Integrated Magneto-Chemical Sensor For On-Site Food Allergen Detection

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ABSTRACT: Adverse food reactions, including food allergies, food sensitivities, and autoimmune reaction (e.g., celiac disease) affect 5–15% of the population and remain a considerable public health problem requiring stringent food avoidance and epinephrine availability for emergency events. Avoiding problematic foods is practically difficult, given current reliance on prepared foods and out-of-home meals. In response, we developed a portable, point-of-use detection technology, termed integrated exogenous antigen testing (iEAT). The system consists of a disposable antigen extraction device coupled with an electronic keychain reader for rapid sensing and communication. We optimized the prototype iEAT system to detect five major food antigens in peanuts, hazelnuts, wheat, milk, and eggs. Antigen extraction and detection with iEAT requires <10 min and achieves high-detection sensitivities (e.g., 0.1 mg/kg for gluten, lower than regulatory limits of 20 mg/kg). When testing under restaurant conditions, we were able to detect hidden food antigens such as gluten within “gluten-free” food items. The small size and rapid, simple testing of the iEAT system should help not only consumers but also other key stakeholders such as clinicians, food industries, and regulators to enhance food safety.

KEYWORDS: food safety, food allergy, allergen, point of care, electrochemical sensing

More than 50 million Americans display food reactions.1–4 Food allergies incur annual costs of 25 billion dollars in the United States alone.5 Even trace amounts of food antigens can trigger acute anaphylaxis, a potentially life threatening hypersensitivity reaction6 requiring epinephrine injections. Although the results of immunotherapeutic trials have been encouraging,6,7 the primary approach continues to rely on food avoidance.8 The Food Allergen Labeling and Consumer Protection Act (FALCPA) mandates food labeling to inform customers about allergenic substances in products.8 Even so, mislabeling or cross-contamination in manufacturing continue to pose regulatory challenges. Furthermore, FALCPA only oversees packaged food, not food served in restaurants. Food labeling outside the United States is less strict, and food allergies often affect travelers. Thus, the ability to rapidly test foods for common allergens remains a major unmet need.

Many existing analytical methods and devices for food testing are designed for sophisticated laboratory rather than consumer use and rely on complex equipment, infrastructure, and advanced training. These analytical methods include enzyme-linked immunosorbent assay (ELISA),10–12 polymerase chain reaction,13 liquid chromatography–mass spectrometry,14 surface plasmon resonance,15,16 and electrochemical sensor.17 Consumer devices, on the other hand, are often slow, insensitive, or have other limitations. Lateral-flow strip tests can achieve on-site detection through direct visualization12,18 but only provide qualitative or semiquantitative information. Moreover, their relatively low sensitivity, caused by insufficient brightness of signal-intensity reporters, may result in false negative results.

We developed a portable, point-of-use technology for rapid, integrated exogenous antigen testing (iEAT). The system consists of a disposable allergen extraction device and an electronic keychain reader for sensing and communication. The extraction kit captures and concentrates food antigens from...
dispersed food. Captured allergens are then quantified using the miniaturized key-chain reader. Overall, the iEAT system enables quantitative allergen detection in a short and actionable time frame (i.e., <10 min for the entire assay) at minimal cost (<$4 per assay). We designed iEAT specifically to promote consumer-based operations: (i) the extraction kit is simple to use, inexpensive, and disposable; (ii) detection is fast, reliable, and accurate; and (iii) embedded communication protocols allow users to record and upload information to a cloud server with time and locale stamps. We optimized the iEAT prototype to detect five representative allergens from wheat, peanut, hazelnut, milk, and egg white. The rapid iEAT assay achieved high sensitivity, far-surpassing the gold standard ELISA. We also show iEAT’s practical use in surveying common foods for these allergens.

