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Aptamer lateral flow assay for the rapid detection of histamine in fish and human blood

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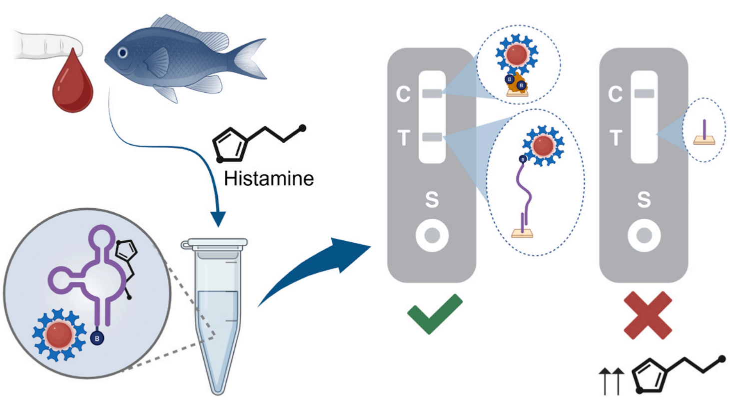
HIGHLIGHTS

- Aptamers selected against histamine have been optimised by truncation.
- Optimised aptamers were implemented in rapid and sensitive lateral flow assays (LFA).
- The LFAs were applied to histamine detection in fish and fingerprick blood samples.
- Analysis of fish extracts achieved recoveries comparable to liquid chromatography.
- Blood analysis shows the suitability of the LFA for management of allergic reactions.

GRAPHICAL ABSTRACT



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ABSTRACT

The ingestion of histamine-rich foods or deficiencies in histamine-degrading enzymes can lead to histamine accumulation and produce intolerance, mimicking an allergic reaction with symptoms ranging from mild to even severe and potentially fatal anaphylaxis. Standard laboratory techniques like liquid chromatography are complex and lengthy, while antibody-based tests are costly and suffer from specificity issues due to the small size of the analyte. In this work, we sought to develop easy-to-use and cost-effective lateral flow assays for the rapid and accurate aptamer-based detection of histamine in fish and human whole blood. To this end, we optimised our previously selected histamine aptamer by truncation and combined it with a short partially complementary DNA probe for assay development. A microplate assay was initially designed for the detection of histamine in fish after a simple extraction procedure and the performance of the assay was comparable to standard liquid chromatographic methods. Aptamer-based lateral flow assays were then developed for the detection of histamine in fish and fingerprick blood. Canned tuna and sardines with known levels of histamine were successfully analysed, and the presence of histamine in spiked fingerprick blood samples was readily assessed by visual inspection. The

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limits of detection achieved were 23 nM (< 0.13 ppm) in fish and 8.4 nM (< 1 ng/mL) in blood, in combination with short duration (less than 10 minutes), in line with assay requirements for food safety control as well as rapid intervention and management of serious allergic reactions.

1. Introduction

Histamine poisoning occurs following the ingestion of foods or beverages containing high levels of this biogenic amine and can affect different organs, with symptoms varying from mild to even severe anaphylaxis and death [44]. The accurate and timely detection of histamine is thus essential to prevent intoxication or to facilitate a rapid response to an allergic reaction and administration of appropriate treatment. The detection and quantification of histamine is, however, a complex task due to its small size, its structural similarity to other biogenic amines, the complexity of sample matrices and the low concentrations present in biological fluids. The methodologies typically used for the quantification of histamine in real samples, including foods and beverages and biological fluids, are based on chromatographic techniques [3,31]. When coupled with spectrometry [52], they provide higher precision and sensitivity but these techniques are inherently laboratory-based resulting in a long turnaround time from sampling to result, and require expensive instrumentation and trained personnel. Moreover, they typically require sample pre-treatment procedures which can be time-consuming and labour-intensive. As an alternative, rapid tests that can be used at the point-of-need by untrained personnel have been developed and commercialised (Supplementary Table S1). The Assay Genie's Histamine Quick Test Strips are semi-quantitative and based on histamine dehydrogenase-catalysed oxidation of histamine where the electron mediator reduces a formazan (MTT) reagent. The intensity of product colour is directly proportional to the concentration of histamine in the sample, with the assay complete within 15 minutes. The assay is designed for use with wine and food samples and has a detection range between 0 and 200 ppm. The same concept is used in a rapid 5-minute assay developed by Kikkoman Biochemifa and the Histamine Quick Test from Sigma Aldrich. In the case of the ProGnosis Biotech kit, the assay time required for the detection of histamine in fish is just 3 minutes with an LOD of 5.3 ppm. Two semi-quantitative kits, one from Biopanda and one from Neogen detect histamine in seafood with a cut-off of 50 ppm. Molecularly imprinted polymer (MIP)-based histamine sensors have also been reported in combination with novel nanomaterials and electrochemical detection for improved sensitivity [1]. MIP-based sensor can however present several disadvantages such as complex polymer preparation, incomplete removal of template and non-specific binding especially in complex samples preventing their effective application for the detection of small molecules. Methods based on the use of antibodies such as enzyme linked immunosorbent assays (ELISA) [26] and radioimmunoassays (RIA) [35] have also been developed and a number of commercial ELISA kits are available. Immunochromatographic detection of histamine in fish [54] or wine [30] has been reported, and there is a commercial kit (HistaSure™ Fish Rapid Test, www.ldn.de), which facilitates implementation at the point-of-need. However, while these assays are robust and simpler than the chromatographic techniques, the requirement of animal hosts to produce the antibodies, storage stability, as well as specificity, especially with regards to small molecule targets, hinder their effective implementation. Coupled with these drawbacks, the cost of antibody production and commercially available antibodies has rapidly increased in recent years and an alternative to antibodies for use in low-cost and specific rapid analytical tests is desirable.

