

# Sensors & Diagnostics

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. Ortiz-Martinez, K. Kourentzi, R. Willson, M. Rito-Palomares and M. González-González, *Sens. Diagn.*, 2026, DOI: 10.1039/D6SD00062B.



This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

## ARTICLE

**Development of a fluorescent lateral flow test for the detection of the metabolic biomarker leptin in human serum**Margarita Ortiz-Martinez,<sup>a,b</sup> Katerina Kourentzi,<sup>a</sup> Richard C. Willson,<sup>a,b,c</sup> Marco Rito-Palomares,<sup>b,c</sup> and Mirna González-González.\*<sup>b,c</sup>eReceived 00th January 20xx,  
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Body mass index (BMI) measurement is the primary method for diagnosing and classifying obesity and metabolic syndrome, despite limitations and variable significance across populations. Metabolic biomarkers, such as adipokines, offer more precise insights and the potential for earlier diagnosis of conditions such as gestational diabetes. Leptin, an adipokine that regulates satiety and energy expenditure, directly correlates with adipose mass, and elevated leptin levels are associated with obesity. Despite its metabolic significance, no commercially available point-of-care (POC) method for leptin detection exists. We developed a sandwich Lateral Flow Immunoassay (LFA) for leptin, immobilizing a capture antibody on a nitrocellulose membrane and conjugating fluorescent europium(III) chelate polystyrene particles to a detection antibody. The LFA prototype successfully detected recombinant human leptin spiked in buffer and 50% calf serum across a range of 0.25–100 ng/mL, with an estimated limit of detection (blank + 3.3 $\sigma$ ) of 0.25 ng/mL in a benchtop and a portable Europium fluorescence reader in both matrices. Additionally, the LFA prototype detected endogenous leptin in samples containing 25% human serum at concentrations as low as 0.21 ng/mL. Signal intensity ratios between test and control lines showed a strong correlation with leptin concentration measured by ELISA (Pearson correlation coefficients for the entire sample set (n=30) were r=0.96 and r=0.95 (both p < 0.0001) for the benchtop and portable reader, respectively). The LFA's range and sensitivity enable the detection of endogenous leptin at physiologically-relevant concentrations. These findings highlight the potential for developing robust LFAs for the detection of leptin and other metabolically-relevant biomarkers in point-of-care settings.

**Introduction**

Metabolic alterations and obesity, mainly diagnosed by a body mass index (BMI) of 30 kg/m<sup>2</sup> or higher, can drive the onset of chronic diseases like Type 2 diabetes; together, they represent a leading global health challenge. Their prevalence has continuously increased and has reached pandemic status globally despite extensive efforts by health organizations and governments.<sup>1–4</sup> According to the World Health Organization, in 2022, 43% of adults were overweight (BMI  $\geq$  25 kg/m<sup>2</sup>), and 16% were living with obesity (BMI  $\geq$  30 kg/m<sup>2</sup>), and among children and adolescents (ages 5 to 19), the prevalence of overweight and obesity was 20% and 8% respectively.<sup>5</sup> The increase in the coming years is expected to be even more accelerated, especially among children and adolescents, as a result of lifestyle changes.<sup>1</sup> This trend is particularly evident in low and middle-income countries with underdeveloped health

systems.<sup>1,3,6</sup> Beyond its direct impact on mortality, morbidity, disability, and quality of life, obesity-associated comorbidities such as cardiovascular disease, several types of cancers, and Type 2 diabetes place immense pressure on healthcare systems and create a severe economic burden.<sup>7–9</sup> The global economic impact of overweight and obesity was estimated to be 2.4% of gross domestic product (GDP) in 2020 and is estimated to increase to 3% by 2035.<sup>7–9</sup> The required global investment for the prevention and early treatment of obesity and its complications is significantly lower than the cost of treating obesity-associated comorbidities.<sup>1,4,10</sup> According to OECD figures, for every dollar invested in obesity prevention, there is a return of up to six dollars.<sup>10</sup> New tools for determining body composition auxiliary to BMI have been proposed and validated recently, including anthropometric measurements (waist circumference, waist-to-hip ratio, among others), electrical impedance analysis (such as total body fat, fat-free mass), densitometry, and imaging-based methods.<sup>2,11</sup> According to the Lancet Diabetes & Endocrinology Commission on Clinical Obesity: “The greatest opportunities for further health improvements lie in targeted efforts that address specific risk factors contributing to metabolic risks”.<sup>2</sup> This emphasizes the need for metabolic biomarkers, used alongside conventional tools to provide reliable information on metabolic health status. Monitoring metabolically-relevant biomarkers at point-of-care (POC) has gained attention as a promising approach for early

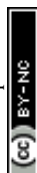
<sup>a</sup> Department of Chemical and Biomolecular Engineering, University of Houston, Houston, TX, USA.

<sup>b</sup> Tecnológico de Monterrey, Institute for Obesity Research, Monterrey, Nuevo León, México.

<sup>c</sup> Tecnológico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey, Nuevo León, México.

\*Corresponding authors E-mail: [mirnagonzalez@tec.mx](mailto:mirnagonzalez@tec.mx)

Supplementary Information available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x



detection and monitoring of metabolic alterations suitable for mass screening.<sup>12,13</sup> Adipokines, hormones secreted mainly by adipose tissue that mediate metabolism and immune response, are particularly interesting due to their correlation with metabolic alterations.<sup>12,14,15</sup> Leptin, the most extensively studied adipokine, plays a key role in regulating satiety and energy expenditure via the hypothalamus.<sup>11,12,16,17</sup> At the center of energy metabolism control is an interplay of three hormones, leptin, ghrelin, and insulin, maintaining the energy balance by regulating food intake and energy expenditure.<sup>18–20</sup> Elevated leptin levels are associated with obesity and other metabolic alterations.<sup>21–23</sup> The relevance of leptin in altered metabolic states has been reported across multiple populations.<sup>19,24–30</sup> Visceral adipose tissue reduction induced by exercise, even when metabolic dysregulation is already present, provides benefits by decreasing leptin levels, increasing circulating adiponectin, and reducing inflammatory cytokines, thereby modulating systemic inflammation.<sup>31,32</sup> Hyperleptinemia is often accompanied by proinflammatory signals associated with metabolic comorbidities such as cardiovascular diseases<sup>32,33</sup> and type 2 diabetes.<sup>21,34</sup> Leptin also plays a role in modulating immune system responses, vascular function, and reproduction.<sup>35</sup> It has been considered a biomarker for breast cancer,<sup>36–38</sup> gynecologic malignancies,<sup>37</sup> and hepatocellular cancer.<sup>39,40</sup> Although strong evidence links leptin to these comorbidities, its clinical utility for routine metabolic health monitoring has yet to be fully realized. Currently, ELISA is the gold standard for leptin determination, but it is performed in centralized laboratories, requires specialized equipment and personnel, and is not commonly included in routine health examinations. The reported physiological range of leptin in adult human serum goes from 4 to 13 ng/mL in individuals of healthy weight and from 15 to 42 ng/mL in individuals with overweight or obesity (Fig. S1 in SI).<sup>19,24–30</sup> These studies show a high inter- and intrapopulation variability in leptin levels. There is no commercially available method for point-of-care (POC) leptin detection, enabling rapid, affordable, and accessible determination of this biomarker.

Lateral flow immunoassays (LFAs) are promising options for POC biomarker detection due to their portability, low cost, and versatility.<sup>41–43</sup> and can be used in remote areas without requiring specialized personnel, equipment, or facilities, making them suitable for POC detection platforms.<sup>42,43</sup> Widely used for infectious disease detection, LFAs are increasingly being adapted for detecting non-communicable disease biomarkers.<sup>44–46</sup> Recent innovations, such as fluorescent reporters and portable readers, have significantly enhanced their sensitivity and enabled quantitative measurements at POC.<sup>47–49</sup> In this study, we developed a highly sensitive, easy-to-use fluorescence-based LFA using carboxylate-modified europium (III) chelate polystyrene particles as labels for leptin detection in serum samples in an immunosandwich format and evaluated its performance by measuring spiked recombinant leptin in buffer and calf serum, as well as endogenous leptin present in human serum samples, using both a benchtop time-resolved fluorescence reader and a portable Europium LFA reader.

