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An integrated, accurate, rapid, and economical handheld consumer gluten detector

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ABSTRACT

Celiac disease, characterized by autoimmune reactions to dietary gluten, affects up to 3 million in the US and approximately 0.5%–1% globally. A strict, lifelong gluten-free diet is the only treatment. An economic, simple, accurate, rapid and portable gluten testing device would enable gluten-sensitive individuals to safeguard their food safety. We developed a novel solution, Nima™, a gluten sensor that integrates food processing, gluten detection, result interpretation and data transmission in a portable device, detecting gluten proteins at or below the accepted 20 ppm threshold. We developed specific monoclonal antibodies, an optimized lateral flow immunoassay strip, and one-step aqueous extraction. Compared with reference R5, Nima™ antibodies (13F6 and 14G11) had 35- and 6.6-fold higher gliadin affinities, respectively. We demonstrated device performance using a comprehensive list of foods, assessing detection sensitivity, reproducibility, and cross-reactivity. Nima™ presented a 99.0% true positive rate, with a 95% confidence interval of 97.8%–100%.

1. Introduction

Celiac disease (CD) is widely accepted as a systemic immune-mediated disorder in genetically susceptible persons, triggered by ingestion of gluten proteins from foods, including *wheat, rye and barley* (Fasano & Catassi 2012; Shan et al., 2002). CD has an estimated prevalence of 0.5%–1% globally, approximately 1% among people of European descent (Han et al., 2013). It affects up to 3 million people in the US and is four times more common today than 50 years ago (Rubio-Tapia et al., 2009). Common symptoms include chronic diarrhea, weight loss and abdominal distention (in 40%–50% patients) with other manifestations present as well (Collin, Vilska, Heinonen, Hällström, & Pikkarainen, 1996; Fasano & Catassi 2012; Shan et al., 2002). Additionally, researchers have reported a population with non-celiac gluten-intolerance, experiencing adverse gluten reactions that are neither allergic nor autoimmune (Sapone et al., 2012).

A strict, life-long gluten-free (GF) diet is the only treatment for CD (Comino et al., 2012; Mena, Lombardía, Hernando, Méndez, & Albar, 2012; Murray, 1999; Shan et al., 2002; Troncone, Auricchio, & Granata, 2008). In August 2013, the FDA ruled that GF foods must have < 20 parts per million (ppm) gluten. It can be very difficult to adhere to a GF diet, due to lack of education, cross-contamination of foods, and inadequate labeling and testing mechanisms (Catassi et al., 2007; Comino et al., 2013; Thompson, Lee, & Grace, 2010; Troncone et al., 2008). These issues can lead to frequent lapses and chronic morbidity in patients (Bethune & Khosla, 2012). Thus, there is a great need for a consumer-friendly, economic, simple, accurate, rapid and portable testing device. Such a device would enable gluten-sensitive individuals to test their foods, ensuring food safety and saving on long-term medical costs. No such device is available, because existing gluten tests are complicated and expensive and, thus, unsuitable for consumers. Current detection methods include mass spectrometry (Dworschak et al., 1998), PCR (Sandberg, Lundberg, Ferm, & Malmheden Yman, 2003) and some immunoassay-based platforms (including plate-based ELISA and lateral flow immunoassay (LFIA) techniques) (Kahlenberg et al., 2006; Mena et al., 2012; Morón, Bethune et al., 2008; Morón, Cebolla et al., 2008; Skerritt & Hill, 1990; Skerritt & Underwood, 1986). Of these, immunoassays are preferred for rapid performance, high sensitivity, and

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ease of implementation in a laboratory. For example, the FDA identified recently two ELISAs for food manufacturers to test GF-labeled foods ("Questions and Answers: Gluten-Free Food Labeling Final Rule", 2013).

To address the need for testing by gluten-sensitive individuals, we developed a novel solution, an all-in-one handheld device called Nima™, which integrates food processing, gluten detection, result interpretation and data transmission. Its small footprint facilitates out-of-the-home use, such as in restaurants, while traveling or at social events. To minimize user errors, the device performs all sample processing steps internally, presenting consumers with a binary positive or negative result. Internal food processing and one-step extraction accelerates gluten extraction and testing, thereby shortening overall detection time (ODT) from at least 20 min (manufacturer's instructions for AgraStrip®, GlutenTox, EZGluten and others) to 2–4 min. Our target for Nima™ was analysis of simple and complex food matrices with an accuracy of 99.0% for ≥ 20 ppm gluten. Here, we report on the development of Nima™ and its performance when tested on a comprehensive range of foods that would be encountered in real-world circumstances.

Prolamin (wheat gliadin, rye secalin, barley hordein) is half the total gluten content (Gessendorfer, Koehler, & Wieser, 2009; Žilić, Barać, Pešić, Dodig, & Ignjatović-Micić, 2011). Similar to other kits developed using the R5 antibody (Valdés, García, Llorente, & Méndez, 2003a), our device detects prolamin (including gliadin, hordein, and secalin) not glutenin. In our study, reference to a concentration of gluten means half the concentration of prolamin was detected, *i.e.* 20 ppm gluten means that 10 ppm gliadin, secalin or hordein was determined analytically.

We constructed a live database of gluten test results in the US. Results are updated automatically via a mobile app, allowing users to store and share their results as well as search those of others. This will help consumers identify GF foods and products with potential issues, thereby aiding the GF community with dietary adherence. While this product focused solely on gluten, the platform is applicable to detecting other common food substances (e.g., allergen sources), including peanuts, tree nuts, dairy products, eggs and soy (Boyce et al., 2010).

2. Materials and Methods

2.1. Materials

Purified prolamins (wheat gliadin, barley hordein, rye secalin), reference material PWG-gliadin, and oat samples were purchased from AromaLAB AG (Planegg, Germany), the German Research Center for Food Chemistry (Freising, Germany) (Gessendorfer et al., 2009; Van Eckert et al., 2006), and USDA (Aberdeen, ID, USA). Information about other materials and chemicals is presented in Supporting Information.

2.2. Monoclonal antibodies, indirect ELISA, surface plasmon resonance (SPR)

Mouse monoclonal antibodies were raised using standard hybridoma techniques (Council, 1999) under a contract with GenWay Biotech, Inc (San Diego, CA, USA). Indirect ELISA was performed using a standard ELISA protocol where plates were coated with wheat, barley, rye prolamins, PWG gliadin, 33-mer or oat samples at equal concentrations, followed by the addition of the antibodies of interest and measurement of their binding in response to food samples. Each condition was analyzed by ELISA in 2 wells and data are expressed as means. SPR data for the three anti-gluten antibodies were obtained under contract with Genscript Corporation (Piscataway Township, NJ, USA). For each antibody, four concentrations were tested, and analyses performed twice for each condition. Further details are in Supporting Information.

2.3. Sensitivity performance of the Nima^{TM} lateral flow immunoassay (LFIA)

For a typical strip, colloidal gold nanoparticles (red) were conjugated with our custom anti-gluten antibody, 14G11. This involved mixing the antibody with the nanoparticles at a ratio of 12 μg antibody/ml gold particle (optical density = 2) in a basic solution (pH = 8.2–8.6) for 30 min at room temperature, and the complex was dispensed onto the conjugate pad (red pad at the base of strip). The test line (TL) containing our other antibody (13F6) was printed onto a nitrocellulose membrane. The hook line (HL) was printed with concentrated wheat and the control line (CL) was printed with an antibody that would recognize the conjugate regardless of the presence of gluten. These components were assembled into lateral flow strips, which is described in the patent application (Zhang, Sundvor, & Shirajian, 2016). Briefly, a 25 mm wide nitrocellulose membrane was glued onto a paper-back card and a wick pad, and the preprinted conjugate pad was assembled on top of the rear card with a 2–3 mm overlap. Cover tape was applied to keep the assembly intact and air was squeezed out by pressing on the assembly. This immunoassay strip is referred to as a "Nima^{TM} strip".