The use of aptamers as biorecognition elements for various applications particularly for small molecules, has been garnering increasing interest in the last few years [36,53]. Aptamers, which are synthetic oligonucleotides, exhibit a unique capability to fold into intricate three-dimensional structures, enabling them to bind with exceptional affinity and specificity to a diverse range of targets. The discovery of aptamers relies on an *in vitro* process termed Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [13,41], allowing for the selection of aptamers with high binding affinity. Whilst in antibody production the obtainable specificity is defined by the host immune system, in the case of aptamers their specificity can be tuned by the inclusion of counter-selection steps during SELEX to eliminate any binders to non-specific molecules and/or by carrying out SELEX in the final matrix the aptamer will be used in.

In our previous work, we reported the development of the H2 histamine-binding aptamer, which exhibited remarkable affinity and specificity and was effectively exploited for the detection of histamine in urine [28]. Subsequently, the same aptamer was used for the detection of histamine in fish employing a label-free gold nanoparticle-based assay [24]. The robustness and versatility of this aptamer is further highlighted by the additional approaches reported in the literature by other research groups detailing its use for the development of different platforms for histamine determination. For example, in a novel platform employing the H2 aptamer, $NaYF_4$:Ce/Tb nanoparticles and gold nanoparticles were exploited for monitoring food freshness using time-resolved fluorescence resonance energy transfer, achieving an LOD of 4.57 nM [49]. The H2 aptamer has also been combined with gold, silver and/or magnetic nanoparticles and surface-enhanced Raman spectroscopy (SERS), achieving low picomolar detection limits [45]. Xu and co-workers developed an assay for the detection of histamine in fish achieving a detection limit of 6.23 pM, using Fe_3O_4@MOF@Pt nanozymes with a complementary H2 histamine aptamer strand to create a colorimetric aptasensor [51]. Zhou et al., reported a highly selective colorimetric aptasensor (LOD of 27 μg/L or 243 nM) based on the enhancement of the affinity of silver citrate, acting as a laccase-mimic, for a chromogenic substrate in the presence of the H2 aptamer [58]. Recently, Wu et al. exploited the H2 histamine aptamer for the development of a fluorescence resonance energy transfer (FRET)-based aptasensor by immobilizing the fluorescently-labelled aptamer on fluorescent polymer dots enabling the detection of histamine in water and tuna [50]. The growing interest in aptamer-based platforms for the detection of histamine is also evidenced by the selection of alternative histamine aptamers, including two DNA aptamers [11,20] and one RNA aptamer [12]. These aptamers were used for the development of an electrochemical impedance aptasensor [20], a colorimetric microplate assay [11], a core-satellite self-assembled SERS aptasensor [6], and fluorescence aptasensors [12,16] for the quantification of histamine in foods and beverages. These reports underscore the continuous advancement and interest in the development of rapid, easy and cost-effective histamine aptasensors.

In this work, we sought to develop simple and rapid histamine tests for food safety control and timely allergy management based on synthetic DNA aptamers as alternatives to the complex and expensive existing methods based on liquid chromatography and antibodies. Aptamer-based lateral flow assays (LFAs) were thus pursued as the most appropriate assay format, facilitating simple, easy-to-use and cost-effective analysis with low time-to-result and suitable for deployment to the point of need. We optimised our previously reported H2 histamine aptamer by truncation and then combined it with a partially complementary capture DNA probe (CCP) to develop a versatile LFA assay for the analysis of different types of samples like food and biological samples (Fig. 1). The assay was first developed on microtitre plates (Fig. 1A) before transferring the same format to an LFA (Figs. 1B and 1C) for final optimization of different parameters. The assays were finally validated with quality control canned fish samples in different matrices with distinct known levels of histamine as well as with histamine-spiked fingerprick blood samples from different individuals.