## Experimental

### Materials and instrumentation

LFA components included a nitrocellulose membrane (CN95, Sartorius), a glass fiber membrane as the sample pad (8980, Ahlstrom), and a cotton/glass blend pad as the absorbent pad (440, Ahlstrom), assembled on plastic backing cards (L-P25, MDI). Buffer components: PBS tablets, Tris base, Tris hydrochloride, Bovine Serum Albumin (BSA), Tween-20, PEG 3350, Sucrose, 2-(N-morpholino)ethanesulfonic acid (MES), sodium hydroxide, and hydrochloric acid solutions were purchased from Millipore Sigma. Carboxylate-modified Europium (III) chelate polystyrene particles (0.19  $\mu\text{m}$ , Excitation: 365 nm, Emission: 610 nm, FCEU002) were purchased from Bangs Laboratories. Goat anti-mouse IgG antibodies were acquired from Arista biologics (ABGAM-0500). Heat-inactivated calf serum (S11210H), mouse anti-human leptin antibodies MAB398 (Clone 44802 validated for capture in ELISA) and BAM398 (Clone 44804 validated for detection in ELISA), recombinant human leptin (CF 398-LP), and Human Leptin Quantikine QuickKit ELISA (QK398) were purchased from R&D Systems. EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) for activation of carboxylated particles was obtained from Thermo Fisher Scientific, and N-Hydroxysuccinimide (NHS) from Millipore Sigma. Equipment used included a water bath sonicator (BRANSON 2800, Branson Ultrasonics Corp., Connecticut, USA), a tube rotator (RT-50, Cole-Parmer, Illinois, USA), and a refrigerated Eppendorf centrifuge (5424 R, Eppendorf, Connecticut, USA). Thirty de-identified human serum samples were acquired from the Gulf Coast Regional Blood Center, Houston, TX. Per the provider's standard safety protocols, all samples were screened for infectious agents prior to receipt and used as anonymous secondary materials. Ten samples were from individuals of unknown sex (U1-U10), ten from males (M1-M10), and ten from females (F1-F10). The samples were aliquoted, labelled, and stored at  $-20\text{ }^{\circ}\text{C}$  until use. All aqueous solutions were prepared using type I ultrapure water generated by a Milli-Q<sup>®</sup> Direct system (Merck Millipore, Massachusetts, USA).

### Conjugation of carboxylated particles with anti-human leptin antibodies

View Article Online  
DOI: 10.1039/D6SD00062B



Fifty microliters of carboxylate-modified Europium (III) chelate polystyrene particles at 1% solids were mixed with 300  $\mu\text{L}$  of 50 mM MES buffer, pH 5.8. The particles were centrifuged at  $20,000\times g$  for 10 min; the supernatant was removed, and the pellet was resuspended in 300  $\mu\text{L}$  of 50 mM MES buffer, pH 5.8, using a water bath sonicator. This process was repeated twice for a total of three washes. After the last centrifugation step, the pellet was resuspended in 93.5  $\mu\text{L}$  MES buffer. Then, 3.2  $\mu\text{L}$  of NHS (50 mg/mL) and 3.3  $\mu\text{L}$  of EDC (10 mg/mL), freshly prepared in MES buffer, were added to achieve a final solids concentration of 0.5%. The molar ratio per carboxyl group was 2.5 for NHS and 20 for EDC, calculated based on the particle acid content provided in the vendor certificate of analysis. The suspension was incubated for 30 minutes at room temperature, protected from light, with continuous mixing on a tube rotator. Following incubation, the activation solution was removed by centrifugation at  $20,000\times g$  for 10 min. The particles were washed three times with 300  $\mu\text{L}$  1 $\times$  PBS following the same resuspension and centrifugation procedure as during initial washing with MES buffer. After the final wash, the particles were resuspended in 60  $\mu\text{L}$  of 1 $\times$  PBS and mixed with 40  $\mu\text{L}$  of monoclonal mouse anti-human leptin antibodies (1 mg/mL). The amount of antibody offered was one monolayer, estimated based on prior capacity studies for IgG binding to polystyrene particles.<sup>50</sup> The suspension was incubated for 4 h at room temperature, protected from light, with continuous mixing in a tube rotator and intermediate mixing by sonication after two hours. The particles were separated by centrifugation and resuspended in 300  $\mu\text{L}$  of 4% BSA in 1 $\times$  PBS for overnight blocking with continuous mixing in a tube rotator at 4  $^{\circ}\text{C}$ , protected from light. After blocking, the particles were washed three times with 1% BSA in 1 $\times$  PBS. The particles were resuspended in 1% BSA at approximately 0.25% solids concentration. The final solids concentration was determined using fluorescence measurements in a microplate reader and adjusted to 0.125% solids with 1% BSA in 1 $\times$  PBS. The conjugated particles were stored at 4  $^{\circ}\text{C}$ , protected from light, until further use.

### LFA assembly and imaging

For strip manufacturing, a 25 mm-wide CN95 nitrocellulose membrane was assembled along the 300 mm length of the backing card and centered along its width. A 20 mm-wide glass fiber sample pad and a 22 mm-wide absorbent pad were placed, overlapping the membrane by 3 mm (Fig. 1). Antibodies at 1 mg/mL in 1 $\times$  PBS /0.25% sucrose were dispensed onto the nitrocellulose membrane using a Biodot lateral flow reagent dispenser (XYZ3060, Biodot, California, USA) at a 1  $\mu\text{L}/\text{cm}$  flow rate. Mouse monoclonal anti-human leptin antibodies (BAM398) were used for the test line, while goat anti-mouse antibodies were used for the control line. The printed cards were dried at 50  $^{\circ}\text{C}$  for 1 h in a convection oven (40AF, Quincy lab, Maryland, USA), followed by overnight drying in a desiccator chamber (Super Dry 151-21, Totech) at room temperature. The dried cards were pressed lightly with a hand roller to ensure complete and even attachment of the

membrane and pads to the backing card and then cut into 3 mm-wide strips using a guillotine cutter (ZQ2000; Kimbio Tech, Shanghai, China). Strips were stored in 50 mL tubes with desiccant sachets in a dry room at room temperature until use. After running, the strips were imaged using a FluorChem gel documentation system (Alpha Innotech Corp., California, USA) attached to a CoolSNAP K4 CCD camera (Photometrics, Blaine, controlled by Micro-manager software (<https://micro-manager.org/>)). The fluorescence intensities of the test line (TL) and the control line (CL) were determined using both a time-resolved europium fluorescence benchtop LFA reader (LTRIC-600, Lumigenex Co., Ltd., Suzhou, China) as well as a portable Europium fluorescence reader (Cube reader, ChemBio Diagnostics GmbH, Berlin, Germany) with a custom adaptor designed and 3D-printed in-house (CAD coordinates for the adapter are available online: ID6961448 <https://www.thingiverse.com/thing:6961448>). The portable reader is a small device (4 cm by 4 cm by 3.6 cm) that can operate autonomously with standard button batteries. It performs on-board data processing to provide immediate results on an integrated screen and also stores data for optional

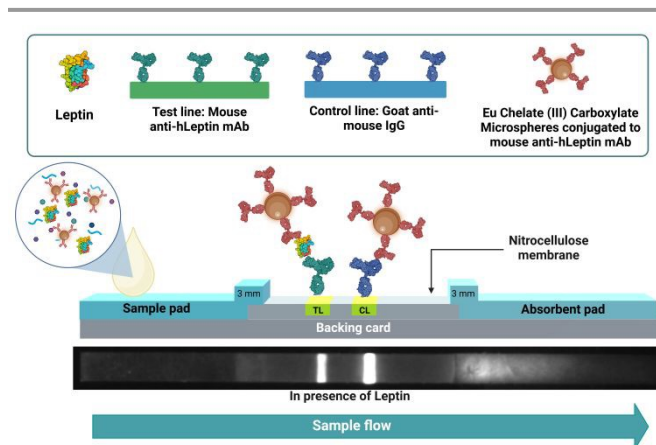


Fig. 1 Schematic representation of the LFA prototype for leptin detection. The sample containing leptin diluted in running buffer is mixed with Europium (Eu) chelate (III) carboxylate microspheres conjugated to mouse anti-human leptin monoclonal antibody. The leptin-bound particles migrate from the sample pad through the nitrocellulose membrane until they reach the test line, where they are captured by immobilized antibodies specific to leptin. The remaining particles continue migrating to the control line, where they are captured by immobilized species-specific antibodies or proceed to the absorbent pad, which collects the liquid that has travelled along the strip. The ratio of the test line (TL)/control line (CL) fluorescence intensity is related to the concentration of leptin in the sample. Created with BioRender.com.

download to a computer for archival purposes.

### Optimization of LFA design and running conditions

Antibody orientation was optimized by testing the pair in both roles: as capture antibodies on the test line and as detection antibodies on reporter particles. 30  $\mu\text{L}$  of recombinant leptin concentrations (0, 0.5, 5, and 50 ng/mL) spiked in running buffer (RB) containing 0.5% BSA, 0.25% Tween-20, and PEG3350 in 1 $\times$  PBS were mixed with 1  $\mu\text{L}$  of conjugated particles (0.125% solids) and applied to the strips. Washes with 20  $\mu\text{L}$  of RB were performed at 5 and 10 minutes, and the strips were imaged 5 minutes after each wash. The best performance was achieved



with BAM398 antibodies on the test line, and MAB398 antibodies conjugated to particles, resulting in high sensitivity, minimal nonspecific binding, and a monotonic signal-to-concentration response. This antibody orientation was chosen for the following experiments (Fig. S2 in SI). Each batch of strips and reporters was validated using a quality control protocol that included a blank and a positive (5 ng/mL) leptin standard (n=3). The coefficient of variation (CV) was calculated to verify that batch-to-batch performance remained within a predefined acceptable range (CV < 10%) before proceeding with clinical or analytical validation.