To validate immunoassay performance, Nima^{TM}strips were tested in a custom-built test fixture, using six Nima^{TM} sensors to perform the same function as the actual Nima^{TM} device. Nima^{TM} strips were exposed to wheat, barley or rye gluten, prepared from the prolamins, at 0, 0.1, 0.2, 1, 25, 100 or 500 ppm in our custom extraction solution (Zhang et al., 2016) for 2 min. For low (0–1 ppm) and high (25–500 ppm) concentrations, 6 and 3 replicates were analyzed, respectively. Control, hook and test line intensities were captured using a linear-array camera inside the Nima^{TM} sensor and analyzed directly using a custom algorithm (Sundvor et al., 2016). A decision (gluten or GF) was determined.

2.4. Nima^{TM} LFIA specificity and compatibility testing

Similar to research conducted by other groups (García, et al., 2005; Morón, Bethune, et al., 2008; Morón, Cebolla, et al., 2008; Valdés, García, Llorente, & Méndez, 2003b), but without any centrifugation, GF flours from various sources were added to 4 ml extraction solution (Zhang et al., 2016), vortexed for 30 s and aliquots of each extract placed in 6 replicate wells in a 96-well plate. A LFIA strip was placed in each well and the solution developed for 2 min. The strips were from three separate lots each run in duplicate. To test specificity, we used the highest possible concentrations, but not so high as to be viscous, which would hinder strip development. Salad dressing, yogurt, vinegar, chocolate, butter and cheese were from local grocery stores. Each sample (0.4–1 g) was vortexed with 3 ml extraction solution for 30 s, then pipetted into a well, the Nima^{TM} strip added, and the reading recorded.

Nima^{TM} strip results were read using a visual scale 0–10 (Fig. S1), with 0–1 considered GF but 2–10 as containing gluten. Each test reading was read blindly by two investigators.

2.5. Food sample preparation and sourcing

We prepared foods following AOAC guidelines (Koerner et al., 2013), and also sourced foods from Food Allergy Research and Resource Program (FARRP, University of Nebraska), Bia Diagnostics, and reputable food companies, of which one, "Company A", wished to remain anonymous. Food preparation and sourcing details are in Supporting Information.

Briefly, a gluten-free version of food dough, burger patty, ice cream or soup was prepared first, and spiked with highly concentrated gluten in 60% ethanol. For burgers and baked goods, samples were cooked fully following their respective recipes (Supporting Information). The amount spiked was determined experimentally to reach a final concentration of 20 ppm in the food as consumed.

2.6. Food testing in the Nima™ device

Each food sample was processed and analyzed in the Nima™ device. Test strip results were read following the visual scale (Fig. S1) and logged by the internal memory. Samples were analyzed in triplicate whenever possible, unless there was a shortage of samples or testing units (as indicated in Fig. 4). The weights of each food tested are listed in Fig. 4. These varied for different foods primarily because amounts that can be placed in the Nima™ are determined by volume rather than by mass.

Test parameters were defined, based on device readings and known gluten content, as follows: True Positive (TP), a *Wheat* sign, and the food contained ≥ 2 ppm gluten; False Positive (FP), a *Wheat* sign, and the food contained < 2 ppm gluten; True Negative (TN), a *Smiley* face, and the food contained < 20 ppm gluten; False Negative (FN), a *Smiley* face, and the food contained ≥ 20 ppm gluten.

True Positive Rate (TPR) = TP/(TP + FN); True Negative Rate (TNR) = TN/(TN + FP); False Negative Rate (FNR) = 1 − TPR; False Positive Rate (FPR) = 1 − TNR. Error Rate was reported as Number of Errors/Total Number of Tests. Accuracy was calculated by the sum of the TP and TN, divided by the total number of tests excluding errors. Accuracy (with errors) was calculated by the sum of the TP and TN, divided by the total number of tests including errors. Precision was defined as TP/(TP + FP).

2.7. Effects of grinding and extraction time on extraction efficiency

To test effects of grinding, yellow cake and bread were spiked at 46 and 26 ppm, respectively (Supplementary Table I). The foods were ground in a coffee grinder, screened through a series of meshes, and particles in each size range extracted in extraction buffer for 30 s, and diluted at least 10-fold before being analyzed using R7001 ELISA. To test the effects of extraction duration, four model foods (FARRP) were directly ground in the Nima™ device itself and extracted directly in the unit for 30, 60 or 90 s at a food:solution (weight: volume) ratio of 1:10. Extracts were centrifuged at 30,000*g* at 4 °C for 2 min, and supernatants assayed by R7001 ELISA. Results were compared against the expected gluten concentrations in the extraction solution to determine extraction efficiencies.

2.8. Data analysis

Unless otherwise indicated, data from replicate samples were averaged and are expressed as mean ± standard error (SE).

3. Results

3.1. Nima™ device use

Fig. 1 shows the Nima™ device with a reusable Nima™ tester and a disposable testing unit (Fig. 1a). Users put foods into the unit (Fig. 1b and c) and, as the top is screwed on, food is mechanically ground, and the extraction solution released (Fig. 1d). The unit is then placed into the Nima™ reader (Fig. 1e) and the user presses a button (Fig. 1f) mixing the contents, by magnetic coupling, for 30 s. As the user screws the cap of the unit all the way down to close it, an internal valve opens automatically, allowing liquid from the extraction chamber to flow on to the LFIA strip. A GF sample is indicated by a “Smiley” face on the screen (Fig. 1g–i) and a gluten-containing one by a “Wheat” symbol (Fig. S2). Test results are also visible through the viewing window on the disposable unit (Fig. 1i), with both control and hook lines shown in this example. More design details were described in a patent application (Sundvor et al., 2014).

3.2. Food grinding

Food particle size post-grinding significantly impacted extraction efficiency, with higher relative surface areas, i.e. smaller particles, improving gluten extraction, as demonstrated with two model foods, gluten spiked yellow cake (46 ppm) and bread (26 ppm). For yellow cake, extraction efficiency decreased from 45 ± 0.1% to 32 ± 2.5% (mean ± SE) as particle size increased from < 0.5 to 1.5–2 mm. For bread, it decreased from 54 ± 7.5% to 27 ± 0.0% (mean ± SE) as particle size increased from < 0.25 to > 2 mm (Fig. S3). Decreasing particle size improved extraction 1.5–2 fold, so effective grinding is desirable. The Nima™ device size and battery power input precluded an electrically-powered grinder, so a manual grinder mimicking that of a garlic grinder was designed. Thickness and spacing of the grinder teeth were determined by grinding efficiency and manufacturing feasibility.

3.3. Food extraction at room temperature

Because gluten has poor water solubility, early extraction solutions employed 60–80% ethanol, which is still used in numerous commercial kits (*e.g.* Romer Labs AgraQuant® ELISA Gluten G12 COKAL0200 and R-Biopharm R7001 ELISA kit), (Sorell et al., 1998). Such solutions, unless diluted, are incompatible with immunoassays. For the Nima™ device, an aqueous gluten-extraction solution that also developed the strip eliminated sample dilution and, thus, simplified the overall device. However, ethanol-based solutions extract gluten from processed foods inefficiently, because inter-protein disulfide bonds form during the baking process (García, et al., 2005; Gessendorfer, Wieser, & Koehler, 2010; Mena et al., 2012). Recent approaches used aqueous combinations of reducing and disaggregating agents, and some claim to extract gluten from heat-processed foods with 93–97% efficiency (García, et al., 2005; Gessendorfer et al., 2010; Mena et al., 2012).