2. Materials and methods

2.1. Materials

Histamine, tryptamine, phosphate-buffered saline tablets (PBS; 10 mM phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4), bovine serum albumin (BSA), maleimide-activated plates and the metal-enhanced 3,3′-diaminobenzidine (DAB) substrate kit were supplied by Fisher Scientific (Spain). Tyramine, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxysuccinimide (NHS), skimmed milk powder, streptavidin-HRP (SA-HRP, 1 mg/mL), 11-mercapto-1-undecanoic acid (MUA) and synthetic urine were provided by Merck (Spain). 3,3′,5,5′-tetramethylbenzidine (TMB) substrate for ELISA was from Surmodics (USA), the polyclonal antibody against histamine from Abcam (Spain) and streptavidin-polyHRP80 (streptavidin-polyHRP, 1 mg/mL) from SDT-Reagents (Germany). The zirconia/silica beads (BioSpec Products Inc., 0.5 mm diameter) were from Palex Medical S.A. (Spain) and the HistaSure™ Fish Rapid Test from LDN (Germany). The FAPAS® quality control (QC) fish samples with different levels of histamine were provided by SETEL Scientific Testing S.L. (Spain). These were tuna containing 37.7 ppm (# T27206QC) or 128 ppm (# T27243QC) histamine and sardines in tomato sauce containing 160 ppm (# T27234QC) or 223 ppm (# T27211QC) histamine. Gold nanoparticles (AuNPs; 40 nm, OD 1) were obtained from BBI Solutions (UK). The FF120HP nitrocellulose membrane and the Whatman CF5 wicking pad were from Cytiva (Spain), the fiberglass sample/conjugate release pad grade 8951 from Ahlstrom (Finland) and the C083 cellulose sample pad (blood separator) was provided by Merck (Spain). Ultra-pure milli-Q water (18.2 MΩ.cm) was used for all experiments. DNA oligonucleotides were purchased from biomers.net (Germany).

2.2. Truncation strategy and structural characterisation

The original full-length H2 aptamer was truncated to remove non-essential bases by initially removing the primer annealing sites (constant regions of the library used in the SELEX process for selection of the aptamer). The H2.1, H2.2 and H2.3 variants were thus obtained by removing the primer annealing site at the 3’ end, the 5’ end or both ends, respectively. A second set of truncated sequences were designed guided by the secondary structure of the full-length H2 aptamer predicted *in silico* at 25ºC, 137 mM NaCl and 1.5 mM $MgCl_2$ by the Mfold webserver (http://www.unafold.org/mfold/applications/dna-folding-form.php). These were a Loop motive (L) structure, a Loop motive with a 6 nucleotide (nt) extension at the 5’ end (L1) and a Loop motive with a 5 nt extension at the 3’ end (L2). All the aptamer sequences are shown in Table 1 and their predicted structures by Mfold in Figure S1. The secondary structures of the truncation variants were also studied using circular dichroism (CD) spectroscopy. All the sequences were prepared at 1 µM in binding buffer (10 mM PBS pH 7.4 with 1.5 mM $MgCl_2$) and equilibrated at 25ºC for at least 30 min prior to spectra acquisition. Two spectra were acquired per aptamer following background (binding buffer) autosubtraction using a 1 cm path length quartz microcuvette in the range of 210 – 330 nm (1 nm step, 0.5 sec time-per-point with adaptive sampling) using a Chirascan CD spectrometer (Applied Photophysics, UK). The spectra were finally averaged and smoothed using the Pro-Data Chirascan software.

