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Scalable electrochemical system for rapid on-site detection of food allergens

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Abstract

Food allergies affect millions of individuals worldwide, significantly impacting personal health and the economy. While avoiding allergenic foods remains the primary management strategy, consumers lack reliable means for immediate allergen detection in everyday dining settings. Here, we present iEAT2 (integrated Exogenous Allergen Test 2), an advanced electrochemical sensing

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Declaration of AI and AI-assisted technologies in the writing process

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

system for rapid, on-site food allergen detection. Building upon our previous assay system, the iEAT2 features technical breakthroughs: i) a complete kit for sample processing, including a torsion device for food grinding, and ii) a new strategy for multi-electrode measurements, which enables the simultaneous detection of multiple allergens in a simplified electronic architecture. We designed a compact iEAT2 prototype capable of 16 electrochemical reactions. Experimental validation confirmed the independent electrochemical measurements in a simultaneous operation. Furthermore, the entire testing protocol was completed within 15 min, from allergen extraction to detection. The platform detected three common food allergens (gliadin, Ara h1, and ovalbumin) at concentrations below established allergic reaction thresholds. It also effectively identified cross-contamination events in real-world food samples. This technology may empower consumers to monitor food safety and improve its management.

INTRODUCTION

Food allergies continue to affect millions of individuals worldwide. For instance, recent estimates indicate that approximately 32 million people in the United States (US) experience adverse reactions to food (Gupta et al., 2019). The economic impact of food allergies in the US has grown substantially, with current annual costs estimated to exceed 30 billion US dollars (Gupta et al., 2013; www.foodallergy.org, 2024). Even trace amounts of food allergens can trigger severe and potentially life-threatening reactions, often requiring immediate medical intervention (Simons et al., 2014; Shaker et al., 2020; Santos et al., 2024). While immunotherapeutic approaches have shown promise in clinical trials, the primary method for managing food allergies remains strict avoidance of allergenic foods (Sim et al., 2020; Sato et al., 2024).

Electrochemical sensing is a promising method for food allergen detection, offering high sensitivity through signal amplification via redox-active reporters. This approach can use small, low-power devices for signal readout, making the assay suitable for on-site uses (Zhang et al., 2020; Park et al., 2021; Kim et al., 2022). Based on this technique, we previously developed a portable sensor, the integrated Exogenous Allergen Test (iEAT), which quantified common allergens (e.g., gluten, peanut) in a compact electrochemical reader (Lin et al., 2017). However, technical drawbacks limited iEAT's practical uses. Namely, the system lacked a preprocessing tool to simplify allergen extraction from food, and the use of a conventional three-electrode scheme restricted the assay throughput (i.e., one reaction at a time) and complicated circuit design for multi-allergen testing.

Here, we report the next-generation iEAT system (iEAT2) with improved applicability in real-world food allergen detection. iEAT2 features key advancements. i) We designed a complete kit for sample processing, including a torsion device for food grinding and a template for assay preparation. ii) More importantly, we devised a new approach to simultaneous multi-electrochemical measurements. This strategy treated an electrochemical cell as a parallel current source, which markedly simplified the electronics and electrical connections. It enabled us to accommodate multiple electrodes in a simple setup, facilitating efficient and scalable allergen detection. We proved the concept by developing a 16-electrochemical cell prototype. Experimental results indicated that the electrical current

generated by each cell was determined solely by its own electrochemical reaction, independent of reactions occurring in other cells. We configured iEAT2 to detect three common food allergens: gliadin (wheat), Ara h1 (peanut), and ovalbumin (egg white) (Seth et al., 2020; Jiang et al., 2023). The platform demonstrated sensitivity below the eliciting dose for allergic reactions in a single food portion. In real-world applications, iEAT2 enabled rapid (15-min) quantitative detection of allergens and identified potential cross-contamination events during food preparation. The speed and ease of use of iEAT2 promise to enhance food safety measures and improve the management of food allergies at the consumer level.

RESULTS AND DISCUSSION

iEAT2 assay flow

We applied an immunomagnetic approach for allergen retrieval, which streamlined the sample handling during the assay. Figure 1 shows the overall workflow (detailed in Methods). We first disrupted a food matrix in the presence of an allergen-extraction buffer. The food extract was then mixed with immunomagnetic beads to capture target allergens. Subsequently, the beads were collected using a permanent magnet and incubated with detection antibodies conjugated with horseradish peroxidase (HRP). Following this labeling process, analytical signals were generated by depositing the beads onto electrodes along with an electron mediator (3,3',5,5'-tetramethylbenzidine, TMB) and applying a reduction potential (0.21 V) (Pérez-Fernández et al., 2020; de Souza Freire et al., 2022). The overall assay was completed within 15 min.

