Supplementary Information:

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

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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 **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 **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 **May contain** gluten containing grains, nuts
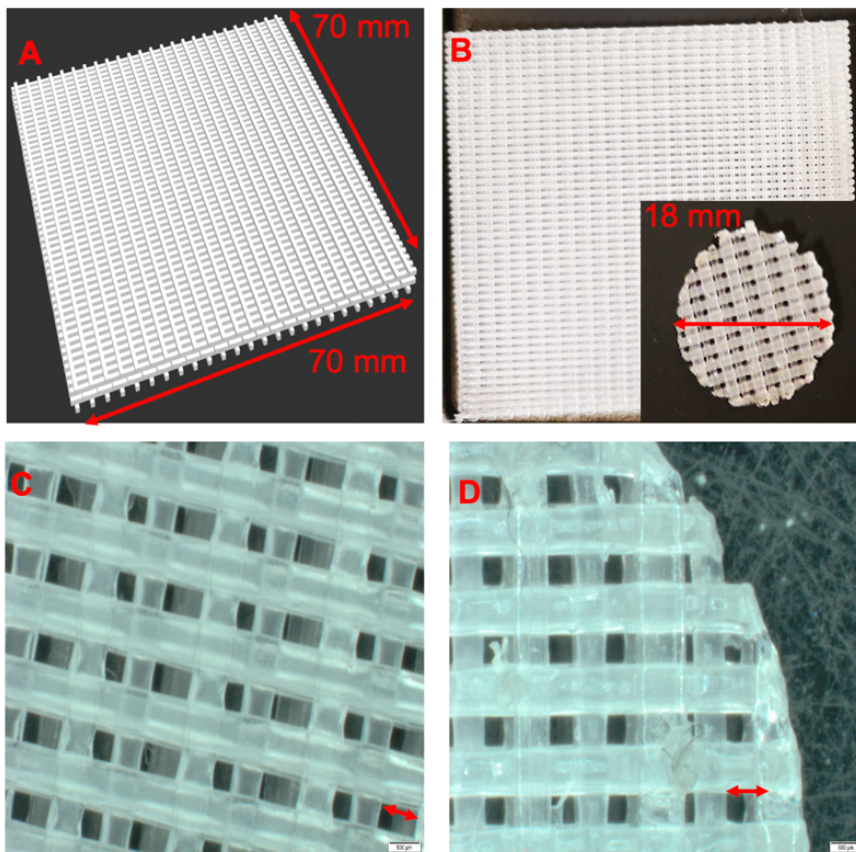
Figure S1. 3D-printed sieves. (*A*) Computer aided design (CAD) of sieve sheet. (*B*) 3D-print of the sieve sheet. Insert showing the 18 mm diameter laser cut sieves. (*C*) Microscope image of sieve, scale bar (500 