To optimize the RB for enhanced sensitivity and reduced nonspecific binding, four formulations varying in protein, surfactant, and polymer concentrations were selected based on literature and preliminary tests (Table S1 in SI). Each was tested by mixing 96  $\mu$ L of blank buffer or 5 ng/mL of recombinant leptin with 3.2  $\mu$ L of vortexed and sonicated conjugated particles (conjugated particles were always vortexed and sonicated prior to dispensing in all experiments). A 31  $\mu$ L sample was dispensed in triplicate onto strips, followed by two 20  $\mu$ L washes at 5 and 10 minutes. Fluorescence was measured five minutes after the second wash. All buffers showed low levels of nonspecific binding. Buffer A (0.5% BSA and 0.5% Tween-20 in 1 $\times$  PBS) gave the highest signal for the positive control and was selected for subsequent experiments in buffer (Fig. S3 in SI). Preliminary optimization was performed using conjugated particles at 0.125% solids, a 30  $\mu$ L wash at 10 minutes, and a final readout 10 minutes later. This protocol was used for all the experiments presented below.

#### Running buffer screening for serum samples

It is well known that the complexity of human serum can affect the performance of immunoassays. These effects can be generally mitigated by dilution in a suitable buffer. Four buffers with varying protein and polymer compositions were tested for the optimal signal-to-noise ratio when used to dilute calf serum (Table S2 in SI). 96  $\mu$ L of calf serum diluted 1:1 with 2 $\times$  RB (E, F, G or H) and spiked with 0 or 5 ng/mL of recombinant leptin was mixed with 3.2  $\mu$ L of conjugated particles (adjusted to 0.125% solids concentration). A 31  $\mu$ L aliquot of the mixture was dispensed onto triplicate strips. Following a 10-minute run, the strips were washed with 30  $\mu$ L of RB. Ten minutes after washing, the strips were imaged, and fluorescence intensity was measured. All buffers showed low nonspecific binding, but the highest signal for the positive control was obtained with buffer F (1 $\times$  composition: 0.25% BSA, 0.5% Tween-20, and 0.5% PEG3350 in 1 $\times$  PBS), which was selected for assay validation with both calf and human serum (Fig. S4 in SI).

#### Experimental validation in buffer and calf serum

The performance of the developed LFA in buffer was assessed by generating a serial dilution curve of recombinant leptin from 0 to 100 ng/mL in the selected buffer A (0.5% BSA and 0.5% Tween-20 in 1 $\times$  PBS) and in calf serum diluted to 50% by mixing in equal parts with RB F 2 $\times$  concentration (0.5% BSA, 1% Tween-20, and 1% PEG 3350 in 1 $\times$  PBS). For each leptin concentration

in both buffer and calf serum 50% and ran on the LFA following the procedure previously described in the buffer screening section.

#### Determination of leptin in human serum samples by ELISA

Leptin levels in the 30 clinical serum samples were measured using a commercial ELISA kit (Human Leptin Quantikine QuickKit ELISA, R&D QK398), with a reported sensitivity of 1.0 pg/mL, according to the manufacturer's instructions. During the ELISA, the plates were washed with a Hydroflex automatic plate washer, and absorbance was measured with an Infinite m200PRO reader (Tecan, Giessen, the Netherlands). All samples were analysed in triplicate. The analysis was repeated once for samples F8 and M6 due to a CV exceeding 10%.

#### Clinical validation with human serum samples

##### Human serum serial dilution

A human serum sample (U7) was diluted two-fold to 50, 25, 12.5, 6.25, 3.125, and 0% in RB F to assess the optimal dilution of human serum. Each dilution was tested in triplicate on LFA strips following the protocol described in the experimental validation section. The 25% dilution of human serum was selected because it allowed detection of samples with low leptin concentrations (0.83 ng/mL) and minimal variability (CV of 3.7% in the benchtop reader and 5.0% in the portable reader).

##### LFA-based detection of endogenous leptin

The 30 human serum samples were assayed in triplicate on the LFA strips at a 25% dilution in 2 $\times$  RB and 1 $\times$  PBS. The final concentrations of the RB components in the sample were 0.25% BSA, 0.5% Tween-20, and 0.5% PEG 3350. The assay followed the previously described protocol in the experimental validation section.

##### Statistical analysis

All experiments were performed in triplicate. Concentrations and test line/control line (TL/CL) ratios are presented as means  $\pm$  standard deviation. ANOVA followed by Tukey's post-hoc test was used to identify significant differences between means, with a significance level set at  $\alpha = 0.05$  for buffer screening experiments. A 4-parameter logistic (4PL) sigmoidal curve was used to model the relationship between leptin concentration and TL/CL ratio in the assay for both buffer and calf serum serial



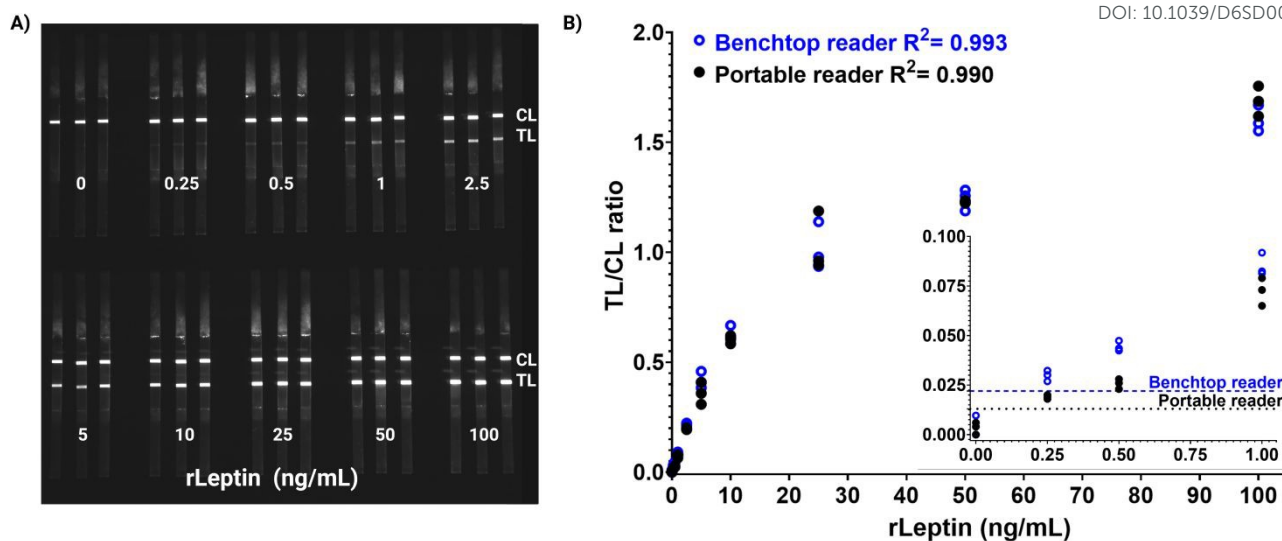


Fig. 2 Serial dilution curve of recombinant leptin from 0 to 100 ng/mL in the selected buffer (0.5% BSA and 0.5% Tween-20 in 1× PBS). A) Grayscale images of LFA strips captured using a FluorChem gel documentation system. In the blanks, the absence of fluorescence on the test line demonstrates low nonspecific binding of the LFA in absence of leptin. B) The graph shows the TL/CL ratio for the 10 leptin concentrations measured with both the benchtop (hollow circles) and portable readers (filled circles). The inset graph highlights the four lowest concentrations, along with the LOD cutoff. BSA: Bovine serum albumin; R<sup>2</sup>: Coefficient of determination. CV: Coefficient of variation; CL: Control line; LFA: Lateral flow assay; LOD: Limit of detection; PBS: Phosphate buffered saline; rLeptin: Recombinant leptin; TL: Test line.

dilutions. The ability of the LFA to detect leptin in human serum dilutions was assessed by comparing the TL/CL means against the blank using ANOVA, followed by Dunnett's post-hoc test ( $\alpha = 0.05$ ). The goodness-of-fit of the serial dilution curves was assessed using the coefficient of determination ( $R^2$ ). The limit of detection (LOD) cutoff for the LFA in buffer and calf serum was calculated using the formula:

$$\text{LOD cutoff} = \mu_{\text{Blank}} + 3.3\sigma$$

The comparative performance of the readers was evaluated using the coefficient of determination from a linear regression analysis. The ELISA values were calculated by interpolation from a 4-parameter logistic (4PL) sigmoidal standard curve. The correlation between the TL/CL ratio in the LFA and leptin concentration measured by ELISA (the gold standard method) was evaluated using Pearson's correlation coefficient. Graphing and statistical analyses were performed using GraphPad Prism 9 software (GraphPad, California, USA), and figures were created or edited using BioRender (<https://BioRender.com>, Toronto, Canada).

