case of CD, to gluten. IgA levels increase gradually after birth until they reach peak levels during adolescence (with normal levels between 61-365 mg/dl) [17].

The cornerstone of CD diagnosis is the laboratory testing of autoantibodies, mainly IgA anti-TG2 and IgA anti-EMA, combined with total serum IgA in order to detect those cases that also present sIgAD [1,17,18]. Anti-DGP (IgA and IgG) and IgG anti-TG2 are also available, the former being considered useful in the diagnosis of CD in infants up to two years of age who have not yet fully developed IgA antibodies [12,18,19]. These types of serological tests have become important due to the fact that they are very convenient to perform (high throughput and automatization) and have lower risks than the duodenal biopsy [20]. Anti-TG2 have also been found to identify adults with mucosal lesions in their duodenum accurately helping clinicians avoid unnecessary invasive procedures [21]. The new 2025 Updated Guidelines on the Diagnosis and Management of Celiac Disease in Adults proposed by the European Society for the Study of Celiac Disease (ESsCD), recommends that routine use of IgA anti-EMA serology is no longer necessary, as long as validated high-performance IgA anti□TG2 assays are used [22]. Due to the labor-intensive and limited availability, IgA anti-EMA could be reserved for unclear cases to ensure diagnostic accuracy [22].

Anti-TG2 are considered pathogenic in CD, meaning they are drivers of the disease and keep perpetuating tissue damage. However, AGA and anti-DGP are not autoantibodies since the human genome does not contain the gene for gliadin (Box 1). This distinction between autoantibodies (anti-TG2 and anti-EMA) and regular antibodies (AGA and anti-DGP) is important because they reflect on different biological and immunological processes, the former are evidence of loss of tolerance by the immune system while the latter are showing a response towards external dietary antigens. As such, AGA and anti-DGP offer more insight into how the body reacts to gliadin when it is not digested properly.

All in all, anti-TG2 and anti-EMA are the primary diagnostic tools for CD, while anti-DGP is used when anti-TG2 results are uncertain, especially in young children or in patients with SIgAD. Usually, a combination of these tests along with a histological evaluation of the small intestine (via biopsy) and a correct anamnesis is the best way to diagnose CD [23]. The standard and most effective way to manage CD is following a gluten free diet (GFD) [24,25]. Considering that the digestion-resistant gliadin peptide p31-43 plays a crucial role in epithelial stress, increased permeability, and innate immune activation, avoiding it will essentially eliminate or considerably put a stop to CD major clinical complications, mainly involvingthe progression of mucosal injury, and perpetuation of inflammation and tissue damage. Follow up of these patients is not always simple. Studies have shown that IgG antibodies (for anti-TG2, anti-DGP and anti-EMA) persist longer than IgA antibodies, which makes sense considering their sources - long versus short-lived plasma cells - and the different roles the antibody isotypes play. IgA antibodies are more predominant in the intestinal mucosa and titers drop rapidly after GFD adherence, while IgG antibodies may show a longer-term immune response, especially if related to extra-intestinal symptoms [18]. In the American Gastroenterology Association clinical practice update of 2019 experts concluded that serology was more useful for follow-up in the case of children than in adults, and that lowering IgA anti-TG2 could help identify those patients with a healing intestinal mucosa [16].

Objective

In this manuscript, we review the methods available for detection of autoantibodies in celiac disease, including anti-transglutaminase and anti-deaminated gliadin, both traditional and emerging technologies.

Box 1: Are AGA and anti-DGP really autoantibodies? The correct answer is no.

We asked different online platforms with “Artificial Intelligence”, made of large language models, what they “thought” autoantibodies were. Responses were along the lines of autoantibodies are antibodies that mistakenly target and attack the body’s own tissues or organs. They are produced by the immune system when it loses the ability to distinguish between “self” and “non-self”, known as loss of immune tolerance, this is a hallmark of autoimmune diseases.

An expert in the field would further argue that autoantibodies actually represent a spectrum within an individual’s *antibodyomics*, including the physiologically important natural autoantibodies, which help with the immune system´s function of eliminating damaged cells and dead-cell remains, as well as other autoantibodies that are found in high titer in a significant proportion of people but are not related to any common autoimmune disease, such as the famous anti-DFS70, discussed elsewhere [26].

Technologies for detection of antibodies, biomarkers in celiac disease

Methodologically speaking, this study is not a systematic review but rather a narrative review. Extensive search was applied to identify studies related to different technologies and platforms for the detection of antibodies in CD, focused on anti-TG2, anti-DGP and anti-EMA. Still, the flowchart for the screening of studies following PRISMA-S method is presented in Supplementary Figure 1.

Traditional Technologies

ELISA (Enzyme-Linked Immunosorbent Assay)

ELISA is the most commonly used method for detecting anti-TG2 and anti-DGP. Based on the principle of antigen–antibody binding, antigens used to coat the wells may be recombinant or native, these can be extracted from sources such as animal tissues, wheat, or other biological materials.

Historically, different generations of ELISA tests have been defined based on the origin of the substrates, with early tests using TG2 derived from guinea pig liver before human derived or recombinant TG2 demonstrated superior performance, Box 2 details the case for DGP.

