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Novel Monoclonal Antibody-Based Immunodiagnostic Assay for Rapid Detection of Deamidated Gluten Residues

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ABSTRACT: Gluten derived from wheat and related Triticeae can induce gluten sensitivity as well as celiac disease. Consequently, gluten content in foods labeled "gluten-free" is regulated. Determination of potential contamination in such foods is achieved using immunoassays based on monoclonal antibodies (mAbs) that recognize specific epitopes present in gluten. However, food-processing measures can affect on epitope recognition. In particular, preparation of wheat protein isolate through deamidation of glutamine residues significantly limits the ability of commercial gluten testing kits in their ability to recognize gluten. Adding to this concern, evidence suggests that deamidated gluten imparts more pathogenic potential in celiac disease than native gluten. To address the heightened need for antibody-based tools that can recognize deamidated gluten, we have generated a novel mAb, 2B9, and subsequently developed it as a rapid lateral flow immunoassay. Herein, we report the ability of the 2B9-based lateral flow device (LFD) to detect gluten from wheat, barley, and rye and deamidated gluten down to 2 ppm in food as well as its performance in food testing.

KEYWORDS: *gluten, prolamins, gliadin, celiac disease, mAb, deamidation, wheat protean isolate, LFD*

INTRODUCTION

Cereal grains are an important class of commercial foods, serving nutritional as well as functional roles in numerous food products.[1,2] Gluten, the principal source of protein, is a complex mixture of proteins accounting for 75−85% of total seed protein and responsible for imparting the rheological properties to dough. Gluten is a composite composed of prolamins and glutelins, each class consisting of numerous closely related proteins characterized by limited solubility in aqueous solution.[3] The prolamin and glutelin fractions of wheat, barley, and rye possess redundant amino acid motifs rich in proline and glutamine that form immunodominant structures capable of eliciting robust humoral and cellular immune responses.[4−6] In particular, these peptide motifs, and their deamidated analogues, bind to select HLA determinants, inducing T cell responses that drive the hallmark features of celiac disease (CD) in genetically susceptible individuals.[7]

CD is a relatively common disorder, affecting roughly 1% of the general population worldwide, with a marked and continuous apparent rise in incidence in recent years.[8] As CD is manifested as a consequence of consuming gluten, disease management is focused on strict dietary avoidance.[9] This need for gluten restriction in conjunction with prevalence and heightened public awareness has prompted an array of gluten-free products. However, it is not correct to assume that naturally "gluten-free" foods are actually free of gluten on account of the potential for contamination via raw ingredients and cross-contact during manufacturing.[8] To address this concern, regulatory authorities have implemented acceptable threshold levels for gluten content in foods labeled as "gluten-free". Assessment of gluten levels in such foods is achieved through the use of immunodiagnostic tools that are highly specific for peptide sequences present in gluten. The current norm for gluten detection is based on the use of the R5 monoclonal antibody (mAb), which recognizes the epitopes QQPFP, QQQFP, LQPFP, and QLPFP that are present in the prolamin fractions of wheat, barley, and rye.[10,11] The presence of glutamine (Q) residues at these binding sites renders the epitopes vulnerable to deamidation. Indeed, when glutamine, a base, is converted to its derivative glutamic acid, the electrostatic charge and spatial complementation involved in the antibody−antigen interaction is affected, in addition to gross changes to the physiochemical properties of gluten. The effect of deamidation at these epitopes is profound; the R5 mAb demonstrates a ≥125-fold reduction in its affinity for deamidated gluten, both industrial and laboratory-generated, relative to its affinity for vital gluten and supplied kit standards based on analyses performed using commercial R5-based competitive ELISAs,[12,13] underscoring the importance of glutamine in defining the antigenicity of these signature R5 epitopes. Similar effects on antibody recognition have been demonstrated for Skerrit and G12, mAbs that are also used in commercial gluten detection kits.[12]

Deamidated gluten, or wheat protein isolate (WPI) as it is more commonly referred to in the food industry, is used ubiquitously as an emulsifier, fortificant, gelling agent, film formation aid, stretchability agent in meat products, baked

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items, pastas, sauces, soups, and cosmetics, and clarifying agent in wine production.[2] It is typically manufactured using heated acidification or, to a lesser degree, transglutaminase treatment, although routine food processing can result in spontaneous gluten deamidation as well. The result of wheat protein deamidation is a highly enriched gluten product containing random or directed deamidation, depending on the manufacturing process, with greatly improved functionality and solubility. Although it is regarded as safe for consumption, WPI may function as a disease-enhancing factor in CD. Evidence for this comes from clinical studies in which T cells obtained from celiac subjects respond preferentially to deamidated gluten compared to native gluten,[14−16] a property that has been attributed to improved binding of deamidated gluten peptides to select HLA-DQ determinants that present antigens to cytopathogenic T cells.[16] Correspondingly, gluten-specific IgG and IgA antibodies obtained from celiac patients have been shown to preferentially bind to deamidated gluten.[17] Whereas the focus of deamidated gluten has been on tissue transglutaminase converting native gluten to its deamidated analogue in the small intestine, little or no attention has been placed on the role of dietary sources of deamidated gluten in CD pathogenesis. Mechanistically speaking, there is no reason to distinguish the two possible exposure routes in CD pathogenesis.

Due to the apparent increasing prevalence of CD and the severity of symptoms associated with consumption of gluten, many countries have adopted food-labeling requirements to protect celiac consumers. In the United States, the Food and Drug Administration (FDA) has recently implemented new regulations mandating that foods labeled "gluten-free" ensure that gluten levels are <20 ppm (20 mg/kg),[18] in keeping with the threshold limits established by WHO and Codex Alimentarius.[19] Given the existing limitations with respect to detection of deamidated gluten, we have developed a monoclonal antibody against deamidated gluten (2B9) and adapted it into a lateral flow device (LFD). Unlike the mAb and corresponding ELISA reported recently by Tranquet et al., which singularly detects deamidated residues,[13] mAb 2B9 and its corresponding LFD can be used to test for both native and deamidated gluten residues, providing more versatile utility and capable of detecting WPI that is only partially deamidated. Application of this test system should aid food manufacturers and regulatory entities in monitoring gluten derivatives that have previously proved challenging to the food diagnostic community.

MATERIALS AND METHODS

Preparation of Prolamin Reference Materials. Prolamins, including wheat gliadins, barley hordeins, rye secalins, and oat avenins, were purified from commercial foods purchased from a local market or at www.nuts.com. Of significance, avenins were isolated from Bob's Red Mill Gluten-Free oats. Reference sample extractions were performed as follows: nonmilled material was ground into a fine powder using a Waring blender. To isolate pure prolamins, globulins and albumins were first removed from flour by repeated extractions with 0.5 N sodium chloride for 1 h at a 1:10 (w/v) ratio. Sample pellets were washed twice with reverse osmosis water at a 1:10 (w/v) ratio for 1 h to remove residual salts. Between washes, sample pellets were centrifuged at 2000 rpm and mechanically redispersed. Prolamins were extracted for 1 h using a 1:10 (solid/liquid) ratio with 60% (v/v) ethanol at room temperature (RT) with moderate agitation. Samples were centrifuged, pellets were discarded, and the soluble fraction was poured into a tray to enable evaporation. Dehydrated prolamin solids were resuspended in 60% (v/v) ethanol and stored at −20 °C. Prolamin concentration was determined by combustion analysis with a Dumas FP-328 instrument (Leco Corp., St. Joseph, MI, USA) and calculated by multiplying the N content by a coefficient of 5.7. To estimate gluten content, a conversion factor of 2 was applied. Deamidated gliadin, 53%, was obtained under MTA with Institute National de la Recherche Agronomique (INRA) and prepared and analyzed according to Gourbeyre et al. by the addition of HCl to purified whole gliadins and heated at 90 °C for 1 h; it was then neutralized with the addition of sodium hydroxide. Percentage deamidation was then calculated from glutamic acid (Glu) residues and diaminobutyric acid (DABA) titration. The level of glutamine (Gln) residues was evaluated from the DABA/norleucine ratio, whereas the level of Glu was determined from the Glu (not converted in DABA)/norleucine ratio. Percentage deamidation was calculated as Glu/(Gln + Glu)/100 as described in ref 20.