## Results and discussion

### Fluorescence-based LFA for leptin detection

While BMI is the most widely used parameter for diagnosing and classifying obesity, it has significant limitations, and its usefulness varies across populations.<sup>51,52</sup> A novel approach consists of monitoring metabolically-relevant biomarkers such as leptin, which is secreted mainly by adipose tissue and plays a crucial role in metabolism and immune response, with detectable alterations in its levels occurring before irreversible damage takes place.<sup>53,54</sup>

We developed a robust, sensitive, and easy-to-use POC lateral flow assay using europium (III) chelate reporter particles for the detection of leptin in serum (Fig. 1). The developed LFA showed a near-zero signal in the absence of leptin, and the fluorescence signal (TL/CL) correlated monotonically with leptin concentration (Fig. 2). Preliminary observations indicate that the LFA strips maintain functional performance for at least four months when stored at room temperature with a desiccant. While the experiments reported here utilized strips within one month of fabrication, ongoing studies are establishing formal long-term expiration limits. Initially, we observed a high fluorescent signal of conjugated europium particles on the absorbent pad and some accumulation at the overlap between the sample pad and the membrane, despite washing. Based on these observations and other preliminary experiments, we adjusted the particle concentration to 0.125% solids (1  $\mu\text{L}$  per strip), increased the wash volume to match the sample volume (30  $\mu\text{L}$ ), and extended the run time to 10 minutes from 5 to improve the flow of the particles on the strip. These conditions enabled leptin detection even at the lowest concentration tested (0.25 ng/mL in buffer and calf serum). A 25% dilution corresponds to a detection limit of 1 ng/mL, as the 4-fold dilution of the sample translates the assay's LOD of 0.25 ng/mL to 1 ng/mL in undiluted serum. This is clinically relevant given that the lowest reported mean leptin concentration, to the best of our knowledge, is  $3.01 \pm 0.58$  ng/mL in lean male samples from Algeria ( $n=70$ ).<sup>55</sup> To validate the reproducibility of the fabrication protocol, representative results from two independent batches were compared. For a 5 ng/mL leptin standard, the batches yielded ratios of  $0.566 \pm 0.03$  and  $0.528 \pm 0.03$  measured in the cube reader (CV = 6.4%), while the blank signal remained consistently low (TL/CL < 0.005).

### Running buffer selection



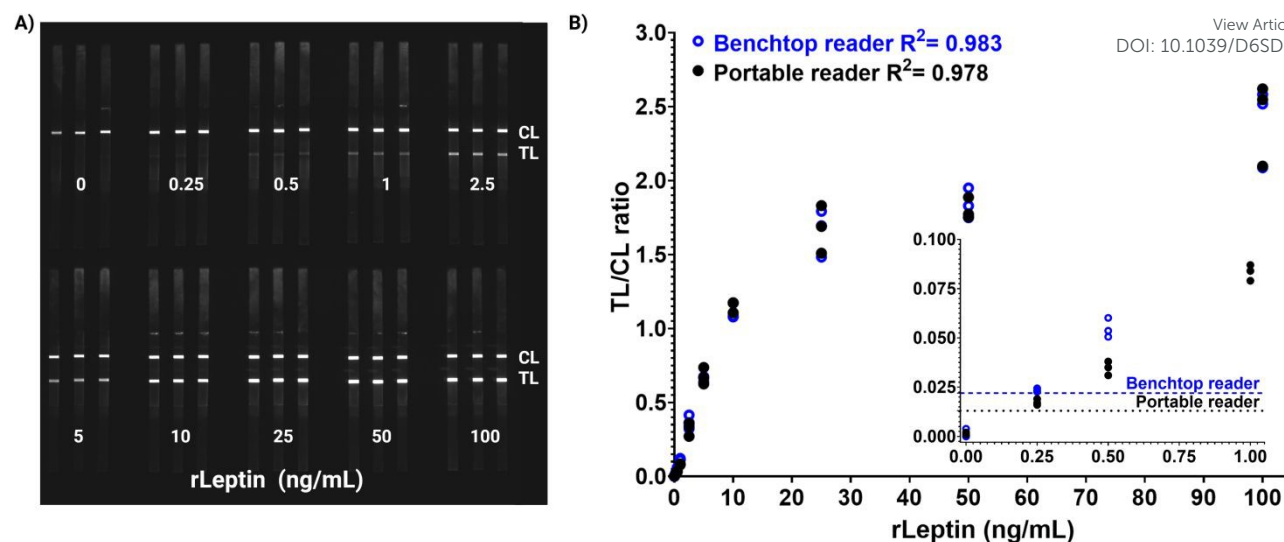


Fig. 3 Serial dilution curve of recombinant leptin from 0 to 100 ng/mL in calf serum, diluted to 50% by mixing with an equal volume of running buffer F 2x (0.5% BSA, 1% Tween-20, and 1% PEG 3350 in 1x PBS). A) Grayscale images of the LFA strips acquired using a FluorChem gel documentation system. B) The graph shows the TL/CL ratio for the 10 leptin concentrations measured with both the benchtop (hollow circles) and portable readers (filled circles). The inset graphs show the four lowest concentrations with the LOD cutoff. BSA: Bovine serum albumin; R<sup>2</sup>: Coefficient of determination; CV: Coefficient of variation; CL: Control line; LFA: Lateral flow assay; LOD: Limit of detection; PEG: Polyethylene Glycol; PBS: Phosphate buffered saline; rLeptin: Recombinant leptin; TL: Test line.

A correctly formulated RB is crucial in developing a selective, specific, reproducible lateral flow assay. The RB ensures optimal interaction between the antigen and antibodies, facilitates the flow, and reduces nonspecific binding.<sup>42,47</sup> Four different RB compositions were evaluated, consisting of BSA as a blocking agent (0.5 and 1%), Tween-20 as a detergent (0.5%), and PEG3350 (0 and 0.5%) as an additive to minimize nonspecific binding (Table S1 in SI). During the initial screening, all buffer compositions yielded fluorescence intensities near zero in the test line in the absence of leptin, with no significant differences among them. However, when recombinant leptin was spiked at 5 ng/mL, the signal for the test line was strongly present in all four buffer compositions tested, while the control line intensities remained consistent among strips (Fig. S3 in SI). Buffer A was selected as the optimal buffer due to its significantly higher TL/CL ratio in a positive sample, as determined by one-way ANOVA with Tukey's post-hoc test for multiple comparisons ( $\alpha = 0.05$ ) (Fig. S2 in SI).

Next, we focused on selecting the optimal buffer for human serum samples. Human serum naturally contains significant endogenous leptin levels, except in cases of congenital leptin deficiency.<sup>18,56,57</sup> To mimic the complexity of human serum without interference from endogenous leptin, we selected calf serum as a surrogate matrix, as the selected antibodies exhibit no cross-reactivity with calf leptin. A similar strategy has been reported previously.<sup>58,59</sup> Four buffer formulations were prepared at 2x strength and mixed in equal volumes with calf serum, resulting in final buffer compositions containing BSA (0, 0.25, and 0.5%), Tween-20 (0.5%), and PEG 3350 (0 and 0.5%) (Table S2 in SI). The LFA demonstrated low nonspecific binding in 50% calf serum without recombinant leptin and successfully detected leptin spiked at 5 ng/mL in all four buffer compositions tested (Fig. S4 in SI). The TL/CL ratio measured using both a benchtop and portable reader was close to zero for blank samples (no leptin added), with no significant differences

among the buffers. All buffers showed a strong positive signal for positive samples spiked with 5 ng/mL leptin, but buffers E and F yielded significantly higher signals by one-way ANOVA with Tukey's post-hoc test for multiple comparisons ( $\alpha = 0.05$ ). Buffer F produced a slightly higher numerical signal and was selected for both calf and human serum samples.

#### Performance of the LFA prototype in buffer and calf serum

Following RB optimization, we evaluated the analytical performance of the leptin LFA prototype using a serial dilution of recombinant leptin ranging from 100 to 0.25 ng/mL, spiked into RB A. No visible signal was observed on the test line for the blank samples. As expected, the test line intensity increased with increasing leptin concentration steadily across the tested range of recombinant leptin concentrations, as shown in the image acquired with the FluorChem (Fig. 2A). This observation was further supported by the TL/CL ratio obtained from both readers with close to zero values for the samples with no leptin added and TL/CL ratios increasing accordingly with the concentration of leptin spiked (Fig. 2B). The LFA demonstrated a high sensitivity in buffer being able to detect even the lowest concentration tested (0.25 ng/mL) in both readers. This concentration falls below the expected physiological levels, so lower concentrations were not tested. The plotted data points were fitted to a 4PL sigmoidal curve, yielding high coefficients of determination ( $R^2$ ) of 0.993 for the benchtop reader and 0.990 for the portable reader, indicating an excellent fit. Across all concentrations tested, there was low variability among replicates, with CVs of less than 11% in the benchtop reader and less than 14% in the portable reader. In the next stage, we evaluated the performance of the leptin LFA prototype in a complex biological matrix, calf serum diluted 50% with RB F, spiked with recombinant leptin. Despite the increased complexity of the calf serum, there was no evidence of nonspecific binding in samples without added leptin (Fig. 3A). A



clear increase in test line intensity was observed with increasing leptin concentration (Fig. 3B). The coefficient of determination assessed the goodness of fit, showing a strong signal-to-concentration relationship with minimal deviation compared to buffer-only conditions with  $R^2$  of 0.983 for the benchtop reader and 0.978 for the portable reader (Fig. 3B). As shown in Fig. 3B, the signals from both readers were closely related. The lowest concentration spiked in 50% diluted calf serum (0.25 ng/mL) was detectable. Across the range, low variability among replicates was observed, with CVs below 13% in the benchtop reader and below 15% in the portable reader. While the values from the blank and lowest concentration tested were similar to those from the buffer serial curve, the remaining leptin concentrations showed a higher TL/CL ratio in calf serum, which may be attributed to matrix effects. To further validate this finding, a linear regression analysis revealed a strong linear relationship between the TL/CL obtained from both readers, with determination coefficients of 0.99 in buffer and 0.98 in 50% calf serum (Fig. S5 in SI). These results confirm that, within the clinically relevant range tested, the portable reader delivers performance comparable to that of the benchtop reader, maintaining accuracy while providing portability. The differences in the TL/CL ratios observed between the two readers are systematic and can be mitigated through device-specific calibration. To ensure diagnostic accuracy during clinical measurements, each reader platform would be calibrated with its own threshold, potentially incorporating an internal standard. Furthermore, while this prototype provides specific values, it can be optimized for semi-quantitative visual or digital assessment by defining specific TL/CL cutoff values corresponding to clinical decision points.

