

Current detection and quantification method for gluten to support the gluten-free claim: an insight about elisa method

Abstract

The global market for gluten-free products has grown in the last few years, and is expected to keep expanding in the future. Considering this trend, it is of paramount importance to critically develop and further enhance control measures to identify and manage contamination issues at all levels of the production chain in order to meet the current regulatory compliance. Detection and quantification methods are one of the widely used tools to monitor the process and ensure full compliance with gluten-free definitions. In this regard, it is important to examine the limitations and challenges of the currently available methods to ensure the compliance of gluten-free labeled products. This short communication reviews some issues associated with the use of commercially available ELISA methods, since they are the current choice for gluten detection.

Keywords: gluten free, elisa, CD, immunological, malabsorption, malnutrition

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Abbreviations: CD, celiac disease; LOQs, limit of quantification; ELISA, enzyme-linked immunosorbent assay; DNA, deoxyribonucleic acid

Introduction

Celiac disease (CD) is an enteropathy mediated by immunological mechanisms triggered by the interaction of gluten with the intestinal mucosa in affected individuals.¹ The consequences of this condition are the intestinal extensions leading to malabsorption and malnutrition.² The only currently effective health strategy for affected consumers is to avoid gluten-containing products, and such the food must be labelled clearly. However, despite unanimously accepted Codex definitions by all member jurisdictions, the national implementation of equivalent laws displays significant variations. With reference to CD and in support of the gluten-free statement, regulatory enforcement, as well as manufacturers' quality controls are typically centered and rely on analytical results. The market for the gluten free is expanding and needs to develop and further improve the detection methods for gluten is of paramount importance. Currently, commercially available antibody-based assays are designed to detect gluten to verify and support gluten-free claims in the context of CD. However, these assays are not developed for the purpose of detecting gluten-containing cereals in the context of food allergies. The other wheat allergens which are water soluble like globulins and albumins are also targeted by commercial ELISA kits. Secondly, there are no thresholds established for food allergens, and the lower limit of Quantification (LOQs) of commercial assays for gluten may not be suitable for testing of allergen. Additionally, there is a gap between gluten free definition and food allergen labeling regulation. For example, gluten-free definitions state the reporting unit as mg gluten/kg product, which is used by the kits. However, food allergen labeling regulations mandate the common name of the cereal to be labeled. Commercial assays report only gluten with no conversion factor from

gluten to cereal available.³ Moreover, because currently available kits indistinguishably detect gluten from wheat, rye, and barley, it is not possible to identify the source of cereal. However, it is to be noted that not all food allergen regulations mandate the labeling of gluten-containing cereals as is the case in Europe and Australia. The United States requires only wheat, and Canada requires wheat and triticale. Japan does, not have gluten-free regulations, but requiring the labeling of the presence of wheat and buckwheat as allergens on the food label. In Japan, they use commercial kits to determine and quantify the presence of soluble wheat protein. It is reasonable to state that, Japanese kits are used to determine the presence of wheat in the context of food allergies in accordance with local regulation food allergies.

Currently, there are many limitations existing methods to detect gluten. For example, ELISA methods are not able to distinguish between gluten from wheat, rye, and barley. Thus, it is unknown if a positive finding by ELISA is may be due to the presence of wheat, barley, or rye. Current commercial ELISA kits for the detection of gluten are calibrated against wheat material (commonly gliadin). This may not be relevant for the gluten-free claim in Europe, but it may be relevant in other jurisdictions like in Canada, where according to their amended labeling requirements, the source of gluten, when present, needs to be indicated on the label. Unless specific ELISA tests are available for each individual cereal, it would be impossible to distinguish sources. In recent study, an innovative approach using immunoassay based on single domain antibodies specific for wheat gliadins was reported.⁴ However, without multiplex assays, testing for all the all regulated sources of gluten may be costly. Only alternative methods, like MS (target cereal gluten proteins) or PCR, can distinguish the source of gluten or cereal proteins in a multiplex fashion. The problem related to the use of PCR is that, since it detects DNA, it may not be very reliable for the quantification of gluten content.⁵

Considering some other jurisdictions, such as Europe and Canada, oats, along with wheat, barley, and rye, do not qualify for the gluten-free labeling when used as ingredients that have not been processed further to reduce gluten content. Current commercial ELISA kits do not cross-react with oats, and contamination of this grain with wheat, rye, and barley can be determined easily. If contamination was the only issue, oats should not be part of the toxic gluten-containing cereals in the regulations. However, it has been shown that oats are toxic to a small percentage of CD patients. This could be one of the major reasons of including oats as a toxic grain along with wheat, rye, and barley.⁶

Because current commercial ELISA tests based on R5 and Skerrit antibodies do not cross-react with oats, the presence of this grain in a product cannot be detected. If the toxicity to oats is lower than to wheat, rye, and barley, then an oat could be excluded from the list of toxic gluten-containing cereals? Conversely, if the oats are to be necessarily included in the list, the appropriate assay should be there available to determine the presence of this grain. In one of the past work previous studies, authors observed a direct correlation between the reactivity of monoclonal antibody G12 (specific for the toxic 33-mer toxic peptide) and the levels of toxicity of oat cultivars shown by in vitro studies that measured cell proliferation and interferon gamma release from isolated peripheral blood mononuclear T cells from patients with CD.⁷ This could be one of the approach which could be used analytically to determine toxic oat residues in food in those jurisdictions where oats are included, along with wheat, rye, and barley, in the regulations as toxic cereals.⁸⁻¹⁰ However, this area needs additionally more research with the patients suffering from CD. Another issue lies in accurately determine the true gluten content in the sample because of several factors like such as sample preparation, to data processing and interpretation of the results.¹¹

Conclusion

Considering the complexities of the large group of proteins called gluten, there have been a number of major improvements and significant progress during the last decades. With the advent of such advances in regulations, analytical techniques have improved the screening methods and label declaration of gluten-free products, which ultimately translate to an increase of food choices for celiac patients and their quality of life.

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Conflict of interest

Author declares no conflict of interest.

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