Dieterich *et al.* identified TG2 as the autoantigen against which the detection of anti-reticulin antibodies (ARA) and anti-EMA tests were made in 1997, ushering in a new era for CD serology testing with the development of specific ELISA testing [27]. This technique is highly sensitive, easy to perform, automatize and can be done at relatively low cost since it doesn't require highly trained personnel or dedicated equipment. However different commercial kits (which usually include all the different reagents and solutions necessary to perform these tests) can have varying specificities, heavily influenced by the source of their antigens. For clinical diagnostics the assays that should be used are those with CE-IVD or FDA approval, or that have been approved by country-specific regulatory agencies. One should not assume that just because the platform is similar (ELISA), assays from different manufacturers will have similar performance as our group has extensively discussed in previous publications [28]. This holds true for any antibody detection kit, production lots may vary within the same brand, kits may vary between brands, and everything varies depending on the quality of the antigen used to coat the wells of each kit. These variabilities may produce false positives or false negatives [14].

Antibody detection can be modulated by modifying either the substrate or the secondary anti-human immunoglobulin (Ig) antibody. Using TG2 as a substrate enables the detection of anti-TG2 antibodies, while coating wells with DGP allows for the detection of anti-DGP antibodies. Similarly, modifying the secondary antibody with specificity for different Ig isotypes allows for the identification of IgG, IgA, or both [29].

Box 2: Obtaining deaminated gliadin for immunoassays.

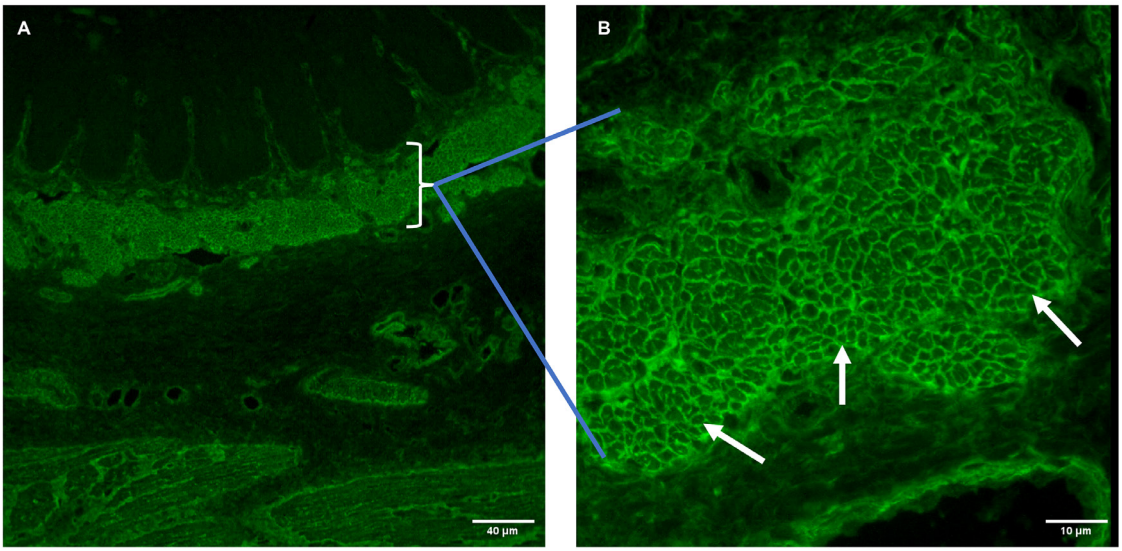
DGP peptides used in solid-phase immunoassays for anti-DGP detection are generated through a multi-step process that begins with the extraction of gliadin from wheat flour. Gliadin - a prolamin protein fraction of gluten - is isolated when using solvent extraction methods, these typically employ ethanol or aqueous alcohol solutions, which separate gliadin from other gluten components such as glutenins [30].

Following extraction, the critical step of deamidation is performed enzymatically by using purified recombinant TG2, which mimics the physiopathological modifications present in patients with CD and ensures the preservation of the conformational epitopes recognized by anti-DGP. This reaction occurs under physiological simulated pH and temperature conditions, with TG2 preferentially targeting glutamine residues in motifs such as QXP or QXXF(Y/W/M/L/I/V), which are essential for HLA-DQ2/DQ8 binding in the pathogenesis of CD [6,7,30].

Indirect Immunofluorescence (IIF) Used for detecting anti-EMA.

The traditional substrate used for detecting anti-EMA by IIF is monkey esophagus, however there are several other alternative substrates such as human umbilical cord, human esophagus or primate liver. Several authors from the 1990s published articles trying to discourage the use of endangered species for organ harvesting to be used as substrate, however all of these current options have problems either with availability or with ethical and environmental concerns [29,31,32]. Volta *et al.* compared results for IgA anti-EMA on monkey esophagus versus human umbilical cord for patients with CD under the premise that the human umbilical cord is: 1. commonly available, 2. rich in reticulin fibers (endomysium) that surround smooth muscle fibers, and 3. that unlike other human tissues it does not contain IgA, therefore limiting cross reactivity [31]. No differences were found between doing the test in either substrate in this study (meaning a similar performance) either with the CD patients or the control groups. This test can be labor intensive if it is done manually. Several semi-automated machines are available to help laboratories run the immunodetection part of the assay. The glass slide is coated with the substrate in several "wells" and on each well the machine pipettes a dilution of the patient's sera. After the slides are ready, the technician can observe the characteristic honeycomb-like immunofluorescence pattern in the microscope (Figure 1). The interpretation is subjective to the technicians' training and skill. High background noise and non-specific staining can hinder readers from confidently emitting a result. As with all IIF techniques, anti-EMA testing has its limitations due to tissue autofluorescence, reagent instability and differences between reagent brands and even kit lots. It is important to highlight that patients must be consuming gluten when this test is performed for diagnosis.