### Endogenous leptin detection in human serum

#### Leptin levels by ELISA

For further clinical validation of our leptin LFA prototype, we tested serum samples, as leptin is typically quantified in serum or plasma. Serum is a more suitable sample for leptin detection, as plasma levels are affected by anticoagulant use and the time elapsed before separation from whole blood.<sup>60</sup> Moreover, leptin remains highly stable in serum even after storage at 4 °C for up to 4 h and undergoing six freeze-thaw cycles.<sup>60</sup> Leptin levels in all 30 human serum samples were first quantified by ELISA against a standard curve of recombinant leptin (per the supplier's protocol), with a CV lower than 10%, and a broad range of leptin content ranging from  $0.83 \pm 0.04$  to  $62.48 \pm 2.68$  ng/mL. The mean leptin level was  $16.62 \pm 15.84$  ng/mL in the unknown sex group,  $21.24 \pm 13.65$  in females, and  $14.48 \pm 17.18$  in males (Fig. S6 in SI). The distribution of leptin levels was wider in the unknown sex and male groups. In agreement with previous findings, the mean leptin level was higher in females than in males.<sup>61,62</sup> However, one male sample (M8) exhibited an unusually high leptin concentration, deviating from the male group's overall trend of lower leptin levels. Adipose tissue distribution and gene expression patterns differ between females and males due to their roles in reproduction, lactation, and other metabolic changes that occur in females during their reproductive phase.<sup>61,63,64</sup> Circulating leptin levels

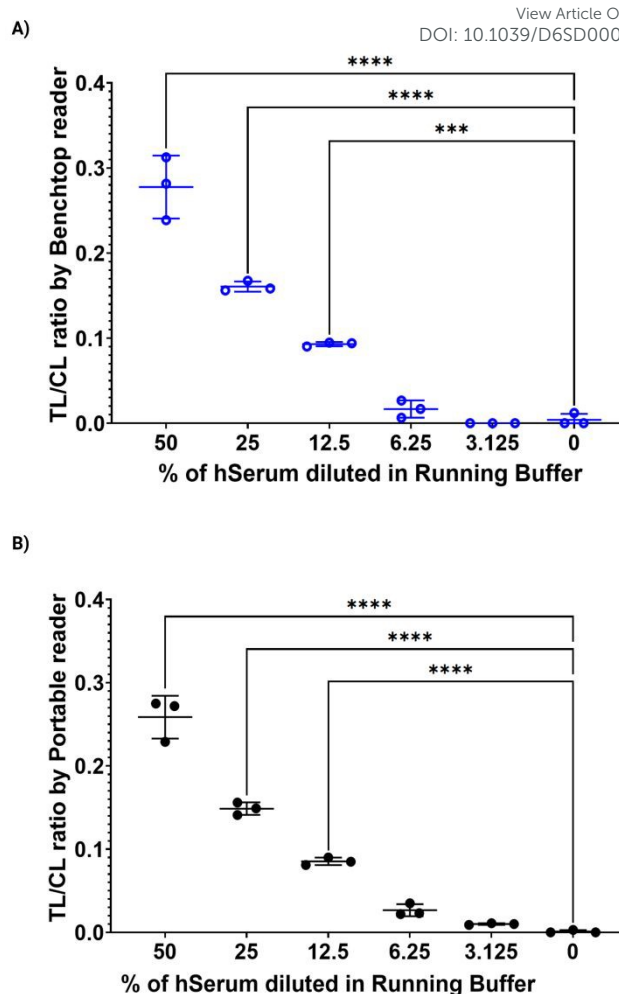


Fig. 4 The human serum sample U7, which had the lowest leptin concentration measured by ELISA (0.83 ng/mL), was serially diluted to 50% (0.42 ng/mL), 25% (0.21 ng/mL), 12.5% (0.10 ng/mL), 6.25% (0.05 ng/mL), and 3.125% (0.03 ng/mL) using running buffer (final composition: 0.25% BSA, 0.5% Tween-20, and 0.5% PEG 3350). The dilutions were tested in triplicate, and the TL/CL ratios obtained using a A) Benchtop reader (hollow circles) and B) Portable reader (filled circles) were plotted. Each point represents one replicate, central lines denote the mean, and error bars indicate the standard deviation. An ANOVA followed by Dunnett's post-hoc test ( $\alpha = 0.05$ ) identified the lowest concentration with a TL/CL ratio significantly different from the blank as 12.5% (0.10 ng/mL) for both readers. BSA: Bovine serum albumin; R<sup>2</sup>: Coefficient of determination; CV: Coefficient of variation; CL: Control line; LFA: Lateral flow assay; PEG: Polyethylene Glycol; PBS: Phosphate buffered saline; rLeptin: Recombinant leptin; TL: Test line. \*\*\*\*p < 0.0001, \*\*\*p < 0.0001.

are generally higher in females than in males. Despite these insights, female animal models remain underutilized in research due to the metabolic variability associated with hormonal cycles.<sup>61</sup> It is crucial to consider physiological sex differences in studies of metabolic imbalances in animal and clinical research. More in-depth research on female populations is needed. Data from these studies will facilitate the development of diagnostic tools and treatments that promote sex-specific equity in medical care.

#### Leptin detection by LFA

Given the ELISA-based leptin level of the lowest concentration sample (U7;  $0.83 \pm 0.04$  ng/mL), we selected that sample to assess the ability of the prototype LFA to detect endogenous leptin and to identify the optimal sample dilution for human serum. The LFA signals at various serum dilution levels measured in the benchtop (Fig. 4A) and portable reader (Fig. 4B)



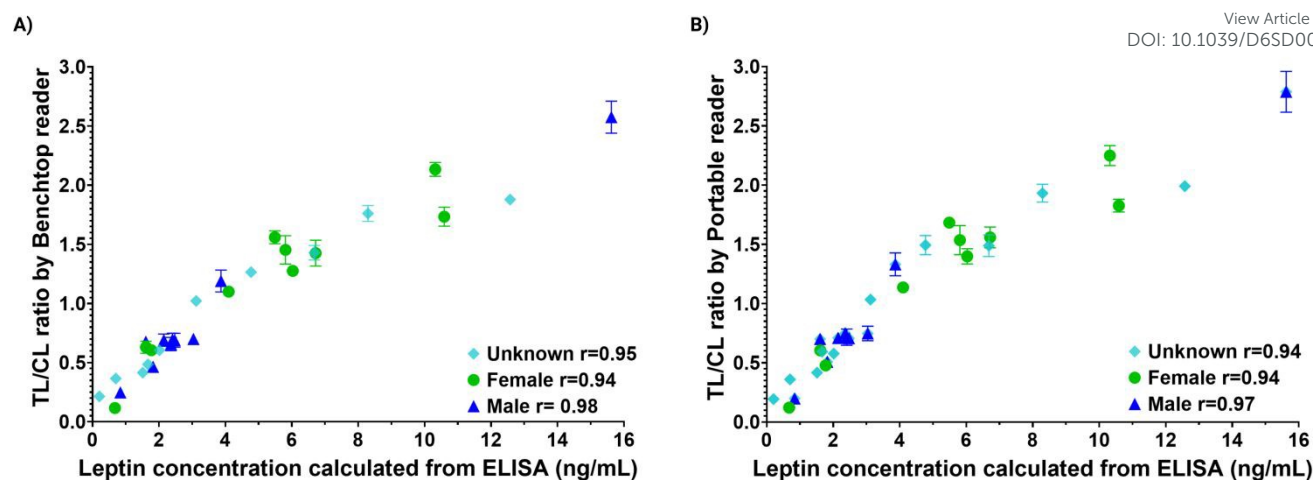


Fig. 5 Correlation between leptin concentration quantified by ELISA and the TL/CL ratio measured by the developed LFA using human serum samples diluted to 25% in running buffer (final concentrations: 0.25% BSA, 0.5% Tween-20, and 0.5% PEG 3350). TL/CL measurements were performed with both A) a benchtop reader and B) a portable reader. A strong correlation is observed between leptin concentration measured by ELISA and TL/CL ratios obtained with the LFA prototype in both readers, as evaluated using Pearson correlation. For the benchtop reader, correlation coefficients were 0.95 ( $p < 0.0001$ ) for the unknown group, 0.94 ( $p < 0.0001$ ) for the female group and 0.98 ( $p < 0.0001$ ) for the male group and 0.96 ( $p < 0.0001$ ) for the whole population ( $n=30$ ). For the portable reader, correlation coefficients were 0.94 ( $p < 0.0001$ ) for the unknown group, 0.94 ( $p < 0.0001$ ) for the female group and 0.97 ( $p < 0.0001$ ) for the male group and 0.95 ( $p < 0.0001$ ) for the whole population ( $n=30$ ). Symbols indicate sex: diamonds for individuals with unknown sex ( $n = 10$ ), circles for females ( $n = 10$ ), and triangles for males ( $n = 10$ ). BSA: Bovine serum albumin; r: Pearson correlation coefficient; CL: Control line; LFA: Lateral flow assay; PEG: Polyethylene Glycol; PBS: Phosphate buffered saline; rLeptin: Recombinant leptin; TL: Test line.