Production of Monoclonal Antibodies. Mouse work was performed according IACUC-approved animal protocols. An 8-week-old female BALB/c mouse (Charles River Laboratories, Wilmington, MA, USA) was immunized subcutaneously at the cervico-dorsal region with duplicated and randomly "deamidated" R5 synthetic, unconjugated peptide (L{Q/E}P{Q/E}{Q/E}PFP{Q/E}{Q/E}L{Q/E}P{Q/E}{Q/E}PFP{Q/E}{Q/E}A (Genscript, Piscataway, NJ, USA). The primary dose was emulsified with Freund's complete adjuvant (Sigma-Aldrich, St. Louis, MO, USA) and subsequent boosts were prepared in Freund's incomplete adjuvant (Sigma-Aldrich) and delivered at 3 week intervals. Following sero-conversion (titer of >1:50000), a final boost consisting of 100 μg of deamidated gliadins (no adjuvant) was administered ip 5 days prior to fusion to clonally expand B cells capable of binding epitopes present in actual protein. To generate hybridomas, SP2/-Ag14 myeloma cells were fused with single cell suspensions of splenocytes using PEG 1500 (Roche, San Francisco, CA, USA) and subsequent hybridomas cultured using HAT selection media (ATCC, Manassas, VA, USA). Ten days postfusion, single colonies were selected and clonally expanded in 96-well culture plates. Hybridomas were screened by indirect ELISA against a panel consisting of native gliadins, deamidated gliadins, hordeins, secalins, R5(−) avenins, orzeins, soy protein, and zeins and isotyped. IgG+ clones that remained stable and sustained high levels of reactivity against secalins, gliadins (native and deamidated), and hordeins were maintained and further characterized. From this, clone 2B9 was identified as a strong candidate clone for assay development.

Purification and Labeling of Monoclonal Antibody. Clone 2B9 was expanded in tissue culture and injected (1×10^6 cells/mouse) ip into pristane-primed BALB/c female mice. Roughly 2 weeks after adoptive transfer, ascitic fluids were collected and centrifuged, and the supernatants were collected, then diluted 1:1 in phosphate buffer, and filtered through a 0.45 μm sterile filter. IgG was purified via protein G affinity column (in-house) using AKTA Prime FPLC (GE Healthcare Lifesciences, Pittsburgh, PA, USA). IgG concentration was determined using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and purity confirmed via SDS-PAGE. Biotin labeling was performed using EZ-Link NHS-Biotin Kit (Thermo Scientific) according to the manufacturer's instructions.

Antibody Activity by Indirect ELISA. To prepare ELISA plates, cereal protein standards were dissolved in 60% (v/v) ethanol at 40 μg/mL and plated in 50 μL aliquots into 96-well plates (Costar 9017, Corning Life Sciences, Tewksbury, MA, USA). Plates were dehydrated and then fixed for 5 min at RT using 10% formaldehyde solution. For soy, protein isolate (Archer Daniels Midland, Decatur, IL, USA) was diluted to 5 μg/mL in 50 mM carbonate buffer, pH 9.8, plated at 100 μL/well, and coated overnight at 4 °C. After antigen coating, plates were washed 4 times with phosphate-buffered saline, 0.05% Tween-20 (PBST; ThermoFisher Scientific) and blocked with 1% BSA (EMD Millipore, Billerica, MA, USA) in PBST. To assess antibody activity, purified IgGs were tested as follows: 3-fold serial dilutions were made in PBST−1% BSA, added to microwells in 100 μL volumes, incubated at 37 °C for 1 h, washed four times with PBST, incubated with 100 μL of goat anti-mouse IgG (H+L) HRP conjugate (1:3,000; KPL, Gaithersburg, MD, USA) diluted in PBST at 37 °C for 1 h, washed

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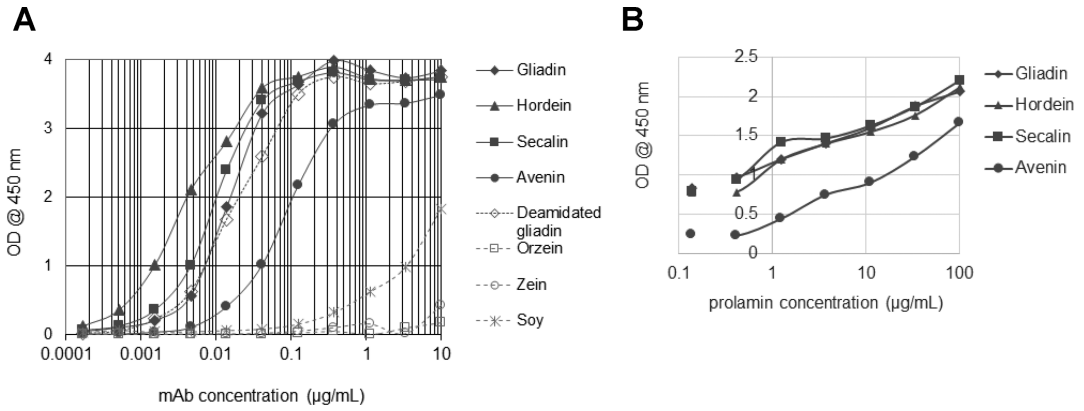

Figure 1. Characterization of hybridoma clone 2B9. (A) Indirect ELISA was performed for 2B9 against prolamins derived from wheat, barley, rye, oats, corn, and rice as well as deamidated gliadins and soy protein extract (plate-bound at 5 μg/mL). The 2B9 mAb was titered starting at 10 μg/mL and using anti-mouse IgG (H+L) HRP conjugate (KPL) at 1:2500. (B) Sandwich ELISA was based on the 2B9 mAb operated with gliadins, hordeins, secalins, and avenin standards as described under Materials and Methods.

four times with PBST, and then resolved using 100 μL of TMB substrate (BD Biosciences, San Jose, CA, USA) for 30 min at RT. Phosphoric acid (50 μL of 1 M) was then added, and the OD values were determined using a Tecan spectrophotometer (Maennedorf, Switzerland) with 450/650 nm filter settings. The raw data were plotted using Microsoft Office Excel. The ELISA was repeated three times to ensure reproducibility, and the results presented here are representative of the three tests.