μm) in bottom right hand corner and indicated by red arrow. (*D*) Microscope image of edge of laser cut sieve, scale bar (500 μm) in bottom right hand corner and indicated by red arrow.
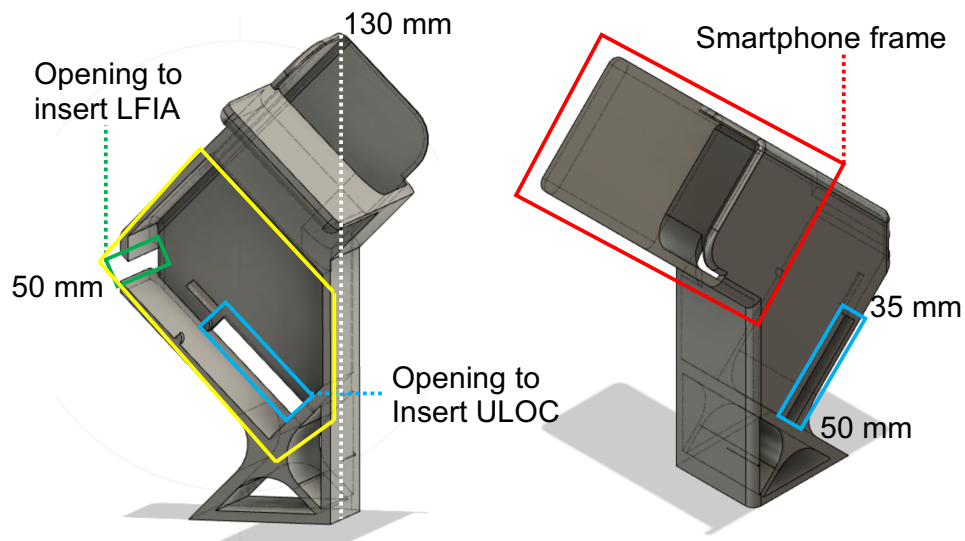
Figure S2. *Computer aided design (CAD) image for smartphone device holder. Slot to insert ULOC is outlined in blue. The frame for clamping the smartphone to is outlined in red. The area to insert LFIA cartridge is outlined in green. The area which is closed by the door is outlined in yellow.*
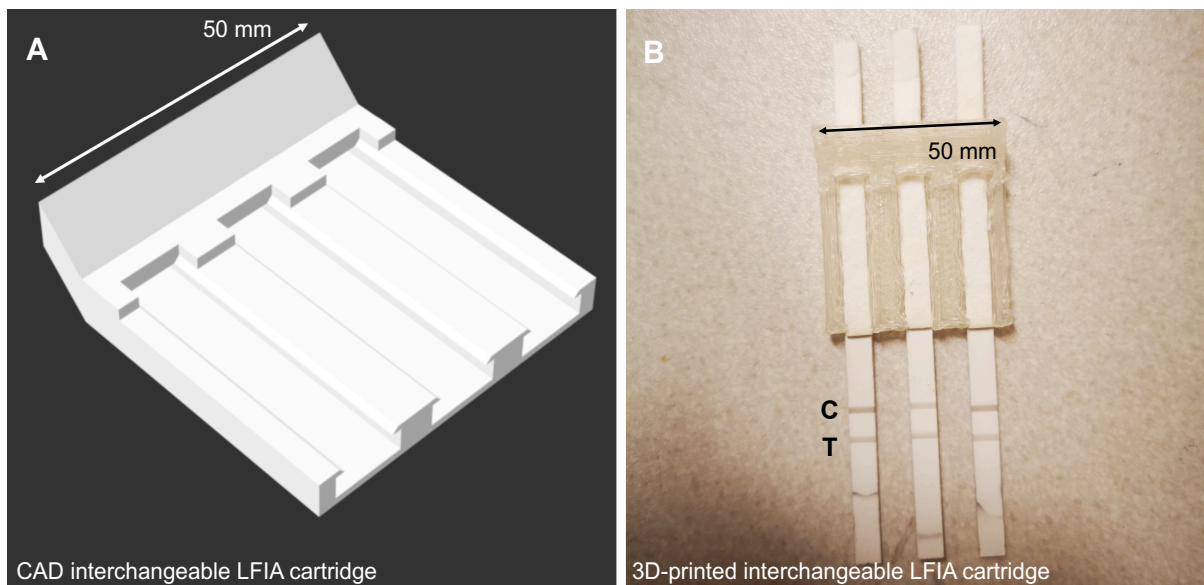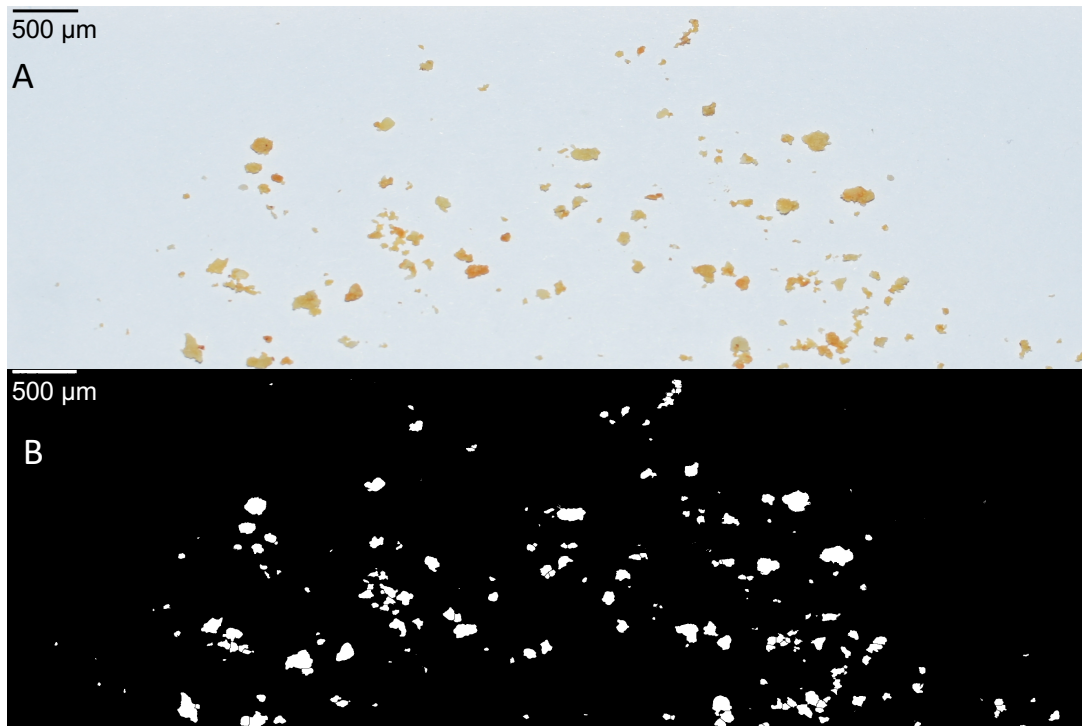

