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Georgina M.S. Ross[a], Daniel Filippini[b] , Michel W.F. Nielen[a,c] & Gert IJ. Salentijn*[a,c]

a. Wageningen Food Safety Research (WFSR), Wageningen University & Research, P.O. Box 230, 6700 AE, Wageningen, The Netherlands.

b. Optical Devices Laboratory, Division of Sensor and Actuator Systems, IFM – Linköping University, S58183, Linköping, Sweden.

c. Laboratory of Organic Chemistry, Wageningen University, Helix Building 124, Stippeneng 4, 6708 WE, Wageningen, The Netherlands

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**Highlights**

- 1-min allergen protein extraction via disposable homogenizer
- 3D-printed unibody lab-on-a-chip (ULOC) for on-chip bioreagent storage and dilution
- Pipette-free sample dilution and transport to multiplex immunosensor
- Fully interconnectable system from sample to smartphone recorded signal

## Abstract

While consumer-focused food analysis is upcoming, the need for multiple sample preparation and handling steps is limiting. On-site and consumer-friendly analysis paradoxically still requires laboratory-based and skill-intensive sample preparation methods. Here, we present a compact, inexpensive, and novel, prototype immunosensor combining sample preparation and on-chip reagent storage for multiplex allergen lateral flow immunosensing. Our comprehensive approach paves the way for personalized consumer diagnostics. The prototype allows for handheld solid-liquid extraction, pipette-free on-chip dilution, and adjustment of sample concentrations into the appropriate assay dynamic working range. The disposable and interconnectable homogenizer allows for the extraction and 3D-sieve based filtration of allergenic proteins from solid bakery products in 1 minute. The homogenizer interconnects with a unibody lab-on-a-chip (ULOC) microdevice, which is used to deliver precise volumes of sample extract to a reagent reservoir. The reagent reservoir is implemented for on-chip storage of carbon nanoparticle labeled antibodies and running buffer for dilution. The handheld prototype allows for total homogenization, extraction, filtration, dilution, mixing, transport, and smartphone-based detection of hazelnut and peanut allergens in solid bakery products with limited operational complexity. The multiplex lateral flow immunoassay (LFIA) detects allergens as low as 0.1 ppm and the system is already consumer-operable demonstrating its potential for future citizen science approaches. The designed system is suitable for a wide range of analytical applications outside of food safety, provided an LFIA is available.

## 1. Introduction

On-site and personalized food safety tests are growing in popularity, with developments in rapid, affordable, sensitive, and disposable handheld assays driving the move from the laboratory to a consumer-based approach [1, 2]. Consumer detection of food allergens is particularly relevant [3, 4], and more so now than ever, with the Food and Drug Administration (FDA) announcing temporary changes to food labeling and ingredients, to prevent disruption to the global food supply chain during the SARS-CoV-2 pandemic [5]. Amendments overlooking hidden or novel allergens, put the allergic individual at risk, exemplifying the necessity for personalized, disposable and simplified analysis of allergens, from sample preparation to detection. To date, the lateral flow immunoassay (LFIA) is the most successful application of consumer diagnostics [6]. Combining LFIAs with smartphones as optical detectors allows for 'on-the-go' decentralized screening [7] and can even provide semi-quantitative results by calibrating test and control line color intensity values towards particular antigen concentrations [8].

Despite these advantages, LFIAs have a limited dynamic range, work only with liquid samples, and predominately target a single analyte. Within a sandwich LFIAs dynamic working range, the test line intensity increases with increasing analyte concentration. However, at high analyte concentrations, the signal intensity can paradoxically decrease as the excess of unlabeled analyte saturates the capture and detector antibodies (mAbs) binding sites [9]. The reduction in test line intensity mimics the signal at much lower analyte concentrations. Dilution within the appropriate concentration range is required to avoid false negative results, which is particularly important for consumer testing. Moreover, when analyzing a complex solid matrix such as food, sample preparation, and reagent storage are pivotal bottlenecks. Even integrated systems often require pre-treatment [10] or heat-assisted actuation in extracting proteins into a liquid [4]. Finally, excluding a few multiplex LFIAs, allergen LFIAs are restricted to singleplex detection, which is limiting for individuals with co-existing allergies. Indubitably, consumers do not have the laboratory skills required for sample preparation and fully integrated analytical systems have mainly been developed for DNA-based analysis [11-13]. Systems with integrated solid phase extraction for aqueous samples are reported [10, 14], but extraction of solid samples is more complex and still requires offline pre-treatment.

In a parallel advancement, the emergence of 3D-printing has revolutionized rapid prototyping of multifunctional lab-on-a-chip [15] and disposable [16] devices for analytical chemistry. Modification of Computer-Aided Designs (CADs) takes little cost and time, and prototypes can be refined iteratively multiple times in a single day, outside of a cleanroom environment. A unibody lab-on-a-chip (ULOC)[15, 17], is a monolithic device with all the analytical functionalities in-built, and takes less than an hour to manufacture [18]. The ULOC's unibody connectors, ending in unidirectional valves, can be connected to silicon tubing as finger pumps [19]; or to detachable devices, for pipette-free, active control of sample actuation with volume metering [20]. Moreover, 3D-printed devices with on-chip

reagent storage [11, 21] can combine with and benefit from the capabilities of paper-based devices [22, 23].

Here we present a multifunctional, consumer-operable prototype immunosensor with ULOC-enabled sample handling for the extraction, dilution and LFIA detection of hazelnut and peanut allergens in the low ppm range in spiked and commercial bakery products.

## 2. Experimental

### 2.1. Reagents and Consumables

Multiplex LFIA and carbon nanoparticle labeled antibodies (CNP-mAbs) against hazelnut and peanut have previously been developed, characterized, and validated [24, 25]. Running buffer (RB)/extraction buffer was 100 mM borate buffer (BB) pH 8.8, composed of 100 mM boric acid (Merck, Darmstadt; Germany) and 100 mM sodium tetraborate (VWR, Leuven; Belgium) with 1% (*w/v*) bovine serum albumin (BSA; Sigma-Aldrich, Zwijndrecht, The Netherlands) and 0.05% tween-20 (*v/v*) (Merck, Darmstadt; Germany). The 10 mL and 1 mL disposable plastic syringes used for the homogenizer and air displacement, were purchased from Becton-Dickinson (Utrecht, The Netherlands) and low binding syringe filters used to filter total protein extracts (5 µm; 1.2 µm; 0.45 µm) were acquired from Pall Life Sciences (Pall Netherlands B.V., Medemblik; The Netherlands). Silicon tubing for ULOC connectors was purchased from Esska-Tech (Arvika; Sweden). ULOCs were sealed on the open side with adhesive tape (3M Ruban Adhesive Scotch Nastro Adhesive, 3M Europe, Diegem; Belgium). Red food dye solution (consisting of water, propylene glycol, and Carmoisine CI 14720) of unknown concentration used for dilution characterization of the ULOC was purchased from a local supermarket. The clamp used for attaching the smartphone to the holder's frame was purchased from Wolfcraft (Wolfcraft, Kempenich; Germany).

### 2.2. Reference Material Preparation

Standardized certified reference materials for food allergens are not currently available; therefore, total hazelnut protein (THP), total peanut protein (TPP), and blank cookie (BC) extracts were prepared in-house [24, 25]. See Supplementary Information (SI) Table S1 for ingredient lists and labeling information. Fresh protein aliquots were defrosted on the day of experiments, and the protein content was checked before use by a NanoDrop (ND 3300, Isogen Life Sciences, De Meern; The Netherlands) protein analyzer. A range of sample types was utilized to characterize each module of the prototype immunosensor (Table 1).