Gluten ELISA in Sandwich Format. To prepare ELISA plates, 2B9 IgG (100 μL, 2 μg/mL) was plate-bound using 50 mM carbonate buffer, pH 9.8, for 3 h at RT. Microwells were washed four times with PBST and then blocked with 1% (w/v) BSA in PBST for 1 h at RT. Prolamins were diluted to 100 μg/mL in PBST, then serially diluted and incubated in the wells for 20 min at RT. Microwells were washed four times with PBST and then incubated with 100 μL of biotin-labeled 2B9 diluted to 2 μg/mL with PBST, for 20 min at RT. Wells were washed four times with PBST and then incubated with 100 μL of streptavidin−HRP (0.12 μg/mL, Pi Bioscientific, Seattle, WA, USA) for 10 min at RT. Wells were washedfour times with PBST and then incubated with 100 μL of TMB substrate (Pi Bioscientific) for 5 min at RT. The reaction was terminated by adding 50 μL of 1 M phosphoric acid. The ELISA was repeated three times to ensure reproducibility, and the results presented here are representative of the three tests.

Preparation of Gold Conjugates. Citrate-capped 40 nm gold nanoparticles were obtained from Pi Bioscientific Inc. 2B9 IgG was diluted in borate buffer to a final concentration of 0.1 mg/mL, and then 7.5 mL was added to 250 mL of gold nanoparticles (A_{530} = 1) in a dropwise fashion with stirring for 30 min. To block, 2.5 mL of 10% BSA (in borate buffer) was added, and the colloid was pelleted by centrifugation at 3000*g* for 1.5 h. Spectral analysis was performed on the resuspended soft pellet, and the absorbance was adjusted to a final reading of A = 20 (at the absorption maxima) using 1% BSA/10% sucrose in 8 mM borate buffer.

Preparation of Lateral Flow Devices and Buffers. Nitrocellulose membrane (Sartorius, Goettingen, Germany) was lined with 2B9 IgG for the sandwich format test line (T1), purified gliadins for the competitive format test line (T2), and goat anti-mouse antibodies for the procedural control line (PC), using an IsoFlow Reagent Dispenser (Imagene Technology, Hanover, NH, USA). To prepare the conjugate pad, the 2B9 IgG gold conjugates were sprayed on strips of glass fiber conjugate pad material (Ahlstrom, Mt. Holly Springs, PA, USA) using the IsoFlow Dispenser. To assemble the test strips, the nitrocellulose membrane, conjugate pad, sample pad (Ahlstrom), and absorbent pad (Advanced Micro Devices, India) were adhered to the adhesive laminate of the backing card (Lohmann, Precision Die Cutting, San Jose, CA, USA) with overlapping surfaces to ensure continuous capillary transfer. The assembled cards were then cut into 5 mm wide strips using a Matrix 2360 programmable shear (Kinematic Automation, Sonora, CA, USA), housed in plastic cassettes (Advanced Micro Devices), and stored with desiccant in sealed foil bags at RT until use. The LFD was configured such that the sample first encounters the T1 line, then the T2 line, and last the PC line. Gluten extraction buffers (containing 60% ethanol) and LFD running buffers were obtained from Pi Bioscientific. In some instances, 1% SDS and 10 mM TCEP-HCl (GoldBio, St. Louis, MO, USA) were added to the gluten extraction buffer to enable denaturing conditions in the assay.

Sample Preparation and Assay Procedure. Samples were mixed and homogenized, and then aliquots of 1 g (for solids) or 1 mL (for liquids) were diluted with 10 and 9 mL of gluten extraction buffer, respectively. The samples were then extracted at 95 °C in a water bath for 1 min, the ensuing extracts were cooled to room temperature and centrifuged (~2500*g*) for 15 min to facilitate phase separation, and then the aqueous phase was collected for use in the assay. In some instances, when denaturing conditions were applied, the sample was extracted in denaturing buffer (described above) for 20 min at 70 °C and treated thereafter as described for native samples. Before the assay was begun, the LFD running buffer and LFDs were equilibrated to room temperature. To operate the LFD, the sample extract was diluted 1/10 in LFD running buffer, and then 100 μL of the mixture was applied to the sample port of the LFD, where it hydrated the gold conjugate and was allowed to wick across the nitrocellulose membrane. The sample was allowed to run for 15 min, after which the results were read using a Qiagen ESE-Quant Gold strip reader (QIAGEN, Stockach, Germany). Kinetic analysis determined that a 15 min operation time was sufficient to allow clear signal (>60 units) at the LOD value for the assay (data not shown).

Method Comparison. Method comparison was performed using the 2B9 LFD on samples extracted using both nondenaturing and denaturing conditions. Commercial kits were based on the sandwich format and included the Neogen Alert for Gliadin R5 ELISA kit[21] and the Romer AgraStrip Gluten LFD kit,[22] which is based on the G12 mAb. Commercial kits were operated and interpreted according to the supplied user manuals.

Interpretation of Results. Unless otherwise mentioned, the results reported are the averages of individual separate runs performed by two independent analysts, with SD (standard deviation) reported parenthetically. Gluten values were calculated as two times the prolamin concentration. The term "ppm" refers to parts per million protein and can be used interchangeably with mg/L or μg/mL units of protein concentration. The LFD is printed with three lines: test line 1, a sandwich format; test line 2, a competitive format; and a procedural control (PC) line to ensure correct fluidics of the assay. The results of the LFD assay were interpreted as follows: In the absence of analyte, test line 1 will not appear, whereas test line 2 will fully appear. When the analyte concentration is at or just above the limit of detection (LOD = 1 ppm of gliadin or 2 ppm of gluten), a clearly visible test line 1 appears along with test line 2. As the concentration of analyte increases, test line 1 also increases in intensity and test line 2 will

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Table 1. Sensitivity and Dynamic Range Testing Using Native Wheat Gliadins[a]

native gliadins (ppm)	strip reader value: test line 1 sandwich	strip reader value: test line 2 competitive	result	Romer G12 AgraStrip	Neogen R5 ELISA
			Nondenatured		
blank	0 (0)	511 (2)	negative	NT[b]	NT
0.01	42 (2)	560 (38)	weak positive	NT	NT
0.1	203 (38)[c]	617 (72)[c]	positive	negative	negative
1	441 (32)	313 (68)	positive	weak positive	weak positive (OD 0.137)
10	542 (79)	70 (5)	positive[d]	positive	positive
100	418 (75)	10 (17)	positive[d]	NT	NT
1000	148 (29)	0 (0)	positive[d]	NT	NT
			Denatured		
blank	0 (0)	756 (33)	negative	NT	NT
0.01	43 (4)	749 (117)	weak positive	NT	NT
0.1	94 (7)	703 (82)	positive	NT	NT
1	423 (33)	553 (16)	positive	NT	NT
10	641 (130)	166 (44)	positive	NT	NT
100	395 (109)	14 (25)	positive[d]	NT	NT
1000	169 (22)	0 (0)	positive[d]	NT	NT

[a]Serially diluted gliadins (non-denatured and denatured) were applied to LFD cassettes and assessed using an electronic strip reader at 15 min. Reported strip reader values are the average and SD calculated from triplicate testing performed by a single analyst. Threshold for determining positivity at the T1 line was set to 60 units. Weak positive was set at 35−59 units or RANN score 2. In select instances, deamidated gliadins were also tested using the Romer G12 AgraStrip and the Neogen R5 Alert for Gliadin kits per the instructions supplied with the kits. [b]NT, not tested. [c]Tested in 20 replicates, where all 20 T1 values exceeded 35 units. [d]High concentrations of analyte result in attenuation (strip reader value <100) of the competitive test line 2 and the sandwich test line 1.