Figure S3. *LFIA cartridge. (**A**) Computer aided design (CAD) of 50 mm LFIA cartridge which houses 3 LFIAs of 4 or 5 mm wide. (**B**) 3D-printed LFIA cartridge with 3 LFIAs inserted.*

Protocol S1. Procedure for particle analysis using ImageJ.

1. Open image of crushed sample in ImageJ (**A**)
2. Set scale. A line was made along the scale of a ruler (5 mm) in the image and **Set Scale** function [Analyze>Set Scale] was selected.
3. Region of Interest (ROI). Using the **Rectangular** tool, a fixed region excluding the scale bar was selected.
4. Crop image. The image was cropped [Image>Crop] based on the defined rectangular ROI.
5. Make image binary. The cropped image was transformed into a binary image (**B**) [Process>Binary>Make Binary]. This makes the outline of the particle visible.
6. Apply **Fill Holes** [Process>Binary>Fill Holes]. The hollow particles are turned into solid particles.
7. Apply **Watershed** [Process>Binary>Watershed]. This breaks closely located particles.
8. Apply **Particle Analysis** [Analyze>Analyze Particles]
9. Copy result of particle sizes to spreadsheet.

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

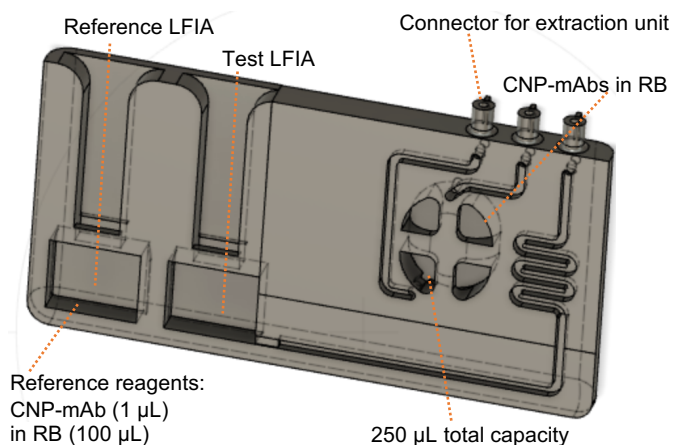| Extraction time	1st	2nd	3rd	%RSD
1	3.94	3.89	4.02	1.5
2	4.64	4.52	4.80	3.0
3	5.58	6.01	5.49	4.9
5	6.59	6.65	6.72	0.9
10	12.42	13.03	12.51	2.5
20	14.68	13.98	14.60	2.6
30	15.11	15.25	14.90	1.1