We designed an aqueous extraction solution (Zhang et al., 2016) enabling gluten extraction at room temperature within 30 s. As assessed by SafeBridge Consultants, Inc, this solution can be disposed of as regular waste. With four model foods, we found that extraction efficiency depended on food matrix and mixing time. Bread was most difficult to extract, with efficiencies of 20.7 ± 3.8%–35.7 ± 6.2% (means ± SE) for 30–90 s mixing; muffin was easiest to extract (37.3 ± 3.4%–61.6 ± 12.2%, Fig. S4). Though longer times increased efficiency, a 30 s extraction, combined with a sensitive LFIA strip, was sufficient to provide the desired sensitivity with our target accuracy.

3.4. Antibody performance

We compared our 13F6 and 14G11 antibodies to R5, a reference antibody used in many existing gluten immunoassays. Using indirect ELISA (Fig. 2a), 13F6 and 14G11 had much better responses to wheat, barley and rye prolamins than R5, and also responded to the 33-mer while R5 did not. None responded to oat avenin, which is not gluten. 13F6 and 14G11 responded similarly to wheat and barley prolamins, but responses to rye prolamin were 8%–20% lower. For R5, responses to barley and rye were 1.4–2.0, and 1.7–2.2 times higher than those for wheat, perhaps because it was originally raised against a rye extract (Sorell et al., 1998). 13F6 and 14G11 responded to wheat prolamin almost identically to PWG gliadin, indicating that both are likely to detect common epitopes within these wheat strains, while R5 showed more differences across wheat strains.

Based on SPR responses (Fig. 2b and c), 13F6 and 14G11 had dissociation constants (K_D) 35- and 6.6-times lower than that of R5 and, thus, bound gliadin with much greater affinity. PWG gliadin is a mixture of α, β, γ, and ω-gliadins, but the SPR kinetic model does not work with multiple antigens. Thus, the absolute K_D value might not be accurate. However, relative differences among the three antibodies were reliable, showing that the newly developed antibodies (13F6 and 14G11) had higher affinities for gliadin and performed better than R5.

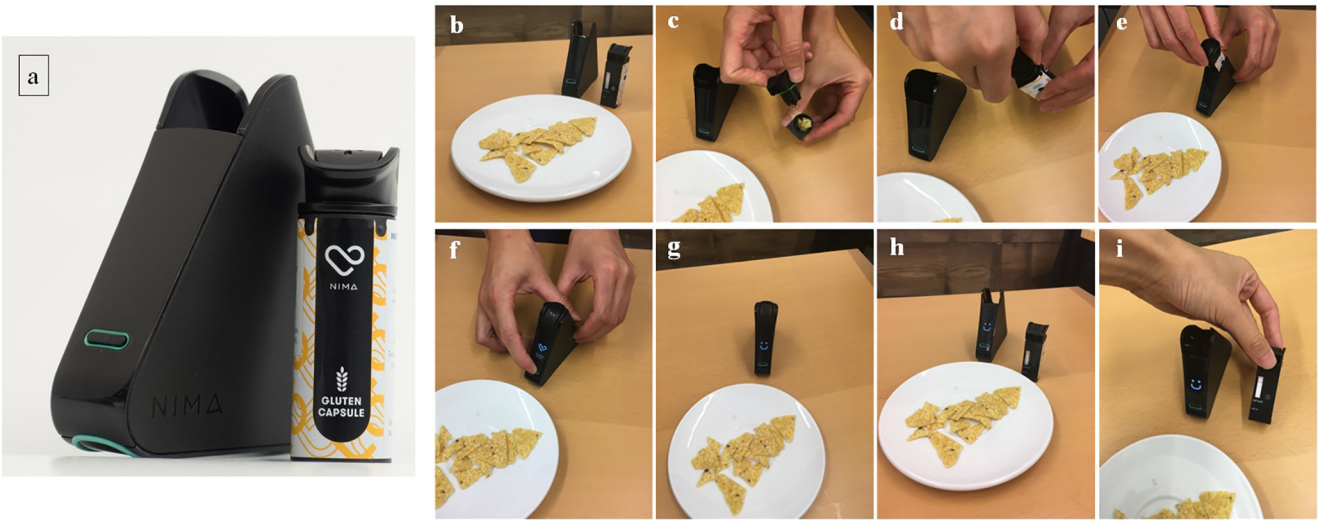
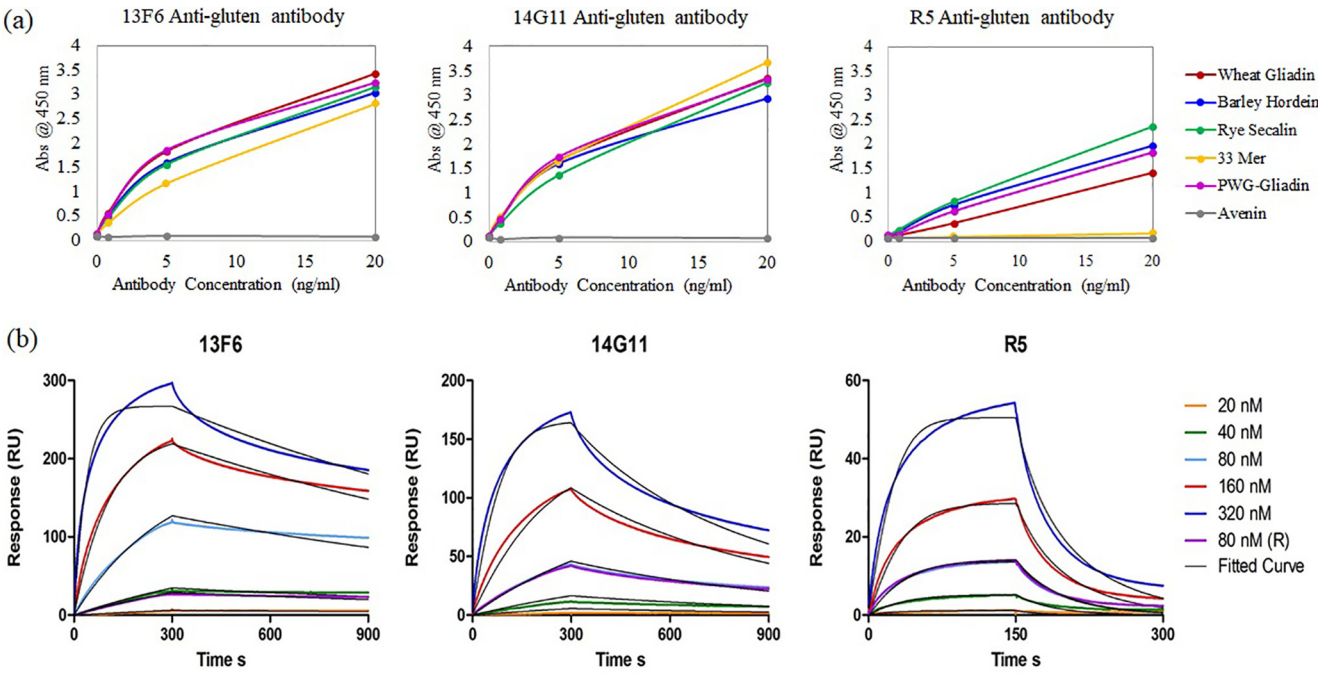


Fig. 1. Current Nima™ device in use. The device has a reusable Nima™ tester and a testing disposable (a). Food is inserted in the disposable (b–c) and, as the top is screwed on, is mechanically ground and extraction solution added (d). The disposable is then placed into the Nima™ reader (e). Pressing a button (f) causes mixing, then a valve allows extract to flow onto the LFIA test strip. A "*Smiley*" face on the screen (g–i) indicates GF and a "*Wheat*" symbol indicates gluten (Fig. S2). Test results are also visible in a viewing window, here showing both control and hook lines (i).