**Table 1. Reference material and sample classification**

| Sample type | Matrix | Spike | Concentration range | Used to Characterize |
|---|---|---|---|---|
| A | Water | Food Dye Solution | N/A | ULOC Dilutor |
| B | Blank cookie extract | Total hazelnut protein (THP) extract | 1 – 1000 ppm (v/v) | LFIA performance |
| C | Blank cookie | Total hazelnut protein (THP) extract | 1 – 1000 ppm (v/w) | Homogenizer |
| D1 | Blank cookie | Hazelnut cookie | 0.1 – 100 ppm (w/w) | Total prototype |
| D2 | Blank cookie | Hazelnut cookie & peanut cookie | 0.1 – 100 ppm (w/w) | Total prototype |

### 2.3. Development and Fabrication

Computer-aided design (CAD) software Autodesk Fusion 360 (Autodesk Inc. San Rafael, CA; USA) was used for designing 3D-printable parts and converting them to printable .stl files. Figure 1 gives a schematic overview of the prototype platform; Figure 2 gives an annotated photographic overview of the disassembled (2A) and assembled (2B) platform. The ULOC dilutor (Figure 2C) was printed with a stereolithography (SLA) printer Form3 (FormLabs, Somerville, MA; USA) at 25 µm layer resolution using proprietary clear resin (Type O4, FormLabs). A fused deposition modeling (FDM) model (Hepheststos 2, BQ, Madrid; Spain) was used to print the sieves (Supplementary Information (SI) Figure S1), device holder (SI Figure S2), and interchangeable LFIA cartridges (SI Figure S3).

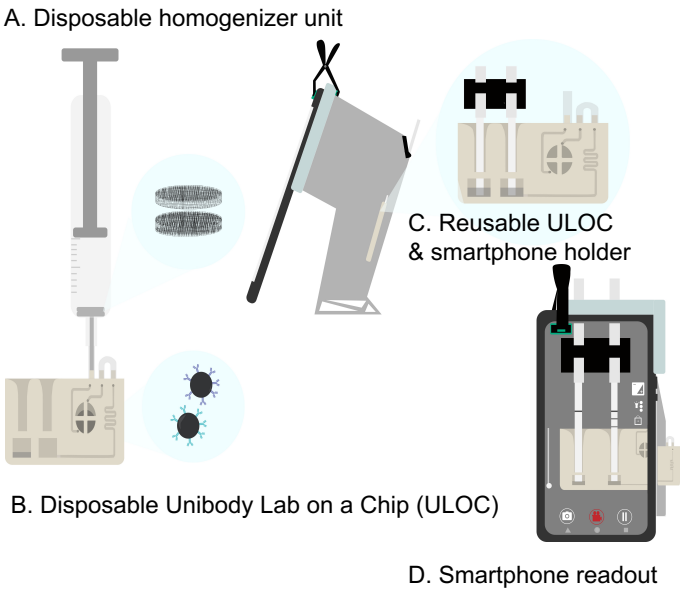


***Figure 1.*** *Overview schematic of parts of the prototype immunosensor. (**A**) Disposable homogenizer unit with 3D-printed sieves (**B**) Disposable unibody lab-on-a-chip (ULOC) for dilution of extracted allergens and mixing with carbon nanoparticle labeled allergen-specific antibodies. (**C**) Reusable smartphone and ULOC holder. (**D**) Smartphone readout, as a result, appears in real-time on the screen.*

### 2.3.1. Homogenizer

The homogenizer module enables solid-liquid extraction from solid food samples. 3D-printed sieves with approximate pore sizes of 0.5 mm were cut by laser (HL40-5g, Full Spectrum Laser LLC, Las Vegas, NV; USA) into discs (18 mm diameter; SI Figure S1). Two sieves (Figure 1A) insert into a 10 mL syringe at an offset to each other. As the plunger pushes solid material against the first 3D-printed sieve, it breaks into smaller pieces which are subsequently blocked by the second sieve that is kept at an offset, preventing particles from blocking microchannels in the ULOC. Silicon tubing (1.5 mm inner diameter, 40 mm length) connects with the ULOC connector. The tubing can be used as a finger pump or is connected by a second larger piece of silicon tubing (2.5 inner diameter, 20 mm length) to the syringe tip, using the syringe pressure for controlled actuation.

### 2.3.2. ULOC Dilutor

The ULOC dilutor (60 mm W x 40 mm L; see SI, Figure S2) has all functional features on a single side. This leaves one side open so uncured resin can be removed from 1 mm deep fluidic channels (1 mm wide) by sonicating (FinnSonic m15, FinnSonic Oy, Lahti, Finland) in ethanol (Sigma Aldrich, Steinheim, Germany) for 30 seconds and air drying. Before sealing the ULOC's open side with adhesive tape, CNP-mAbs and RB were pre-loaded into the reagent reservoir (volume capacity of 250 µL) and into the reference well (1 µL of CNP-mAb and 100 µL of RB) for control measurements. The RB both stabilized CNP-mAbs for on-chip storage and acted as a dilution buffer for injected samples. The test and reference wells had a total volume capacity of 200 µL each. Silicon tubing secured the first unibody connector with the homogenizer syringe tip. The remaining two connectors were joined together by silicon tubing (see Figure 1B).

### 2.3.3. ULOC & Smartphone Holder

The ULOC could be inserted into an opening (50 mm W x 35 mm L) in the 3D-printed device holder, which shielded the assay from ambient light (see SI; Figure S2). The LFIA cartridge, which fits 2 LFIAs (4 or 5 mm wide), aligned the LFIAs with the test and reference wells of the ULOC. A smartphone was clamped to the outer frame of the holder overlaying the rear-facing camera and flash.

## 2.4. Characterization of Prototype Immunosensor

### 2.4.1. Extraction time

Pre-ground raw hazelnut was incubated in the homogenizer syringe with RB for different periods (1, 2, 3, 5, 10, 20, or 30 minutes) to optimize extraction time and assess sieve efficiency. The total protein concentration was quantified (n=3) using the NanoDrop.

### 2.4.2. ULOC Dilutor

Before characterization, 5 or 10 µL dye was actively injected via a disposable syringe into the manifold. This was repeated multiple times for distance verification and the distances were marked on the ULOC (see SI Figure S4). The ULOC was characterized for dilution by mixing dye with water (sample type A) at various dilution factors (DFs). Adjustable water volumes were pipetted into the ULOC reservoir. This yielded DFs of x10, x15, x20 and x40 when 10 µL of aqueous dye solution was injected up to the mark on the ULOC. For comparison with a manually pipetted sample, the same DF dye/water was pipetted into the ULOC reference well. Smartphone images of the ULOC were acquired using OpenCamera (v1.47.3) to keep exposure and focus constant on a Google Pixel 2 XL (Google, California; USA). On and off-chip dilutions were evaluated by comparing the color intensities in the test and reference wells at the end of the manifold [20]. For image analysis, ImageJ [26] split images into their RGB (red, green, blue) color channels. In the blue channel pixel intensity readings were taken from the test and reference wells for direct comparison.