Figure S4. Annotated computer aided design (CAD) of the ULOC device indicating where the reference and test LFIAs are inserted, where the extraction unit connects to, where the carbon-nanoparticle labelled antibodies (CNP-mAbs) and running buffer (RB) are pre-loaded and where the reference reagents are pre-loaded.
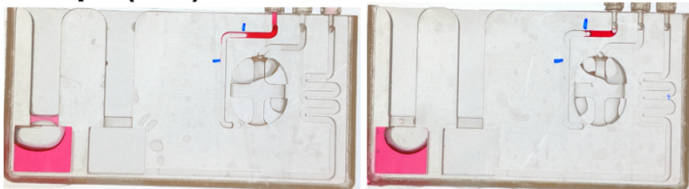
A. 10 µL (n=2)
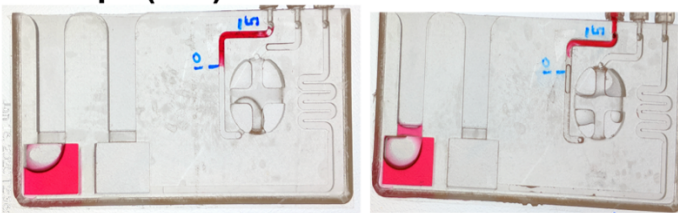
B. 5 µL (n=2)