RESULTS AND DISCUSSION

**iEAT Assay.** Figure 1A depicts the portable iEAT system comprising a keychain reader, an extraction kit, and a smartphone app. The first step in sensing is extracting the allergen via a specially designed disposable kit that uses immunomagnetic enrichment (Figure 1B). Allergens are captured on magnetic beads and labeled with a second antibody conjugated with an oxidizing enzyme (horseradish peroxidase, HRP). When mixed with chromogenic electron mediators (3,3′,5,5′-tetramethylbenzidine, TMB), the enzyme catalyzes the oxidation of TMB and the reduction of H2O2. The oxidized TMB is then reduced by receiving electrons from the electrode. This process generates electrical current, which is measured by an electrode (Figure 1C). The electrical detection scheme makes it possible to perform quantitative measurements with a miniaturized electronic device. Furthermore, using magnetic beads as a solid substrate improves the assay performance in two ways. First, the extraction process and sample handling can be simplified via magnetic actuation. To enable portable operations, we designed a simple sheathed magnetic bar for bead collection and resuspension (Figure S1A), which obviates the need for specialized equipment (e.g., centrifuge, pipettes). Second, electrochemical signals can be amplified by magnetically concentrating beads on top of the electrode. For this, we designed an electrode holder fitted with a small magnet (Figure S1B).

We custom-built the iEAT signal reader (Figure 2A and Figure S1C) with the following features: (i) a keychain-size device for easy portability and yet having analytical capacities comparable to a benchtop system; and (ii) easy extension to multichannel electrodes. The mini-reader not only detects and displays results but also wirelessly communicates with smartphones via Bluetooth to transmit test results and other information to a cloud server for web-based data collection and sharing among users (Figure S2). Furthermore, this communication capability provides an extended user interface for system control, data storage, and wireless battery charging. The device houses multiple components including potentiostats for current measurements, a microcontroller unit (MCU) for signal processing, a mini display screen, a rechargeable battery, and a card-edge connector to insert an electrode board. This miniaturized device is a standalone unit, measuring electrical
currents and displaying allergen concentrations according to preloaded lookup tables.

Figure 2B shows the details of the iEAT reader. Custom-designed potentiostats connect to a digital-to-analog converter for potential control and an analog-to-digital converter for signal digitization. The microcontroller unit (MCU) converts current levels to allergen concentrations according to preloaded lookup tables. The MCU also communicates with a smartphone to provide an extended user interface and to wirelessly charge the battery. ADC, analog-to-digital converter; DAC, digital-to-analog converter; Amp, amplifier; OLED, organic light-emitting diode; MUX, multiplexer. Both the iEAT reader and a commercial electrochemical system were used to measure buffer solutions with varying concentrations of ferrocyanide in 0.1 M KCl solution. The correlation between the two systems' performance was 0.995. The coefficients of variation (CVs) were <5%. CSB logo is used with permissions.

Figure 3. Optimized protocol for antigen extraction. (A) Allergen extraction was measured under different assay conditions in 2-ME buffer. Model samples were prepared by spiking rice with peanut allergen (Ara h1, 10 mg/kg). The extraction yield was defined as the ratio between detected and spiked allergen amounts. The yield was already >60% with 2 min emersion in the extraction buffer at 20 °C and further increased with added incubation time and temperature. Brief preheating (20 s) with a microwave (MW) oven significantly shortened the extraction time (~1 min) at 20 °C. (B) We tested three different extraction buffers: 2-ME, TECP/GUA, and TECP/sarkosyl. The extraction time was 10 min, but the temperature was varied. All three buffers showed a similar extraction yields. 2-ME, 2-mercaptoethanol; TECP/GUA, tris(2-carboxyethyl)phosphine with guanidine; TECP/sarkosyl, TECP with N-lauroylsarcosine. (C) Lyophilized reagents for the iEAT assay (immunomagnetic beads, antibodies) were stored at ambient condition. No significant changes in reagents' reactivities were observed. All measurements are in duplicate, and the data are displayed as mean ± SD.
3A shows an example of Ara h1 extraction with 2-ME bu and temperature, and we monitored the recovery yield. Figure 4. Characterization of the iEAT assay. (A) Samples with varying doses of antigens were analyzed by the iEAT system, and response curves were generated. The detection limit was below the respective elicit dose (ED) for all antigens tested. These curves were uploaded to the iEAT reader for data conversion. A curve for the peanut allergen (Ara h1) is shown as an example. (B) The iEAT was highly reproducible. Both the intra-assay and the interassay variations were <5%. (C) When compared with the gold standard ELISA, the iEAT results showed an excellent match ($R^2 = 0.995$). (D) Testing for specificity. All five antigens (5 mg/kg) were spiked into a food matrix. Specific signals were >20-fold larger than nontarget samples (note the low noise and high target-to-background ratio). curves. We observed an excellent match between two systems ($R^2 = 0.995$; Figure 2C). The iEAT reader also showed good precision: the coefficient of variations (CVs) from five repetitive measurements were <4.1%, comparable to CVs obtained with the benchtop system (<4.9%). The iEAT reader, however, had a much smaller form factor (5.5 × 2.5 × 2.4 cm$^3$, 35 g) than the benchtop system (38 × 21 × 17 cm$^3$, 6 kg) and was capable of 8 parallel measurements.