(c)

Analyte	Ligand	k_a $(M \cdot s)^{-1}$	k_d (s^{-1})	K_D (M)
13F6	Gliadin	1.14×10^{5}	6.63×10^{-4}	5.81×10^{-9}
14G11	Gliadin	7.36×10^{4}	2.25×10^{-3}	3.06×10^{-8}
R5	Gliadin	2.66×10^{5}	5.39×10^{-2}	2.03×10^{-7}

Fig. 2. Performance of Nima™ 13F6 and 14G11 and reference R5 anti-gluten antibodies. (a) Indirect ELISA results on plates coated with wheat gliadin, barley hordein, rye secalin, 33-mer, PWG gliadin, or avenin (oat prolamin). Results are means of 2 wells per sample. Error bars (SE) are so small that they are invisible on the graph. (b) Surface Plasmon Resonance (SPR) responses. Gold surfaces were conjugated with gliadin and binding of various concentrations of antibody monitored. (c) k_a, k_d and K_D values, determined by SPR. Further details in Supporting Information.

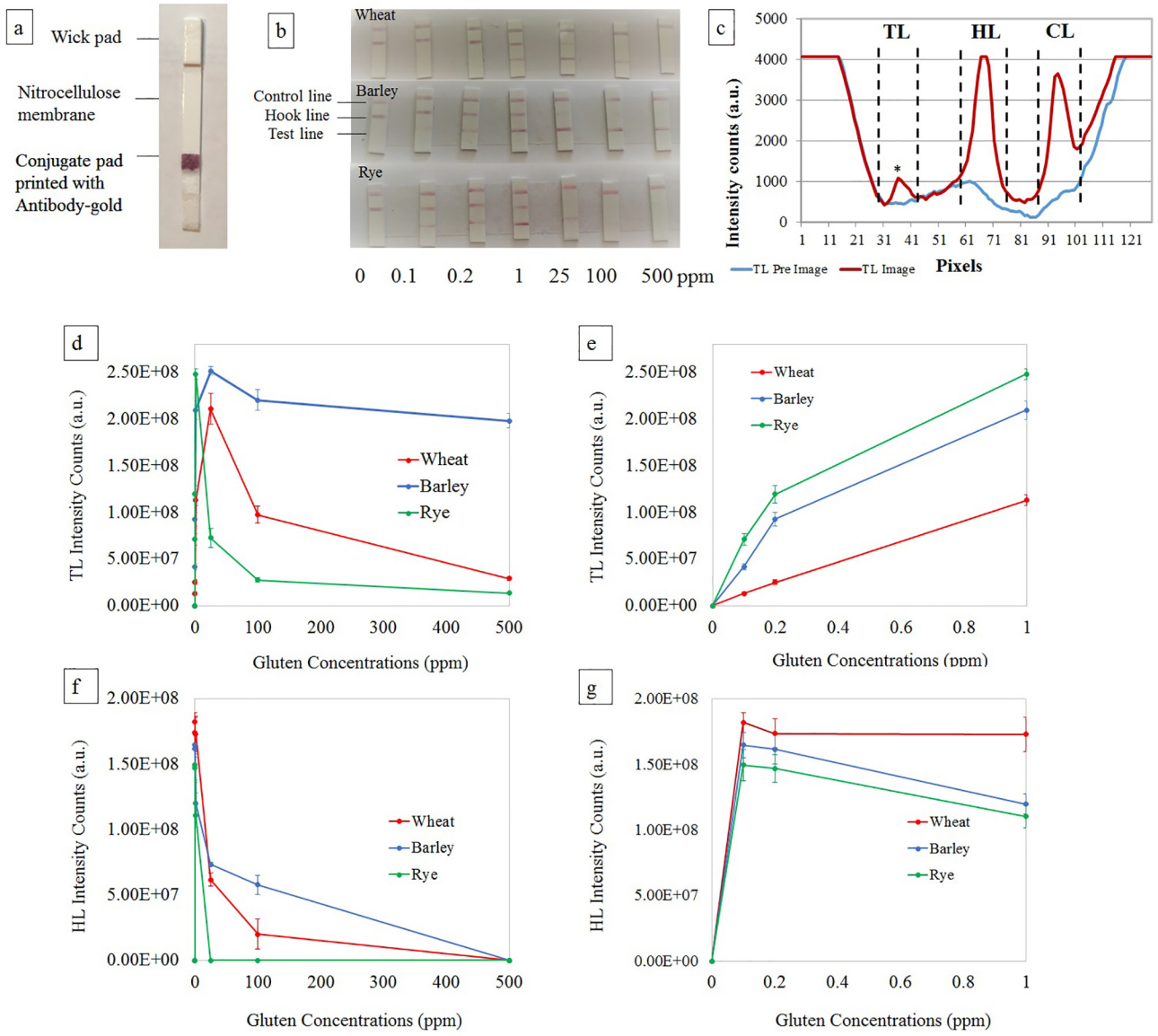


Fig. 3. Sensitivity of LFIA chemistry. (a) A representative Nima™ strip. (b) Nima™ strips exposed to wheat, barley, and rye gluten at indicated concentrations in extraction solution for 2 min. Control line (CL), hook line (HL) and test line (TL) on strips are shown. (c) Light intensity as a function of position along the strip, captured by the camera, shows peak intensities at the line positions. The blue line shows the output of a blank strip (reference) and the red line shows the output of the strip exposed to gluten, with 3 lines. TL, HL and CL detection regions are indicated with dotted lines and the actual test line peak by "*". (d, f) Intensities of TL (d) and HL (f), indicated by the convolution values of the curves, at various gluten concentrations. (e, g) Magnified views of lower concentration TL (e) and HL (g) readings, from plots d and g, respectively. In (d–g), data are means ± SE. For low (0–1 ppm) and high (25–500 ppm) concentrations, 6 and 3 replicates were analyzed, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Thus, in our device, 14G11 was conjugated to the gold nanoparticles and 13F6 was used to prepare the TL on the LFIA strip (Zhang et al., 2016).

3.5. LFIA strip sensitivity

To determine the limit of detection (LoD), we performed a simulation to calculate gluten concentration distribution in a food extract - details are presented in Supporting Information. In a user study, volunteers filled the capsule with 0.67 ± 0.39 (SD) g food. Based on this and various assumptions, we simulated food weight distributions (Fig. S5) and found that, with a test strip LoD of 0.1 ppm, 99% of the extracted gluten concentration was detected, an FNR of 1.07%. The simulation focused on detecting 20 ppm gluten foods, which was our primary goal, but we anticipate higher TPRs for foods with ≥20 ppm gluten. Therefore, with a target strip LoD of 0.1 ppm, TPR was > 99%. Our analysis indicated a need to ensure a certain degree of extraction efficiency across all food matrices, but also showed that complete extraction was unnecessary for device performance.

To examine LFIA sensitivity, we prepared wheat, barley and rye prolamin standards at 0–250 ppm (or 0–500 ppm gluten) in extraction solution. LFIA reactions for these were determined in a 96-well plate. Fig. 3d–g show that, with all three prolamin samples, HL intensity monotonically decreased, while TL intensity first increased but decreased at higher prolamin concentrations. As expected, CL remained constant, regardless of gluten concentration. The combination of TL and HL intensities enabled the device to distinguish among GF (HL positive, TL negative), relatively low gluten (HL and TL both positive), high gluten (HL negative, TL positive; or, HL and TL both negative). The HL discriminated between GF and high-gluten cases, both having a negative TL signal.