Figure 1: Characteristic honeycomb-like pattern in samples with anti-TG2 antibodies.



Indirect immunofluorescence assay with monkey Esophagus as substrate (Kit NOVA Lite, Ref. 704155, Inova Diagnostics). A representative sample with anti-TG2 antibodies, positive for both IgG and IgA, was applied and the assay was performed following manufacturer recommendations. (A) 10x objective. (B) 40x objective. The characteristic honeycomb-like pattern (arrows) can be observed with 400x magnification

The results of this test can be reported as either qualitative (positive or negative) or in a semi-quantitative form by reporting titers from double dilutions. Despite differences in initial screening dilutions, Murray *et al.* found that across several laboratories in the USA IgA anti-EMA testing usually reports high sensitivities (60.0-100 %) and specificities (80.0-100 %), however both of these parameters are affected by the initial screening dilution [29,33–36]. Even though the concordance between laboratories was high (kappa coefficient: 0.739), the laboratory that used the highest screening dilution (1:20) had a statistically lower sensitivity than the rest [33]. Anti-EMA values are usually linked to higher degrees of histological lesions and villous atrophy and its titers also change once the patient has implemented a GFD [24,29,37].

The main difference between an IgA and IgG anti-EMA is the FITC conjugate immunoglobulin used, if one is testing IgG then an immunoglobulin against the Fc portion of an IgG antibody should be used. When using monkey esophagus as a substrate the pattern observed is the same. Nevertheless, as we have stated before, it is recommended to use this test exclusively in the case of sIgAD as is the case for any other IgG antibody used for diagnosis in this disease [38].

Anti-EMAs are not the only identified targets for CD-related autoantibodies, others have been described as well, but their use has dwindled in the past decades since ease of use and automatization of the more commonly used tests increased. Other examples of targets are: calreticulin, zonulin, desmin, reticulin and antibodies against jejunum [39].

Anti-reticulin antibodies (ARA) were originally described in 1977 in patients with DH, CD and Crohn's disease in rat stomach, kidney, and liver tissue (SKL) where the R1 pattern (peritubular staining in the kidney or periportal tissue in the liver) was associated with CD [40]. Both IgA and IgG ARA can be detected [29] and seem to be closely related to anti-EMA. Hällström *et al.* found positive IgA antibodies for both ARA and anti-EMA in 91.0 % of an untreated adult CD patient cohort, while 83.0 % of a cohort of DH patients had both autoantibodies [40]. Even so, the study could not confidently differentiate both diseases (CD and DH) with these (or any other) tests. Both types of antibodies were found to decrease with a GFD. Although both autoantibodies seem to be closely related, they do not appear to have the same antigen specificity. Mäki described these tests as rodent-type reticulin (reticulin) and primate-type reticulin (endomysium) in the early 90s [39]. In 2003 Koroponay-Szabó *et al.* reported a study with TG2 knockout mouse tissue in which they conclude that diagnostically relevant anti-EMA, ARA and jejunal antibody (JEA) found in patients with CD and DH were clearly and exclusively anti-TG2 autoantibody dependent [41]. More recent papers have declared ARA obsolete due to its failure to measure up to other tests used for routine diagnostics [42]. Rodent substrate offers a lower sensitivity and larger inherent subjectivity due to the fact that the R1 pattern in SKL must be detected in the three different rodent tissues. With the later identification of TG2 as the target antigen for anti-EMA, and that anti-DGP proved to be a more specific CD biomarker, and their subsequent test developments, the algorithm for CD diagnosis changed thus, ARAs fell further in disuse [27,43]. As Korponay-Szabó and collaborators state in their 2003 publication, ARA and anti-EMA are not antigen dependent or even species dependent but rather detect the same phenomena in different tissue types, as both detect anti-TG2 autoantibodies [41]. These autoantibodies that have not endured the test of time have not demonstrated to be S3MA2RT2, as Fritzler *et al.* described in 2021 [44]. They were either not Specific, Sensible or Scalable, Measurable using conventional technologies, did not *Add value* to clinical management or were not Actionable or Realistic, or were neither Titrable nor addressed the Temporal Timing of the disease, and so fell into the "death-valley" of autoantibodies [44].

Caja et al. describe how anti-EMA is a superior test for anti-TG2 ELISA due to the fact that it shows a greater correlation to the presence of HLA DQ2 or DQ8, which is not always the case for individuals who are anti-TG2 seropositive [14]. This, and because anti-EMA is a "more stable test" makes it hold its place as the "gold-standard" serology test, although the concept of gold-standard changes over time. A 2006 review by Lewis and Scott discusses how anti-EMA more often has a higher specificity while tests for anti-TG2 antibody have a higher sensitivity [45]. Thus, the majority of the current guidelines such as the 2020 European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the 2023 American College of Gastroenterology (ACG) guideline, recommend performing solid-phase assays such as anti-TG2 and anti-DGP as screening and confirming antibody positivity with the more specific anti-EMA assay. Yet, as previously mentioned, some 2025 updated guidelines, as from ESsCD, recommends the decrease of routine IgA anti-EMA in adult CD diagnostics [22,34,46–48].