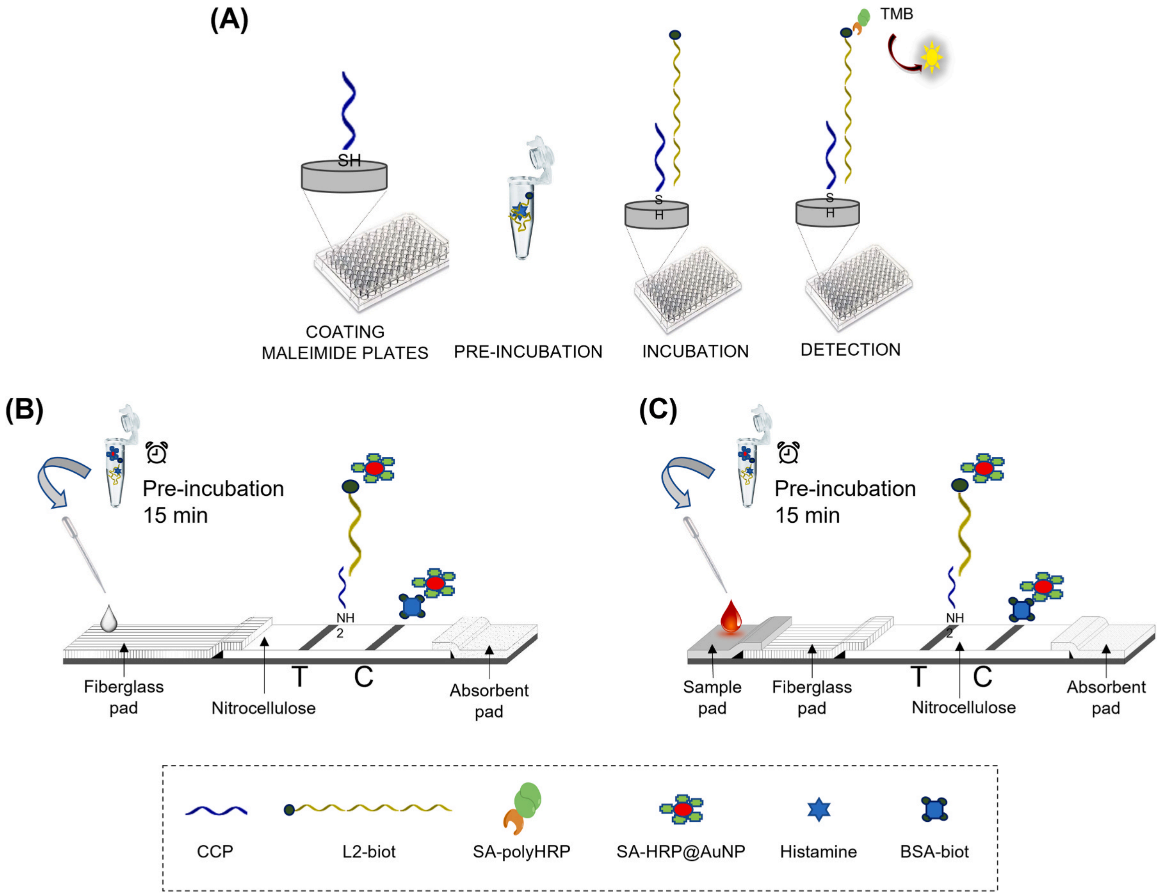

Fig. 1. Schematic representation of the different aptamer-based assays developed for histamine detection. (A) Assay design on microtitre plates. (B) Lateral flow assay (LFA) for analysis of fish samples. (C) LFA for analysis of blood samples.

Table 1
Oligonucleotide sequences used in this work.

ID	Oligonucleotide sequence (5' to 3')	Length (nt)
H2	AGCTCCAGAAGATAAATTACAGGGAACGTGTTGGTTGCGGTTCTTCCGATCTGCTGTGTTCTCTATCTGTGCCATGCAACTAGGATACTATGACCCCGG	99
H2.1	AGCTCCAGAAGATAAATTACAGGGAACGTGTTGGTTGCGGTTCTTCCGATCTGCTGTGTTCTCTATCTGTGCCATG	76
H2.2	GAACGTGTTGGTTGCGGTTCTTCCGATCTGCTGTGTTCTCTATCTGTGCCATGCAACTAGGATACTATGACCCCGG	76
H2.3	GAACGTGTTGGTTGCGGTTCTTCCGATCTGCTGTGTTCTCTATCTGTGCCATG	53
L	ACAGGGAACGTGTTGGTTGCGGTTCTTCCGATCTGCTG	38
L1	TAAATTACAGGGAACGTGTTGGTTGCGGTTCTTCCGATCTGCTGT	45
L2	ACAGGGAACGTGTTGGTTGCGGTTCTTCCGATCTGCTGTGTTCT	44
CCP	CACGTTCCCTGT	12

2.3. *Affinity studies using enzyme-linked aptamer assay (ELAA)*

Histamine was immobilized on maleimide-activated microplate wells through a 11-mercaptoundecanoic acid (MUA) crosslinker as described previously [28], with minor modifications. Briefly, 50 μL of 500 μM of MUA in PBS (10 mM pH 7.4) were added to the wells of a maleimide-activated microtitre plate and incubated overnight at 4°C. The carboxylic acid head groups of the immobilized MUA were activated using an EDC/NHS mixture (50 μL of 10 mg/mL of each of EDC and NHS in 25 mM MES pH 5) and 50 μL of 10 mM of histamine was then added, followed by incubation at 22ºC for 2 h. The wells were finally blocked with ethanolamine (200 μL of 1 M, pH 8.0) for 15 min. Immobilization of histamine on the plate was verified by ELISA using a polyclonal anti-histamine antibody. To evaluate the binding affinity of the H2 aptamer and its truncated variants for histamine, different concentrations of each biotinylated aptamer sequence were prepared in binding buffer in the range of 0.78 –100 nM with serial 1/2 dilutions and were directly added to the wells with the immobilized histamine. Following a 30-min incubation, the plate was washed thoroughly with PBS containing 0.05 % v/v Tween-20 (PBST) and streptavidin-polyHRP (50 μL of 1/20,000 dilution in PBST) was added, followed by a 30-min incubation. After a final washing step, 50 μL of TMB substrate were added. The absorbance was read at 450 nm after the addition of 50 μL of 1 M H_2SO_4 using the SpectraMax 340PC384 microplate reader (Molecular Devices, USA). Unless otherwise stated, all the incubation steps were performed at 22°C under mild shaking conditions. The measurements were performed in triplicate. The affinity dissociation constants (K_D values) of the aptamers were calculated after fitting the absorbance values at 450 nm for each aptamer concentration to the "One site - Specific binding" model of GraphPad Prism.

2.4. *Extraction of histamine from quality control (QC) fish samples*

Each FAPAS QC canned fish sample (100 ± 5 mg) was mixed with 5 mL of milli-Q water (pH 5.7) and 50 mg of zirconia/silica beads. The suspensions were hand-shaken vigorously for 1 min and then let to rest for 5 min at ambient temperature (22–25°C). Finally, the suspensions were filtered through 0.22 μm nylon syringe filters and the extracts obtained were either used immediately or stored at −20°C until use. In parallel, fresh mackerel fish was bought from the local market and was used to prepare a histamine-negative extract for use as the histamine diluent in reverse-phase high-performance liquid chromatography (RP-HPLC) quantification experiments.