were plotted and analysed by an ANOVA followed by Dunnett's post-hoc test ( $\alpha = 0.05$ ). There was a signal on the test line at all dilution levels tested in the portable reader and at up to 6.25% serum dilution in the benchtop reader. However, the lowest dilution level at which the TL/CL ratio was significantly different from the blank was 12.5% (estimated from ELISA to be 0.10 ng/mL). It is worth noting that the estimated concentration was below the LOD of 0.25 ng/mL in both buffer and calf serum. The 50% dilution was not selected in further testing mainly because of the observed high variability among replicates. These results demonstrate that the LFA prototype can detect concentrations slightly below the tested range. However, such low concentrations fall outside physiologically-relevant ranges in the majority of samples, according to previous reports. We selected a 25% dilution of human serum for experimental validation of the developed LFA, as it provided low variability (CV of 3.7% for the benchtop reader and 5% for the portable reader) and a clearly detectable signal in a low-concentration sample. This dilution may enable the detection of even lower concentrations than the U7 sample. Additionally, our proposed protocol requires minimal serum volume and allows prompt leptin detection in triplicate via a simple dilution step, without complex equipment or specialized supplies. The developed fluorescence-based LFA for leptin successfully detected endogenous leptin in 30 human serum samples diluted at 25% in RB with low variability (CV  $< 10\%$  in both readers). Unlike previous POC tests for leptin, which reported insufficient clinical sample testing, we validated our LFA prototype using 30 human serum samples and compared its performance with the gold-standard ELISA, yielding promising results.

All test strips displayed clear signals on the test lines, as observed in FluorChem images (Fig. S7 in SI). As expected, both the benchtop (Fig. 5A) and portable reader (Fig. 5B) data demonstrated a direct relationship between ELISA-measured

leptin concentrations and the TL/CL ratios obtained with the LFA prototype. Pearson correlation analysis confirmed a strong positive correlation, with coefficients on the benchtop reader of 0.96 ( $p < 0.0001$ ) for the whole population ( $n=30$ ), 0.95 ( $p < 0.0001$ ) for the unknown group, 0.94 ( $p < 0.0001$ ) for the female group and 0.98 ( $p < 0.0001$ ) for the male group. Similarly, for the portable reader, the coefficients were 0.95 ( $p < 0.0001$ ) for the whole population ( $n=30$ ), 0.94 ( $p < 0.0001$ ) for the unknown group, 0.94 ( $p < 0.0001$ ) for the female group and 0.97 ( $p < 0.0001$ ) for the male group.

Future improvements to the assay could include immobilizing the fluorescent reporters to enable direct sample addition and standardization of the fluorescence signal relative to leptin concentration, allowing for semi-quantitative or even quantitative determination of leptin in human serum. These results further support the feasibility of developing similar POC devices for rapid and accurate detection of metabolic biomarkers in human serum. Further implementation will require extensive evaluation of clinical samples, as well as the development of standardized protocols for sample handling and storage, which must be finalized during clinical testing. Integrating serum separation membranes or centrifuge-free devices, as previously proposed, could further enhance the usability of this assay in decentralized settings and improve accessibility to leptin analysis. In our literature review, we identified only one study focused on the development of a lateral flow assay for leptin detection.<sup>65</sup> Zhou and collaborators tested leptin at 100 ng/mL spiked into a serum sample diluted 1:4 in PBS; no further testing was conducted.<sup>65</sup> We compared published state-of-the-art leptin point-of-care detection assays in the last 10 years that evaluated human serum samples and reported a limit of detection (Table S3 in SI). The majority of these studies describe electrochemical sensors that, despite their high sensitivity potential, are not currently designed for



POC use due to the requirement for specialized equipment for signal acquisition. Also, some reports present only results from a single serum sample spiked with a known leptin concentration.<sup>66–69</sup> Ultrasensitive LFAs, as opposed to electrochemical-based biosensors, offer rapid and robust sample-to-answer workflows, reduced fabrication costs, are deliverable to end users, and require no expensive equipment for result readout.<sup>70</sup> Although some electrochemical devices reported lower detection limits, even at femtogram levels (Table S3 in SI), the LOD achieved in this study showcases the potential of our leptin LFA to detect leptin in physiologically-relevant concentrations at POC.<sup>66,69,71–73</sup> Furthermore, as demonstrated during the COVID-19 pandemic, the infrastructure for the mass production of lateral flow devices already exists and can be further leveraged to address other globally relevant targets.<sup>47,74</sup>

Further LFA integration with a panel of markers, such as adiponectin, other adipokines, or inflammatory signalling molecules, could enable comprehensive metabolic monitoring. Such a tool could facilitate population screening and direct the limited health resources more efficiently. Additionally, an easy-to-use, rapid leptin test could be highly valuable for research applications, such as leptin determination in cell culture supernatants to study metabolic changes following interventions or treatments.

## Conclusions

Fluorescence-based LFAs represent a promising approach for point-of-care detection of metabolic biomarkers in serum, combining sensitivity, versatility, robustness, cost-effectiveness, and portability. The prototype LFA developed in this study successfully detected leptin at physiologically-relevant concentrations (0–100 ng/mL) spiked into a running buffer, and in 50% diluted calf serum, with a calculated LOD of 0.25 ng/mL in both matrices, demonstrating low nonspecific binding and low variability. It also detected endogenous leptin in diluted human serum at 25% without requiring additional leptin spiking, while maintaining low variability. The TL/CL ratios obtained using both benchtop and portable LFA readers strongly correlated with the leptin levels measured by ELISA, even in low-concentration samples. The assay workflow requires minimal sample preparation, a simple dilution (1:4), and delivers rapid, robust, and consistent results using the portable reader, highlighting its potential as a point-of-care tool for leptin detection, especially in decentralized and resource-limited settings.

Even before individuals are classified as obese by BMI, they may present metabolic alterations that put them at high risk of developing comorbidities such as cardiovascular diseases, type 2 diabetes, and cancer, among others. Thus, measuring biomarkers that more accurately monitor metabolic health, such as leptin at the point of care, can reduce the risk of developing comorbidities that increase mortality and morbidity by enabling timely lifestyle changes and other interventions for those at higher risk. The developed LFA serves as a robust screening and research tool for monitoring leptin levels at the

point of care. While the platform currently provides quantitative correlation with ELISA, its transition to routine clinical diagnostics will require further clinical trials to establish standardized screening and/or diagnostic thresholds for the relevant populations. Future work will focus on clinical validation of the leptin LFA prototype with human samples and expanding the platform to include additional metabolically relevant biomarkers for comprehensive metabolic health monitoring.

## Author contributions

Margarita Ortiz-Martinez: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, visualization, writing – original draft and writing – review & editing. Katerina Kourentzi: methodology, project administration, resources, supervision, validation, and writing – review & editing. Richard C. Willson: conceptualization, methodology, funding acquisition, project administration, resources, supervision, validation, and writing – review & editing. Marco Rito-Palomares: funding acquisition, resources, and writing – review & editing. Mirna Gonzalez-Gonzalez: funding acquisition, project administration, resources, and writing – review & editing.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

The data supporting this article are included in the Supplementary Information files:

1. Supplementary data Leptin LFA.xlsx (Raw data)
2. Supplementary Information LFA Leptin.docx (Additional tables and figures)

## Acknowledgements

The authors gratefully acknowledge the financial support of the Challenge-Based Research Funding of Tecnológico de Monterrey (IJXT070-22TE57001) and the support and facilities provided by Dr. Willson's team at the University of Houston. We also acknowledge Dr. Frank Eduardo Melendez Anzurez and MSc. Patricia Elizabeth Alvarez Ortiz at Tecnológico de Monterrey for their support during the project's conceptualization and funding acquisition stages, and Caleb Goodson for designing the 3D-printed adaptor for the POC fluorescent reader.