To enable on-site sample processing, we designed a disposable allergen extractor and an assay template. The allergen extractor (Fig. 1B) used a torsion coil for the mechanical breakdown of food specimens. A food sample was inserted into a main well along with an extraction buffer. The specimen was ground through the manual activation of a string attached to the coil (Movie S1), which promoted allergen dissolution into the extraction buffer. We evaluated the extraction efficiency using a model food system (gliadin spiked in rice porridge; Fig. 1C). The extractor demonstrated superior performance, achieving an extraction yield of 61%, compared to 32% for vortexing and 13% for buffer incubation only. We further applied the extractor to food matrices of different textures: liquid (gliadin spiked in rice milk) and solid (gliadin added to a chocolate block). The extraction yield declined as the food matrix became challenging to disintegrate (Table S1), indicating the need to fine-tune the grinding protocol, particularly for hard-solid food items. Nevertheless, the overall yield values remained within the same order of magnitude.

The assay template kit incorporated a magnet-bar array and a reaction tray (Fig. 1D). The top-row chambers of the tray were prefilled with immunomagnetic beads, into which food extracts from the allergen extractor were introduced. The magnet-bar array facilitated bead collection and transfer to the bottom-row chambers containing detection antibody solutions (Movie S2). The array's permanent magnets were encased in a removable plastic sheath, enabling magnet detachment for bead release. This method demonstrated high efficiency, recovering >80% of the initial immunomagnetic beads for electrochemical measurements (Fig. S1).

Scheme for parallel electrochemical detection

iEAT2 utilized a two-terminal, two-electrode setup for electrochemical detection (Bezing et al., 2023; Costa et al., 2024). We modeled the reaction cell as a circuit block comprising a current source, a source resistor (R_s), and a double-layer capacitor (C_d) at the electrode-electrolyte interface (Fig. 2A) (Lazanas and Prodromidis, 2023). An array of electrochemical cells was represented as these circuit blocks connected in parallel. The R_s and C_d values were assumed to be similar among the reaction cells, given the uniform buffer conditions across measurements. However, the electrical current (i) in each cell was expected to vary, corresponding to the specific allergen concentration in that particular cell.

We tested the hypothesis by comparing currents from an array of four reaction cells (Fig. 2B). Each cell was configured to generate a distinct current by a varying amount of HRP in electrochemical reactions. We initially measured current values from isolated reactions in individual cells. In a subsequent test, currents were measured from all four cells undergoing simultaneous electrochemical reactions. The current values exhibited an excellent match between separate and concurrent measurements (Fig. 2B), indicating that each cell could execute independent reactions with minimal inter-cell interference. This finding had notable implications for the design of a high-throughput assay system: i) a multi-cell electrode array is effectively a two-terminal device, simplifying electrode design and electrical connections, and ii) the signal reader could be implemented as a standard current meter, bypassing the need for more complex potentiostats.

iEAT2 detection system

Based on the two-terminal device strategy, we designed the iEAT2 hardware capable of 16 parallel measurements (Fig. 2C and Fig. S2). A disposable detection chip (Fig. 2C, **top**) was fabricated using printed circuit board technology, featuring a 2×8 array of two-terminal, gold-plated electrode cells (Park et al., 2021; Cho et al., 2022). We constructed a plastic fluidic chamber and bonded it to the chip's surface using adhesive, isolating each electrochemical cell. The chamber remained securely attached to the chip during the electrochemical reactions. The measured signals exhibited no significant differences between the chips with and without the chamber (Fig. S3). An accompanying base station (Fig. 2C, **bottom**) interfaced with the chip for signal readout, utilizing spring-loaded pins to establish electrical connections. Disc magnets were positioned beneath the electrochemical cell locations to concentrate magnetic beads.

We simplified the detection circuit by incorporating a multiplexed switching array (Fig. 2D and Fig. S4). Electrochemical reactions were initiated in all 16 cells simultaneously by applying a regulated 0.21 V potential difference between paired electrodes. Each cell reached a steady-state current within 30 seconds (Fig. S5). Upon the completion of the reaction, the microcontroller sequentially connected individual cells to an ammeter through the multiplexed switching array. The ammeter circuit, comprising a transimpedance amplifier and a voltage amplifier, converted current signals into voltage outputs. The microcontroller read the voltage outputs and converted them into allergen concentrations using calibration lookup tables. The processed data were visualized on an onboard monitor,

with a warning triggered when concentrations exceeded predefined allergy-inducing thresholds (Table 1).

Assay characterization

We adapted iEAT2 to detect three prevalent allergens, Ara h1 (peanut), gliadin (wheat), and ovalbumin (egg white), and evaluated its analytical performance. For each target allergen, we prepared a pair of reagents (Methods for details): magnetic beads (2.7 μm) conjugated with target-specific antibodies and HRP-labeled detection antibodies. Test samples were produced by introducing known quantities of allergen proteins into a standardized food matrix (boiled rice flour). The rice flour was selected because it was free from our allergen targets and facilitated their even distribution. The iEAT2 assay protocol comprised allergen extraction (2 min), allergen capture on magnetic beads (5 min), and labeling with detection antibodies (5 min). The labeled beads were then deposited onto electrochemical cells along with the TMB substrate. Electrical currents were measured by applying an electrical potential of 0.21 V between electrodes (Methods).