decrease in intensity. Above a certain analyte concentration, test line 1 will start to diminish due to Prozone effects; thus, instances where the test line 1 is weak or absent and test line 2 is absent denote high target concentrations. In instances where target analyte is highly hydrolyzed, test line 1 may not register any signal; however, test line 2 will remain fully operational, thereby providing additional assurance for analyte that may have been hydrolyzed as a consequence of fermentation or acid treatment, which can occur during deamidation under pH extremes.[13] For basic assay parameter analyses, an ESE reader value of 60 units was used for determining the threshold for the T1 sandwich line, an intensity that is clearly visible by eye. T1 line values between 35 and 59 were regarded as weak positives to allow for direct comparison with the Romer Laboratories AgraStrip, which relies on the use of a RANN score card due to the rapid evolution of false positives that limit the use of strip readers on account of time constraints. T2 intensity values <100 units were used to denote the threshold for the T2 competitive line. Please note, the Romer Laboratories AgraStrip does not include this competitive line; thus, it was not considered in the analysis.

RESULTS AND DISCUSSION

Antibody Characterization. Following preliminary screening using hybridoma tissue culture supernatants against wheat, barley, and rye prolamins (data not shown), a candidate IgG+ clone, 2B9, was expanded in vivo and purified from ascitic fluids. The relative binding affinities of 2B9 for plate-bound antigens including deamidated gliadins, native gliadins, hordeins, secalins, avenin, zein, oryzein, and soy protein were established using streptavidin indirect ELISA (antimouse IgG_{H+l} conjugated HRP) (Figure 1A). 2B9 demonstrated high avidity for gliadins (native and deamidated forms), hordeins, and secalins, with half-binding activities ranging from 0.003 to 0.01 μg/mL and modest cross-reactivity against avenins derived from R5(−) oats (half-binding activity of ∼0.1 μg/mL), no activity against soy protein, zein, or oryzein, and very weak activity against native soy protein.

To confirm suitability for gluten detection from barley, rye, and wheat sources, clone 2B9 was further tested in sandwich ELISA using a biotin/streptavidin-based detection system against gliadins, hordeins, secalins, and avenin standards. The ELISAs were operated at room temperature, using 20 min of incubation with analyte, 20 min with 2B9−IgG−biotin, and 10 min with SA−HRP. The curves obtained using this preliminary ELISA for the gliadin, hordein, secalis, and avenin standards diluted in 60% ethanol (not denatured) are presented in Figure 1B. 2B9 demonstrated essentially overlapping detection curves for gliadins, hordeins, and secalins, with the ability to detect down to 1 ppm of prolamins for all three targets, corresponding to 10 μg/g prolamin content in food. The sandwich ELISA exhibited >10-fold less detection of avenin. Collectively, these features indicate that the 2B9 mAb is suitable for further assay development.

Lateral Flow Sensitivity and Dynamic Range Testing. The analytical limit of detection (LOD) for the LFD assay was tested using various prolamin extracts of known protein concentration prepared at log-fold dilutions in gluten extraction buffer to desired levels and then diluted again 1/10 in gluten LFD running buffer. Each target analyte was tested using the native extraction buffer based on 60% ethanol solution and a denaturing extraction buffer based on 60% ethanol solution plus SDS and a reducing agent. In each instance, 100 μL of each diluted sample was applied to the sample port of the LFD and was permitted to migrate for 15 min, at which time the test result was read using an electronic strip reader. The threshold of definite positivity for the T1 line was set at 60 units, with T1 line values of 35−59 defined as weak positive (faint, but visible to the naked eye) to allow comparison with the Romer LFD kit. At 0 and 0.01 ppm, none of the nondenatured prolamin analytes registered values exceeding the threshold value range using the electronic reader. At 0.1 ppm, for both nondenaturing and denaturing conditions, gliadins, hordeins, and secalins, all

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Table 2. Sensitivity and Dynamic Range Testing Using Chemically Deamidated Wheat Gliadins[a]

deamidated gliadins, 53% (ppm)	strip reader value: test line 1 sandwich	strip reader value: test line 2 competitive	result	Romer G12 AgraStrip	Neogen R5 ELISA
Nondenatured					
blank	0 (0)	709 (22)	negative	NT[b]	NT
0.01	0 (0)	730 (25)	negative	NT	NT
0.1	49 (6)[c]	722 (64)[c]	weak positive	negative	negative
1	206 (13)	629 (36)	positive	negative	negative
10	658 (42)	395 (23)	positive	weak positive	weak positive (OD 0.188)
100	609 (38)	36 (5)	positive[d]	positive	positive
1000	520 (19)	0 (0)	positive[d]	NT	NT
Denatured					
blank	0 (0)	759 (41)	negative	NT	NT
0.01	0 (0)	760 (24)	negative	NT	NT
0.1	48 (2)	679 (19)	weak positive	NT	NT
1	107 (13)	650 (43)	positive	NT	NT
10	448 (14)	444 (23)	positive	NT	NT
100	568 (11)	82 (6)	positive[d]	NT	NT
1000	278 (25)	0 (0)	positive[d]	NT	NT

[a]Serially diluted, chemically deamidated gliadins (non-denatured and denatured) were applied to LFD cassettes and assessed using an electronic strip reader at 15 min. Reported strip reader values are the average and SD calculated from triplicate testing performed by a single analyst. Threshold for determining positivity at the T1 line was set to 60 units. Weak positive was set at 35−59 units or RAAN score 2. In select instances, deamidated gliadins were also tested using the Romer G12 AgraStrip and the Neogen R5 Alert for Gliadin kits per the instructions supplied with the kits. [b]NT, not tested. [c]Tested in 20 replicates, where all 20 T1 values exceeded 35 units. [d]High concentrations of analyte result in attenuation (strip reader value <100) of competitive test line 2 and sandwich test line 1.

registered electronic values at the T1 line exceeding the 35 unit cutoff values, whereas avenin did not register any signal. Although native gliadins were weakly positive at 0.01 ppm gliadins, replicate testing consisting of 20 tests revealed 90% positive outcomes at 0.01 ppm gliadins and 100% positive outcomes at 0.1 ppm for both native and deamidated gliadins levels, confirming the designation of LOD for 0.1 ppm native and deamidated gliadins for the assay. The competitive test line was more variable, generally attenuating at 100 ppm prolamins, except for native gluten (nondenatured), where attenuation (defined as signal <100 units) was observed at 10 ppm of gliadins. As the competitive test line was more variable across targets and increased standard deviation values (generally), the competitive test line exerts more application to the detection of highly hydrolyzed residues (not tested) or scenarios where the concentration of target analyte is sufficiently high so as to essentially ablate the signal at the T1 sandwich line due to Prozone effects (Tables 1−5). The rate of T2 signal attenuation was similar for samples extracted under both nondenaturing and denaturing conditions (Tables 1−5). The T1 sandwich line detected R5(−) avenins at 100 ppm (Table 5).