We conducted a similar test, using a custom-built test fixture with

an internal camera identical to that in the Nima™ device, to record line intensities at 2 min. Intensity counts, determined using our custom algorithm, are plotted in Fig. 3d–g.

The peak identification algorithm was described in our patent application (Sundvor et al., 2016). Briefly, it looked for a difference in light intensities of the blue pre-image (blank strip, Fig. 3c) and red post-image (developed strip, Fig. 3c). The peak value of that difference was compared to a set threshold. Peak location was checked to confirm it was in the zone where a line is expected, by convolving data with a preset mathematical kernel. The convoluted value obtained through the kernel represented peak intensity, with additional noise filtering functions applied.

TL intensities are plotted against gluten concentration in Fig. 3d and e. HL intensities are similarly plotted in Fig. 3f and g. In both cases, a concentration of 0 gave an absolute "0" reading, after post-processing, using our algorithm.

While both antibodies responded similarly to gluten proteins from the three grains using ELISA, the device had a slighter lower TL signal toward wheat (Fig. 3d–e) at low prolamin concentrations. Because the majority of gluten contaminations in foods is from wheat, this provided a safe threshold for gluten detection. Fig. 3f–g also show that rye prolamin bound to 14G11 conjugates rapidly, leaving no conjugates to bind to HL at high rye concentrations, indicating a higher k_a for rye than for wheat and barley. The TL dynamic reading was more complicated, resulting from combined effects of antibody avidity toward the antigen and competition from the two antibodies for the same epitopes.

3.6. LFIA strip specificity and compatibility

Fig. 4a shows various GF flours, spices and nut samples, based on AOAC gluten testing guidance (Koerner et al., 2013), used to test LFIA strips (see Methods). We extracted 0.8 g of each flour in 4 ml solution to maximize detection of cross-reactivity and provide enough liquid to analyze multiple replicates simultaneously.

All flour samples were tested independently by Bia Diagnostics (R7001 ELISA) to verify gluten concentrations < 2 ppm. All GF flours, nuts and spices produced a GF response; we could not obtain GF buckwheat and flax seed samples, which was confirmed by the ELISA.

Fat-containing food matrices can clog pores and hinder capillary flow in LFIAs. Our user data indicated that salad dressings and yogurts, sometimes containing high fat, are of particular interest. We tested GF salad dressing, yogurt, chocolate, butter and cheese samples (Fig. 4b). The strips ran properly with medium weights, but some larger amounts clogged the test strip. In such cases, the CL did not develop, giving instead an "Error" reading. Threshold weights differed for each food (Fig. 4a or b with "*"). However, with a pea sized amount, as specified in our user manual (~400 mg yogurt or salad dressing), all read correctly as GF.

We also tested distilled and rice vinegars, with 1 ml (maximum volume) yielding the correct results. While vinegar is potentially problematic for lateral flow gluten testing, by limiting sample size and controlling extraction volume, the Nima™ device overcame these issues.

3.7. Food testing

We tested a wide variety of GF and gluten-containing foods. We prepared foods following AOAC guidelines (Koerner et al., 2013), spiking GF food matrices with 20 ppm wheat gluten. "Company A", Bia Diagnostics and FARRP provided samples containing known amounts of gluten. We also sourced foods from local restaurants, grocery stores and farms. See Methods and Supplementary Table II for information about food sourcing and preparation.

The samples tested are listed in Fig. 5a, with results summarized in Fig. 5b–d. Fig. 5b shows results for all foods listed, with subcategories in Fig. 5c and d. Fig. 5c should more accurately describe the device performance, because gluten in the foods was distributed homogenously and spiked with a known amount of gluten. In contrast, restaurant foods and packaged foods (Fig. 5d) might intrinsically contain non-homogenously distributed gluten. For these foods, known gluten concentrations were determined in a portion of sample by external testing, so results were more likely to be influenced by sampling.

As defined in the Methods, TP, TN, FN and FP values were determined for each food. These values enabled us to determine TPR, TNR, FNR and FPR, which were 99.0% (97.8%–100%), 92.2% (87.4%–96.8%), 1.0% (0%–2.2%) and 7.8% (3.2%–12.4%), respectively, for all foods (Fig. 5b–d, presented in means (95% confidence intervals)). The values for "Nima", "Bia", "FARRP" and "Company A" foods were 99.5% (98.6%–100%), 88.1% (80.3%–95.9%), 0.5% (0%–1.4%) and 11.9% (4.1%–19.7%), respectively. The primary focus of protecting consumers is FNR and a rate of 1.0% is acceptable for our device. Ongoing testing includes expanding the range of foods analyzed.

3.8. Testing device reproducibility using gluten spiked bread

We prepared GF bread, and spiked some with 5, 10, 20 or 30 ppm gluten (Supplementary Table III). We tested these using the Nima™ at 25% (107 mg), 37.5% (161 mg), 50% (215 mg) and 100% (430 mg) of the maximal weight filling the chamber, analyzing each preparation 15 times at each weight and each spiked concentration. A total of 300 data points were collected, giving TPR of 98.9% (97.4%–100%), FNR of 1.1% (0%–2.6%) and TNR of 100% (Fig. 6a). As expected, the percentage of samples detected correlated with spiked gluten concentrations and food weights (Fig. 6b). For the 20 ppm bread, 2 of 15 read FN at 107 mg but, with its fluffiness, this sample was at the lower limit of our permitted weight range (most foods are denser than bread) and, at 161 mg, this risk was mitigated. With increased weight, gluten levels < 20 ppm were also detected. Results for 10 ppm bread at 25% weight did not align with those for 5 ppm bread at 50% weight, indicating that extraction efficiency was affected by other factors, such as grinding efficiency. Overall, the FNR was in the acceptable range for device performance.

4. Discussion

4.1. Factors affecting Nima™ device performance and accuracy

The Nima™ device showed performance characteristics, such as sensitivity, specificity and rapid results, suitable for gluten screening by consumers. The test process was comprised of multiple steps, food input, grinding, mixing and gluten extraction, fluid delivery, strip development, result recording and interpretation, each affecting overall assay performance. Several possible factors, therefore, can contribute to variability and accuracy of the test results.

4.1.1. Food types

The device was designed to test as many food types as possible, including solid and liquid, and cooked and uncooked, with food type and cooking status introducing a range of complexities to gluten testing. We emphasized cooked foods to enable our consumers to eat more safely in restaurants. Baked and broiled foods are more challenging for gluten testing (Koerner et al., 2013; Mena et al., 2012), increasing the stringency of product design and testing. We followed AOAC guidelines (Koerner et al., 2013) to prepare a comprehensive list of Nima™ foods. Of 20 AOAC recommended food matrices, we eliminated alcoholic beverages and combined others, ultimately testing 14 matrices (Fig. 5a). Our detection, based on a sandwich immunoassay, was not designed to detect fermented products with partially hydrolyzed gluten. We also added restaurant and packaged foods and flours used commonly in GF cooking. More flours were added to test specificity, using LFIA strips (Fig. 4). We obtained additional foods from various sources in order to increase sample diversity, including "Company A", FARRP

(a)