Figure 3A shows the titration results for gliadin in boiled rice flour. The iEAT2 assay achieved the limit of detection (LOD) of 7.5 ppm and a dynamic range spanning four orders of magnitude. The iEAT2 results correlated with those from conventional ELISA, validating iEAT2's quantitative accuracy (Fig. 3B and Fig. S6). Additional titration experiments corroborated iEAT2's high sensitivity (Fig. 3C), yielding LODs of 0.08 ppm (Ara h1) and 0.07 ppm (ovalbumin). The iEAT2 assay of these targets showed a good correlation with conventional ELISA (Fig. S7).

The observed LODs were below the threshold concentration of each allergen; this threshold was estimated from the eliciting dose (ED_{01}) that is predicted to trigger an allergic reaction in 1% of the allergic population (Table 1) (Food and Drug Administration, HHS, 2016; Houben et al., 2020; Remington et al., 2020). We also selected antibodies with minimal cross-reactivity (see Table S2 for the antibody list). The iEAT2 assays exhibited high target specificity with off-target signals comparable to IgG control levels (Fig. 3D and Fig. S8).

We further evaluated iEAT2 using incurred samples, a more challenging matrix where a known quantity of a food allergen is incorporated into a food matrix and processed (cooked) (Huet et al., 2022; Holcombe et al., 2024). Specifically, we added Ara h1 to chocolate mixes and produced chocolate bars. Subsequent iEAT2 analysis of these bars revealed a LOD of 0.19 ppm (Fig. S9), approximately 2.4-fold higher compared to the LOD with rice flour matrix samples. This discrepancy could be attributed to the lower yield of allergen extraction from the solid food matrix (Table S1), which should be considered when analyzing complex food items.

iEAT2 field testing

To assess iEAT2's practical utility, we analyzed commercial food items, including bread, protein bars, sandwiches, and rice crackers. We sampled a food portion with a disposable scoop and processed about 250 mg of the sample with the allergen extraction kit (2 min). The food extract was aliquoted into the assay template (10 μL per well), and each aliquot was immunolabeled (10 min) for Ara h1, gliadin, ovalbumin, or control, followed by the

iEAT2 measurements. The overall assay time was 15 min. Figure 4A summarizes the profiling results, with the allergen amount scaled for a single-serving portion of each food item (see Table S3). iEAT2 correctly detected and quantified expected allergens across various food items, aligning with their known ingredient compositions. The results were also consistent with the allergen warnings provided on food packaging.

We further used iEAT2 to assess allergen cross-contamination. As an illustrative case, we prepared fried rice using kitchen utensils (knife, cutting board, and pan) previously used to make a peanut-containing dish (Kung Pao chicken). iEAT2 could detect trace amounts of Ara h1 in the fried rice prepared with these utensils (Fig. 4B and Table S4). This application supported iEAT2's extended utility of monitoring allergen transfer that may arise from shared processing environments or accidental contact during food preparation.

CONCLUSIONS

The iEAT2 system can facilitate the onsite detection of multiple food allergens. Its design features a disposable kit for sample preprocessing and an array of electrochemical cells, enhancing assay simplicity and throughput. The assay scheme is particularly innovative. iEAT2 uses a parallel array of two-terminal electrochemical cells, each functioning as an independent current source. The system could perform concurrent assays within the array by measuring these currents. This strategy allowed us to increase the number of reaction cells with minimal complexities in electrical connection and detection electronics. While our prototype device implemented 16 parallel measurements, this detection concept would be scalable to larger systems, expanding the analytical capacity of electrochemical sensors.

The rising prevalence of food allergies and intolerances has heightened the demand for onsite food allergen testing (Kwon et al., 2020; Muthukumar et al., 2020). The iEAT2 system, with its rapid analysis time (15 min) and high sensitivity, can empower individuals to make informed choices regarding food consumption, reducing the risk of adverse immune reactions while minimizing unnecessary food restrictions. The quantitative nature of the iEAT2 assay could allow consumers to establish personalized allergen thresholds, potentially facilitating individualized dietary management. The system's utility may extend beyond individual uses. The food industry, for instance, may leverage iEAT2's high throughput and speed to assess ingredient composition, evaluate cross-contamination risks, and improve menu labeling accuracy.