To confirm the LOD values, the LFD was tested 10 times by a single analyst at 0.1 and 1.0 ppm of gliadins to ensure that the test yielded positive outcomes in each instance (data not shown). The overall analytical T1 line LOD for gliadins, hordeins, and secalins was determined to be 0.1 ppm of prolamins or 0.2 ppm of gluten for surface analysis and 1 ppm of prolamin or 2 ppm of gluten for foods due to a 10-fold dilution factor incurred as a consequence of sample extraction. For deamidated gliadins, as mentioned, a weak positive was observed at the T1 line; thus, analytical LOD was set at 0.1 ppm of prolamin, or 0.2 ppm of gluten, translating to 2 ppm of gluten in food. It is important to note that the rate of attenuation for the T2 line was not as rapid for deamidated gluten as it was for the native Triticeae-derived prolamins (Tables 1−4). The T2 line was more variable in outcomes and could not be reliably used to establish defined LOD values for samples of unknown prolamin concentration.

In contrast, when the Romer LFD (G12) and Neogen ELISA (R5) kits were used to compare outcomes for native and deamidated gliadins (Tables 1 and 2), the G12-based Romer AgraStrip and R5-based ELISA were 100-fold less sensitive in detection of deamidated and native gliadins, compared to the LOD for the 2B9 detection system. Moreover, both the R5- and G12-based detection systems were ∼1 log less sensitive in detection of deamidated gliadins compared to native gliadins (Tables 1 and 2).

Cross-Reactivity Analysis. To determine the specificity of the LFD assay, full-strength extracts prepared using non-denatured and denatured conditions were prepared from a panel of select commodities using the gluten extraction buffer. As summarized in Table 6A, limited cross-reactivity was detected for the non-denatured targets, with an occasional weak signal (<35 units) reported. Significant and reportable cross-reactivity was observed for almond protein (down to 1 ppm, data not shown) and teff (Table 6A). The cross-reactivity toward teff was fully eliminated when the extract was denatured prior to testing, whereas a very weak residual signal (30 ± 6 units was observed for denatured almond extract (Table 6B). Blasting of the cannonical R5 epitope QQPFP reveals that the sequence occurs in *Prunus* species, suggesting that the occurrence of a minimal epitope footprint in the context of an ordered tertiary structure is sufficient to cause cross-reactivity in the native form. Alternatively, it is possible that bivalent almond lectins capable of binding oligosaccharides present on the mAb IgG are responsible for the cross-reactivity seen with almond.

Furthermore, the LFD reported distinct varieties of rye, wheat, and barley, including spelt and kamut wheat prolamins. Analysis of denatured commodity extracts revealed significant

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Table 3. Sensitivity and Dynamic Range Testing Using Native Barley Hordeins[a]

native hordeins (ppm)	strip reader value: test line 1 sandwich	strip reader value: test line 2 competitive	result
Nondenatured			
blank	0 (0)	512 (10)	negative
0.01	21 (18)	508 (30)	negative
0.1	93 (20)	534 (50)	positive
1	419 (49)	353 (27)	positive
10	696 (95)	107 (22)	positive
100	481 (47)	0 (0)	positive[b]
1000	139 (14)	0 (0)	positive[b]
Denatured			
blank	0 (0)	751 (66)	negative
0.01	76 (8)	754 (118)	positive
0.1	187 (5)	675 (83)	positive
1	505 (81)	503 (127)	positive
10	677 (24)	105 (77)	positive
100	380 (16)	0 (0)	positive[b]
1000	80 (19)	0 (0)	positive[b]

[a]Serially diluted hordeins (non-denatured and denatured) were applied to LFD cassettes and assessed using an electronic strip reader at 15 min. Reported strip reader values are the average and SD calculated from triplicate testing performed by a single analyst. Threshold for determining positivity at the T1 line was set to 60 units. Weak positive was set at 35−59 units. [b]High concentrations of analyte result in attenuation (strip reader value <100) of competitive test line 2 and sandwich test line 1.

Table 4. Sensitivity and Dynamic Range Testing Using Native Rye Secalins[a]

native secalins (ppm)	strip reader value: test line 1 sandwich	strip reader value: test line 2 competitive	result
Nondenatured			
blank	0 (0)	751 (66)	negative
0.01	41 (5)	755 (118)	weak positive
0.1	182 (32)	676 (83)	positive
1	653 (44)	502 (127)	positive
10	773 (57)	105 (77)	positive[b]
100	518 (63)	0 (0)	positive[b]
1000	267 (27)	0 (0)	positive[b]
Denatured			
blank	0 (0)	755 (46)	negative
0.01	61 (10)	743 (40)	positive
0.1	142 (40)	747 (86)	positive
1	511 (96)	542 (99)	positive
10	689 (73)	81 (17)	positive[b]
100	552 (112)	0 (0)	positive[b]
1000	340 (71)	0 (0)	positive[b]

[a]Serially diluted secalins (non-denatured and denatured) were applied to LFD cassettes and assessed using an electronic strip reader at 15 min. Reported strip reader values are the average and SD calculated from triplicate testing performed by a single analyst. Threshold for determining positivity at the T1 line was set to 60 units. Weak positive was set at 35−59 units. [b]High concentrations of analyte result in attenuation (strip reader value <100) of competitive test line 2 and sandwich test line 1.

cross-reactivity at the T1 line with poppy seed (negative at 1/10 dilution), sesame (negative at 1/100 dilution), and sunflower (negative at 1/100 dilution), although the reactivity toward these three commodities appeared to be lectin-mediated, as the T2 line was not involved (Table 6B). Mild cross-reactivity was seen with denatured walnut using full-strength extract. As indicated, cross-reactivity toward almond and teff extract was lost under denaturing conditions. Significant cross-reactivity to oats (tested and reported to be negative for gluten based on R5-ELISA in sandwich format) that disappeared at 1/100 dilution was seen under denaturing conditions. Under both conditions (Table 6), no cross-reactivity was observed for soy, rice, millet, corn, sorghum, or flaxseed using full-strength extracts prepared per the test method.

Table 5. Sensitivity and Dynamic Range Testing Using Native Oat Avenins[a]

native avenins (ppm)	strip reader value: test line 1 sandwich	strip reader value: test line 2 competitive	result
Nondenatured			
blank	0 (0)	511 (10)	negative
0.01	0 (0)	512 (69)	negative
0.1	0 (0)	564 (59)	negative
1	0 (0)	479 (61)	negative
10	0 (0)	411 (54)	negative
100	13 (22)	267 (15)	negative
1000	113 (16)	13 (23)	positive[b]
Denatured			
blank	0 (0)	759 (33)	negative
0.01	0 (0)	749 (43)	negative
0.1	0 (0)	714 (77)	negative
1	0 (0)	788 (47)	negative
10	0 (0)	660 (64)	negative
100	54 (48)	330 (92)	weak positive
1000	0 (0)	47 (45)	positive[b]

[a]Serially diluted avenins (non-denatured and denatured) were applied to LFD cassettes and assessed using an electronic strip reader at 15 min. Reported strip reader values are the average and SD calculated from triplicate testing performed by a single analyst. Threshold for determining positivity at the T1 line was set to 60 units. Weak positive was set at 35−59 units. [b]High concentrations of analyte result in attenuation (strip reader value <100) of competitive test line 2 and sandwich test line 1.