## References

- 1 World Obesity Federation, *World Obesity Atlas 2023*, World Obesity Federation, London, England, 5th edn., 2023.
- 2 GBD 2021 Forecasting Collaborators, Burden of disease



- scenarios for 204 countries and territories, 2022–2050: a forecasting analysis for the Global Burden of Disease Study 2021, *Lancet*, 2024, **403**, 2204–2256. 20
- 3 NCD Risk Factor Collaboration, Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults, *Lancet*, 2024, **403**, 1027–1050.
- 4 C. Boutari and C. S. Mantzoros, A 2022 update on the epidemiology of obesity and a call to action: as its twin COVID-19 pandemic appears to be receding, the obesity and dysmetabolism pandemic continues to rage on, *Metabolism*, 2022, **133**, 155217. 21
- 5 World Health Organization, *Obesity and overweight Factsheet*, WHO, Geneva, 2024. 22
- 6 M. Blüher, Obesity: global epidemiology and pathogenesis, *Nat. Rev. Endocrinol.*, 2019, **15**, 288–298. 23
- 7 A. Okunogbe, R. Nugent, G. Spencer, J. Ralston and J. Wilding, Economic impacts of overweight and obesity: current and future estimates for eight countries, *BMJ Glob. Heal.*, 2021, **6**, e006351. 24
- 8 J. Ling, S. Chen, N. R. Zahry and T. A. Kao, Economic burden of childhood overweight and obesity: A systematic review and meta-analysis, *Obes. Rev.*, 2023, **24**, e13535. 25
- 9 World Obesity Federation, *World Obesity Atlas 2024*, World Obesity Federation, London, 6th edn., 2024. 26
- 10 Y. Goryakin, S. Vuik and M. Cecchini, *The Heavy Burden of Obesity*, OECD, Paris, 2019. 27
- 11 P. González-Muniesa, M.-A. Martínez-González, F. B. Hu, J.-P. Després, Y. Matsuzawa, R. J. F. Loos, L. A. Moreno, G. A. Bray and J. A. Martinez, Obesity, *Nat. Rev. Dis. Prim.*, 2017, **3**, 17034. 28
- 12 K. Nimptsch, S. Konigorski and T. Pischon, Diagnosis of obesity and use of obesity biomarkers in science and clinical medicine, *Metab. Clin. Exp.*, 2019, **92**, 61–70. 29
- 13 A. E. Urusov, A. V. Zherdev and B. B. Dzantiev, Towards Lateral Flow Quantitative Assays: Detection Approaches, *Biosensors*, 2019, **9**, 89. 30
- 14 Z. Ma, R. L. Gingerich, J. V. Santiago, S. Klein, C. H. Smith and M. Landt, Radioimmunoassay of leptin in human plasma, *Clin. Chem.*, 1996, **42**, 942–946. 31
- 15 M. A. Larsen, V. T. Isaksen, O. S. Moen, L. Wilsgaard, M. Remijn, E. J. Paulssen, J. Florholmen and R. Goll, Leptin to adiponectin ratio – A surrogate biomarker for early detection of metabolic disturbances in obesity, *Nutr. Metab. Cardiovasc. Dis.*, 2018, **28**, 1114–1121.
- 16 A. M. DePaoli, 20 Years of leptin: Leptin in common obesity and associated disorders of metabolism, *J. Endocrinol.*, 2014, **223**, T71–T81.
- 17 M. S. Poetsch, A. Strano and K. Guan, Role of Leptin in Cardiovascular Diseases, *Front. Endocrinol. (Lausanne)*, 2020, **11**, 1–13.
- 18 J. M. Friedman and J. L. Halaas, Leptin and the regulation of body weight in mammals, *Nature*, 1998, **395**, 763–770.
- 19 L. T. van Egmond, E. M. S. Meth, J. Engström, M. Ilemosoglou, J. A. Keller, H. Vogel and C. Benedict, Effects of acute sleep loss on leptin, ghrelin, and adiponectin in adults with healthy weight and obesity: A laboratory study, *Obesity*, 2023, **31**, 635–641. DOI: 10.1039/D6SD00062B
- R. H. Lustig, D. Collier, C. Kassotis, T. A. Roepke, M. J. Kim, E. Blanc, R. Barouki, A. Bansal, M. C. Cave, S. Chatterjee, M. Choudhury, M. Gilbertson, D. Lagadic-Gossmann, S. Howard, L. Lind, C. R. Tomlinson, J. Vondracek and J. J. Heindel, Obesity I: Overview and molecular and biochemical mechanisms, *Biochem. Pharmacol.*, 2022, **199**, 115012.
- Z. Al-Hamodi, M. AL-Habori, A. Al-Meerri and R. Saif-Ali, Association of adipokines, leptin/adiponectin ratio and C-reactive protein with obesity and type 2 diabetes mellitus, *Diabetol. Metab. Syndr.*, 2014, **6**, 99.
- G. Frühbeck, V. Catalán, A. Rodríguez and J. Gómez-Ambrosi, Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk, *Adipocyte*, 2018, **7**, 57–62.
- A. A. Kumar, G. Satheesh, G. Vijayakumar, M. Chandran, P. R. Prabhu, L. Simon, V. R. Kutty, C. C. Kartha and A. Jaleel, Plasma leptin level mirrors metabolome alterations in young adults, *Metabolomics*, 2020, **16**, 87.
- M. Rosická, M. Krsek, M. Matoulek, Z. Jarkovská, J. Marek, V. Justová and Z. Lacinová, Serum ghrelin levels in obese patients: the relationship to serum leptin levels and soluble leptin receptors levels, *Physiol. Res.*, 2003, **52**, 61–66.
- S. Wang, D. B. Reed, S. Goli and D. Goswami, Blood leptin and C-reactive protein provide more sensitive assessment than blood lipids and other inflammatory biomarkers in overweight university students, *Nutr. Res.*, 2011, **31**, 586–593.
- S. Mishra, V. Gupta, S. Mishra, R. Sachan and A. Asthana, Serum level of orexin-A, leptin, adiponectin and insulin in North Indian obese women, *Diabetes Metab. Syndr. Clin. Res. Rev.*, 2017, **11**, S1041–S1043.
- H. S. Al-Amodi, N. A. Abdelbasit, S. H. Fatani, A. T. Babakr and M. M. Mukhtar, The effect of obesity and components of metabolic syndrome on leptin levels in Saudi women, *Diabetes Metab. Syndr. Clin. Res. Rev.*, 2018, **12**, 357–364.
- N. M. Rashad, S. E. Sayed, M. H. Sherif and M. Z. Sitohy, Effect of a 24-week weight management program on serum leptin level in correlation to anthropometric measures in obese female: A randomized controlled clinical trial, *Diabetes Metab. Syndr. Clin. Res. Rev.*, 2019, **13**, 2230–2235.
- A.-V. Sitar-Tăut, A. Cozma, A. Fodor, S.-C. Coste, O. H. Orasan, V. Negrean, D. Pop and D.-A. Sitar-Tăut, New Insights on the Relationship between Leptin, Ghrelin, and Leptin/Ghrelin Ratio Enforced by Body Mass Index in Obesity and Diabetes, *Biomedicines*, 2021, **9**, 1657.
- A. Korac, B. Srdic-Galic, A. Kalezic, A. Stancic, V. Otasevic, B. Korac and A. Jankovic, Adipokine signatures of subcutaneous and visceral abdominal fat in normal-weight and obese women with different metabolic profiles, *Arch. Med. Sci.*, 2021, **17**, 323–336.
- A. M. Gonzalez-Gil and L. Elizondo-Montemayor, The role of exercise in the interplay between myokines, hepatokines, osteokines, adipokines, and modulation of