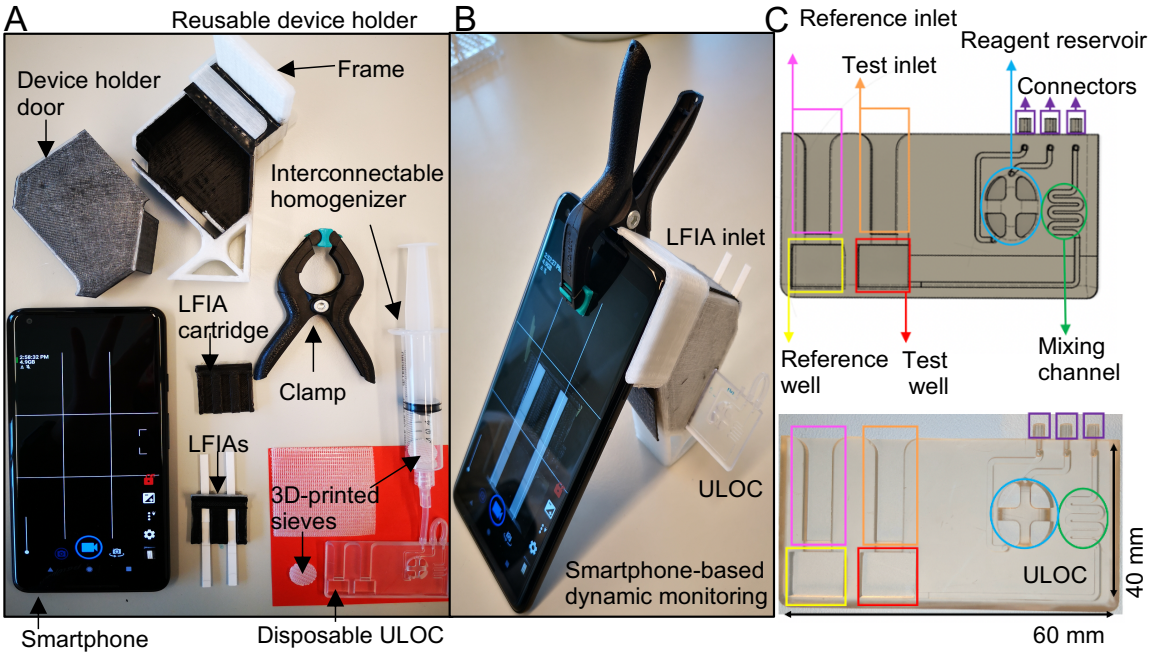


***Figure 2.*** *Overview of prototype immunosensor. (**A**) Annotated Computer-Aided Design (CAD) of the Unibody Lab on a Chip (ULOC) dilutor. (**B**) Photo of 3D-printed ULOC with color code matching (**A**). (**C**) Disassembled prototype immunosensor showing all components. (**D**) Side view of the assembled device where the ULOC slots into smartphone holder, the smartphone clamps to the holder's frame, the LFIAs insert into the opening, and the results are viewed on the phone. (**E**) Front view of prototype immunosensor where real-time LFIAs development is visible on-screen.*

### 2.5. Dynamic Data Acquisition

Images and videos were acquired by smartphone, attached to the holder frame, using OpenCamera to ensure fixed acquisition conditions (fixed focus, locked exposure, controlled illumination, for videos: 30 frames per second, 720 x 480 pixels). LFIA results appeared on the screen as they emerged. Recorded time point images were analyzed in ImageJ by splitting images into their color channels. A blue channel pixel intensity (BCPI) reading was taken from below the test line at t=0 as a background response; the BCPI measurements from the test and control lines were then subtracted from this to give the corrected BCPI (cBCPI) value. In the assay dynamic working range, cBCPI increases as test and control lines increase in intensity. The T/C ratio is a standard metric used for normalizing sandwich format LFIA results [27].

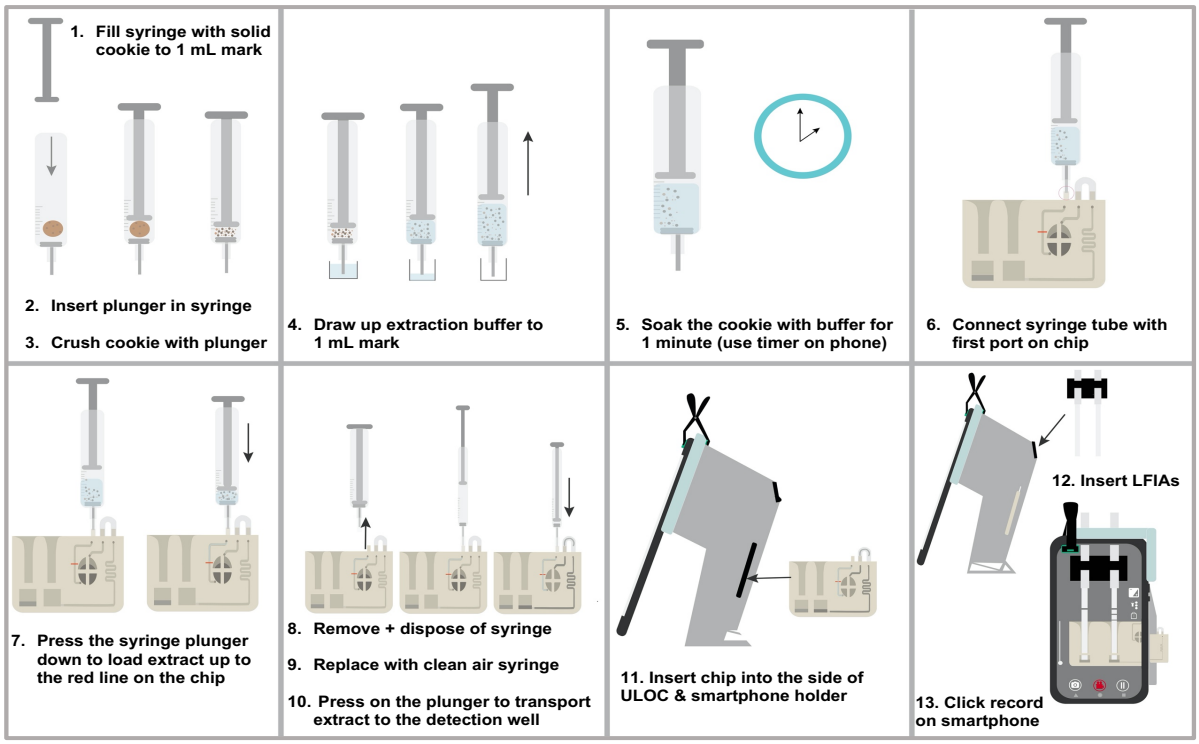


***Figure 3.*** *Pictogram instructions for operating prototype immunosensor for homogenization, extraction, ULOC-based dilution, transport, and LFIA based detection of food allergens with on-screen smartphone readout. The guidelines have been designed to guide the consumer during citizen science experiments.*

### 2.6. Prototype Immunosensor Characterization

Experiments were performed in triplicate; see Figure 3 for the pictogram operation procedure. Before sealing the ULOC with adhesive tape, the reagent reservoir was filled with 2 µL CNP-mAb and 190 µL RB, resulting in a Dilution Factor (DF) x 20 when 10 µL of sample is actively injected into the reservoir. Sample type B was used to characterize the LFIA immunochemistry and smartphone readout. Pre-weighed sample type C was used to evaluate the extraction and filtration by the homogenizer by comparing the LFIA result against results obtained with sample type B. For sample types C and D (approximately 0.25 g), solid samples were first crushed in the homogenizer and then incubated with 1 mL RB for 1-minute, before filtering through the 3D-sieves. Finally, to characterize the system for detecting real-life incurred and processed allergens, sample type D was investigated. The volume of pre-loaded CNP-mAb was increased to 4 µL (2 µL for anti-hazelnut mAb-CNP and 2 µL for anti-peanut mAb-CNP) for multiplex analysis. Here, we consistently injected sample up to the 10 µL mark to assure the reproducibility of results, however, the user actively controls the sample injection, and can simply inject further into the ULOC if a greater volume is required. Air displacement transported the sample to the detection well. The ULOC was then inserted into the device holder, the LFIAs inserted into the ULOC, and the smartphone acquired the data. Here, the immunochromatographic limit of detection (LOD) is the lowest concentration at which two lines (test and control) can be visually, or by smartphone, distinguished compared to a blank sample (n=3).