Antigen Extraction. We first optimized the antigen extraction protocol. Our goal was to minimize both extraction time and cost while maximizing recovery yield. We used five major protein antigens as extraction targets: gliadin (wheat), Ara h1 (peanut), Cor a1 (hazelnut), casein (milk), and ovalbumin (egg white). We spiked a known amount (10 mg/kg) into homogenized food extracts, collected with the sheathed magnetic bar (Figure S1) and transferred to fresh buffer. Control beads were conjugated to isotype-matched IgG antibodies (see Materials and Methods section for details). We programmed the iEAT reader to average the current level from control beads were about 110 nA. The current reached a plateau within 60 s after the reduction bar (Figure S5B). We therefore opted to use the odorless, low-cost TECP/sarkosyl extraction buffer (Table S1). To speed up the extraction process, we also built a small heating device (Figure S4). The extraction condition was set for 2 min incubation at ∼60 °C, which was shown to recover >80% antigens (Figure S3A).

Measurement Optimization. To capture the predetermined antigens, we prepared immunomagnetic beads (2.8 μm in diameter) by conjugating monoclonal antibodies to magnetic beads. Control beads were conjugated to isotype-matched IgG antibodies. An optimal bead concentration, determined using peanut allergen titration, was ∼8 × 10$^6$ beads/mL (Figure S5A). Following incubation at room temperature (3 min) with food extracts, beads were collected with the sheathed magnetic bar (Figure S1) and transferred to fresh buffer for washing. Subsequently, the beads were labeled with an HRP-conjugated antibody (10 μL; 20 μg/mL) for 3 min at room temperature, washed, and mixed with TMB for signal generation (see Materials and Methods section for details). The electrical current reached a plateau within 60 s after the reduction potential (∼0.1 V) was applied (Figure S5B). We therefore programmed the iEAT reader to average the current level between 50 and 60 s. For the 8-channel system, the total detection time was 2 min through multiplexing. Electrical current levels from control beads were about ∼110 nA across different allergens, and the current difference between targeted sample and background was defined as the net signal. The total assay time, including the allergen extraction, was <10 min.

For a given extraction buffer, we changed the incubation time and temperature, and we monitored the recovery yield. Figure 3A shows an example of Ara h1 extraction with 2-ME buffer. The extraction was efficient even at room temperature; more than 60% of originally spiked antigens were recovered within 2 min of incubation. The extraction yields increased with temperature. For example, after 20 s of heating in a microwave oven (1100 W), yields increased to 80% with 1 min of incubation in the extraction buffer. We observed no adverse effect (e.g., protein denaturation) from heat treatment, and all three extraction buffers showed similar performance for five tested antigens (Figure 3B and Figure S3A). We therefore opted to use the odorless, low-cost TECP/sarkosyl extraction buffer (Table S1). To speed up the extraction process, we also built a small heating device (Figure S4). The extraction condition was set for 2 min incubation at ∼60 °C, which was shown to recover >80% antigens (Figure S3A).
conditions (refrigeration, room temperature). There were no significant differences in reagent activities; all lyophilized reagents retained their activity (>96%) after 4 weeks in storage (Figures 3C and S6). Optimal amounts of final reagents can be preloaded during kit assembly, thus simplifying assay steps. Given its ready availability, we chose PBS as an excipient, and for ease of use, we chose ambient conditions for stored lyophilized reagents.