We envision enhancing iEAT2's analytical capabilities through system engineering. The detectable targets can be expanded to encompass major allergen sources by using different antibodies in iEAT2's bead-based assay format. For instance, commercial antibodies are available to target allergens in hazelnuts (Cor a 9), almonds (Pru du 6), shellfish (tropomyosin), finned fish (parvalbumin), and latex-cross-reactive fruits such as avocado, banana, chestnut, kiwi, papaya, passionfruit, and strawberry (Ibero et al., 2007; Kuehn et al., 2017; Lin et al., 2017; Torre et al., 2022; Civera et al., 2023). We also need to refine the iEAT2 assay protocols, considering that allergen extraction becomes less efficient with rigid food items. One approach is to predetermine optimal assay parameters (extraction protocols, calibration curves) for common food items (e.g., bread, cooked rice, cooked

pasta, peanut butter). These parameters can be preloaded to the iEAT2 software, allowing users to select appropriate settings. Besides protein antigens, the assay can be modified to detect small molecules, toxins, or nucleic acids by employing alternative affinity ligands (e.g., aptamers, molecular imprints) (Lin et al., 2016; Bai et al., 2023; Fan et al., 2023; He et al., 2024; Nie et al., 2024). This expansion will open up opportunities for broader applications in food safety, such as mold detection, pesticide quantification, and food source identification. Moreover, given its platform technology nature, the core assay can be adapted for point-of-care clinical diagnostics, further expanding its utility across multiple fields.

MATERIALS AND METHODS

Materials.

We purchased superparamagnetic beads (6.7×10^7 beads/mg, Dynabeads M-270 Epoxy, Invitrogen); bovine serum albumin ($\geq 98\%$, BSA), ammonium sulfate and Tween 20 (Sigma); 1 M sodium phosphate buffer (PB, TeKnova, pH 7.2); 1-step ultra 3,3',5,5'-tetraethyl benzidine (TMB) ELISA substrate solution (Abcam). Extraction buffer (Food allergen ELISA kit, Morinaga Institute of Biological Science, Inc). Unless otherwise stated, all solutions were prepared at 25 °C using ultrapure water with an electrical resistivity of 18.2 M Ω ·cm. Antigen and antibody information is in Table S2.

Preparation of immunomagnetic beads.

Two milligrams of magnetic beads coated with epoxy were suspended in 500 μ L of 0.1 M PB solution at room temperature. Beads were magnetically washed three times using 0.1 M PB solution and resuspended in 100 μ L 0.1 M PB solution. Twenty micrograms of antibodies against a target allergen were added into 100 μ L of beads solution, and 100 μ L of 3 M ammonium sulfate was added into the mixture. The mixture was incubated on a rotating mixer for 8 hours at 4°C. The reacted beads were then magnetically washed three times with 1 $\times$ PBS solution and suspended in 50 μ L of 1 $\times$ PBS containing 1% BSA. Prepared beads were stored at 4 °C.

Standard food matrix.

We mixed 1 g of rice flour and 10 mL of 1 $\times$ PBS and boiled the solution for 10 min. After the boiling and cooling step, we added allergen proteins to the matrix and homogenized them. This procedure created controlled samples for standardized allergen testing.

Construction of the iEAT2 electrochemical cell array.

The electrode chip was constructed in a double-layer printed circuit board (PCB). The top conducting layer contained patterned electrodes, while the bottom layer had electrical contacts. Electrodes were coated with gold through an electroless plating process (nickel immersion gold). Using a laser cutter (Fusion Edge 24, Epilog Laser), we fabricated a separate fluidic chamber from an acrylic sheet (thickness, 4.5 mm) laminated with an adhesive layer. The chamber was bonded to the PCB chip to complete the iEAT2 electrochemical cell array. As an alternative bonding method, silicone-based glues can be applied and thermally cured for improved long-term durability.

iEAT2 device.

We built a portable iEAT2 reader designed to accommodate sixteen 2-terminal electrochemical cells (see Fig. S3 for the circuit schematic). An array of permanent neodymium magnets (N52, 1/8 inch in diameter and thickness, 5862K101, McMaster-Carr) were used to concentrate magnetic beads inside the electrochemical cells. Gold-plated pogo pins (575–0985015207114110, Mouser Electronics) were used to make electrical connections between the reader and the PCB chip. The core electronic components of the reader device included a microcontroller (Arduino Mega2560), voltage-controlled analog switches (ADG884, Analog Devices), a multiplexer (MAX336, Analog Devices), and operational amplifiers. The system operated on a 9-V battery, which provided converted voltages of 0.21 V for electrochemical cells, ± 15 V for amplifiers, and 5 V for additional components. The analog switches connected the electrochemical cells either to the ground or to the multiplexer for current measurements. The multiplexer (16-to-1) sequentially routed each electrochemical cell to the current measurement circuit, which consisted of a transimpedance amplifier (OPA170, Texas Instruments) and a voltage amplifier (LT6105, Analog Devices). The microcontroller digitized the output voltage at 10-bit resolution, measuring the current of each electrochemical cell for 1 sec at the 0.1-second sampling rate. Following measurement, the microcontroller averaged the results and converted them into allergen concentrations according to the pre-established calibration curves. The onboard display (Arduino OLED 128 $\times$ 32) presented test outcomes, which employed emoji indicators to represent allergen concentrations in relation to eliciting doses.

iEAT2 assay.

We cut food samples and combined a fragment (about 250 mg) with the extraction buffer (1 mL) in a manual extractor. We disrupted the sample through a torsion spring mechanism. Following a 2-min process, a sandwich-type immunoassay was conducted to determine allergen concentrations. We added magnetic beads and mixed the solution for 5 min. We washed the beads after capturing allergens, added detection antibodies with HRP, and mixed the solution for 5 min. Subsequently, we moved the beads using a magnet array and resuspended the beads in 100 μ L of TMB solution. To measure the current signal, we applied the potential (0.21 V) and measured the current.