## 3. Results and Discussion

### 3.1. Extraction time

A major restriction of allergen analysis is the lengthy extraction process, including weighing, heating, grinding, and numerous filtering steps [2, 27]. Extended extractions delay rapid screening tests such as LFIA. While [4] reported a 2-min magneto-assisted allergen antigen extraction, this still required microwave pre-heating. Previously, [24] described a method for extracting total proteins from cookies and peanut flour at room temperature in 30 minutes; even then samples required extensive dilution to comply with the LFIA working range, indicating a much shorter extraction time could still be appropriate. To test this, we evaluated different extraction times to attempt to reduce the overall assay duration, see Figure 4 and SI (Table S2).

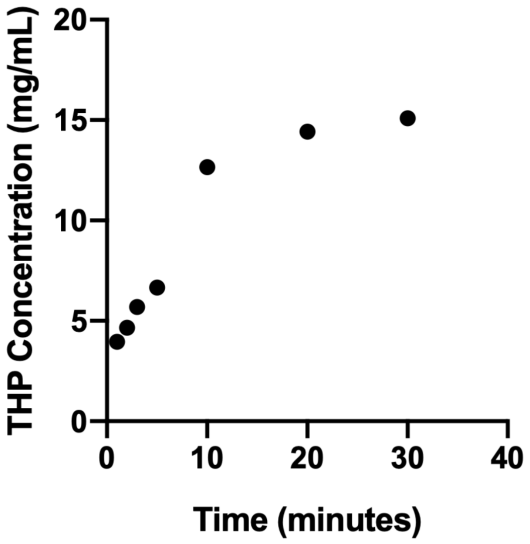


***Figure 4.*** *Graph showing effects of different buffer incubation times (1, 2, 3, 5, 10, 20 & 30 minutes) on the total hazelnut protein (THP) concentration (mg/mL) in the extract from raw hazelnut (n=3 extractions). Error bars are displayed but are too small to see, for standard deviation, see SI Table S2.*

High protein concentrations are extracted within the first minute (RSD = 1.5%, *n=3*), with the concentration increasing with longer buffer incubation time (first 10 min). The disposable 3D-sieves circumvented the need for further sample filtration (see 2.1). Unlike other integrated microdevices [4, 10, 11], our extraction requires no sample pre-treatment or heating, and the detachable unit interconnects with the ULOC which then executes all outstanding sample handling. Only one other reported allergen screening device offers solid-liquid internal extraction in less than 4-min [1]. These experiments used pre-weighed samples but to improve consumer-operability, the user can instead simply fill the homogenizer syringe with the solid sample to the 1 mL visual mark (see SI Figure S5), avoiding the need for weighing equipment. While this would not result in quantitative results, such an

approach is adequate for semi-quantification. Currently, in this early prototype, extraction buffer is provided in a pre-measured vial containing 1 mL. However, future refinement could include an additional ULOC chamber for on-chip extraction buffer storage.

### 3.2. ULOC Dilutor

Allergenic proteins exist in foods over a broad dynamic range and must be detected at trace levels for protecting sensitive individuals. Still, it is vital to understand that highly concentrated samples can yield paradoxically low signal intensities, and it is reported that sample dilution (DF x10-100) can minimize the occurrence of false-negatives[27, 28]. While sample dilution is a prerequisite for allergen analysis, we cannot expect the consumer to do this. To circumvent the issue, we have created a system allowing for arbitrary sample dilution by pre-storing adjustable volumes of RB in the reservoir. When the extracted sample is injected into the reservoir, it efficiently mixes with the pre-stored CNP-mAbs by air displacement and is also diluted in RB.

Figure 5A compares BCPIs for on-chip versus off-chip dilutions (dye in water, sample type A, n=3) using different DFs. Figure 5B indicates where to take the pre-dilution (DF x0), mid-dilution (5C), and off- and on-chip (5D) BCPIs measurements from. For consistency, measurements were always taken below the dye's meniscus. The ULOC DFs invariably matched the manually pipetted DFs, suggesting that the ULOC delivers well-defined sample volumes (see SI Figure S4). Other ULOC devices have already been extensively characterized for integrated actuation (2-15 µL) with comparable accuracy to pipettes [20, 22]. Injecting sample causes turbulent mixing (Figure 5C) because of the co-injection of air bubbles. Air metering is also documented elsewhere [13]. The current combined immunosensor benefits from nitrocellulose, when the LFIA touches the turbid liquid, the nitrocellulose wicks the fluid, displacing the air from its pores and the optical measurement is not disrupted.

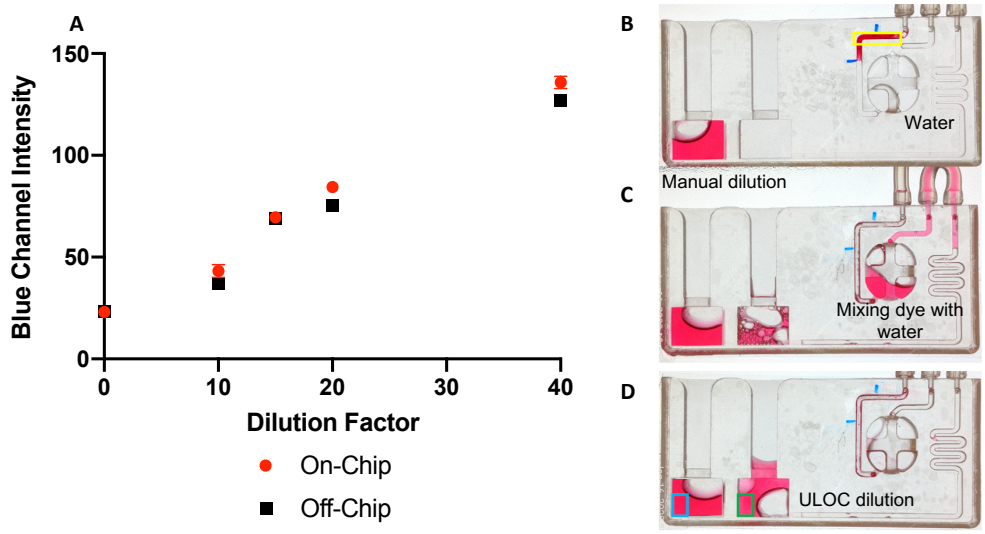


***Figure 5**. ULOC dilutor. (**A**) Graph depicting the performance of ULOC for unidirectional sample dilution as a function of blue channel intensity. The red circle represents ULOC dilutions. The black square represents manual dilutions. (**B**) ULOC device before dilution, the area where the volume metering reading is taken from is outlined in yellow. (**C**) ULOC during dilution, the dye mixes with water in the reagent reservoir and is delivered to the test area by the fluidic system. (**D**) ULOC after dilution, intensity reading for the manual dilution (outlined in blue), and the intensity reading for the ULOC dilution (outlined in green).*