**Analytical Performance.** We next evaluated iEAT’s analytical performance for rapid detection.20 We first generated response curves, varying the target allergen dose (Figure 4A for Ara h1 and Figure S7 for others); these curves were uploaded to the iEAT reader as lookup tables. The iEAT assay was highly sensitive, precise, and quantitative. The limit of detection (LOD), defined as 3σ × m−1 (where σ and m are the standard deviation and the slope of the calibration curve, respectively), was lower than the eliciting dose (ED) thresholds for typical food servings (Table 1 and Table S2).21,22 The intra-assay variations, estimated by measuring three different concentrations of standard (1, 5, and 10 mg/kg) with six duplicates, were <5% (Figure 4B), and the interassay variations were <5%. For comparison, we also tested the same samples by ELISA. The iEAT results correlated well with ELISA measurements (Figure 4C, R² = 0.995). The iEAT assay, however, was much faster (10 min vs 2 h for ELISA). To test the assay specificity and crosstalk, we applied target probes to different allergens, such as gluten in hamburgers and pizza, but we also detected unexpected antigens contributed by food processing. For example, salad contained gluten, likely from the salad dressing. We also identified ovalbumin and casein in beer, which is presumably added as food additives; egg-white is used to improve the foam characteristics, and casein is used to stabilize beer during the brewing process.23,24

Utilizing iEAT’s interface with a smartphone, we tracked personal dietary intake, recording antigen data with time stamps in a cloud server (Figure S2). As one example, we surveyed gluten-free menu items from seven local restaurants and logged the results (e.g., food name, gluten contents) with locale information. Among “gluten-free” items, we observed a wide spectrum of antigen levels (1 μg/mg to >100 mg/kg food); three items had gluten far exceeding the regulatory limit (Figure 5C). These results were then used to create an evidence-based restaurant map that can be shared online.

## Table 1. iEAT Detection Limits for the Five Food Antigens

<table>
<thead>
<tr>
<th>uploaded antigen</th>
<th>limit of detection (mg/kg)</th>
<th>reference dose (mg/kg)</th>
<th>1 serving size (g)</th>
<th>action level 1 (mg/kg)</th>
<th>action level 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gliadin</td>
<td>0.075</td>
<td>0.20</td>
<td>30 (peanut)</td>
<td>6.67</td>
<td>20°</td>
</tr>
<tr>
<td>Ara h1</td>
<td>0.007</td>
<td>0.20</td>
<td>30 (hazelnut)</td>
<td>3.33</td>
<td></td>
</tr>
<tr>
<td>Cor a1</td>
<td>0.089</td>
<td>0.10</td>
<td>30 (milk)</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>casein</td>
<td>0.170</td>
<td>0.10</td>
<td>233 (milk)</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>ovalbumin</td>
<td>0.003</td>
<td>0.03</td>
<td>50 (egg white)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

“Reference doses are from the revised Voluntary Incidental Trace Allergen Labeling (VITAL 2) guideline.6 One serving size is from Code of Federal Regulations Title 21 by the U.S. Food and Drug Administration. Action level 2 indicates a significant chance of adverse reaction, requiring a precautionary statement. For gliadin, the minimum level is 20 mg/kg regardless of the portion size.

Field Testing. We next applied the iEAT system for testing of consumer food products. We first examined a panel of packaged staple foods (bread, milk, cereal) and desserts (cookie, ice cream). We sampled a small portion of food using a disposable scoop. For solid foods (e.g., cookies, breads), we crumbled samples to fill the scoop. Approximately 1 g of food sample was then processed over 2 min, as described above, and extracts were assayed for gliadin, Ara h1 (peanut), Cor a1 (hazelnut), casein (milk), and ovalbumin (egg white). The profiling results, extrapolated for one-serving size, are summarized in Figure 5A. As expected, products with specific labeling (e.g., “gluten-free,” “nut-free”) were largely devoid of the listed allergen. Most products, however, contained at least one unspecified antigen; for instance, brands of nut-free cookies contained gluten, whereas a gluten-free brand contained peanut allergen. We next assayed foods (burger, salads with dressing, pizza, and beer) obtained from local restaurants (Boston, MA area). The profiling results (Figure 5B) showed the expected allergens, such as gluten in hamburgers and pizza, but we also detected unexpected antigens contributed by food processing. For example, salad contained gluten, likely from the salad dressing. We also identified ovalbumin and casein in beer, which is presumably added as food additives; egg-white is used to improve the foam characteristics, and casein is used to stabilize beer during the brewing process.23,24