Samples	Source	Weights (g)	Samples	Source	Weights (g)
Almond flour	Nuts.com	0.8	Oat flour	Trader Joe's	0.4
Amaranth flour	Nuts.com	0.2	Potato flour / starch	Bob's Red Mill	0.8
Arrowroot flour	Nuts.com	0.8	Quinoa flour	Quinoa.com	0.8
Black bean, ground in-house	Local organic farm	0.8	Sesame seeds	Nuts.com	0.8
Brown rice flour	Nuts.com	0.8	Sorghum flour	Bob's Red Mill	0.8
Buckwheat	Nuts.com	0.6	Soybean, ground in-house	Whole Foods	0.4
Chestnut flour	Nuts.com	0.8	Sweet rice flour	Nuts.com	0.4
Coconut flour	Nuts.com	0.4	Tapioca flour	Nuts.com	0.8
Coffee, ground in-house	Neapolitan Roast, Mrespresso.com	0.8	Tea, ground in-house	Longjing Tea, Hangzhou	0.8
Masa harina corn flour	Nuts.com	0.8	White bean flour	Local organic farm	0.8
Dried fruit	Goji berries, Navitas Naturals	0.8	White rice flour	M206, California Cooperative Rice Research Foundation	0.8
Egg powder	Hoosier Hill Farm	0.145	Yellow split pea, ground in-house	Whole Foods	0.8
Fava bean flour	Local organic farm	0.4	Cayenne powder	Simply Organic	0.4
Flax seed flour	Nuts.com	0.8	Tumeric powder	Spicely	0.8
Garfava flour	Nuts.com	0.8	Garlic powder	Spicely	0.8
Green pea flour	Nuts.com	0.8	Ground cumin	Spicely	0.4
Hazelnut flour	Nuts.com	0.8	Ground ginger	Spicely	0.8
Lentil bean, ground in-house	Whole foods	0.8	Vanilla extract	Spicely	0.4
Lima bean flour	Nuts.com	0.8	Vanilla bean powder	Spicely	0.8
Milk powder	Nuts.com	0.145	Allspice	McCormick	0.8
Millet flour	Nuts.com	0.8			

(b)

Samples	Weight (g)	Samples	Weight (g)
Salad Dressing, Newman's Own Sesame Ginger, 5% Fat	0.2, 0.4, 0.6*	Yogurt, Siggi's Plain 0% Fat	0.4, 0.6, 0.8
Salad Dressing, Newman's Own Light Balsamic, 13.3% Fat	0.2, 0.4, 0.6*	Yogurt, Siggi's Plain 4% Fat	0.4, 0.6, 0.8
Salad Dressing, Newman's Own Raspberry Walnut, 16.7% Fat	0.2, 0.4, 0.6, 0.8*	Yougurt, Greek Yogurt, Plain, non-fat	0.2, 0.4, 0.6*
Salad Dressing, Newman's Own Light Italian, 20% Fat	0.2, 0.4, 0.6, 0.8*	Yogurt, Wallaby Plain Greek 0% Fat	0.4, 0.6, 0.8
Salad Dressing, Newman's Own Balsamic Vinaigrette, 30% Fat	0.2, 0.4, 0.6, 0.8	Yogurt, Wallaby Vanilla Australian 1.5% Fat	0.4, 0.6, 0.8
Salad Dressing, Newman's Own Caesar, 53.3% Fat	0.2, 0.4, 0,6, 0.8	Yogurt, Wallaby Plain Greek 2% Fat	0.4, 0.6, 0.8*
Salad Dressing, Newman's Own Olive Oil and Vinegar, 59.3% Fat	0.2, 0.4, 0.6, 0.8	Yogurt, Wallaby Vanilla Greek 4% Fat	0.4, 0.6, 0.8*
Salad Dressing, Annie's Organic Asian Sesame, 38.7% Fat	0.2, 0.4, 0.6, 0.8	Yogurt, Dannon Coffee Yogurt 1.5% Fat	0.4, 0.6, 0.8
Salad Dressing, Annie's Tuscan Italian, 30% Fat	0.2, 0.4, 0.6*	Distilled Vinegar (Safeway)	1
Salad Dressing, Annie's Organic Roasted Garlic, 36.7% Fat	0.2, 0.4, 0.6, 0.8	Mizkan Rice Vinegar	1
Salad Dressing, Wishbone Italian Dressing, 23.3% Fat	0.2, 0.4, 0.6, 0.8*	Ghirardelli 72% Dark Chocolate	1
Salad Dressing, Ken's Balsamic Vinaigrette, 33.3% Fat	0.2, 0.4, 0.6, 0.8	Clover Organic Unsalted Butter	1
Salad Dressing, Marie's Balsamic Vinaigrette, 15% Fat	0.2, 0.4, 0.6*	Tillamook Sharp Cheddar Cheese	1
Salad Dressing, Marie's Creamy Caesar, 44.8% Fat	0.2, 0.4, 0.6, 0.8*		

Fig. 4. Specificity of the LFIA chemistry. (a) GF flour, spice, nut samples tested on strips, with sources and weights tested. (b) GF salad dressing, yogurt, vinegar, chocolate, butter and cheese samples and weights tested. All tested GF except flax seed and buckwheat, confirmed by ELISA to contain low levels of gluten. All samples were tested in 6 times, including duplicates for each lot of strips and three independent lots. "*" shows the highest acceptable weight for each sample beyond which the sample could start clogging the system.

and Bia, which were quantified previously for gluten.

4.1.2. Food weights

Sample weights affected test results but, unlike laboratory-based tests where foods are weighed, consumers using a portable device can, at best, control only volume. Actual sample weight was affected by food type, sampling scheme, volume and density, and, therefore, was largely uncontrolled. Because of the wide range of foods users will test, and the range of volumes that could potentially be put into the device, weight was a necessary variable for consideration in device design. Results from our user study identified amounts of foods consumers are likely to place in the device, which not only enabled us to choose a target LFIA LoD, but also helped us to understand how much was too little, preventing gluten detection, and how much was too much, causing system malfunction. Therefore, we specified clear instructions in our *User Manual* and the *Start Guide,* encouraging users to put in a pea sized amount. However, as with other consumer devices, not all users followed the instructions, leading to wider weight variations in the field. While we took this into account in our testing and design, inserting too much food into the device remains a risk, best mitigated through user education and proactive marketing efforts. Excessive amounts of certain foods interfered with the LFIA because of issues such as viscosity, liquid absorption or pore clogging. In such cases, the device indicated an "Error", preventing misleading false results.

4.1.3. Sample inhomogeneity

Only a small portion of foods can be tested per dish or per package. If gluten contamination is not distributed evenly within the same sample (homogenous), the portion tested will not reflect the entire serving. Instructions for R7001 ELISA and Romer COKAL0200 kits