Figure S5. Unibody Lab on a Chip (ULOC) devices showing loading of dye to (*A*) 10 µL mark (n=2) (*B*) 5 µL mark (n=2).
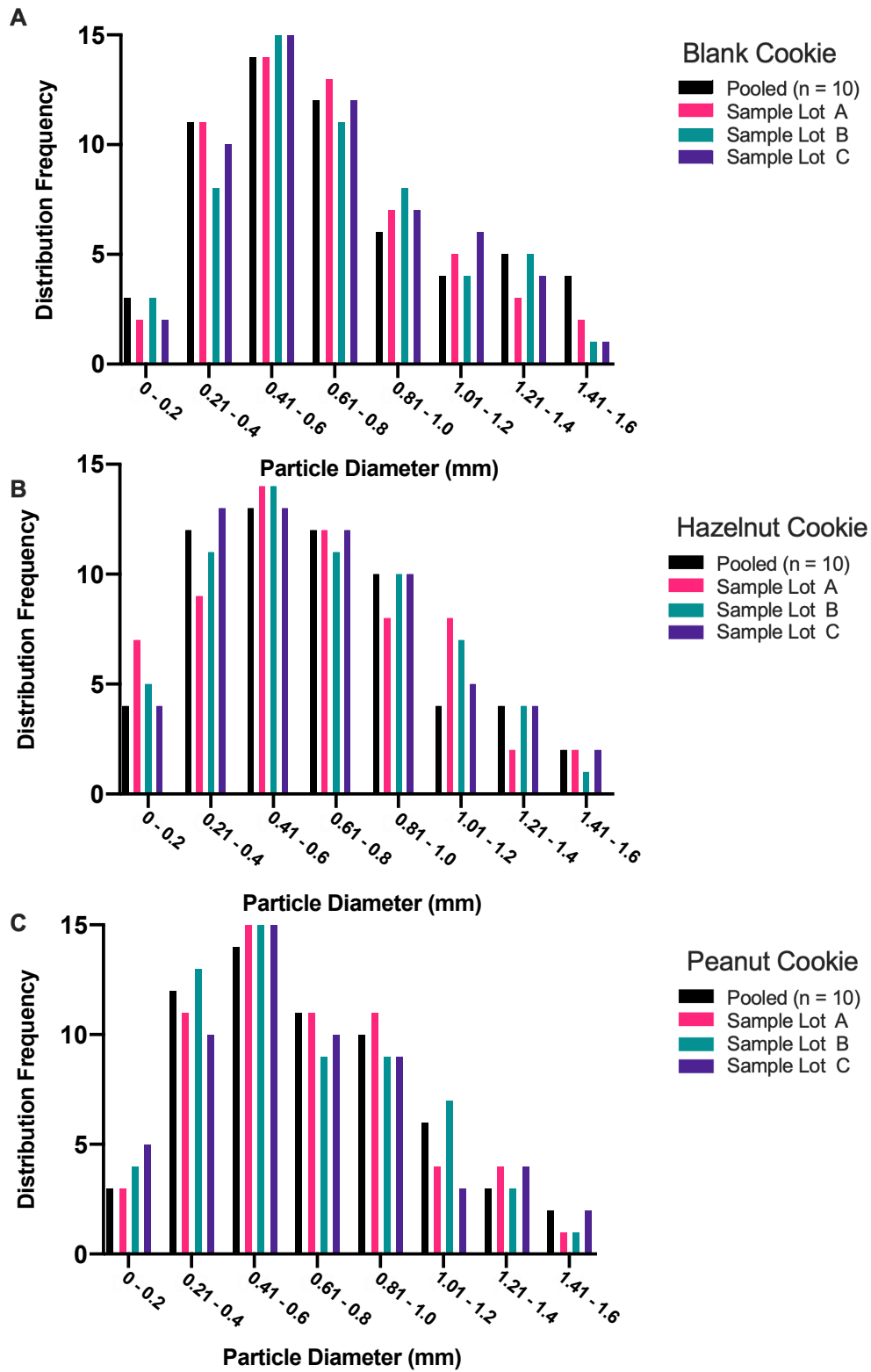
Figure S6. *ImageJ Particle size distribution of 3 varieties of cookie sample (**A**) blank, (**B**) hazelnut and (**C**) peanut cookies, crushed by the homogenizer unit, where the black bars represent the size distribution from a pooled sample (n=10), and the pink, teal and purple bars represent 3 individual homogenizations.*
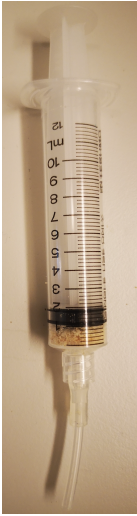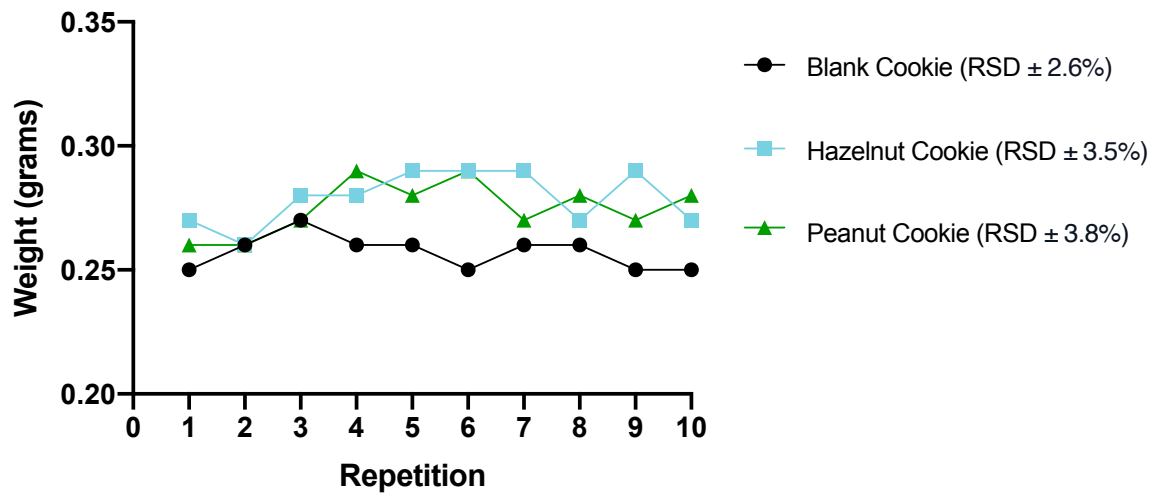
A