Recent wide-spread Technologies

Chemiluminescence Immunoassay (CLIA): Provides enhanced diagnostic accuracy with better automation and sensitivity.

CLIA applies the same antigen–antibody principles as ELISA but detects immune complexes through light emission expressed as relative luminescence units (RLU). The antigen, usually human recombinant TG2 is immobilized on paramagnetic or glass microbeads This technique requires specialized equipment to be able to run the test and detect the light emitted. Compared with conventional colorimetric ELISA or fluorescence based FEIA (fluorescence enzyme immunoassay) (described ahead), CLIA offers enhanced analytical sensitivity, broader dynamic range, and full automation. Paramagnetic-bead chemistry provides a larger reactive surface and lower background noise, improving precision and reducing inter-assay variability.

A systematic review and meta-analysis found pooled sensitivity 0.98 (95.0 % CI 0.95–0.99) and specificity 0.97 (95.0 % CI 0.94–0.99) for IgA anti-TG2 by CLIA, values comparable or slightly superior to ELISA and FEIA [49]. The analysis also noted delayed normalization of anti-TG2 titers during GFD follow-up when measured by CLIA, an important consideration for long term monitoring. Elsewhere, in a large biopsy-correlated series using a CLIA platform for IgA anti-TG2 (QUANTA Flash by Werfen), sensitivity and specificity reached 98.2 % and 98.4 %, respectively [50]. The optimal biopsy-saving thresholds were ≈ 15 × ULN (upper limit of normal) in adults (350 CU) and ≈ 28 × ULN in children (560 CU), higher than the 10 × ULN rule adopted for ELISA assays [23]. This data supports CLIA's diagnostic reliability but indicates that method-specific cut-offs must be applied.

In summary, CLIA provides standardized, quantitative detection of anti-TG2 and anti-DGP antibodies with high automation and throughput, though it requires dedicated instrumentation and calibration traceability [51].

Fluorescence Enzyme Immunoassay (FEIA): Offers quicker response times and higher throughput than ELISA.

FEIA is a variant of ELISA, the difference being that instead of a colorimetric reaction at the end of the test, the secondary antibody is attached to a fluorescent molecule (usually FITC), thus fluorescence intensity is detected. This change enables the use of different detection methods, improving sensitivity and allowing for the measurement of lower concentration of antibodies. FEIA could be considered as an evolution of the ELISA principle, offering shorter turnaround times and higher analytical throughput. The use of fluorescence-based detection also facilitates multiplexing capabilities and broader dynamic range, contributing to improved quantification and reproducibility. Commercial FEIA platforms (e.g., Phadia EliA systems) are fully automated, enabling continuous sample loading and standardized calibration protocols. Both CLIA and FEIA surpass ELISA in speed, automation, and traceability, but CLIA generally achieves slightly higher sensitivity at very low antibody titers. The main limitation in both is higher costs since they are closed systems with dedicated equipment.

According to the meta-analysis by Pjetraj et al., FEIA for IgA anti-TG2 reached pooled sensitivity of 0.97 and specificity of 0.99, comparable to CLIA (0.98 / 0.97) and ELISA (0.96 / 0.97) [49]. Despite this, FEIA showed faster response times and better reproducibility in multicenter evaluations. These results confirm FEIA as a robust, high-performance alternative suitable for both screening and follow-up of CD.

Emerging technologies

Multiplex Flow Immunoassay (MFI) and microarray: Emerging as an alternative with greater sensitivity for different antibody types.

Multiplex assays enable the detection of multiple autoantibodies in a single test and can be classified regarding the ability that they have for specific autoantibody identification [28,52]. For example, if one had a test for detecting IgA and IgG anti-DGP simultaneously (as in the case for INOVA CLIA anti-DGP screen test) one could not identify if a positive sample was positive for antibody isotype IgA, IgG, or both. In the case of a Line Blot, one could identify every individual autoantibody through the antigen adsorbed in a line on the nitrocellulose membrane that would appear in the case of sample positivity.

More modern formats for MFIs are bead-based (microspheres with unique fluorescent signatures covered with the antigens such as BioPlex 2200 platform [53]), or microarray-based technologies that use "spots" of antigens adsorbed on a nitrocellulose membrane, polymer, or hydrogel. These assays have been developed thanks to the use of molecular biology cloning and micro-printing technologies and have been adapted to large-scale laboratories through technology that allows for these assays to run automatically and with a higher throughput [28]. The conservation of the selected proteins in their native conformation is essential for optimal autoantibody detection. The test principle is the same as with ELISA, CLIA and IIF; involving the formation of immune complexes between the substrate antigen, patient autoantibody and the secondary detection antibody, which according to the preferred detection method, can be conjugated to a fluorophore or chemiluminescent dye.

A recent example of this technological evolution is the MosaiQ AiPlex Celiac Disease Microarray Solution, an IVDR-CE–marked microarray system designed for the MosaiQ platform. A analytical comparison conducted in a reference laboratory in Wales, UK evaluated the MosaiQ AiPlex CD microarray from AliveDx - a fully automated, single-use multiplex immunoassay - for the simultaneous semi-quantitative detection of IgA and IgG anti-TG2 and anti-DGP antibodies [54]. When compared with routine ELISA, FEIA, and CLIA methods (EliA Celikey, QUANTA Flash, QUANTA Lite, and ORGENTEC assays), the AiPlex system demonstrated a positive percent agreement (PPA) of 96.0

% and negative percent agreement (NPA) of 98.0 % for IgA anti-TG2, and PPA 78.0 % / NPA 93.0 % for IgG anti-TG2. Overall concordance with standard assays was high (90.0 % positive and 95.0 % negative agreement), confirming the analytical equivalence and reliability of this microarray platform for CD serology. This platform is presented as a robust and efficient alternative for high-throughput laboratories, capable of simultaneously analyzing multiple antibody isotypes while minimizing sample handling and operator variability [54].