a

Foods	Source	Gluten Conc. (ppm)	Weight (g)	Foods	Source	Gluten Conc. (ppm)	Weight (g)
Nima				**Restaurant**			
Meat burger	Nima	20 & 0	0.34, 0.67, 1	Oil fried plantain	Pica Pica	< 2	0.37, 0.75, 1.5
Veggie burger	Nima	20 & 0	0.34, 0.67, 1	Chocolate fudge	Pica Pica	< 2	0.24, 0.48
Cupcake	Nima	20 & 0	0.26, 0.52, 0.78	Carrot currant donut	Dynamo Donut	15	0.34, 0.67, 1
Salad Dressing	Nima	20 & 0	0.25, 0.5, 1	Biscuit	Proposition Chicken	84	0.29, 0.58, 0.87
Soup	Nima	20 & 0	0.42, 0.84, 1.26	Big steak omelette	IHOP	19	0.25, 0.51, 0.75
Oatmeal	Nima	20 & 0	0.20, 1, 1.5	Soft pretzel	Mariposa	0.40	0.26, 0.52
Pasta	Nima	20 & 0	0.37, 0.74, 1.1	Linguini pan seared ahi tuna	Pasta Moon	➢ 84	0.30, 0.60, 1.20
Banana bread	Nima	20 & 0	0.14, 0.27, 0.41	French fries	Radish	30.5	0.24, 0.48, 0.72
Granola	Nima	20 & 0	0.22, 0.45, 0.68	BBQ pork sandwich	Radish	9.57	0.27, 0.55, 1.10
Ice cream	Nima	20 & 0	0.1, 0.4	**Packaged**			
Sauce	Nima	20 & 0	0.4, 0.8, 1.2	Betty crocker brownie mix	Safeway	< 2	0.3, 0.6
Muffin	Nima	20 & 0	0.14, 0.28, 0.42	Bull's eye Kansas BBQ sauce	Safeway	2.44	0.2, 0.7, 1.4
Pie crust	Nima	20 & 0	0.14, 0.56, 0.84	Safeway signature lemon pepper marinade	Safeway	<2	0.3
Tortilla chips	Nima	20 & 0	0.10, 0.20, 0.30	Rice thins brown rice sea salt & peppers crackers	Safeway	< 2	0.22, 0.4, 0.5
Company A				Trader Joe's GF Joe Joe's cookie	Trader Joe's	< 2	0.14, 0.4, 0.8
Cracker	Comp. A	31	0.1, 0.3	Societe Roquefort Cheese	Trader Joe's	< 2	0.25, 0.5, 1.0
Biscotto	Comp. A	21	0.1, 0.3	**Flour Samples**			
Flour 1	Comp. A	3	0.1, 0.3	Red lentil	Local organic farm	42	0.3
Flour 2	Comp. A	11	0.1, 0.3	Chestnut flour	Nuts.com	< 2	0.45
Flour 3	Comp. A	5	0.1, 0.3	Rice flour	California Cooperative Rice Research Foundation	< 2	0.45
Pasta 1, boiled	Comp. A	3	0.1, 0.3	**FARRP Samples**			
Pasta 2, boiled	Comp. A	5	0.1, 0.3	Yeast Extract	FARRP	891	0.1, 0.3, 0.6
Pasta 3, boiled	Comp. A	11	0.1, 0.3	Yellow grits	FARRP	69	0.1, 0.3, 0.6
Sugo albasilico pastoriz	Comp. A	50	0.1, 0.3	Hemp Protein Powder	FARRP	466	0.1, 0.3, 0.6
Sugo al basilico steriliz zato	Comp. A	50	0.1, 0.3	White Powder (Oat Flakes)	FARRP	158	0.1, 0.3, 0.6
B sauce	Comp. A	50	0.1, 0.3	Brown Rice Syrup	FARRP	131	0.1, 0.3, 0.6
Sugo pestato verdure	Comp. A	0	0.1, 0.3	White Rice Flour	FARRP	92	0.1, 0.3, 0.6
Bia Diagnostic Samples				Tortilla chips	FARRP	494	0.1, 0.3. 0.6
Crackers (pre-ground)	Bia	20	0.1, 0.3, 0.6	Nutty Rice	FARRP	16	0.1, 0.3, 0.6
Lentil pellet (pre-ground)	Bia	20	0.1, 0.3, 0.6	Creamy Buckwheat	FARRP	32	0.1, 0.3, 0.6
Lentil pellet (pre-ground)	Bia	20	0.1, 0.3, 0.6	Cocoa Powder	FARRP	190	0.1

b All foods

Nima Results	True condition		
	Gluten	Gluten Free	Total
	>= 2 ppm	< 2 ppm	
Gluten Found	284	10	294
	>= 20 ppm	< 20 ppm	
Smile	3	119	122
Subtotal	287	129	416
Error	15	16	31
Total	302	145	447

	%	+/-
True Found (TP)	99.0%	1.2%
True Smile (TN)	92.2%	4.6%
False Smile (FN)	1.0%	1.2%
False Found (FP)	7.8%	4.6%
Error Rate	6.9%	2.4%
Accuracy	96.9%	1.7%
Accuracy (w/ errors)	90.2%	2.8%
Precision	96.6%	2.1%

c Nima, FARRP, Bia, Barilla

Nima Results	True condition		
	Gluten	Gluten Free	Total
	>= 2 ppm	< 2 ppm	
Gluten Found	214	8	222
	>= 20 ppm	< 20 ppm	
Smile	1	59	60
Sub Total	215	67	282
Error	14	4	18
Total	229	71	300

	%	+/-
True Found (TP)	99.5%	0.9%
True Smile (TN)	88.1%	7.8%
False Smile (FN)	0.5%	0.9%
False Found (FP)	11.9%	7.8%
Error Rate	6.0%	2.7%
Accuracy	96.8%	2.1%
Accuracy (w/ errors)	91.0%	3.2%
Precision	96.4%	2.5%

d Restaurant and Packaged

Nima Results	True condition		
	Gluten	Gluten Free	Total
	>= 2 ppm	< 2 ppm	
Gluten Found	70	2	72
	>= 20 ppm	< 20 ppm	
Smile	2	60	62
Sub Total	72	62	134
Error	1	12	13
Total	73	74	147

	%	+/-
True Found (TP)	97.2%	3.8%
True Smile (TN)	96.8%	4.4%
False Smile (FN)	2.8%	3.8%
False Found (FP)	3.2%	4.4%
Error Rate	8.8%	4.6%
Accuracy	97.0%	2.9%
Accuracy (w/ errors)	88.4%	5.2%
Precision	97.2%	3.8%

Fig. 5. Performance of the Nima™ device. (a) Foods, sources, gluten concentrations and weights tested in our Nima™ device. Foods were sourced and prepared as described in Materials and Methods, Supporting Materials and Methods and Supplementary Table II. (b–d) Summary of test results for (b) all foods tested, (c) Nima, Bia, "Company A" and FARRP foods only and (d) restaurant and packaged foods only. TP, TN, FP, FN and error rates, determined as described in Materials and Methods. Each sample at each weight was tested in triplicate.

a

Nima Results	True condition		
	Gluten	Gluten Free	Total
	>= 2 ppm	< 2 ppm	
Gluten Found	184	0	184
	>= 20 ppm	< 20 ppm	
Smile	2	117	119
Subtotal	186	117	303
Error	5	0	5
Total	191	117	308

	%	+/-
True Found (TP)	98.9%	1.5%
True Smile (TN)	100.0%	0.0%
False Smile (FN)	1.1%	1.5%
False Found (FP)	0.0%	0.0%
Error Rate	1.6%	1.4%
Accuracy	99.3%	0.9%
Accuracy (w/ errors)	97.7%	1.7%
Precision	100.0%	0.0%

b

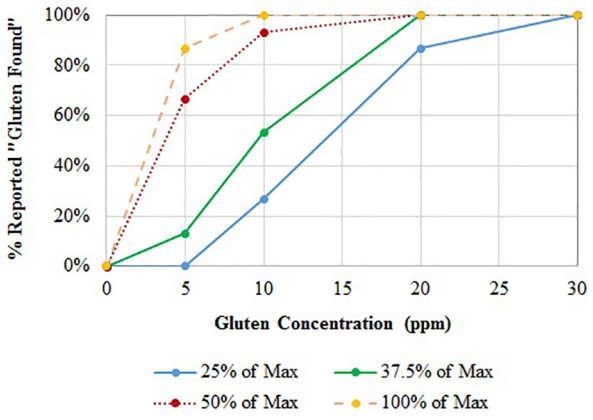


Fig. 6. Reproducibility testing. Breads were prepared GF, with some spiked at 5, 10, 20 or 30 ppm gluten. Bread samples were tested at indicated sample weights for each spiking level, with each sample tested 15 times. Sample weights were: 25% (107 mg), 37.5% (161 mg), 50% (215 mg) and 100% (430 mg) of the maximal weight filling the device chamber. (a) TP, TN, FP, FN and error rates from testing. (b) Percentage of samples detected increased as spiked gluten level and food weights increased.

recommend grinding at least 5 g and testing 0.25 g of the resulting homogenate. However, when 50 g oats containing one wheat kernel was ground, ELISA results varied from 5 to a few hundred ppm (Fritz, Chen, & Contreras, 2017). For such a heterogenous sample, if the true sample average was 20 ppm gluten, the ELISA assay was estimated to have a 63% chance of indicating < 20 ppm, even with current best laboratory sampling and grinding practices. Thus, sampling remains a challenge, regardless of assay method, and is an inherent limitation of gluten or any other food testing. Larger sample sizes were preferred but impractical, as consumers wish to enjoy their food and because of the requirement for a portable device.