### 3.3. Dynamic Data Acquisition

To investigate the influence of assay duration on the signal development, LFIAs were readout after 5 (SI Figure [S6A]), 10 [S6B], 15 [S6C], and 20 [S6D] minutes with sample type B (0.1-1000 ppm). From these images, the test and control line development [S6E] and the T/C ratio development [S6F], are each plotted as a function of time (min) in independent calibration curves. At 5 min, the lowest concentration visibly readable is 10 ppm, with signal improving with increased duration, at 10 min a 1 ppm signal is readable. However, at 1000 ppm no signal is generated on either line, even after 10 min. Even with ULOC-enabled sample dilution (DF x20), high-concentration LFIA effects are observed, affirming the necessity of dilution. Comparatively, running the LFIAs for 15-min allows signals to develop for all concentrations. Considering that 20-min is the recommended reaction time for LFIA for high signal stability and sensitivity [8], a 15-min readout is suitable for assessing LFIAs, without neglecting highly concentrated samples. In future versions, the ULOC could benefit from having multiple dilution wells for running 3 LFIAs simultaneously across an entire assay dynamic working range, further limiting the occurrence of concentration-dependent effects.

### 3.4. Total Allergen Protein Detection

Allergenic proteins can be subject to conformational alterations during food processing [29]. Therefore, biosensors must demonstrate proficiency in detecting allergens in both raw and processed products. Here, solid cookie samples were pre-weighed (0.25 g), but the user can instead fill the homogenizer with the cookie to the 1 mL mark to approximately obtain the same sample weight (see SI Figure S5). Though this method is less precise, it would suffice for qualitative assessment of bakery products for the presence of allergens. Previously, we found allergen samples need extensive dilution before LFIA analysis [24]. Here, a manual DF x 20 (5 µL of THP in 95 µL RB) gave clear results at all tested concentrations (see SI Figure S7), without compromising detection at the lowest levels, so a DF x 20 was always applied for ULOC-enabled dilutions.

#### 3.4.1. Total Allergen Protein Extract Screening

See Figure 6A for signal development under optimum conditions (e.g., THP extract spiked into RB). For sample B (THP extract spiked into BC extract (v/v); Figure 6B), the T/C ratio detection limit is 1 ppm (n=3). Despite using the prototype for analysis, the LOD here is not much higher than in our previous work (0.5 ppm; n=20)[24], which was obtained using standardized laboratory conditions and equipment. Sample B measurements are reproducible (RSD ± 2.9%), indicating the ULOC mixes well and delivers determined volumes, and that the LFIA still works when combined with the ULOC. To reflect a real solid-liquid extraction, solid sample type C (BC spiked with THP extract (w/v); Figure 6C) was extracted and analyzed, with a LOD of 1 ppm (RSD at 1 ppm ± 3.7%). The slight increase in T/C deviation could be due to the crushing efficacy of the homogenizer. Small differences in buffer incubation times between repeat measurements and non-uniform dispersion of liquid THP extract could be consequential to the somewhat higher variation.

### 3.4.2. Incurred Single Allergen Screening

Thermal processing can affect allergen detectability [29]. See Figure 6 to compare signal development for samples using total allergen protein extracts (6E) with samples containing incurred allergens (6D&F). Testing commercial hazelnut cookies mixed with blank cookies (sample type D1), exemplifies the effectiveness of extracting and detecting incurred proteins. Sample D1 has a LOD of 0.1 ppm (n=3, RSD ± 3.03%; see Figure 6D and F) for processed hazelnut. Compellingly, the D1 LOD is lower than the LOD for sample C. The LFIA is evidently more sensitive towards processed hazelnut. This sensitivity has been also indicated in our singleplex hazelnut LFIA where the same mAb reached the same LOD for HC extract in BC extract [25].

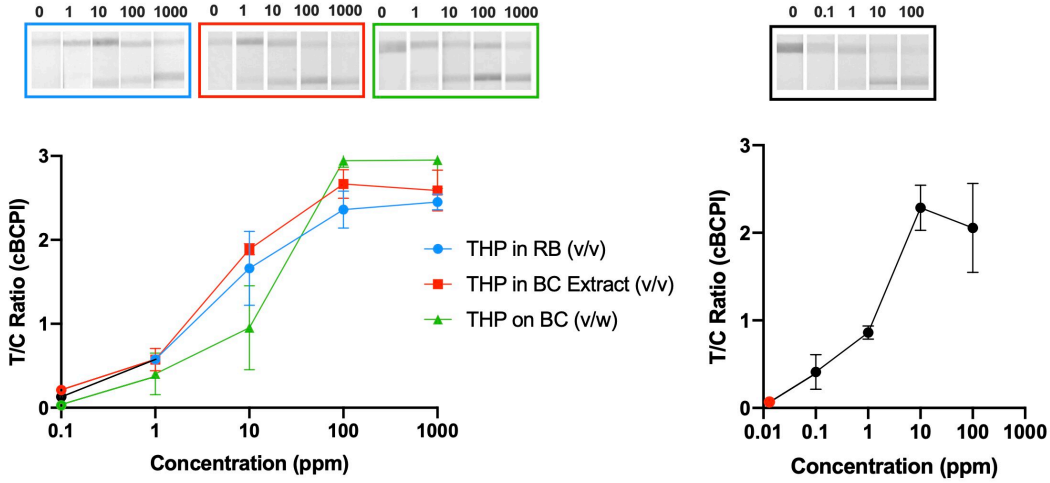


***Figure 6.*** *Photographs and calibration curves showing LFIA signal development in increasing concentration of analyte where error bars represent standard deviation (n=3) (A) 1-1000 ppm, total hazelnut protein (THP) extract spiked into running buffer (RB)(v/v); (B) 1-1000 ppm, THP extract spiked into blank cookie (BC) extract (v/v); (C) 1-1000 ppm, BC spiked with THP extract (w/v); (D) 0.1-100 ppm, BC spiked with hazelnut cookie (HC) (w/w); (E) Calibration curve for [A; blue circle], [B; red square] and [C; green triangle] where hollow circles represent the signal at 0 ppm; (F) Calibration curve for D, red circle represents 0 ppm measurement.*

In Figure 6, high-concentration effects (1000 and 100 ppm) are evident. Even with the faster extraction time and ULOC-dilution concentration-dependent effects still occur, affirming the necessity to dilute allergen samples before analysis [27, 28]. For consumer testing, the loss of the control line could be problematic and some tests have additional target lines to limit this [1]. Here, we included a reference well in the ULOC pre-containing RB and CNP-mAb for a blank control. The consumer can use this to directly compare the appearance of the test and control lines in real-time. Of course, in a dedicated smartphone-app, any human error would be avoidable triggering an alert when the LFIA falls outside normalcy.