2.5. *Analysis of fish extracts by reverse-phase high-performance liquid chromatography (RP-HPLC)*

The fish extracts were initially derivatized with dansyl chloride prior to reverse-phase high-performance liquid chromatography (RP-HPLC) analysis, the gold standard for histamine detection. Specifically, 200 μL of extract or histamine standard (prepared in the histamine-negative mackerel fish extract) were transferred to 1.5 mL microtubes each containing 35 μL of NaOH (2 M in water), 65 μL of saturated $NaHCO_3$ (approximately 1 M in water) and 400 μL of dansyl chloride (20 mg/mL in acetonitrile). The samples were vortexed for 30 sec, and then incubated for 15 min at 40°C. Subsequently, 20 μL of 25 % w/v NH_4OH (in water) were added and the tubes were incubated for 30 min at ambient temperature (22–25°C). The volume of the samples was adjusted to 1 mL with acetonitrile, followed by centrifugation for 5 min at 3000 x g. The organic phase containing the histamine-dansyl derivatives was recovered and filtered through a 0.2 μm PTFE syringe filter. Analysis of the extracts was performed on an Agilent 1100 Series HPLC system equipped with a diode array detector (DAD) and a quaternary pump system (Agilent Technologies). A Kromasil C18 5 μm, 100 Å (4.6 mm ID x 250 mm, Phenomenex) reverse-phase column thermostatted at 25°C was used for separation and was fitted with a SecurityGuard cartridge (4 ×3.0 mm ID, Phenomenex). The injection volume was 100 μL and the flow rate was 1 mL/min. The mobile phase was acetonitrile in water and a gradient elution was performed as detailed in Table S2. The elution peaks were monitored at 254 nm. A calibration curve with histamine standards in the histamine-negative mackerel fish extract (0, 12.5, 25, 50, 100, 250, 500 and 1000 ppm) was constructed to quantify the histamine level in the fish extracts. Four replicates of each sample extract were analysed.

2.6. *Histamine detection in microtitre plates*

A DNA probe was designed to partially hybridize with the truncated histamine aptamer for use as a capture probe in the assays. This partially complementary DNA capture probe (Table 1, CCP) was modified with a 5'-T15 spacer and a 5'-SH and was used (50 μL of 500 nM in PBS 10 mM pH 7.4) to functionalise maleimide-activated microplate wells via an overnight incubation at 4°C, which was followed by blocking with 2 % w/v skimmed milk in PBS (10 mM pH 7.4). A range of different concentrations of histamine (50 μL of 0–400 nM) were pre-incubated with 50 μL of a range of concentrations of biotinylated L2 aptamer in binding buffer (0.5 – 10 nM) for 10 – 30 min at 22°C under rotation. The mixtures were then transferred to the wells of the microtitre plate with immobilized capture probe and incubated for 0 – 20 min at 22°C under mild agitation. The wells were washed four times with PBST and streptavidin-polyHRP (50 μL of 1/20,000 dilution in PBST) was added and incubated for 30 min. Following another washing step, 50 μL of TMB substrate were added and the absorbance was read at 450 nm following the addition of 50 μL of 1 M H_2SO_4 using the SpectraMax 340PC384 microplate reader (Molecular Devices, USA). Optimized conditions with regards to biotinylated aptamer concentration and duration of the off-plate and on-plate incubation steps were finally employed for the detection of histamine in the fish extracts following dilution with PBS. All standards and samples were analysed at least in duplicate.

2.7. *Preparation and characterization of the SA-HRP@AuNP conjugate*

The 40 nm gold nanoparticles (1 mL of OD 1) were sonicated for 5 min in an ultrasonic bath (M2800-E, Branson Ultrasonics, USA), followed by the addition of SA-HRP to a final concentration of 12.5 μg/mL. The suspension was incubated for 30 min at 22°C under tilt rotation on a Dynal MX1 mixer (Life Technologies, USA) and BSA was then added to a final concentration of 1 % w/v and incubated for another 30 min at 22°C under tilt rotation. The conjugate was centrifuged at 15000 rpm for 30 min at 4°C using an Eppendorf 5417 R refrigerated centrifuge (Eppendorf, Germany), the supernatant was removed and the pellet was resuspended in 1 mL of washing/storage buffer (5 mM sodium borate pH 9, 1 % w/v BSA, 10 % w/v sucrose). This process was repeated 3 times and finally the pellet was resuspended in the same washing/storage buffer and stored at 4°C until use. The conjugate was characterized by visible spectroscopy using a Varian Cary 100 Bio UV–VIS spectrometer and by transmission electron microscopy (TEM) using a JEM-1011 microscope (Markel Jeol) operating at 80 kV. The Zeta potential of the nanoparticles was measured using the Zetasizer Nano-ZS (Malvern Instruments).