Method Comparison and Spiking Recovery Analyses. The LFD assay was additionally tested using wheat flour (WF) and commercial wheat protein isolate (WPI) from MGB prepared by acid and thermal treatment by serially diluting both types of commercial gluten sources into gluten-free bread mix (Table 7). The protein concentration for neat WPI was 810,000 ppm (81% gluten based on label) and for the wheat flour 140,000 ppm (14% protein based on label, assume 12% gluten content). Under the non-denaturing conditions, both the 0.00001 and 0.0001% WPI and WF spikes were clearly negative at the T1 line (0.08 and 0.8 as well as 0.012 and 0.12 ppm of gluten, respectively). The 0.001% WPI spike, corresponding to 8.1 ppm of gluten was positive, although the corresponding 0.001% WF spike (1.2 ppm gluten) was negative at the T1 line. In comparison, under the denaturing conditions, both the 0.001% WPI spike (8.1 ppm of gluten) and the 0.001% WF spike (1.2 ppm of gluten) were clearly positive at the T1 line, with the 0.0001% WPI demonstrating a weak positive (0.8 ppm gluten) at the T1 line. Under both non-denaturing and denaturing conditions, the 0.01% WPI,

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Table 6. Cross-Reactivity Analysis[a]

commodity	strip reader value: test line 1 sandwich	strip reader value: test line 2 competitive	result	commodity	strip reader value: test line 1 sandwich	strip reader value: test line 2 competitive	result
(A) Native Conditions							
lima bean	11 (19)	523 (3)	negative	egg	0 (0)	598(37)	negative
mung bean	11 (19)	581 (52)	negative	wheat, Primadur	244 (65	19 (23)	positive[b]
green pea	0 (0)	544 (21)	negative	wheat, IMP	374 (61)	7 (5)	positive[b]
lupin	0 (0)	560 (23)	negative	wheat, Cadenza	259 (53)	2 (4)	positive[b]
kidney bean	0 (0)	489 (13)	negative	wheat, Venusla	240 (30)	6 (10)	positive[b]
adzuki bean	0 (0)	610 (36)	negative	wheat, Kamut	210 (29)	4 (7)	positive[b]
lentil	0 (0)	533 (11)	negative	wheat, King Arthur	296 (46)	0 (0)	positive[b]
chick pea	0 (0)	543 (38)	negative	wheat, Claire	266 (71)	6 (10)	positive[b]
poppy seed	0 (0)	657 (19)	negative	wheat, spelt	228 (65)	22(33)	positive[b]
banana	0 (0)	677 (49)	negative	wheat, Axonia	266 (42)	12 (13)	positive[b]
apple	0 (0)	595 (78)	negative	wheat, semolina	325 (14)	10 (9)	positive[b]
raw chicken	12 (21)	623 (38)	negative	rye, Picasso	545 (70)	5 (8)	positive[b]
sesame	26 (22)	545 (20)	negative	rye, commercial	262 (48)	0 (0)	positive[b]
sunflower	0 (0)	569 (29)	negative	barley, commercial	425 (51)	0 (0)	positive[b]
peanut	0 (0)	645 (73)	negative	barley, Triumph	233 (18)	0 (0)	positive[b]
almond	682 (32)	64 (11)	positive[b]	barley, Optic	420 (47)	0 (0)	positive[b]
macadamia	0 (0)	587 (46)	negative	barley, Halcyon	378 (74)	41 (14)	positive[b]
walnut	12 (21)	659 (16)	negative	barley, Marris Otter	443 (14)	0 (0)	positive[b]
hazelnut	10 (18)	649 (70)	negative	rice, jasmine	0 (0)	512 (98)	negative
cashew	0 (0)	597 (76)	negative	millet	26 (9)	528 (112)	negative
pistachio	0 (0)	606 (29)	negative	corn	22 (11)	472 (23)	negative
soy	9 (17)	565 (30)	negative	sorghum	0 (0)	507 (66)	negative
celery	0 (0)	610 (88)	negative	flaxseed	0 (0)	523 (78)	negative
mustard	0 (0)	568 (39)	negative	oats (R5−)	12 (21)	244 (178)	negative
gum, arabic (1/10)	10 (17)	636 (83)	negative	teff	524 (17)	0 (0)	positive[b]
milk, 2%	0 (0)	626 (30)	negative				
(B) Denaturing Conditions							
lima bean	12 (20)	873 (44)	negative	celery	0 (0)	831 (37)	negative
mung bean	0 (0)	843 (30)	negative	mustard	0 (0)	855 (29)	negative
green pea	41 (3)	872 (20)	weak positive	gum, arabic (1/10)	0 (0)	832 (30)	negative
lupin	0 (0)	831 (25)	negative	milk, 2%	0 (0)	850 (30)	negative
kidney bean	13 (22)	854 (37)	negative	egg	0 (0)	882 (30)	negative
adzuki bean	12 (21)	859 (30)	negative	wheat, Primadur	147 (46)	44 (14)	positive[b]
lentil	0 (0)	817 (31)	negative	wheat, IMP	407 (54)	47 (15)	positive[b]
chick pea	12 (21)	844 (38)	negative	wheat, Cadenza	309 (78)	0 (0)	positive[b]
poppy seed	132 (20)	731 (16)	positive	wheat, Venusla	144 (52)	0 (0)	positive[b]
poppy seed 1/10	0 (0)	816 (35)	negative	wheat, Kamut	39 (40)	0 (0)	positive[b]
banana	0 (0)	825 (26)	negative	wheat, King Arthur	171 (41)	0 (0)	positive[b]
apple	0 (0)	819 (29)	negative	wheat, Claire	254 (47)	0 (0)	positive[b]
raw chicken	0 (0)	863 (13)	negative	wheat, spelt	213 (14)	0 (0)	positive[b]
sesame	343 (130)	806 (11)	positive[c]	wheat, Axonia	164 (34)	72 (29)	positive[b]
sesame 1/10	109 (10)	895 (34)	positive[c]	wheat, semolina	221 (57)	92 (29)	positive[b]
sesame 1/100	0 (0)	776 (32)	negative	rye, Picasso	111 (31)	0 (0)	positive[b]
sunflower	258 (32)	687 (13)	positive	barley, Triumph	250 (44)	0 (0)	positive[b]
sunflower 1/10	103 (23)	895 (34)	positive	barley, Optic	319 (25)	95 (11)	positive[b]
sunflower 1/100	0 (0)	779 (37)	negative	barley, Halcyon	335 (33)	15 (26)	positive[b]
peanut	0 (0)	830 (26)	negative	barley, Marris Otter	190 (12)	100 (22)	positive
almond	30 (6)	207 (32)	negative[c]	rice, jasmine	0 (0)	800 (22)	negative
almond 1/10	0 (0)	546 (44)	negative	millet	0 (0)	874 (26)	negative
macadamia	0 (0)	727 (56)	negative	corn	0 (0)	815 (33)	negative
walnut	96 (16)	862 (47)	positive	sorghum	0 (0)	823 (23)	negative
walnut 1/10	0 (0)	883 (77)	negative	flaxseed	0 (0)	853 (47)	negative
hazelnut	0 (0)	836 (39)	negative	oats (R5−)	0 (0)	0 (0)	positive
cashew	0 (0)	859 (34)	negative	oats (R5−) 1/10	86 (10)	490 (0)	positive
pistachio	0 (0)	857 (39)	negative	oats (R5−) 1/100	0 (0)	670 (15)	negative
soy	0 (0)	844 (21)	negative	teff	0 (0)	778 (443)	negative

[a]Full-strength extracts prepared from common commodities and cereal grains were prepared and tested per Methods using (A) native conditions and (B) denaturing conditions and then applied to the LFD cassettes and assessed using an electronic reader at 15 min after application. Reported

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Table 6. continued

strip reader values are the average and SD calculated from triplicate testing performed by a single analyst. R5(−) refers to oat avenins that have been tested with R5 sandwich ELISA and found to be negative for gluten using this test system. [b]High concentrations of analyte result in attenuation (strip reader value <100) of competitive test line 2 and sandwich test line 1. [c]Putative lectin-mediated bridging of the T1 line to gold conjugate.