### 3.4.3. Incurred Multi-Allergen Screening

Sample type D2 (HC and PC in BC (w/w); see SI Figure S8) demonstrates the prototype's effectiveness for simultaneously co-extracting and detecting unrelated processed allergens. Both analytes were detectable at 0.1 ppm (n=3, RSD ± 2.5% and 1.6% for hazelnut and peanut, respectively). There is a slightly lower deviation in multiplex measurements owing to increased control

line stability, from using two different CNP-mAbs compared with singleplex analysis. The sensitivity is even better than when using the same LFIAs to detect THP and TPP spiked into BC extract (v/v, LOD 0.5 ppm)[24] and has similar or greater sensitivity compared to other LFIAs [27, 28].

### 3.5. Consumer Diagnostics Potential

To demonstrate the consumer-operability, the prototype was tested by an independent person with no scientific or technical background. According to the European Citizen Science Association (ECSA), citizen science should involve generating new knowledge that is beneficial to both the citizen and the researcher with results being made public through open access [30]. We provided the participant with a blank and spiked cookie and two vials with pre-contained volumes of RB, 4 LFIAs, and the prototype immunosensor. Following a 5-minute explanation and using the pictogram-based standard operating procedure (see Figure 3), the participant performed the assay (n=2; see SI Figure S9). He then placed the ULOC in the device holder, inserted the reference and test LFIAs, and recorded the result on the smartphone. The participant differentiated between the positive and negative results for the spiked and blank samples, signifying the early prototype is already is operable by non-skilled individuals after only a short explanation.

## 4. Conclusions

The reported immunosensor allows for solid sample preparation, protein extraction, dilution, delivery, detection, and smartphone readout of multiple allergens in bakery products. The detachable homogenizer efficiently co-extracts and filters two major but distinct allergens from solid samples in record time. Extracted liquid sample can be actively injected into the ULOC where it is mixed with RB for arbitrary sample dilution, and with labeled bioreagents before delivery to the detection chamber. This pipette-free dilution limits the occurrence of false-negative results in LFIA. Real-time results are automatically readable as they develop on the phone screen. While results can be read within 5 minutes, the results are optimum after 15. The interchangeable LFIA cartridge means the reported system with ULOC-enabled sample dilution can easily be applied to test different LFIAs targeting various food contaminants, affirming the value of such a simplified, adjustable, and multifunctional system.

The immunosensor is inexpensive, with current material costs of less than 1$/USD. The prototype is already consumer-operable, and advancements will continually improve the usability of the system. The presented handheld system is an encouraging development for affordable, simplified multiplex consumer immunodiagnostics.

## Conflicts of interests

There are no conflicts to declare


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**Supplementary Information:**

# Interconnectable solid-liquid protein extraction and chip-based dilution for multiplexed consumer immunodiagnostics

Georgina M.S. Ross[a], Daniel Filippini[b] , Michel W.F. Nielen[a,c] & Gert IJ. Salentijn*[a,c]

a. Wageningen Food Safety Research (WFSR), Wageningen University & Research, P.O. Box 230, 6700 AE, Wageningen, The Netherlands.

b. Optical Devices Laboratory, Division of Sensor and Actuator Systems, IFM – Linköping University, S58183, Linköping, Sweden.

c. Laboratory of Organic Chemistry, Wageningen University, Helix Building 124, Stippeneng 4, 6708 WE, Wageningen, The Netherlands

**Table S1.** Blank biscuit and allergen biscuit ingredients

| Sample | Ingredients | Allergen Information |
|---|---|---|
| Blank Cookie | Wheat flour, Sugar, palm oil, salt, glucose–fructose syrup, raising agent, (ammonium carbonate [E503]), natural flavor | **Contains:** wheat gluten<br><br>**May contain** milk, sesame |
| Hazelnut Cookie | Whole grains, oat flakes, wheat flour, sugar, vegetable oil, glucose fructose syrup, raising agents, salt, emulsifiers, cane sugar molasses | **Contains:** Hazelnut (10.8%), gluten<br><br>**May contain** milk, egg and sesame |
| Peanut Cookie | Wheat flour, sugar, vegetable oil, center, salt, milk proteins, invert sugar syrup, dextrose, aroma | **Contains:** Peanut (25%), egg, milk proteins<br><br>**May contain** gluten containing grains, nuts |

**Table S2.** Effect of different incubation times (minutes) on the protein concentration (mg/mL) in the extract of whole raw hazelnut (*n=3*)

| Extraction time | 1st | 2nd | 3rd | %RSD |
|---|---|---|---|---|
| 1 | 3.94 | 3.89 | 4.02 | 1.5 |
| 2 | 4.64 | 4.52 | 4.80 | 3.0 |
| 3 | 5.58 | 6.01 | 5.49 | 4.9 |
| 5 | 6.59 | 6.65 | 6.72 | 0.9 |
| 10 | 12.42 | 13.03 | 12.51 | 2.5 |
| 20 | 14.68 | 13.98 | 14.60 | 2.6 |
| 30 | 15.11 | 15.25 | 14.90 | 1.1 |

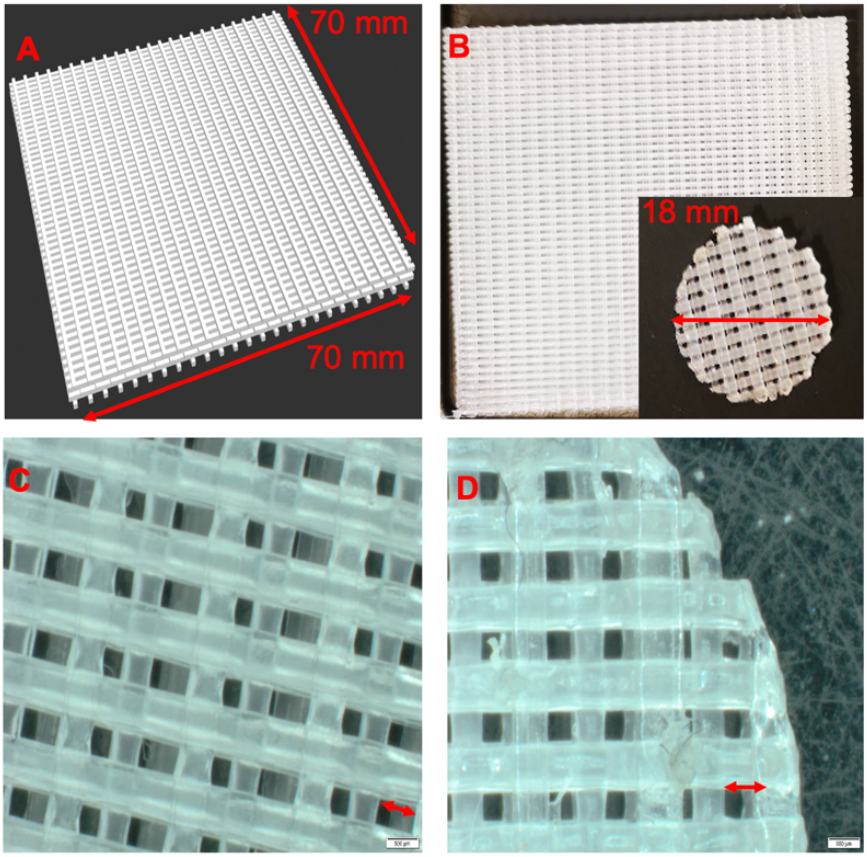


***Figure S1.*** *3D-printed sieves. (**A**) Computer aided design (CAD) of sieve sheet. (**B**) 3D-print of the sieve sheet. Insert showing the 18 mm diameter laser cut sieves. (**C**) Microscope image of sieve, scale bar (500 µm) in bottom right hand corner and indicated by red arrow. (**D**) Microscope image of edge of laser cut sieve, scale bar (500 µm) in bottom right hand corner and indicated by red arrow*