2.8. Preparation and assembly of the lateral flow assay (LFA) devices

Biotinylated BSA required for constructing the control line of the LFA was prepared in-house by incubating 1 mL of BSA (6 mg/mL in PBS) with 225 μL of sulfo-NHS-biotin (10 mM) for 30 min at 22°C. The biotinylated BSA was purified using a 3 kDa MWCO centrifuge filter. Both the biotinylated BSA (3 mg/mL in water) and an amine-modified capture probe partially complementary to the L2 truncated aptamer (CCP with a 5'-T15 spacer and a 5'-aminolink C6 modification; 80 μM in water) were automatically dispensed onto an FF120HP nitrocellulose membrane to construct the control and test lines, respectively, using the ALFRD lateral flow reagent dispenser (Claremont BioSolutions) at 0.25 mL/min and 50 mm/sec. Following dispensing, the membrane was air dried for 10 min, placed in a CL-1000 UV 254 nm crosslinker (UVP) and exposed for 5 min to 90 mJ/cm^2. The strips were assembled on a backing card with a cotton linter absorbent pad (Whatman CF5) and fiberglass sample pad (Ahlström 8951) with or without the blood separator sample pad (Millipore C083) (Figs. 1B and 1C) ensuring a minimum of 2 mm overlap of the material. The assembled strips were cut to a 4 mm width using a strip cutter (Advanced Sensor Systems P. Ltd), inserted into plastic cassettes (TV Plastic, India) and either used immediately or stored at 4°C in vacuum-sealed bags with desiccant until use.

2.9. Detection of histamine with the lateral flow assay (LFA) devices

Two different calibration curves were constructed, one in buffer for the detection of histamine in the fish samples and one in blood for detection in this biological fluid. For analysis in buffer, 3 μL of histamine (0–2 μM in water with serial 1/3 dilutions) were mixed with biotinylated L2 aptamer (final 5 nM) and SA-HRP@AuNPs (final OD 1) in a total volume of 20 μL of LFA running buffer (0.5 % w/v PEG-20000, 1 % w/v sucrose, 0.1 % v/v Tween-20 in PBS). The mixtures were incubated for 15 min at 22°C before analysis. In the case of blood, 2 μL of fingerprick blood collected with EDTA-coated glass capillaries were added to the mixtures prepared as detailed above. The 20 μL pre-incubated mixtures were then added to the sample window of the cassettes with strips containing only the fiberglass sample pad for analysis of the fish samples (Fig. 1B) or to the strips with both the fiberglass pad and the blood separator sample pad for detection in blood (Fig. 1C). After addition of each mixture to the strip, 100 μL of LFA running buffer were added and left to run. For signal amplification, 20 μL of DAB substrate (prepared according to the kit's manufacturer) were directly added on top of the cassette's result viewing window and the results were recorded after 1 min. A smartphone camera was used to image the strips, and the intensity of the bands at the test and control lines were measured with the ImageJ software. The calibration curves were constructed by plotting the ratio of the intensity of the test lines in the presence and absence of histamine [%(B/B$_0$)] against the logarithmic concentrations of histamine. A sigmoidal four-parameter logistic model was used to fit the data using GraphPad Prim 8. Quality control (QC) fish samples from FAPAS® were used to validate the LFA. The fish extracts (prepared as detailed earlier) were diluted five times with PBS (10 mM pH 7.4) and analysed with the LFA devices as described above. In parallel, the same extracts were analysed using the commercial HistaSure™ Fish Rapid Test kit following the manufacturer's instructions. For detection in blood, histamine (200 nM) was spiked in fingerprick blood samples from ten healthy subjects, mixed with the biotinylated L2 aptamer, the SA-HRP@AuNPs conjugate and the LFA running buffer as described above and analysis was performed as described for the fish samples. Each sample was analysed in duplicate.

3. Results and discussion

3.1. Structural and binding properties of the H2 histamine aptamer variants after truncation

With the aim of developing a simple and cost-effective aptamer-based LFA device for the detection of histamine in diverse sample types, the H2 aptamer previously reported by our group [28] was optimized by truncation. We sought to eliminate non-essential nucleotides and in this way, facilitate assay design and lower the overall costs. Several variants of the original full-length aptamer were thus designed (Table 1) employing two strategies. The first truncation strategy was based on the removal of the constant regions of the original library used for the selection of aptamer. This was achieved by eliminating the 3' (H2.1), the 5' (H2.2) or both ends (H2.3) of the aptamer sequence, corresponding to the library primer annealing sites. The second truncation approach was based on the secondary structure of the original H2 full-length aptamer as predicted by the Mfold webserver at binding conditions in terms of salt (NaCl) and magnesium chloride concentrations and temperature. This approach resulted in the design of three different variants: the loop motifs L, L1 and L2, with the two last sequences L1 and L2 containing additional nucleotides either at the 5' or 3' ends, respectively to stabilize the stem. The predicted structures of all the variants are characterized by multiple stem-loop structures (Fig. S1), which are also observed in the predicted structures of the other histamine DNA [11,20] and RNA [12] aptamers previously reported in the literature. The folding of the variants into stem-loop structures was confirmed experimentally by Circular Dichroism (CD) spectroscopy. As can be seen in Fig. 2 and Fig. S2, all the variants exhibited CD maximum peaks at 282 – 284 nm and CD minimum peaks at 245–250 nm, characteristic of hairpins [23]. The intensity of these peaks was significantly decreased for the shorter loop motif sequences compared to the full-length parent H2 aptamer and the variants lacking one or both primer binding sites. These findings suggest changes in the folding of these sequences with lower extent of secondary structure and potentially lower stability, which are also consistent with the higher ΔG values of the loop motifs calculated with the Mfold