## CONCLUSIONS

Adverse food reactions can be categorized as food allergies (both IgE and non-IgE mediated), food sensitivities, food intolerances, and autoimmune reactions (e.g., celiac disease). IgE-mediated food reactions occur when a food allergen binds allergen-specific IgE present on mast cells and basophils. This leads to the release of histamine and other mediators, within minutes to hours, which results in respiratory, cardiovascular, or gastrointestinal symptoms. Conversely, non-IgE mediated food allergies, including enterocolitis, food protein-induced enteropathy, allergic proctolitis, and eosinophilic esophagitis, are relatively rare and less defined clinical entities affecting mainly children.25,26 The prevalence of food allergies is estimated to be 2–10% of the population with rates increasing27 and self-reported rates climbing much higher than reported prevalence.28 In the United States, 30% of allergic children suffer from multiple food allergies.29 Each year there are an estimated 203,000 food allergy-related emergency department visits in the United States, including 90,000 cases of anaphylaxis.6 Novel therapeutic approaches, including oral immunotherapy with or without anti-IgE antibody (Omalizumab), are being pursued.7 However, the accepted standard of care is to strictly avoid allergenic foods and administer emergency medication upon accidental exposure. Food sensitivities, including nonceliac gluten sensitivity, are immune reactions causing symptoms often indistinguishable from food allergies, whose pathogenesis and prevalence are still poorly defined.31 Finally, celiac disease is a systemic immune-mediated disorder affecting 0.6–1% of the general population triggered by the ingestion of gluten in genetically susceptible individuals.32 While the gluten-free diet remains the cornerstone of celiac disease treatment, its implementation remains the major challenge for patients, mainly due to frequent cross-contamination.

The ever-present threat of accidental exposure has a considerable negative impact on the wellbeing of patients and their families.7 Food avoidance by patients and families is commonplace and may have unintended consequences, such as nutritional deficiencies. For example, in one recent study of 125 children, 80–100% of foods being avoided were shunned unnecessarily and could be reintroduced into the children’s diets.33 To reduce its negative impact, a food allergy must be properly diagnosed and managed, including properly identifying food allergens in real-time.
We developed the point-of-care food testing system (iEAT) to sensitively detect multiple food antigens, clear “safe” foods, and eliminate unnecessary avoidance to empower consumers. We also envision the system being leveraged by food industries and their regulators as well as food reaction clinics. The signal detection, based on electrochemical reactions, is fast, scalable, well-suited to compact electronic devices, and amenable to multiplexing. We prototyped the keychain reader to be operable as a standalone that can also be charged wirelessly and enable Bluetooth communication with cloud servers. The device is inexpensive (<$40; Table S3) with assay costs of <$4 per antigen (Table S1). With scale-up and the ability to produce lyophilized kits, we expect these costs would decrease considerably. With these features, the iEAT system closely aligns with the WHO guideline for point-of-use devices, ASSURED, which is defined as affordable, sensitive, specific, user-friendly, rapid and robust, equipment free (i.e., no large electricity-dependent instruments), and deliverable (see Table S4 for comparison with other methods).

We expect to further strengthen iEAT’s analytical capacities by addressing the following aspects. First, calibration measurements should be expanded to assess how food matrices and processing affect extraction yields and detection sensitivity. Of particular interest is to use incurred samples wherein a known amount of standard allergens is incorporated during food processing. By better mimicking normally manufactured products, incurred samples would generate more accurate calibrators for antigen quantification and aid in selecting optimal antibodies for target antigens. Second, more allergen targets can be included for comprehensive food testing. We chose to quantify five representative model antigens that are commonly found in consumer foods and responsible for the majority of food reactions. Many other potential antigens remain such as those in shellfish (shrimp, lobster), finned fish (tuna, salmon), tree nuts (walnuts, pecan, cashew), pollen, and fruits, among others; these antigens can be readily incorporated into the detection list. The iEAT reader features both a single and a multichannel electrode (Figure 2A), and the latter can measure eight allergens simultaneously. Sequential measurements or scaling up the channel number are both feasible ways to allow broader testing.

While we focused on specific protein antigens, the current assay format could also be modified to detect small molecules, toxins, or nucleic acids by changing affinity ligands (e.g., aptamers, oligonucleotides); creating detection panels for food safety (e.g., pesticides) and for food-source identification (e.g., DNA-based testing). The device could have many interesting applications, such as verifying food origins, confirming the absence of contaminants, or supporting dietary restrictions for religious purposes. These and other applications could be further enhanced through system integration, for example, by developing disposable fluidic cartridges to simplify and automate sample processing and by converting smartphones’ pressure-sensitive screens into scales to accurately measure sample weight. Irrespective of the specific application, we envision that the portable iEAT technology will allow for more rigorous and evidence-based analysis of food products, enhance consumer protection, reduce accidental allergy exposure, and identify problems in our food supply chain.