Enzyme-linked immunosorbent assay (ELISA).

Antibodies were prepared at a concentration of 5 μ g/mL, and 100 μ L was added to each well of a 96-well (Nunc MaxiSorp flat-bottom plate, Thermo Fisher Scientific) for adsorption of antibodies on the wellplate for 12 hrs at 4°C. Following this, the plate was washed twice with 200 μ L of PBS containing 0.1% BSA. The plate was then treated with 1% BSA solution, 200 μ L per well, and incubated for 2 hours at 20 °C. After two more washes, varying concentrations of food allergen standard were added (100 μ L per well) and left for target capture for 2 hrs at 20 °C. The plate was washed again as before, and then HRP-labeled detection antibodies were added (500 ng/mL, 100 μ L per well) and incubated for 1 hr at 20 °C. After incubating time, the plate was washed twice with 1xPBS. Finally, 3,3',5,5'-tetramethylbenzidine (TMB, 50 μ L, BioLegend) was added to each well

and allowed to react for 30 min at 20 °C. The reaction was stopped with 50 µL of stop solution, and the optical absorbance was measured at 450 nm using a plate reader.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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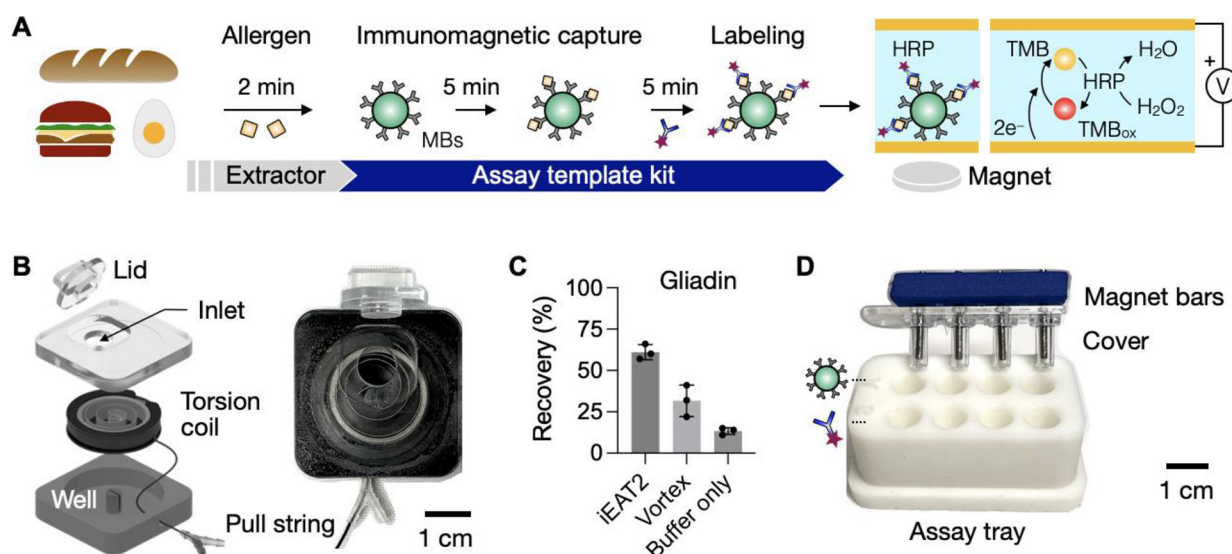


Figure 1. iEAT2 for onsite food allergen detection.

(A) Assay process. Food specimens were processed to facilitate allergen extraction. Subsequently, extracted allergens were immunocaptured on magnetic beads (MBs) and labeled with detection antibodies. The labeled MBs were deposited onto an electrode for electrochemical reactions, and the resulting electrical currents were measured. HRP, horseradish peroxidase; TMB, 3,3',5,5'-tetramethylbenzidine. **(B)** A mechanical grinder was constructed around a torsion coil. Food specimens and an extraction buffer were introduced to a well through an inlet. Pulling the attached string activated the coil to mechanically break down the food specimens, enhancing allergen extraction efficiency. **(C)** Comparison of allergen extraction efficiencies of different methods: iEAT2 (mechanical grinder), vortexing (30 sec), and extraction buffer-incubation only. A standard sample was prepared by spiking a rice porridge (1 g) with gliadin (1 mg). The iEAT2 method showed the highest extraction yield (61% of the input). Data are displayed as mean $\pm$ s.d. from triplicate measurements. **(D)** The disposable assay kit featured a tray preloaded with MBs and detection antibodies. A magnet-bar array facilitated the bead transfer throughout the assay process.