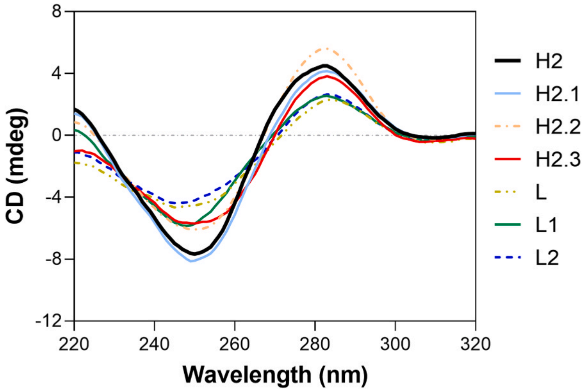

Fig. 2. Circular Dichroism (CD) spectra of the histamine H2 aptamer variants after truncation. Each aptamer solution was prepared at 1 μM in binding buffer (10 mM PBS pH 7.4 with 1.5 mM MgCl$_2$) and the CD spectra were acquired at ambient temperature (22 – 25 °C).

webserver compared to the other variants (Fig. S1).

Different techniques can be used to evaluate the affinity of aptamers for their ligands, including surface plasmon resonance (SPR) [18], isothermal calorimetry (ITC) [39] and microscale thermophoresis (MST) [33] among others. When the target is a small molecule, this task can be challenging due to low sensitivity, problems with target immobilization as well as non-specific adsorption. To address this, an enzyme-linked aptamer assay (ELAA) was designed to evaluate the affinity of the histamine aptamer variants. Histamine was immobilized on microtitre plates via a long carbon chain crosslinker and a range of different concentrations of biotinylated aptamers added. As can be seen in Table 2 and Fig. S3, the affinity dissociation constants for all truncated sequences were similar and in the low nanomolar range (0.818 – 8.691 nM), suggesting that the primer annealing sites are not essential for histamine binding and that the L is the minimum variant among the ones tested, maintaining high binding affinity for histamine. The L2 variant was finally chosen for further assay development as it was considerably shorter than the H2.1, H2.2 and H2.3 variants, which would translate into lower assay cost, while maintaining high binding affinity for histamine and facilitating assay development considering its predicted stem-loop structure.

3.2. Histamine aptamer-based assay development

The development of a robust, rapid assay for the detection of small molecules is often a complicated task, due to the small size of the target, which does not typically allow simultaneous binding by two biorecognition molecules in a sandwich-type assay. Competitive assays have been designed to surpass this limitation, but the low number of target functional groups required for both immobilization and biorecognition can hinder assay development. On the other hand, when the small molecule target is immobilized, blocking agents used to prevent non-specific adsorption can produce steric hindrance effects, thus impeding aptamer binding. Despite these drawbacks, competitive assays are the predominant choice for detecting small molecules. In this work, the first assay design tested for histamine detection was based on the competition between histamine in solution and histamine immobilized on microtitre plate wells through a long carbon chain spacer for binding to a limited concentration of labelled aptamer. Even though it was possible to detect histamine with this assay format (data not shown), the lack of reproducibility and issues of compatibility with a lateral flow assay format encouraged us to explore an alternative strategy, exploiting the use of a capture probe partially complementary to the aptamer. The correct design of this complementary probe is critical be able to attain the desired levels of sensitivity and selectivity in the detection process [46,47]. A short sequence with limited complementarity may yield weak hybridization, leading to potential false-positive signals, whilst an overly long oligonucleotide with strong binding to the aptamer may result in false negatives. Achieving the right balance requires a complementary sequence with comparable or slightly lower affinity for the aptamer than the target. In this work, a 12-nucleotide capture complementary probe (CCP) expected to hybridize with bases of the stem-loop part of the L2 histamine aptamer was designed (Table 1, Fig. S1). For microtitre plate detection, it was modified with a 5'-thiol group to allow immobilisation on wells of a maleimide-activated microtitre plate (Fig. 1A), whilst for LFA a 5'-amine moiety was used for immobilization on nitrocellulose strips via UV-crosslinking (Figs. 1B and 1C). The assay relied on pre-incubation of histamine with the biotinylated L2 truncated aptamer in solution, followed by addition of the mixture to wells or strips with immobilized CCP for capturing free biotinylated aptamer (not complexed with the target) and finally detection with SA-HRP, directly for plate detection (SA-polyHRP) or conjugated to gold nanoparticles for the LFA platform.