Figure 5. Onsite food surveillance. (A) The iEAT system was used to screen for five major food antigens in manufactured foods. The antigen amounts per one serving size (e.g., 50 g for bread) were plotted. Products with explicit labeling (i.e., gluten-free, nut-free) were devoid of the specified allergen but contained other allergen types. For each antigen, the heatmap scale is up to action level 2 dose set by VITAL 2. (B) Tests on foods from a local cafeteria revealed unexpected antigens: gliadin in salad dressing and ovalbumin and casein in beer. These proteins are used as food additives during manufacturing. ND, nondetected. (C) In testing gluten-free menu items from seven local restaurants (color-coded), we observed widely varied gluten levels, and some items had gluten levels far exceeding the regulatory limit. The test results were stored in the cloud server to create a customized restaurant map.
MATERIALS AND METHODS

**Materials.** The following chemical and biochemical reagents were used as received: superparamagnetic beads (6.7 × 10⁹ beads/mg, Dynabeads M-270 Epoxy, Invitrogen); bovine serum albumin (≥98%, BSA, Sigma); Pierce high-sensitivity streptavidin-horseradish peroxidase (strept-HRP, Thermo Scientific); sulfuric acid (1 N, Fluka); sucrose (≥99.5%, Sigma); 1-step ultra 3,3′,5,5′-tetra ethy lbenzidine (TMB) ELISA substrate solution (Thermo Scientific); tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl, ≥99%, Sigma); 2-mercaptoethanol (2-ME, ≥99%, Aldrich); guanidine hydrochloride (GUA, ≥99%; Sigma); sodium dodecyl sulfate (SDS, ≥99%, Sigma-Aldrich); N-lauroylsarcosine (≥95%, Sigma); tris(2-carboxyethyl)phosphine hydrochloride (TCEP, ≥98%, Aldrich); Tween 20 (Sigma), and potassium ferrocyanide (298.5%, Sigma-Aldrich). Unless otherwise stated, all solutions were prepared at 25 °C, used ultrapure water with an electrical resistivity of 18.2 MΩ*S* cm, and discarded. This washing step was repeated twice. Next, beads were washed twice with 1 M ammonium sulfate in sodium phosphate buffer (pH 7.4). We then added each of the beads to 50 μL Ab-MB solution (8 × 10⁹ beads/mL) and incubated for 3 min at room temperature. For washing, the beads were collected using a glass sheathed magnetic bar and released in PBS (100 μL). The beads were then incubated with HRP-conjugated antibodies (10 μL; 20 μg/mL) for 3 min and washed as described above. The HRP-bead complex was mixed with TMB substrate and loaded on the electrode. After 1 min, the chronoamperometry measurement was started. The current levels between 50 and 60 s were averaged.

**Enzyme-Linked Immunosorbent Assay (ELISA).** The stock capturing antibody and reference IgG antibody were diluted to ~3 μg/mL in carbonate-bicarbonate buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, pH 9.6) and added to 96-well polystyrene sterile flat bottom microplates (100 μL/well) for overnight incubation at 4 °C. The coated plates were washed three times with PBS with 0.05% Tween 20 (PBST) to remove unbound antibody. Then PBS with 1% BSA was added (100 μL/well) to block unoccupied binding sites. Plates were rinsed three times with PBST. Allergen standards and sample extracts were dispensed (100 μL/well) into plate wells in duplicate for 1 h incubation at room temperature. Plates were washed three times with PBST. Then a 100 μL aliquot of biotinylated detection antibody was added and incubated for 1 h at room temperature. After washing three times with PBST, 100 μL strept-HRP solution was added (100 μL/well) into plate wells and left for 30 min at room temperature. Plates were rinsed three times with PBST. The chemiluminescence signal was developed by adding 100 μL TMB substrate. After 10 min incubation at room temperature, 100 μL sulfuric acid (1 N) was added to each well to stop the enzyme reaction. The optical absorbance of each well was measured at 450 nm using a plate reader (TECAN).