B

Figure S7. *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).*
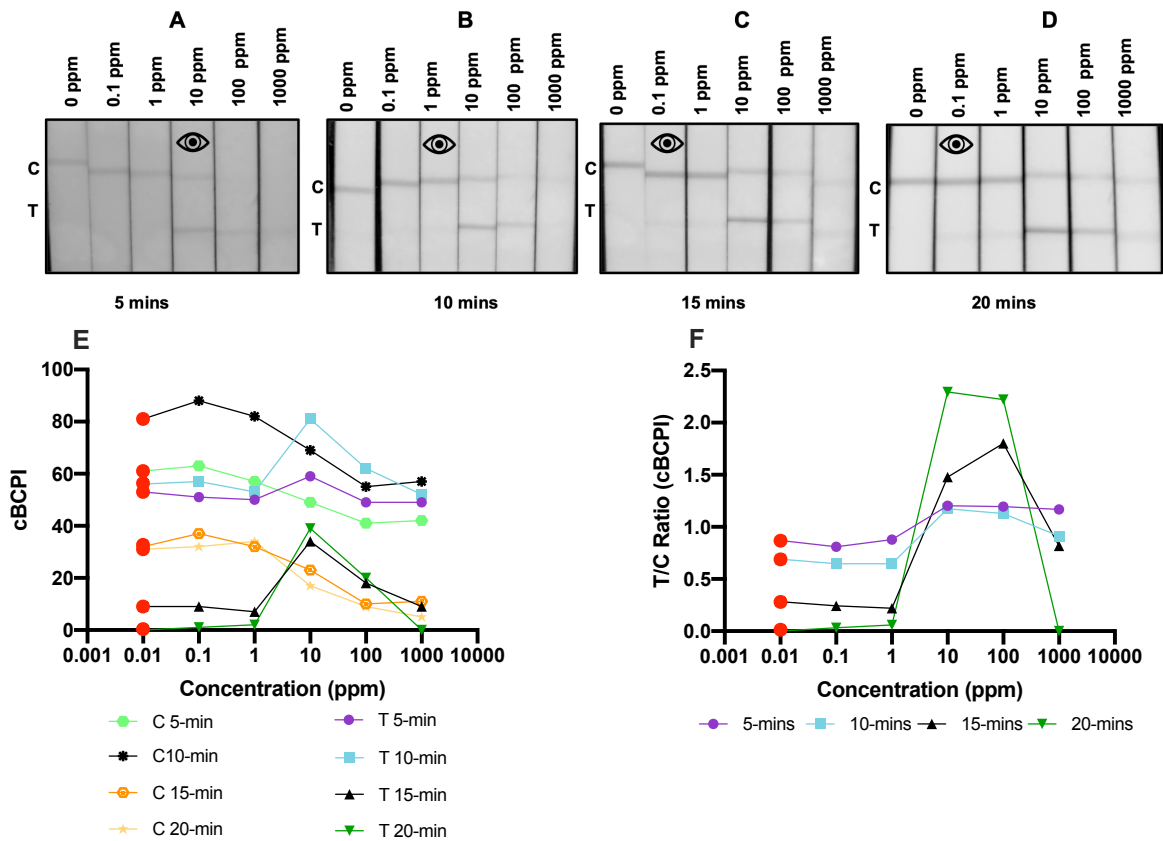
Figure S8. *End point images and calibration curves showing development of LFIAs tested in an increasing concentration of total hazelnut protein (THP) extract in blank cookie (BC) extract in the range of 0.1-1000 ppm, C represents the control line and T represents the test line, the eye symbol represents the visual 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 development (**F**) Calibration curve showing the test line divided by control line (T/C ratio) development.*
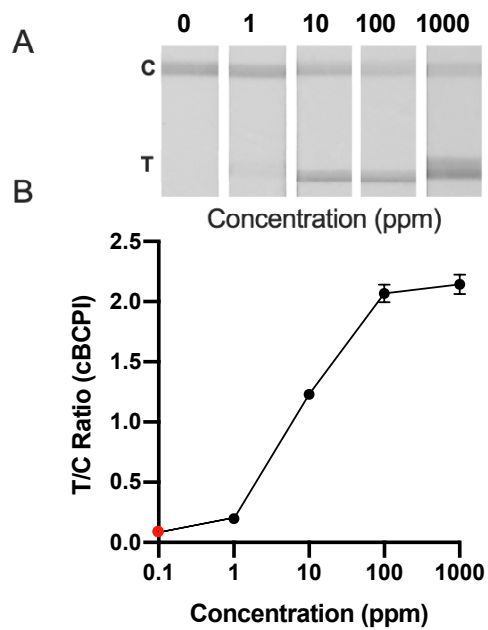
Figure S9. *Calibration curve for 5 µL of total hazelnut protein (THP) in 95 µL running buffer (RB), a dilution factor (DF) of x20. Corrected blue channel pixel intensity is plotted by subtracting the blank response and dividing the test line intensity by the corresponding control line intensity value (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 S10. *Citizen science experiments (n=2). Participant (age 15 years) was provided with a short demo, pictogram instruction sheet, and the prototype to perform experiments.*