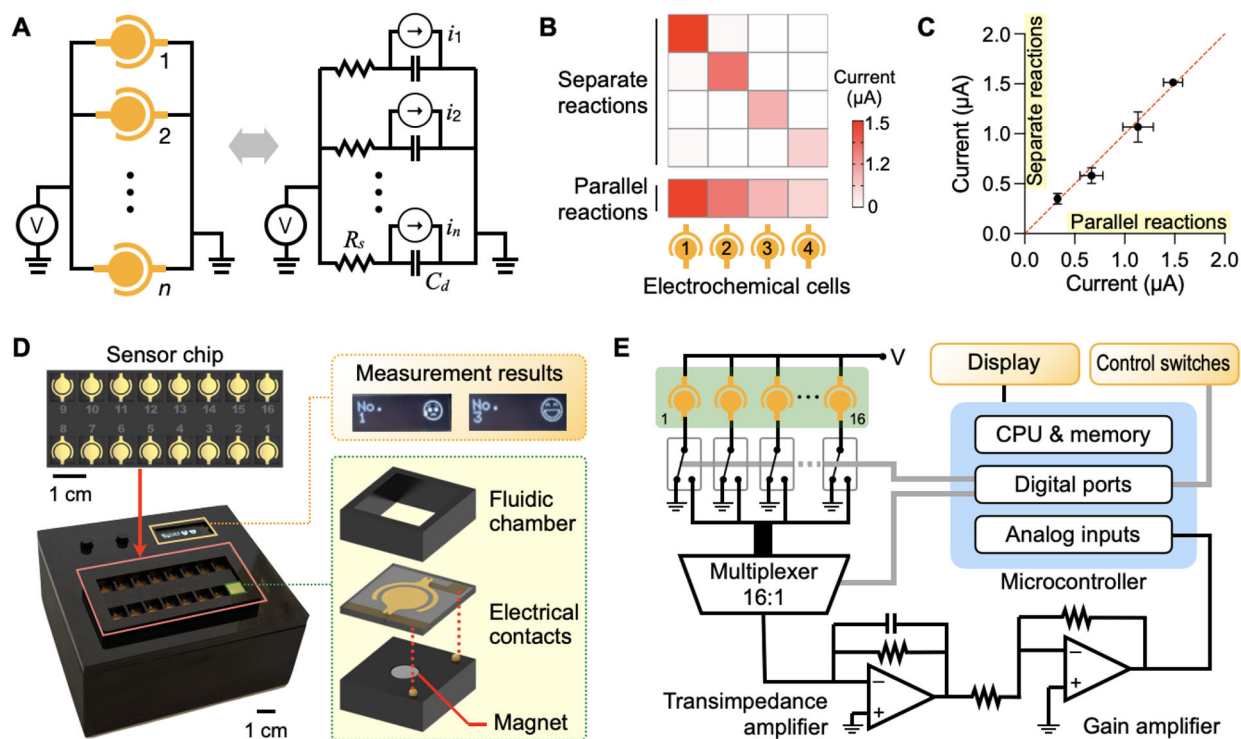


Figure 2. iEAT2 detection system for multi-electrochemical cells.

(A) An array of two-terminal electrochemical cells were approximated as independent equivalent circuits connected in parallel. Each cell included a solution resistor (R_s), a double-layer capacitor (C_d), and a current source. (B) Electrochemical currents were measured from four electrochemical cells in a parallel array. Reactions were conducted either separately (one reaction per cell) or simultaneously in all cells. Crosstalk between cells was negligible. Each tile shows a mean value from triplicate measurements. (C) An excellent match was observed between individual and parallel electrochemical detection. Each data point indicates measured currents from active electrochemical cells. The reference line (dotted) passes through the origin with a slope of 1. Data are shown as mean $\pm$ s.d. from triplicate measurements. (D) An iEAT2 prototype system was implemented. A disposable sensor chip (top) consisted of an array of electrodes fabricated in a printed circuit board and an acrylic fluid chamber attached to the top. The chip was placed on a signal reader (bottom), secured by a magnet-embedded lid. The reader made electrical contact with the chip through spring-loaded pins and had a magnet array to concentrate magnetic beads on the chip surface. The reader contained control electronics, a display, and a 9 V battery self-contained operation. The device was powered by a 9 V battery. (E) Schematic of the detection electronics. A microcontroller controlled the connection of each electrochemical cell to either ground or a current measurement circuit through a 16-to-1 multiplexer and analog switches. The measured current was converted to voltage by a transimpedance amplifier, followed by amplification using a gain amplifier. The microcontroller digitized these voltage signals and processed them into allergen concentration values. The results were displayed on an external display using emoji icons: smiling for safe allergen levels and sad for dangerous levels. System operation was controlled by two discrete switches:

the “Measurement” button initiated sequential current measurements, and the “Summary” button displayed the results.