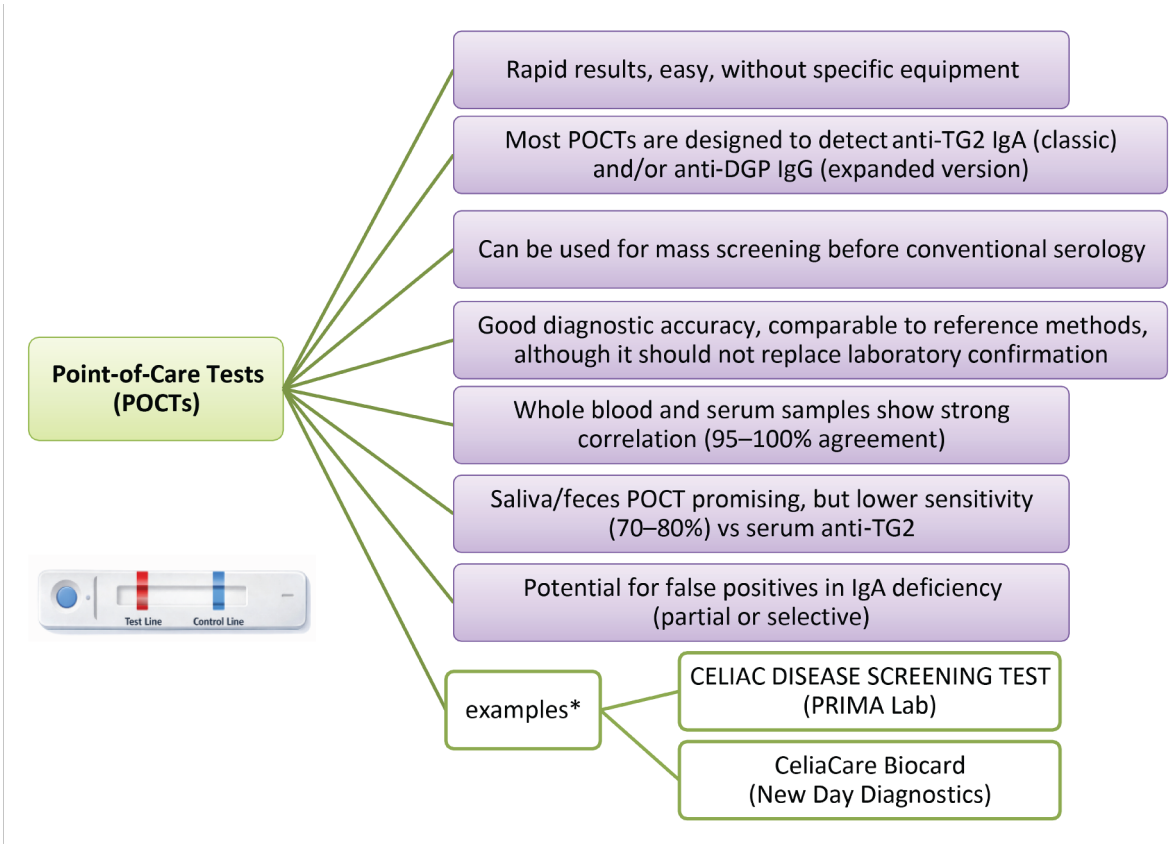
Point-of-care tests (POCT): Rapid tests developed for use in settings with limited resources or in a general physician's office. POCTs offer immediate results with moderate sensitivity with no need of laboratory equipment or expert personnel [29,55], such as the CeliaCare Biocard (from New Day Diagnostics) and the CELIAC DISEASE SCREENING TEST (from PRIMA Lab) among others. These are single-use immunochromatographic or dot-blot assays in which patient antibodies bind immobilized recombinant TG2, forming visible immune complexes detected without instrumentation [56].

Baldas et al. first described a new dot-blot assay specifically designed for ambulatory settings based on the recognition of human, recombinant TG2 and compared it to IIF (anti-EMA) and ELISA methods [57]. The test could be performed in 20 minutes and presented a sensitivity of 100 % and a specificity of 98.0 %, for which they concluded it was highly accurate in detecting untreated patients (Figure 2). This test is able to detect patients with sIgAD due to concomitant detection of IgA and IgG antibodies, has a low cost and can be performed with minimal handling. Another advantage is that it can be performed with either serum or whole blood drop [58], although in both cases it is diluted and not used directly. Nemec et al. used lateral flow tests and reported that when they used the whole blood drop test the sensitivity was slightly lower, 96.0 % than in the test that required serum (100 %) in comparison to ELISA tests [58]. They were unable to identify the patients that had sIgAD since that commercial brand of POCT could not measure IgG anti-TG2.