Table 7. Spiking Recovery and Method Comparison[a]

	strip reader value (for non-denatured)			strip reader value (for denatured)				
% spike in gluten-free bread mix	test line 1	test line 2	result	test line 1	test line 2	result	Romer G12 AgraStrip	Neogen R5 ELISA
blank (gluten-free bread mix)	0 (0)	621 (36)	negative	24 (20)	681 (80)	negative	NT[b]	NT
10 ppm of native gliadins	627 (58)	60 (15)	positive[c]	563 (71)	68 (4)	positive[c]	NT	NT
0.00001% WPI (0.08 ppm of gluten)	7 (12)	664 (24)	negative	43 (16)	695 (79)	weak positive	NT	NT
0.00001% WF (0.01 ppm of gluten)	0 (0)	706 (79)	negative	0 (0)	772 (66)	negative	NT	NT
0.0001% WPI (0.8 ppm of gluten)	21 (37)	642 (45)	negative	44 (17)	703 (21)	weak positive	NT	NT
0.0001% WF (0.12 ppm of gluten)	0 (0)	655 (33)	negative	0 (0)	748 (8)	negative	NT	NT
0.001% WPI (8.0 ppm of gluten)	177 (28)	632 (26)	positive	197 (44)	714 (32)	positive	negative	positive
0.001% WF (1.2 ppm of gluten)	13 (14)	633 (23)	negative	94 (33)	744 (19)	positive	negative	negative
0.01% WPI (80 ppm of gluten)	580 (27)	383 (52)	positive	607 (70)	393 (34)	positive	positive	positive
0.01% WF (12 ppm of gluten)	252 (47)	734 (7)	positive	248 (63)	662 (67)	positive	positive	positive
0.1% WPI (800 ppm of gluten)	547 (46)	60 (23)	positive[c]	476 (49)	43 (13)	positive[c]	NT	NT
0.1% WF (120 ppm of gluten)	587 (74)	403 (15)	positive	649 (66)	156 (32)	positive	NT	NT
1.0% WPI (8000 ppm of gluten)	245 (27)	0 (0)	positive[c]	154 (56)	0 (0)	positive[c]	NT	NT
1.0% WF (1200 ppm of gluten)	536 (60)	56 (55)	positive[c]	372 (47)	25 (22)	positive[c]	NT	NT
10% WPI (80000 ppm of gluten)	36 (63)	0 (0)	positive[c]	68 (7)	0 (0)	positive[c]	NT	NT
10% WF (12000 ppm of gluten)	246 (59)	0 (0)	positive[c]	130 (44)	0 (0)	positive[c]	NT	NT

[a]Gluten-free bread dough was serially spiked with wheat protein isolate (WPI, Honeyville, Arise 8000 from MGB) or wheat flour (WF, King Arthur Brand, whole wheat) at defined w/w %, extracted per the test method and then tested on the 2B9 LFD. Select contaminations were additionally tested using the Romer AgraStrip for Gluten detection based on G12 and the Neogen Gluten ELISA based on R5. Reported values are the average and SD calculated from triplicate testing performed by a single analyst. [b]NT, not tested. [c]High concentrations of analyte result in attenuation (strip reader value <100) of competitive test line 2 and sandwich test line 1.

corresponding to 81 ppm of gluten was strongly positive, and the 0.01% WF spike (12 ppm of gluten) was positive. Unlike the results reported in Tables 1 and 2, where laboratory-prepared deamidated gliadins resulted in modestly weaker signals relative to native gliadins, in this instance, using commercially sourced deamidated gluten (WPI), comparable line intensities were observed for both the native and deamidated test analytes. This may relate to differences in degree of deamidation, as industrially performed deamidation is controlled to roughly 20−30% compared to 53% using the chemically defined deamidated gliadins. Importantly, these data establish comparable LODs for both native and deamidated targets of commercial products.

When the Romer (G12) and Neogen (R5) kits were assessed using the WPI and WF spiked into gluten-free bread mix, the R5-based ELISA reported levels of WPI contamination that were on par with the 2B9-based LFD, with OD 450 nm in the range of 0.56 (compared to 0.65 for the 10 ppm control) for the 0.001% contamination level (corresponding to 8.1 ppm of gluten). In comparison, the G12-based LFD exhibited a log-fold reduction in sensitivity toward the WPI spike compared to the WF spike, with a positive outcome observed at 0.01% WPI contamination or 81 ppm of gluten. These outcomes for commercially prepared deamidated gluten (WPI) differ from the results obtained from the laboratory-prepared deamidated gluten, in that the R5-based ELISA appeared adequate in its ability to detect deamidated product, whereas neither the G12- nor the R5-based gluten detection system was efficient at reporting the laboratory-prepared deamidated gluten. As the degree of gluten deamidation is determined by application, with WPI generally having lower percent deamidation, these comparative data suggest that R5-based systems are not adequate for detection of all types of deamidated gluten, and G12 is not efficient in detecting mildly deamidated gluten including WPI.

Matrix Effects and Spiking Recovery Analyses. The LFD assay was assessed for the effects of food matrices on assay selectivity by spiking a panel of complex foods with increasing amounts of purified gliadins. In particular, the six model foods chosen for this study were selected on the basis that they present distinct challenges to allergen recoverability, assay fluidics, and immune-based detection of allergen residues including high polyphenol, increased osmolarity and ionic strength, pH, high starch content, and viscosity. A secondary consideration was utility, for example, commodities that were likely candidates for gluten testing. Spiking known amounts of target analyte into complex matrices enables more comprehensive assessment of how effective the buffer/extraction method is at recovery of target analyte. Furthermore, given the homogeneous assay format for LFD and resultant limitations with respect to matrix effects, it allows for determination of how complicated matrices affect the performance of the device as it pertains to fluidics and antigen−antibody immune complex formation. As shown in Table 8, the reported LOD (10 ppm gliadin) was achieved for all test matrices except dark chocolate, for which only weak-positive outcomes were detected at this level, thereby indicating reasonable resistance of the assay to matrix effects.