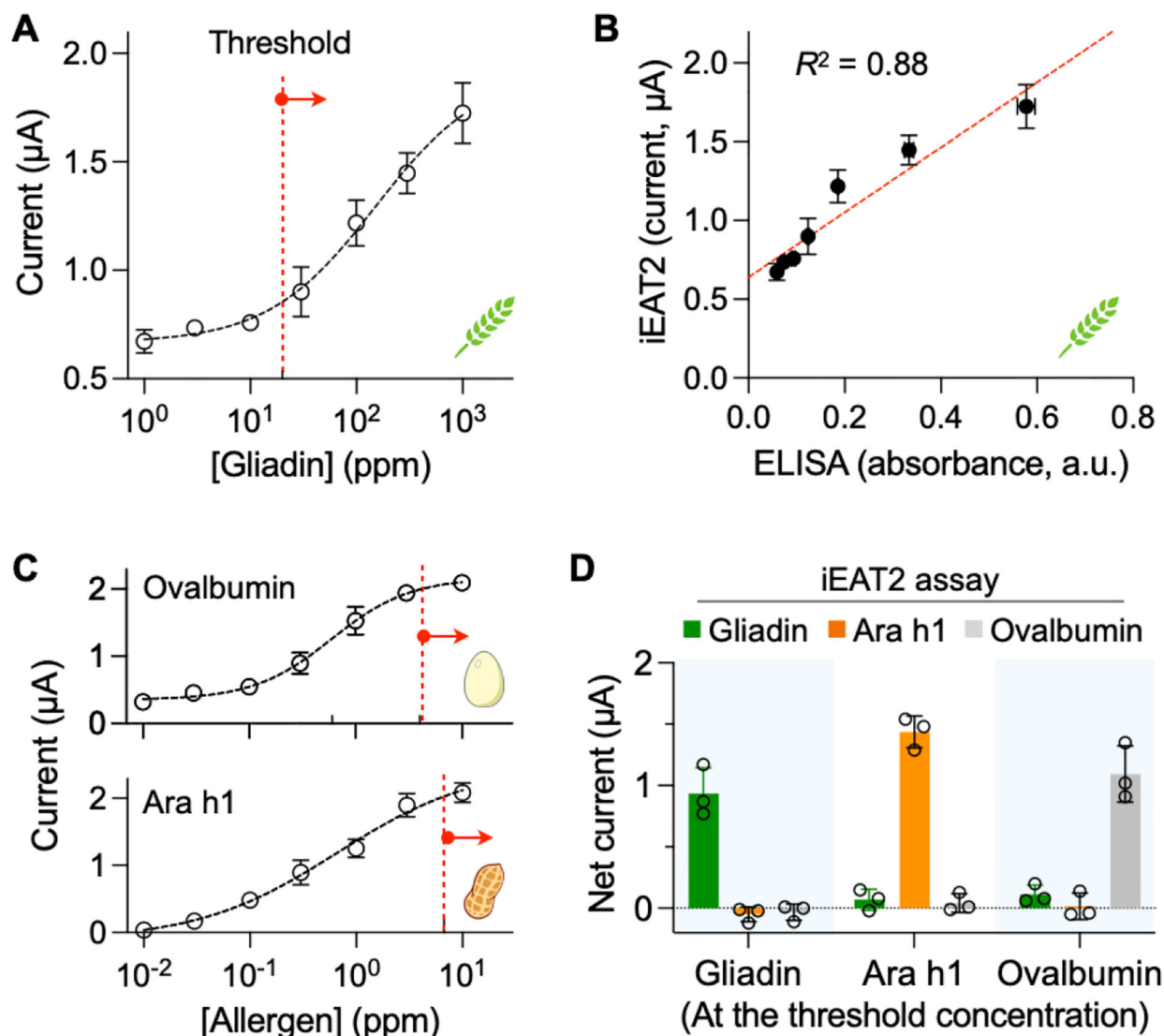


Figure 3. iEAT2 assay characterization.

(A) iEAT2 quantified varying amounts of gliadin spiked in a standard food matrix (rice porridge). The detection limit (7.5 ppm) was below the estimated eliciting dose of 14 ppm (indicated by the red dotted line), which would trigger an allergic reaction in a single food portion (see Table 1). Data are displayed as mean $\pm$ s.d. from triplicate measurements. **(B)** The iEAT2 results for gliadin showed a strong linear correlation ($R^2 = 0.88$) with those by conventional ELISA. The assay time was 15 min for iEAT2 and 210 min for ELISA. Each data point shows mean $\pm$ s.d. from duplicate (ELISA) and triplicate (iEAT2) measurements. **(C)** Titration experiments were performed for peanut (Ara h1) and egg white (ovalbumin) allergens. The detection limits were below their respective eliciting doses (6.7 ppm for Ara h1 and 4 ppm for ovalbumin, indicated by red dotted lines). Data are displayed as mean $\pm$ s.d. from triplicate measurements. **(D)** The detection probes demonstrated high on-target specificity while generating negligible signals with off-target samples. The test samples (rice porridge) contained specific allergens at their respective threshold doses. Net current values

are presented with background values subtracted. Data are displayed as mean $\pm$ s.d. from triplicate measurements.