This methodology was tested in both adults [59] and children [60] and has improved since Baldas's description in the 2000s. In the study by Korponay-Szabó et al. [60] 2690 children of 6 years of age were tested with a 5-minute immunochromatographic card detecting IgA anti-TG2 antibodies in whole blood. In this study the test yielded 78.1 % sensitivity and 100 % specificity, while sensitivity decreased to 65.1 % when compared to combined laboratory testing for IgA and IgG anti-EMAs by IIF. Alarida et al. also reported a low sensitivity in their study performed on Libyan children in a cohort of low pre-test probability [61] concluding that rapid determination of IgA or IgG anti-TG2 on whole blood drop was not the test of choice for screening purposes.

One of the advantages of this kind of testing is that it could be useful for population screening, especially in countries that have routine mandatory screening for children. However, there are still concerns regarding the cost-effectiveness of these measures as public policy, and regarding the adequate follow up and continued care for these families and patients [55]. Other issues are that POCT may have quality control concerns when handled by users with little experience, not to mention that rapid results are rarely decisive in CD [16]. POCTs should not replace laboratory confirmation in CD (Figure 2).

Figure 2: Summary of the main advantages and limitations of POCTs for CD.



*It is illustrating its operational simplicity and clinical applicability compared with conventional laboratory-based serological methods. *Two examples of rapid lateral-flow or dot-blot assays are mentioned in the Figure; however, numerous commercial POCTs are available from different manufacturers, such as Simtomax (Augurix Diagnostics), Simple CD1WB (Operon), MyTest Celiac (Cooper Consumer Health) and Xeliac Pro (EUROSPITAL SpA), to mention a few examples. While some POCTs are well studied and clinically validated, others are consumer-oriented kits with limited or no independent clinical validation.*

Strengths and weaknesses of different platforms

Various publications go over comparisons between different techniques. However, before going into detail, it is important to remember that even if one compares different techniques and platforms (CLIA vs IIF, IIF vs ELISA, etc.) one should always consider that there can be significant variability within the same technique among different brands, especially for ELISAs, as discussed above.

A recent systematic review by Pjetraj *et al.* evaluated the diagnostic accuracy of IgA anti-TG2 assays across different technologies, including CLIA, ELISA, and FEIA [49]. Pooled results demonstrated excellent overall performance for all methods, with CLIA sensitivity 0.98 (95.0 % CI 0.95–0.99) and specificity 0.97 (95.0 % CI 0.94–0.99), FEIA 0.97/0.99, and ELISA 0.96/0.97. No statistically significant differences in diagnostic accuracy were found between the three assay types, indicating that each provides reliable detection of IgA anti-TG2 antibodies when validated and properly calibrated. The authors also noted minor inter-method variability in antibody-titer normalization during gluten-free diet follow-up, underscoring the need for harmonized cut-off definitions and traceable calibration standards (Table 1). Taken together, this evidence supports the analytical equivalence between modern immunoassay platforms and reinforces their collective value in the accurate, non-invasive serologic diagnosis of CD.

For the diagnosis of CD, anti-TG2 IgA represents the most sensitive marker and the basis of current screening algorithms, while IgA anti-EMA remains the most specific assay, closely correlating with the presence of villous atrophy. Anti-DGP antibodies, particularly of the IgG isotype, serves as useful complementary markers in younger children or in patients with sIgAD. Comparative studies and meta-analyses have quantified the relative diagnostic performance of these antibodies, providing the basis for modern serologic algorithms and biopsy-sparing criteria.

A study by Volta *et al.* (2023) mentions the high variability in accuracy and tracks diagnostic precision among several serologic markers for CD such as IgA anti-TG2, IgA anti-EMA and both IgA and IgG for anti-DGP [62]. With a total of 5.098 pediatric and adult patients among all 44 original studies, they found that IgA anti-TG2 had a slightly higher sensitivity than IgA anti-EMA (93.4 % vs 92.8 %) and IgG/IgA anti-DGP had lower sensitivities (81.8 % and 83.8 % respectively). The highest specificity was as expected for IgA anti-EMA (99.0 %), followed by IgG anti-DGP (96.4 %), IgA anti-TG2 (95.8 %) and IgA anti-DGP (92.1 %) [59,62].

Lewis and Scott (2006) compared anti-EMA and anti-TG2 finding very high sensitivities (93.0 %) and specificities (more than 99.0 % and 98.0 % respectively) for both assays in the context of patients with CD that presented villous atrophy [45].

They also reviewed the difference between using human recombinant TG2 vs guinea pig TG2 (human recombinant tests had high sensitivities and specificities with a 95.0 % CI) as well as the difference between using monkey esophagus vs human umbilical cord for anti-EMA studies (they were both very similar, 93.1 % vs 92.9 % in sensitivities and 99.1 % vs 99.7 % specificities, with a 95.0 % CI) [45].

Table 1 summarizes the characteristics, advantages, and limitations of the main serological technologies for CD. Traditional methods such as ELISA and IIF remain the most widely used, with ELISA predominating as an open, versatile, and cost-effective platform adaptable to different reagents and instruments. IIF has exceptional specificity for anti-EMA antibodies but remains labor-intensive and operator-dependent,meaning higher costs as well. In contrast, automated methods such as CLIA and FEIA offer enhanced analytical sensitivity, broader dynamic range, and random-access automation, aligning with modern high-throughput laboratory workflows. The advent of multiplex and microarray systems has enabled simultaneous multi-isotype antibody detection within a single reaction, combining efficiency with excellent analytical concordance to reference assays. POCTs extend diagnostic accessibility to low-resource or primary-care settings, providing rapid, equipment-free results suitable for preliminary screening (Figure 2). Collectively, these technologies illustrate a clear transition from traditional manual assays toward integrated, automated, and multiplexed solutions that improve diagnostic precision, workflow efficiency, and patient accessibility.