- inflammation for energy substrate redistribution and fat mass loss: A review, *Nutrients*, 2020, **12**, 1.
- 32 A. Vecchié, F. Dallegrì, F. Carbone, A. Bonaventura, L. Liberale, P. Portincasa, G. Frühbeck and F. Montecucco, Obesity phenotypes and their paradoxical association with cardiovascular diseases, *Eur. J. Intern. Med.*, 2018, **48**, 6–17.
- 33 S. Zhao, C. M. Kusminski and P. E. Scherer, Adiponectin, Leptin and Cardiovascular Disorders, *Circ. Res.*, 2021, **128**, 136–149.
- 34 M. Ortiz-Martínez, M. González-González, A. J. Martagón, V. Hlavinka, R. C. Willson and M. Rito-Palomares, Recent Developments in Biomarkers for Diagnosis and Screening of Type 2 Diabetes Mellitus, *Curr. Diab. Rep.*, 2022, **22**, 95–115.
- 35 V. Francisco, J. Pino, V. Campos-Cabaleiro, C. Ruiz-Fernández, A. Mera, M. A. Gonzalez-Gay, R. Gómez and O. Gualillo, Obesity, fat mass and immune system: Role for leptin, *Front. Physiol.*, 2018, **9**, 1–20.
- 36 I. Haque, A. Ghosh, S. Acup, S. Banerjee, K. Dhar, A. Ray, S. Sarkar, S. Kambhampati and S. K. Banerjee, Leptin-induced ER- $\alpha$ -positive breast cancer cell viability and migration is mediated by suppressing CCN5-signaling via activating JAK/AKT/STAT-pathway, *BMC Cancer*, 2018, **18**, 99.
- 37 S. Tewari, R. Vargas and O. Reizes, The impact of obesity and adipokines on breast and gynecologic malignancies, *Ann. N. Y. Acad. Sci.*, 2022, **1518**, 131–150.
- 38 A. L. Strong, J. F. Ohlstein, B. A. Biagas, L. V. Rhodes, D. T. Pei, H. A. Tucker, C. Llamas, A. C. Bowles, M. F. Dutreil, S. Zhang, J. M. Gimble, M. E. Burow and B. A. Bunnell, Leptin produced by obese adipose stromal/stem cells enhances proliferation and metastasis of estrogen receptor positive breast cancers, *Breast Cancer Res.*, 2015, **17**, 112.
- 39 C. D. Mann, C. P. Neal, G. Garcea, M. M. Manson, A. R. Dennison and D. P. Berry, Prognostic molecular markers in hepatocellular carcinoma: A systematic review, *Eur. J. Cancer*, 2007, **43**, 979–992.
- 40 Y. Rajesh and D. Sarkar, Association of Adipose Tissue and Adipokines with Development of Obesity-Induced Liver Cancer, *Int. J. Mol. Sci.*, 2021, **22**, 2163.
- 41 K. M. Koczula and A. Gallotta, Lateral flow assays, *Essays Biochem.*, 2016, **60**, 111–120.
- 42 C. Parolo, A. Sena-Torralba, J. F. Bergua, E. Calucho, C. Fuentes-Chust, L. Hu, L. Rivas, R. Álvarez-Diduk, E. P. Nguyen, S. Cinti, D. Quesada-González and A. Merkoçi, Tutorial: design and fabrication of nanoparticle-based lateral-flow immunoassays, *Nat. Protoc.*, 2020, **15**, 3788–3816.
- 43 E. B. Bahadır and M. K. Sezgintürk, Lateral flow assays: Principles, designs and labels, *TrAC - Trends Anal. Chem.*, 2016, **82**, 286–306.
- 44 D. W. Jekarl, H. Choi, E. S. Kim, S. Lee, H. Il Park, M. Kim and Y. Kim, Analytical evaluation and clinical application of insulin and C-peptide by a whole blood, lateral flow, point of care (POC) assay system, *Scand. J. Clin. Lab. Invest.*, 2019, **79**, 347–353.
- 45 M. Ali, M. Sajid, M. A. U. Khalid, S. W. Kim, J. H. Lim, D. Huh and K. H. Choi, A fluorescent lateral flow biosensor for the quantitative detection of Vaspin using upconverting nanoparticles, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, 2020, **226**, 117610.
- 46 B. M. Gannon, M. J. Glesby, J. L. Finkelstein, T. Raj, D. Erickson and S. Mehta, A point-of-care assay for alpha-1-acid glycoprotein as a diagnostic tool for rapid, mobile-based determination of inflammation, *Curr. Res. Biotechnol.*, 2019, **1**, 41–48.
- 47 K. Kourentzi, K. Brosamer, B. Vu and R. C. Willson, Accelerated Development of a COVID-19 Lateral Flow Test in an Academic Setting: Lessons Learned, *Acc. Chem. Res.*, 2024, **57**, 1372–1383.
- 48 T. Salminen, E. Juntunen, S. M. Talha and K. Pettersson, High-sensitivity lateral flow immunoassay with a fluorescent lanthanide nanoparticle label, *J. Immunol. Methods*, 2019, **465**, 39–44.
- 49 S. Kasetsirikul, M. J. A. Shiddiky and N.-T. Nguyen, Challenges and perspectives in the development of paper-based lateral flow assays, *Microfluid. Nanofluidics*, 2020, **24**, 17.
- 50 L. A. Cantarero, J. E. Butler and J. W. Osborne, The adsorptive characteristics of proteins for polystyrene and their significance in solid-phase immunoassays, *Anal. Biochem.*, 1980, **105**, 375–382.
- 51 M. G. Swainson, A. M. Batterham, C. Tsakirides, Z. H. Rutherford and K. Hind, Prediction of whole-body fat percentage and visceral adipose tissue mass from five anthropometric variables, *PLoS One*, 2017, **12**, e0177175.
- 52 R. Pray and S. Riskin, The History and Faults of the Body Mass Index and Where to Look Next: A Literature Review, *Cureus*, 2023, **15**, e48230.
- 53 J. H. Stern, J. M. Rutkowski and P. E. Scherer, Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk, *Cell Metab.*, 2016, **23**, 770–784.
- 54 L. Scheja and J. Heeren, The endocrine function of adipose tissues in health and cardiometabolic disease, *Nat. Rev. Endocrinol.*, 2019, **15**, 507–524.
- 55 H. Benbaibeche, A. Bounihi and E. A. Koceir, Leptin level as a biomarker of uncontrolled eating in obesity and overweight, *Ir. J. Med. Sci.*, 2021, **190**, 155–161.
- 56 C. T. Montague, I. S. Farooqi, J. P. Whitehead, M. A. Soos, H. Rau, N. J. Wareham, C. P. Sewter, J. E. Digby, S. N. Mohammed, J. A. Hurst, C. H. Cheetham, A. R. Earley, A. H. Barnett, J. B. Prins and S. O’Rahilly, Congenital leptin deficiency is associated with severe early-onset obesity in humans, *Nature*, 1997, **387**, 903–908.
- 57 J. S. Flier and R. S. Ahima, Leptin physiology and pathophysiology: knowns and unknowns 30 years after its discovery, *J. Clin. Invest.*, 2024, **134**, e174595.
- 58 X. Chen, T. Dong, X. Wei, Z. Yang, N. M. Matos Pires, J. Ren and Z. Jiang, Electrochemical methods for detection of biomarkers of Chronic Obstructive Pulmonary Disease in serum and saliva, *Biosens. Bioelectron.*, 2019, **142**, 111453.
- 59 D. Y. Joh, A. M. Hucknall, Q. Wei, K. A. Mason, M. L. Lund, C. M. Fontes, R. T. Hill, R. Blair, Z. Zimmers, R. K. Achar, D.



- Tseng, R. Gordan, M. Freemark, A. Ozcan and A. Chilkoti, Inkjet-printed point-of-care immunoassay on a nanoscale polymer brush enables subpicomolar detection of analytes in blood, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, E7054–E7062.
- 60 L. Flower, R. H. Ahuja, S. E. Humphries and V. Mohamed-Ali, Effects of Sample Handling on the Stability of Interleukin 6, Tumour Necrosis Factor- $\alpha$  and Leptin, *Cytokine*, 2000, **12**, 1712–1716.
- 61 T. G. Valencak, A. Osterrieder and T. J. Schulz, Sex matters: The effects of biological sex on adipose tissue biology and energy metabolism, *Redox Biol.*, 2017, **12**, 806–813.
- 62 J. Cheng, Y. Luo, Y. Li, F. Zhang, X. Zhang, X. Zhou and L. Ji, Sex- and body mass index-specific reference intervals for serum leptin: a population based study in China, *Nutr. Metab.*, 2022, **19**, 1–8.
- 63 A. B. Zavalza-Gómez, R. Anaya-Prado, A. R. Rincón-Sánchez and J. M. Mora-Martínez, Adipokines and insulin resistance during pregnancy, *Diabetes Res. Clin. Pract.*, 2008, **80**, 8–15.
- 64 C. N. Boyle and C. Le Foll, Amylin and Leptin interaction: Role During Pregnancy, Lactation and Neonatal Development, *Neuroscience*, 2020, **447**, 136–147.
- 65 G. Zhou, X. Mao and D. Juncker, Immunochromatographic Assay on Thread, *Anal. Chem.*, 2012, **84**, 7736–7743.
- 66 İ. Uludağ and M. K. Sezgintürk, A direct and simple immobilization route for immunosensors by CNBr activation for covalent attachment of anti-leptin: obesity diagnosis point of view, *3 Biotech*, 2022, **12**, 33.
- 67 C. Erkmen, G. A. Tiğ and B. Uslu, First label-free impedimetric aptasensor based on Au NPs/TiO<sub>2</sub> NPs for the determination of leptin, *Sensors Actuators B Chem.*, 2022, **358**, 131420.
- 68 Q. Zhang, Y. Qing, X. Huang, C. Li and J. Xue, Synthesis of single-walled carbon nanotubes–chitosan nanocomposites for the development of an electrochemical biosensor for serum leptin detection, *Mater. Lett.*, 2018, **211**, 348–351.
- 69 C. Erkmen, G. Aydoğdu Tig and B. Uslu, Evaluation of aptamer and molecularly imprinted polymers as a first hybrid sensor for leptin detection at femtogram levels, *Talanta*, 2023, **265**, 124809.
- 70 V. T. Nguyen, S. Song, S. Park and C. Joo, Recent advances in high-sensitivity detection methods for paper-based lateral-flow assay, *Biosens. Bioelectron.*, 2020, **152**, 112015.
- 71 B. Özcan and M. K. Sezgintürk, A novel and disposable GP-based impedimetric biosensor using electropolymerization process with PGA for highly sensitive determination of leptin: Early diagnosis of childhood obesity, *Talanta*, 2021, **225**, 121985.
- 72 C. Zhang, L. Liu, H. Li, J. Hu, J. Zhang, H. Zhou, Z. Zhang and X. Du, An oriented antibody immobilization based electrochemical platform for detection of leptin in human with different body mass index, *Sensors Actuators B Chem.*, 2022, **353**, 131074.
- 73 J. Cai, X. Gou, B. Sun, W. Li, D. Li, J. Liu, F. Hu and Y. Li, Porous graphene-black phosphorus nanocomposite modified electrode for detection of leptin, *Biosens. Bioelectron.*, 2019, **137**, 88–95. DOI: 10.1039/D6SD00062B
- 74 Y. Liu, L. Zhan, Z. Qin, J. Sackrison and J. C. Bischof, Ultrasensitive and Highly Specific Lateral Flow Assays for Point-of-Care Diagnosis, *ACS Nano*, 2021, **15**, 3593–3611.



The data supporting this article are included in the Supplementary Information files:

1. Supplementary data Leptin LFA.xlsx (Raw data)
2. Supplementary Information LFA Leptin.docx (Additional tables and figures)