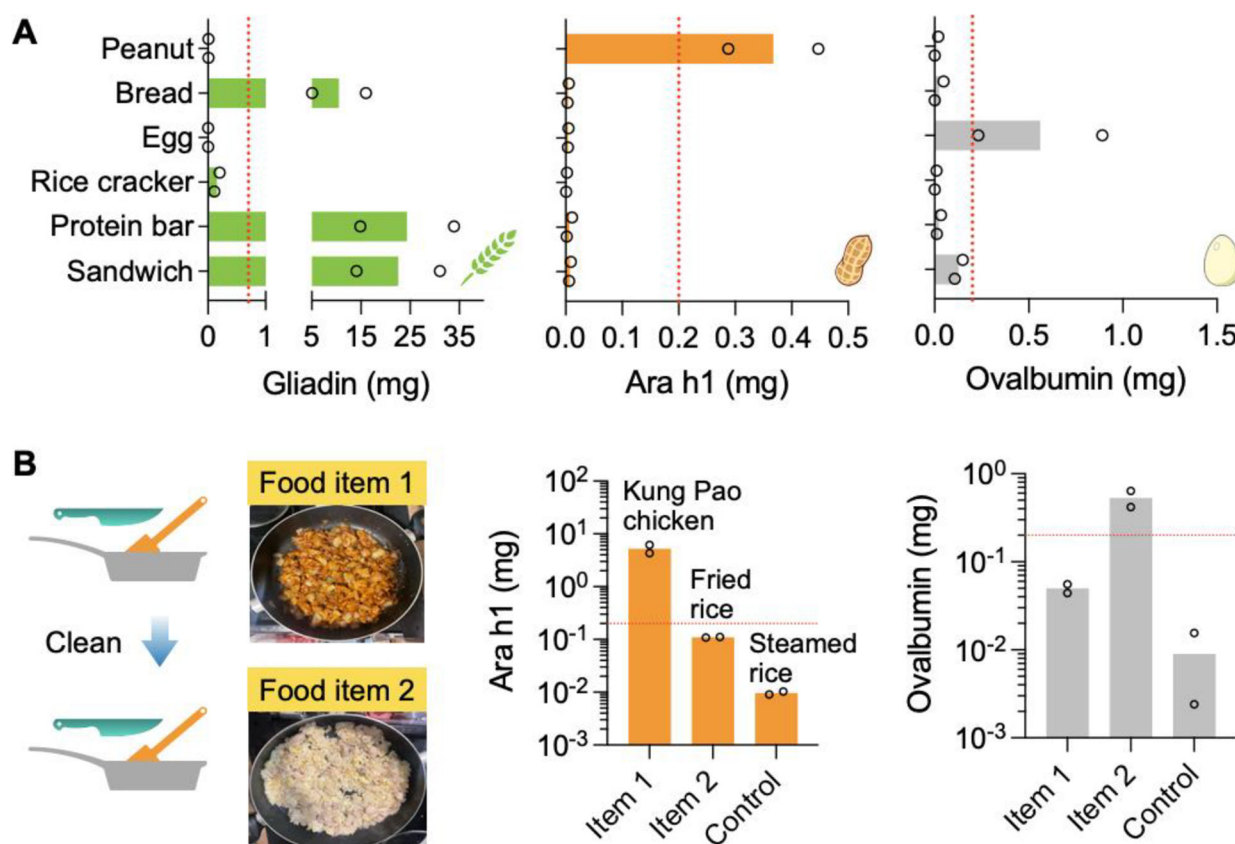


Figure 4. iEAT2 surveillance of real food items.

(A) iEAT2 was used to quantify the amounts of gliadin, Ara h1, and ovalbumin in commercial food items. The assay detected expected allergens in these products, confirming the declared ingredient compositions. Red dotted lines indicate the eliciting doses estimated for a single-serving portion of each food item (Table S3). Bars indicate mean values from duplicate measurements. (B) Cross-contamination detection. A dish of Kung Pao chicken (food item 1), containing peanuts, was prepared. The same cleaned utensils were used to prepare a second dish of egg-fried rice (food item 2). The iEAT2 assay accurately identified the primary allergens in both dishes. Additionally, it detected a trace of peanut allergen (Ara h1) in the egg-fried rice, indicating potential cross-contamination during the cooking process. Steamed rice, prepared separately, served as a negative control. All allergen quantities were normalized to standard serving sizes (Table S4), with respective eliciting doses indicated by red dotted lines. Bars indicate mean values from duplicate measurements.

Table 1.

iEAT2 detection limits and reference doses.

Allergen	Eliciting dose ED ₀₁ (mg) [†]	Suggested 1 serving size (g) [‡]	Threshold (ppm)	iEAT2 detection limit (ppM)
Peanut	0.2	30	6.7	0.08
Egg	0.2	50	4.0	0.07
Wheat	0.7	50 (bread)	14.0	7.50

[†]The eliciting dose (ED₀₁) is the threshold amount of consumed protein predicted to trigger an allergic reaction in 1% of the allergic population.

[‡]The single serving size values are from the U.S. Food and Drug Administration's Code of Federal Regulations Title 21.