Table 1: Comparison of Traditional and Emerging Technologies.

Technology	Characteristics	Strengths	Platform weaknesses and critical points of the experimental procedures	Marker	Diagnostic Performance*		References
					Sensitivity Range	Specificity Range	
ELISA	Quantitative solid-phase assay for detecting anti-TG2 and anti-DGP antibodies. The most widely used platform for CD serology.	- Open platform, compatible with multiple reagents and instruments. - Cost-effective, simple, and well validated. - Extensively standardized and accepted by regulatory agencies.	- Requires batch testing and manual handling. - Variable performance across manufacturers and antigen sources. - Limited to single-analyte detection. - Must be careful with standard curve calibration and definition of cut-offs.	IgA anti-TG2	90.0% - 98.0%	95.0% - 99.0%	[1,23,36,62,63]
				IgG anti-TG2	~70% or lower in IgA-competent	94.0% - 100%	[1,18,22]
				IgG anti-DGP	80.0% - 97.0%	86.7% - 99.0%	[1,7,64]
Indirect Immunofluorescence (anti-EMA test)	Detects anti-EMA antibodies using tissue substrates (monkey esophagus or human umbilical cord).	- High specificity (“gold standard” for anti-EMA). - Good confirmatory test. - Detects antibodies against TG2 in their native conformational state.	- Subjective pattern interpretation requiring skilled personnel. - Semi-quantitative. - Requires fluorescence microscopy. - Labor-intensive; ethical and supply issues with animal substrates.	IgA anti-EMA	83.8% - 98.0%	97.5% - 100%	[1,22,23,62,65,66]
CLIA (Chemiluminescence Immunoassay)	Fully automated, quantitative detection based on light emission from acridinium- or isoluminol-labeled complexes.	- High analytical sensitivity and dynamic range. - Rapid, random-access workflow and excellent reproducibility.	- Closed system, higher instrument cost and potential procurement issues in some countries, vendor lock-in. - Requires calibration traceability and maintenance. - High dynamic range (0.1-4965 CU); requires method-specific cut-offs.	IgA anti-TG2	95.3% - 98.3%	94.0% - 99.0%	[49,50,67]

Technology	Characteristics	Strengths	Platform weaknesses and critical points of the experimental procedures	Marker	Diagnostic Performance*		References
					Sensitivity Range	Specificity Range	
FEIA (Fluorescence Enzyme Immunoassay)	Variant of ELISA using fluorescent detection.	- Faster turnaround and higher throughput than ELISA. - Broad dynamic range and multiplexing potential.	- Closed automated system with higher per-test cost and potential procurement issues in some countries. - Limited flexibility for antigen/cut-off customization. - Slightly lower sensitivity than CLIA at very low titers.	IgA anti-TG2	~97%	98.0% - 99.0%	[21,49,62]
Multiplex Flow Immunoassay / Microarray	Detects multiple autoantibodies (anti-TG2, anti-DGP) simultaneously on bead- or microarray-based platforms.	- Enables multiplexing of IgA/IgG isotypes in a single test. - May even incorporate internal IgA-deficiency check. - High throughput, minimal sample volume, and automation. - Comparable accuracy to reference methods (PPA 96.0%, NPA 98.0% for IgA anti-TG2).	- High setup cost and need for complex panels and robust assay standardization. - Limited clinical availability. - Interpretive software required.	Combined (anti-TG2/ DGP)	77.0% - 96.0%	98.0% - 100%	[53,68,69]
POCT (Point-of-Care Tests)	Lateral-flow or dot-blot rapid assays detecting anti-TG2 IgA/IgG +/- anti-DGP IgG.	- Rapid, low-cost, and equipment-free. - Suitable for field use and primary-care screening. - Whole-blood and serum results strongly correlated (95.0-100% agreement).	- Moderate sensitivity compared with laboratory assays. - Not reliable to rule out CD in high-suspicion clinical settings. - Potential false results in IgA deficiency. - Positive results should be confirmed with standard laboratory testing. - Limited quality control in untrained hands. - Many consumer-oriented kits with little-to-no clinical validation.	IgA/IgG anti-TG2	70.0% - 98.0%	90.0% - 98.0%	[56,65,67,70]

**Diagnostic performance of CD serological platforms in comparison with reference standards such as duodenal histology and/or gold-standard antibody assays.*

Conclusion

Advances in serologic technologies over the past decade - and how those are applied - have improved considerably, resulting in significant changes benefiting the patients such as allowing the diagnosis of CD without duodenal biopsies in defined scenarios. In children, the ESPGHAN 2020 pathway allows a biopsy-sparing diagnosis when IgA anti-TG2 is ≥10× ULN, confirmed on a second sample with anti-EMA and made within a shared decision-making process [64,71]. Large prospective validation studies have confirmed the safety of this approach: Wolf *et al.* demonstrated that children with IgA anti-TG2 ≥10× ULN plus anti-EMA positivity can be diagnosed without biopsy, achieving a PPV ≈ 99.0 %; Chokkalla *et al.* reported recently (2024) comparable accuracy for the biopsy-free approach versus the traditional method in North American pediatric cohorts [72,73].