**Extraction Buffers.** Three extraction buffers were prepared: (1) 50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, 250 mM 2-ME, 2 M GUA, 1% SDS, pH 7.4; (2) 50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, 20 mM TCEP, 2 M GUA, pH 7.4; and (3) 50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, 5 mM TCEP, 2% N-lauroylsarcosine, pH 7.4.

**Food Sample Preparation.** Food samples were obtained from local supermarkets and restaurants. Food samples (~1 g) were cut into small pieces and mixed with 19 mL of extraction buffer. Following the allergen extraction, the supernatant was taken as a sample extract for subsequent IEAT detection. An amount of allergen in a sample was reverted by multiplying 20 dilution fold.

**EAT Reader.** The keychain detector (5.5 × 2.5 × 2 cm³) was built around a microcontroller unit (MCU, ATSAMD21G18, Atmel Corporation). A digital-to-analog converter (DAC8852, Texas Instruments) was used to set the potential between the reference and the working electrodes. For the current measurements, a digital-to-analog converter (ADS1115, Texas Instruments) and a potentiostat were connected to MCU’s peripheral interface. The potentiostat consisted of two operational amplifiers (AD8608, Analog Devices): one amplifier maintains the potential difference between a working electrode and a reference electrode, and the other one works as a transimpedance amplifier to convert a current to a voltage signal. Other peripherals include a communication module (Bluefruit EZ-Link) for Bluetooth connection with external devices, a display module, and a rechargeable battery. Screen-printed gold electrodes were purchased (220AT and 8 × 220AT, Metrohm). Other parts (e.g., device housings, printed circuit boards for eight electrodes) were custom-built. With a fully charged battery, the device continuously worked for 2 h. The estimated energy consumption of the reader was about 0.05 mW. The typical noise level was 0.3 nA.

**Smartphone Application.** Using MIT App Inventor 2, we created an Android application to facilitate system operation and data recording. The application allowed users to control the device, take sample photos, and record measurement details (time stamps, current measurements) was used to set the potential difference between the reference and the working electrodes. For the current measurements, a digital-to-analog converter (ADS1115, Texas Instruments) and a potentiostat were connected to MCU’s peripheral interface. The potentiostat consisted of two operational amplifiers (AD8608, Analog Devices): one amplifier maintains the potential difference between a working electrode and a reference electrode, and the other one works as a transimpedance amplifier to convert a current to a voltage signal. Other peripherals include a communication module (Bluefruit EZ-Link) for Bluetooth connection with external devices, a display module, and a rechargeable battery. Screen-printed gold electrodes were purchased (220AT and 8 × 220AT, Metrohm). Other parts (e.g., device housings, printed circuit boards for eight electrodes) were custom-built. With a fully charged battery, the device continuously worked for 2 h. The estimated energy consumption of the reader was about 0.05 mW. The typical noise level was 0.3 nA.

**Preparation of Antibody-Tagged Immunomagnetic Beads (Ab-MBs).** Magnetic beads (∼3.4 × 10⁹) were resuspended in 1 mL of sodium phosphate buffer. The bead solution was briefly vortexed, and the beads were collected by using a magnet. The supernatant was reverted by multiplying 20 dilution fold.

**Lyophilization of Immunomagnetic Beads and Detection Antibody.** Sucrose and PBS were used in preparing the lyophilization of Ab-MBs and antibodies. An amount of 300-fold higher molar concentration of sucrose (in PBS) with respect to antibody concentration or same volume of PBS solvent (as preparing sucrose) was added to Ab-MB or antibody stock solutions. The mixture was frozen in liquid nitrogen and then dried in a VirTis Freezezombie 25EL freeze-dryer (SP Scientific). The lyophilized reagents were stored at room temperature or at 4 °C and reconstituted before use by adding 200 μL of ultrapure distilled water.

**Antigen Standards.** We used white rice flour as a model food matrix. 1.0 g of rice flour was prepared in 10 mL PBS and boiled for 10 min. We then added each of five allergens (gliadin, Ara h1, Cor a1, casein, ovalbumin) and homogenized the mixture using a vortex mixer.

**Statistical Analysis.** All data obtained were presented as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 6. A p-value of under 0.05 was considered significant.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.7b04318.

Additional figures and tables (PDF)

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