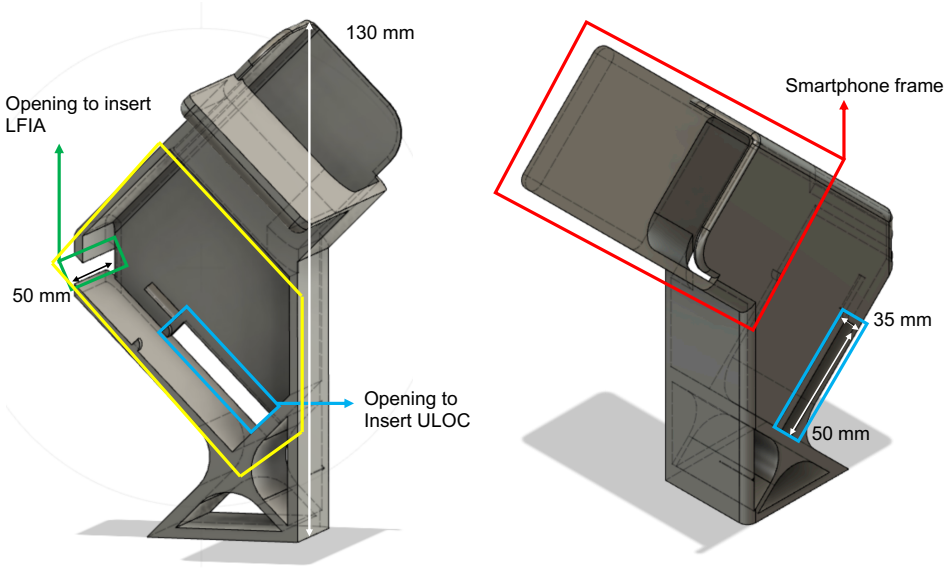


***Figure S2.*** *Computer aided design (CAD) image for smartphone device holder. Slot to insert ULOC is outlined in blue. The frame for clamping the smartphone to is outlined in red. The area to insert LFIA cartridge is outlined in green. The area which is closed by the door is outlined in yellow.*

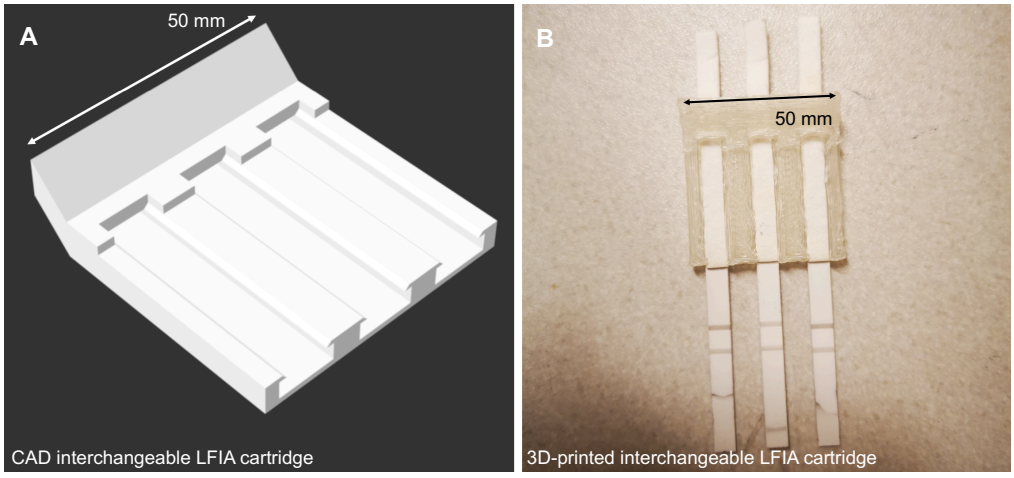


***Figure S3.*** *LFIA cartridge. (**A**) Computer aided design (CAD) of 50 mm LFIA cartridge which houses 3 LFIAs of 4 or 5 mm wide. (**B**) 3D-printed LFIA cartridge with 3 LFIAs inserted.*


***Figure S4.*** *Unibody Lab on a Chip (ULOC) devices showing loading of dye to (**A**) 10 µL mark (n=2) (**B**) 5 µL mark (n=2)*

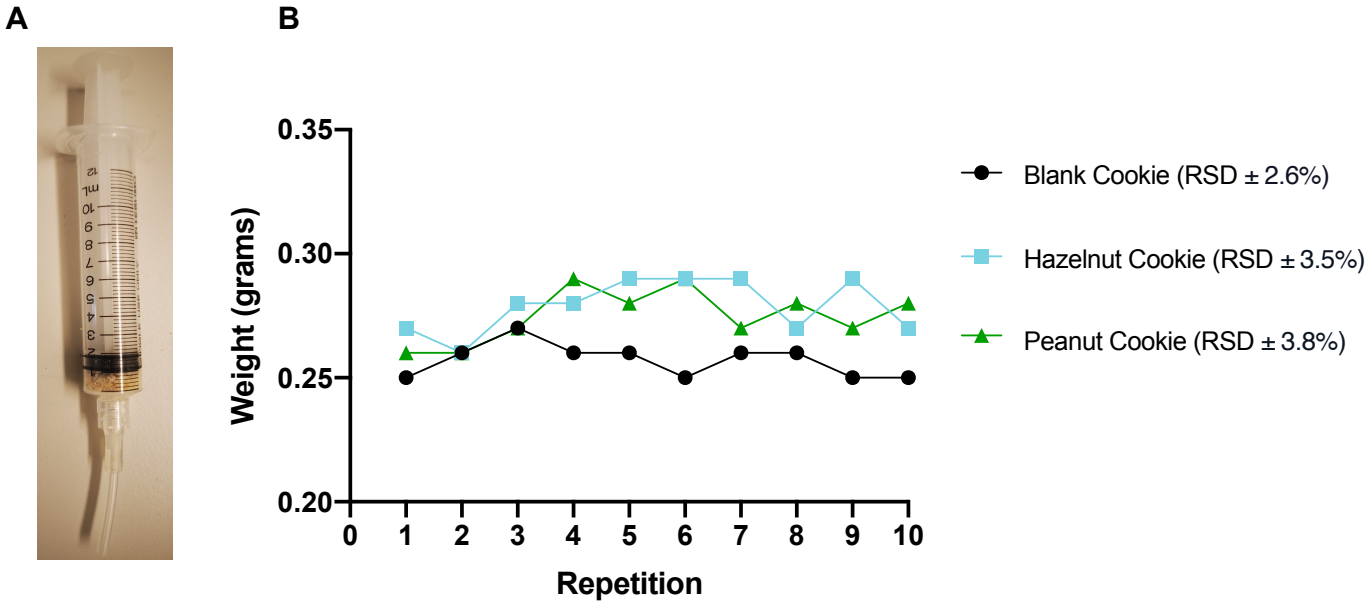


***Figure S5.*** *Filling the homogenizer syringe to 1 mL mark with cookie (**A**) photo of syringe filled to 1 mL with blank cookie. (**B**) Graph showing the variation in weight from filling the syringe to 1 mL mark with blank cookie (black), hazelnut cookie (blue) and peanut cookie (green).*