Analytical innovations have also clarified assay-specific cut-offs and limitations. Previtali *et al.* (2018) showed that CLIA may require higher diagnostic thresholds than ELISA, while Pjetraj *et al.* (2024) confirmed that CLIA achieves sensitivity 0.98 and specificity 0.97 - comparable to conventional platforms [49,50]. Multiplex and multi-analyte systems also follow the same logic: in 2025 Gambino *et al.* validated a multiparametric IgA blot combining several gliadin and TG-related epitopes with 100 % specificity when ≥ 6 antigen markers were positive; the same year Zingone *et al.* showed that concurrent positivity for IgA anti-TG2 and IgG anti-DGP above 10×ULN predicted Marsh 3 histology in adults with 100 % PPV [68,69].

As is the case for many AIDs, assay standardization - in particular for autoantibody measurement - remains a major challenge, and CD is no exception. The lack of international standardization for antibody concentrations means that results obtained across different platforms are not directly comparable and often require local validation to support clinical decisions. A significant step toward addressing this gap is the recent international effort to harmonize CD serology through certified reference materials. In 2025, the Joint Research Centre of the European Commission, in collaboration with the IFCC, released the first certified reference material for anti–TG2 antibodies, both IgA and IgG (ERM-DA487_IFCC). This initiative provides a metrologically traceable calibration anchor, representing an important advance toward more reliable clinical cut-offs and standardized diagnostic pathways in CD.

Viewed together, these developments point towards a future in which most CD diagnoses will be biopsy-free, achieved through the integration of quantitative autoantibody profiles, HLA-DQ2/DQ8 genotyping, and emerging T-cell or mucosal-immune biomarkers [74]. Machine-learning and multi-omics tools, such as the XGBoost-based prescreening model of Dreyfuss *et al.*, demonstrate how artificial intelligence can recognize latent serologic or biochemical signatures before clinical diagnosis [75,76]. In this precision-diagnostic framework, intestinal biopsy will likely remain a problem-solving procedure, reserved for seronegative, equivocal, or refractory presentations rather than routine.

Another recent development, showcasing a future trend for laboratory testing in CD, is related to the new 2025 ESsCDupdated guidelines that further consolidate IgA anti-TG2 at the center of CD diagnosis in adults, recommending that routine anti-EMA is not required when high-performance anti-TG2 assays are used, reserving anti-EMA for equivocal cases [22]. However, we must emphasize that in the current guidelines from ESPGHAN and ACG, the recommendation is to perform solid-phase assays such as anti-TG2 and anti-DGP as screening and confirm positivity with the more specific anti-EMA assays [34,46,47].

List of Abbreviations

ACG: American College of Gastroenterology
AID: Autoimmune Disease
AGA: Anti-gliadin Antibodies
ARA: Anti-reticulin Antibodies
CD: Celiac Disease
CE-IVD: Conformité Européenne – In Vitro Diagnostic
CLIA: Chemiluminescence Immunoassay
CU: Conventional Units
DGP: Deamidated Gliadin Peptides
DH: Dermatitis Herpetiformis
ECM: Extracellular Matrix
ELISA: Enzyme-Linked Immunosorbent Assay
EMA: Anti-endomysium Antibodies
ESPGHAN: European Society for Paediatric Gastroenterology, Hepatology and Nutrition
ESsCD: European Society for the Study of Coeliac Disease
FDA: Food and Drug Administration
FEIA: Fluorescence Enzyme Immunoassay
GFD: Gluten-Free Diet
HLA: Human Leukocyte Antigen
IIF: Indirect Immunofluorescence
JEA: Jejunal Antibody
MFI: Multiplex Flow Immunoassay
NPA: Negative Percent Agreement
NPV: Negative Predictive Value
POCT: Point-of-Care Testing
PPA: Positive Percent Agreement
PPV: Positive Predictive Value
sIgAD: Selective IgA Deficiency
SKL: Stomach, Kidney, and Liver Tissue
TG2: Transglutaminase 2
ULN: Upper Limit of Normal
USA: United States of America

Declarations

Declaration of Conflict of interests

The authors declare that they have no conflicts of interest in relation to this manuscript.

Ethical Approval

Not applicable.

CRediT Author Statement

DL: Conceptualization, Methodology, Investigation, Writing – Original Draft.
GDK: Conceptualization, Methodology, Supervision, Writing – Review & Editing.

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Disclosure on the use of AI tools
The manuscript was originally written in Spanish and Portuguese, the mother language of the authors, and translated to English using online AI tools such as ChatGTP (by OpenAI) and Gemini (by Google/Alphabet). The same tools were also applied to improve the structure and clarity of parts of the text. Figure 2 was partially generated using NotebookLM and ChatGTP, followed by manual adjustments. The online platforms with "Artificial Intelligence" mentioned in Box 1 for the question about autoantibodies were ChatGTP, Gemini and Elicit (https://elicit.com/). Last, as detailed in Supplementary Figure 1, identification and filtering of entries and references were carried out with the help of automation tools, such as Google's Gemini, ChatGPT, Zotero, among others, in other to build the list of studies that went for manual screening to be part of the reference list, following PRISMA-S method.

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Supplementary file

Supplementary Figure 1: Flowchart according to the PRISMA-S method for the selection, screening, and inclusion of studies in the review.