4.1.4. Grinding, mixing and gluten extraction

To keep the device portable, we could not include an electric grinder but, instead, used a manual grinder where the motion of closing the cap effectively engaged the grinding mechanism. Such an action produced more torque than a battery of reasonable size and worked effectively for loose foods, such as cakes and burgers, and hard foods, such as chocolates and nuts, though grinding of certain foods, such as chicken meat, was more challenging.

Food type, particle size, extraction time and temperature impacted extraction efficiency. Particle size after grinding varied significantly among foods, affecting extraction efficiency. A heating unit was impractical in the consumer unit and, based on user requirements, was extraction time was limited to 30 s. Extraction efficiency, tested with four model foods, was 20.7%–37.3%, though extraction efficiencies will likely vary more in the field. We assumed incomplete extraction in our design, targeting detection of 20 ppm with 99.0% accuracy.

Validation studies using ELISA typically focus on a few model food matrices, such as corn and rice flours (Alvarez & Boye, 2014; Immer & Haas-Lauterbach, 2012), and proficiency testing uses uncooked foods, such as cake mix and infant formulae. However, again, we expanded our testing well beyond these matrices because our device is intended for testing a wide variety of foods as consumed.

4.2. 20 ppm gluten threshold

The 20 ppm gluten threshold was originally proposed by Catassi et al. (2007). Among 49 adults studied, one responded to 10 mg gluten/day but the others were unaffected up to 50 mg. The investigators estimated total daily food consumption of 500 g, establishing the 20 ppm threshold (10 mg/500 g). Codex Alimentarius defined GF as ≤20 ppm in 2008 (Commission, 2008) and the standard remains, adopted by many countries (Koerner et al., 2013). In August 2013, the FDA ruled that gluten levels < 20 ppm were required for a GF label. However, certain GF certification bodies adopted more conservative criteria, with GF Certification Organization (GFCO) and Celiac Support Association (CSA) requiring ≤10 and ≤5 ppm gluten, respectively.

While a threshold is useful for food manufacturers and assay companies, the food testing community has no gluten standards for assays, sample preparation or testing protocols. Based on the FDA ruling, foods containing 21 ppm gluten would be categorized as having gluten, while ones with 19 ppm would be GF. In any assay kit, the standard deviation for 20 ppm is about 2 ppm, with many factors contributing to greater variability (Immer & Haas-Lauterbach, 2012). Thus, the 20 ppm threshold should be used only as a guideline and any amounts of gluten greater than the assay LoD should be investigated further.

We aimed to detect foods containing ≥20 ppm gluten with at least 99.0% accuracy and validated this in multiple studies. The probability of detecting gluten depended on gluten concentration and sample weight (Fig. 6). The device was designed so that, as long as a food contains 20 ppm gluten, it could be detected (*Gluten Found*, "*Wheat*" sign) in a 0.1–2 g sample with a 99.0% chance. Gluten-containing foods generally have ≥20 ppm, increasing device accuracy in the field. For foods without gluten, which we defined as < 2 ppm, the device should report a GF result ("*Smiley*"). For foods containing 3–19 ppm, gluten might still be detected and, in such cases, the entire food serving might have spots with ≥20 ppm, which would not be considered a FP result. The likelihood of a *Gluten Found* report is proportional to gluten concentration so, if the concentration is very low, the likelihood of detection is also low. Food weight might also affect results in this 3–19 ppm zone. For example, if a food containing 15 ppm gluten gives a *Smiley* reading at 300 mg, doubling the weight might yield *Gluten Found*. Similarly, if a 200 mg sample containing 20 ppm gluten triggered *Gluten Found*, 300 mg of a food containing 15 ppm gluten might yield the same reading. These cases are neither FP or FN. The Nima™ device was designed to report *Gluten Found* when the food contained ≥20 ppm gluten in samples between 0.1 and 2 g. The need to guarantee detection of 20 ppm with a wide range of foods and weights meant the device was not able to distinguish among foods containing gluten at 3–19 ppm. We performed user studies to better understand the impact of such uncertainty on consumers and concluded that most would be happy for the device to report any gluten.

4.3. Immunoassay cross-reactivity

LFIA cross-reactivity was tested on a wide range of GF foods (Fig. 4). With a few exceptions, because we could not source GF (romano beans) or they interfered with LFIA development (pure guar and xanthan gums), we tested all 38 AOAC recommended categories (testing meat in the complete device (Fig. 5)). Although tested at high concentrations to increase detection of any cross-reactivity, all produced a negative response. Two flax seed and one buckwheat sample, sold as GF, tested positive, and ELISA confirmed that they contained some gluten. Thus, although considered GF by the FDA, trace levels were detected using our device, as the extracted gluten was above the LoD. While some salad dressing and yogurt samples (Fig. 4b) interfered with strip development, because of their viscosity, the recommended pea sized amount (0.4 g) worked well and tested GF. Vinegar, at the maximum volume for the device, caused no pH artifacts. Thus, overall, the LFIA showed negligible cross-reactivity with a wide range of GF foods.

4.4. False positive and negative results and errors

Of 447 tests conducted (Fig. 5), there were three FN reports, two from a restaurant biscuit (7/9 positive readings) with, possibly, uneven gluten distribution. One FN was for a buckwheat sample from FARRP (8/9 positive readings). There were also 10 FP cases. We believe that LFIA cross-reactivity is an unlikely explanation, because these foods did not consistently test positive. Again, sample heterogeneity is a more likely potential explanation.

In 447 tests, 31 errors (6.9%) were reported by the device as “Error”. We examined these closely to see if they were potentially avoidable. Thirteen involved slow test strip development (*e.g.* because of viscosity), 10 were caused by no mixing, five did not eject the extract and three had inadequate liquid for LFIA development. Mechanical issues, such as valve malfunction, are being addressed in ongoing device optimization. Sample-dependent issues are being addressed primarily by understanding device limitations and modifying user instructions to ensure that problems caused by quantities of certain foods are understood and avoided. As discussed earlier, these problems can be caused by viscosity, excess liquid absorption and LFIA pore clogging, or even physical restriction of the valve mechanism. For most problematic foods, a pea sized amount avoided such errors. For others (*e.g.* pure guar gum), it was clear that users should be instructed not to test the product. Importantly, the “Error” indication alerted users that a test was unreliable. Our engineers continue to incorporate user feedback to improve the product.

Overall, the many complications inherent in gluten testing in a wide variety of foods make development of any consumer device challenging. Our goal is to provide a device with a high degree of reliability that will give gluten-sensitive consumers a useful guide in evaluating the safety of their foods.

5. Conclusions

For the first time, we developed a Nima™ device to integrate food processing, gluten detection, result interpretation and data transmission in a portable device for consumers. Based on our testing, the device showed many of the properties needed to help gluten-sensitive individuals navigate better a GF lifestyle. These included high sensitivity and specificity and rapid result reporting. It also had a low FN rate, enabling consumers to feel confident about gluten contents, particularly if concentrations exceed the FDA defined threshold of 20 ppm. We have, therefore, begun offering the Nima™ device to the public while continuing with optimization.

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Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflicts of interest

All authors, except for Dr. Alim Seit-Nebi performed this work as employees of Nima. Dr. Seit-Nebi is an employee of GenWay Biotech, Inc. and managed the contract research for this project, producing the 14G11 and 13F6 antibodies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.08.117.

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