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Table 8. Selectivity Analysis[a]

commodity	spiking level[b] (gliadins)	strip reader value: test line 1 sandwich	strip reader value: test line 2 competitive	result
70% dark chocolate	blank	0 (0)	709 (4)	negative
	1× LOD	52 (5)	134 (17)	weak positive
	2× LOD	64 (4)	181 (39)	positive
orange juice	blank	0 (0)	669 (125)	negative
	1× LOD	79 (15)	454 (107)	positive
	2× LOD	135 (31)	439 (132)	positive
gluten-free pasta	blank	0 (0)	539 (43)	negative
	1× LOD	153 (22)	182 (15)	positive
	2× LOD	182 (52)	116 (11)	positive
soup base	blank	11 (19)	618 (93)	negative
	1× LOD	79 (15)	248 (76)	positive
	2× LOD	135 (31)	235 (35)	positive
rice flour	blank	0 (0)	584 (62)	negative
	1× LOD	93 (10)	373 (47)	positive
	2× LOD	125 (11)	443 (30)	positive
gluten-free bread mix	blank	0 (0)	640 (99)	negative
	1× LOD	87 (13)	381 (100)	positive
	2× LOD	113 (8)	354 (10)	positive

[a]The performance of the LFD was evaluated by spiking six complex foods with native gliadin protein at increments of LOD (1.0 ppm), extracting under non-denaturing conditions, then testing the sample extracts and reading the results using an electronic strip reader. Reported strip reader values are the average and SD calculated from triplicate testing performed by a single analyst. [b]Limit of detection (LOD) is 1.0 ppm of prolamins or 2.0 ppm of gluten in food.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

CD, celiac disease; LFD, lateral flow device; mAb, monoclonal antibody; ppm, parts per million; WPI, wheat protein isolate; WHO, World Health Organization; FDA, U.S. Food and Drug Administration; ELSA, enzyme-linked immunosorbent assay; LOD, limit of detection; rpm, rotations per minute; PBST, phosphate-buffered saline, Tween-20; BSA, bovine serum albumin; HRP, horseradish peroxidase; IgG, immunoglobulin G; RT, room temperature; PC, procedural control; SD, standard deviation

REFERENCES

(1) Arendt, E.; Zannini, E. *Cereal Grains for the Food and Beverage Industries*; Woodhead Publishing: Cambridge, UK, 2013.

(2) Day, L.; Augustin, M. A.; Batey, I. L.; Wrigley, C. W. Wheat-gluten uses and industry needs. *Trends Food Sci. Technol.* **2006**, *17*, 82−90.

(3) Wieser, H. Chemistry of gluten proteins. *Food Microbiol.* **2007**, *24*, 115−119.

(4) Ciccocioppo, R.; Di Sabatino, A.; Corazza, G. R. The immune recognition of gluten in coeliac disease. *Clin. Exp. Immunol.* **2005**, *140*, 408−416.

(5) Reilly, N. R.; Green, P. H. Epidemiology and clinical presentations of celiac disease. *Semin. Immunopathol.* **2012**, *34*, 473−478.

(6) van Heel, D. A.; West, J. Recent advances in coeliac disease. *Gut* **2006**, *55*, 1037−1046.

(7) Kim, C. Y.; Quartsen, H.; Bergseng, E.; Khosla, C.; Sollid, L. M. Structural basis for HLA-DQ2-mediated presentation of gluten epitopes in celiac disease. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 4175−4179.

(8) Lebwohl, B.; Ludvigsson, J. F.; Green, P. H. Celiac disease and non-celiac gluten sensitivity. *BMJ* **2015**, *5*, h4347.

(9) Hischenhuber, R.; Crevel, R.; Jarry, B.; Makis, M.; Moneret-Vautrin, D. A.; Romano, A.; Troncone, R.; Ward, R. Review article: safe amounts of gluten for patients with wheat allergy or coeliac disease. *Aliment. Pharmacol. Ther.* **2006**, *23*, 559−575.

(10) Osman, A. A.; Uhlig, H. H.; Valdes, I.; Amin, M.; Mendez, E.; Mothes, T. A monoclonal antibody that recognizes a potential coeliac-toxic repetitive pentapeptide epitope in gliagins. *Eur. J. Gastroenterol. Hepatol.* **2001**, *13*, 1189−1193.

(11) Kahlenberg, F.; Sanchez, D.; Lachmann, I.; Tuckova, L.; Tlaskalova, H.; Mendez, E.; Mothes, T. Monoclonal antibody R5 for detection of putatively celiac-toxic gliadins peptides. *Eur. Food Res. Technol.* **2006**, *222*, 78−82.

(12) Kanerva, P. M.; Brinck, O.; Sontag-Stohm, T.; Salovaara, H.; Loponen, J. Deamidation of gluten proteins and peptides decreases the antibody affinity in gluten analysis assays. *J. Cereal Sci.* **2011**, *53*, 335−339.

(13) Tranquet, O.; Lupi, R.; Echasserieau-Laporte, V.; Pietri, M.; Larre, C.; Denery-Papini, S. Characterization of antibodies and development of an indirect competitive immunoassay for detection of deamidated gluten. *J. Agric. Food Chem.* **2015**, *63*, 5403−5409.

(14) Anderson, R. P.; Degano, P.; Godkin, A. J.; Jewell, D. P.; Hill, A. V. S. In vivo antigen challenge in celiac disease identifies a single transglutaminase-modified peptide as the dominant A-gliadin T-cell epitope. *Nat. Med.* **2000**, *6*, 337−342.

(15) Vader, L. W.; de Ru, A.; van der Wal, Y.; Kooy, Y. M.; Benckhuijsen, W.; Mearin, M. L.; Drijfhout, J. W.; van Veelen, P.; Koning, F. Specificity of tissue transglutaminase explain cereal toxicity in celiac disease. *J. Exp. Med.* **2002**, *195*, 643−649.

(16) Qiao, S. W.; Bergseng, E.; Molberg, O.; Jung, G.; Fleckenstein, B.; Sollid, L. M. Refining the rules of gliadin T cell epitope binding to the disease-associated DQ2 molecule in celiac disease: importance of proline spacing and glutamine deamidation. *J. Immunol.* **2005**, *175*, 254−261.

(17) Aleanzi, M.; Demonte, A. M.; Esper, C.; Garcilazo, S.; Waggener, M. Celiac disease: antibody recognition against native and selectively deamidated gliadin peptides. *Clin. Chem.* **2001**, *47*, 2023−2028.

(18) Department of Health and Human Services, Food and Drug Administration. Food Labeling; Gluten-Free Labeling of Foods. *Fed. Regist.: Rules Regul.* **2013**, *78*, 47154−47179.

(19) Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten, Codex Standard 118 − 1979; www.codexalimentarius.org (accessed July 8, 2015).

(20) Gourbeyre, P.; Denery-Papini, S.; Larre, C.; Gaudin, J. C.; Brossard, C.; Bodinier, M. Wheat gliadins modified by deamidation are more efficient than native gliadins in inducing a Th2 response in Balb/

DOI: 10.1021/acs.jafc.5b06085
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

c mice experimentally sensitized to wheat allergens. *Mol. Nutr. Food Res.* **2012**, *56*, 336−344.
(21) Lupo, A.; Roebuck, C.; Walsh, A.; Mozola, M.; Abouzied, M. Validation study of the Veratox R5 Rapid ELISA for detection of gliadin. *J. AOAC Int.* **2013**, *96*, 121−132.
(22) Radcliffe, S.; Sutzko, M.; Jiang, Z.; Freitag, D.; Swoboda, C.; Frank, L.; Rogers, A. Validation of the AgraStrip® gluten G12 test kit. *J. AOAC Int.* **2014**, *97*, 1638−1650.

DOI: 10.1021/acs.jafc.5b06085
J. Agric. Food Chem. XXXX, XXX, XXX−XXX