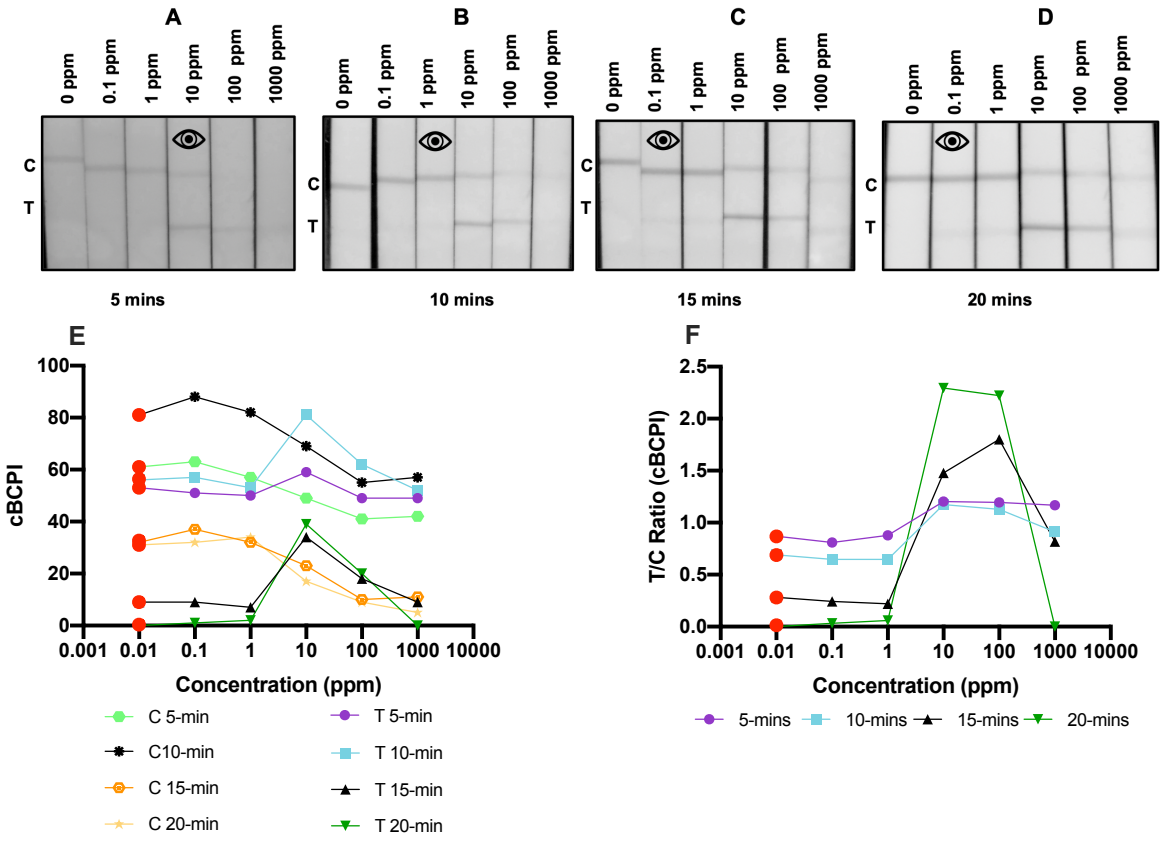


***Figure S6.*** *End point images and calibration curves showing development of LFIAs tested in an increasing concentration of total hazelnut protein (THP) extract in blank cookie (BC) extract in the range of 0.1-1000 ppm, C represents the control line and T represents the test line, the eye symbol represents the LOD at that given time point. All intensities are measured in the blue channel of RGB corrected by subtracting the background response of a blank test. (**A**) LFIAs after 5 minutes. (**B**) LFIAs after 10 minutes. (**C**) LFIAs after 15 minutes. (**D**) LFIAs after 20 minutes. (**E**) Calibration curve showing the corrected blue channel pixel intensity (cBCPI) response for the control (C) and test (T) line develop of the LFIAs in S4A-D. (**F**) Calibration curve showing the test line divided by control line (T/C ratio) for the LFIAs in S4A-D and curves in S4E.*

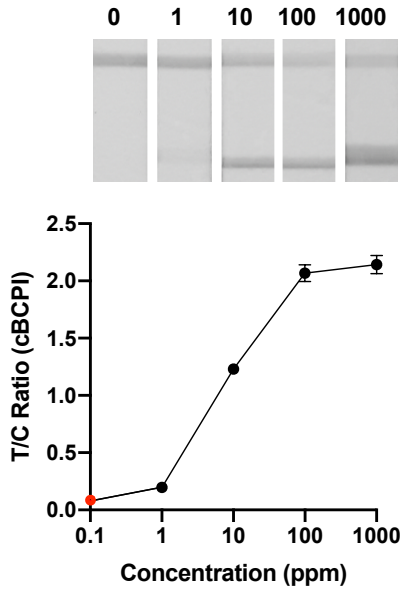


***Figure S7.*** *Calibration curve for 5 µL of total hazelnut protein (THP) in 95 µL running buffer (RB), a dilution factor (DF) of x20. Corrected blue channel pixel intensity is plotted by subtracting the blank response and dividing the test line intensity by the corresponding control line intensity value (T/C ratio). The red circle represents the T/C ratio in a blank (0 ppm) sample. Error bars represent the standard deviation (n=3).*

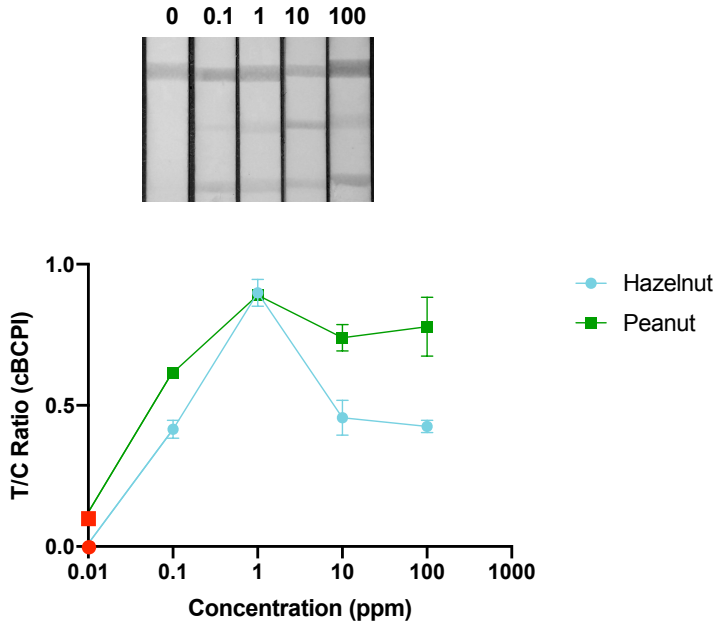


***Figure S8.*** *Multiplex calibration range for hazelnut and peanut cookie mixed with a blank cookie. (**A**) Photo of multiplex range. (**B**) Corrected blue channel pixel intensity is plotted by subtracting the blank response and dividing the test line intensity by the corresponding control line intensity value (T/C ratio). The red circle represents the T/C ratio in a blank (0 ppm) sample. Error bars represent the standard deviation (n=3).*

***Figure S9.*** *Citizen science experiments (n=2). Participant (age 15 yrs) was provided with a short demo, pictogram instruction sheet, and the prototype to perform experiments.*