Table 2
Affinity dissociation constants (K_D values) of the H2 full-length aptamer and its truncated variants determined by Enzyme-Linked Aptamer Assay (ELAA). Standard deviations from triplicate measurements are shown.

Aptamer	Length (nt)	K_D (nM)	R^2
H2	99	0.818 ± 0.103	0.862
H2.1	76	1.841 ± 0.269	0.882
H2.2	76	1.012 ± 0.160	0.813
H2.3	58	7.352 ± 0.578	0.983
L	38	8.691 ± 0.948	0.969
L1	45	6.051 ± 0.747	0.952
L2	44	3.121 ± 0.397	0.927

3.2.1. Detection of histamine using enzyme-linked aptamer assay (ELAA) on microtitre plates

The assay format was initially evaluated on microtitre plates using an enzyme-linked aptamer assay (ELAA). The thiolated CCP was immobilized on maleimide-activated plates, while the biotinylated L2 aptamer was pre-incubated in solution with histamine. This pre-incubation step was performed in aptamer binding buffer at pH 7.4. For effective detection, it is critical to maintain the pH since any changes can affect the ionization of nucleotides, thus altering the hydrogen bonding patterns crucial for histamine recognition, as previously demonstrated [11]. Optimal pH conditions are also vital to maintain the three-dimensional structure of the aptamer and ensure the formation of stable complexes with the target molecules. Different parameters of the assay were optimized, including the concentration of the aptamer and the duration of the incubation steps, off-plate and on-plate. As can be seen in Fig. S4A, a biotinylated aptamer concentration of 1.25 – 2.5 nM led to the highest signal difference between samples with and without histamine, and 1.25 nM of L2-biotin was finally chosen for further experiments. Regarding the duration of the pre-incubation step, 15 min was considered as optimal since it provided the highest differentiation in signal (Fig. S4B). Finally, 0 – 20 min were tested for the on-plate incubation of the pre-incubated mixtures. As can be observed in Fig. S4C, more than 5 min were required for efficient histamine detection, with optimal differentiation achieved after a 10 min incubation. The sensitivity of the assay was finally evaluated under these optimized conditions (1.25 nM of L2-biotin, 15 min of pre-incubation and 10 min of incubation on-plate). Increasing histamine concentrations led to decreasing signals as less aptamer was available to hybridise with the immobilized CCP, reaching approximately 70 % of signal decrease with the highest concentration of histamine (400 nM) (Fig. S5). The half-maximal inhibitory concentration (IC50) was calculated to be 41 nM (4.56 ng/mL) and the LOD to be 13.8 nM (1.4 ng/mL). A recent study detailing the detection of histamine using an antibody-based competitive microplate assay reported an IC50 of 21.51 ng/mL [54]. The LOD achieved in this work compares well with those achieved with commercially available ELISA kits, which have LODs between 0.068 – 7 ng/mL (Table S3). In some cases, the aptamer-based ELAA resulted in higher sensitivity than antibody-based assays, in addition to the other advantages provided by aptamers such as versatility, ease of modification, and lower cost. Furthermore, the developed ELAA is completed within just 25 minutes, whilst the commercially available ELISA kits require a minimum of 1 hour to be carried out.

The microplate Enzyme-Linked Aptamer Assay (ELAA) was validated with quality control fish samples (FAPAS®) containing different levels of histamine. A simple and rapid procedure requiring minimum infrastructure was developed for the extraction of histamine from these samples to facilitate analysis. It was based on the use of water as the solvent and microbeads for rapid homogenization and extraction, followed by syringe filtering to remove insoluble material and proteins which could potentially interfere with detection (Fig. 3). The histamine content of the extracts was first quantified with reverse-phase high-performance liquid chromatography (RP-HPLC), the gold standard for histamine detection, to ensure efficient extraction from the samples. HPLC analysis indeed confirmed that histamine was efficiently extracted from the fish samples with the rapid method developed in this work,

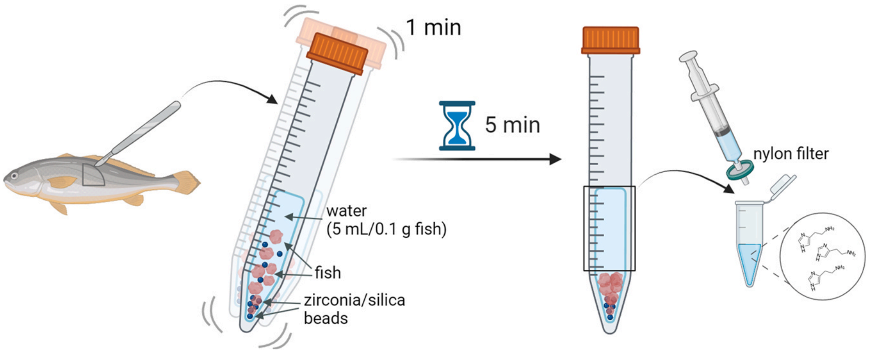

Fig. 3. Schematic illustration of the rapid histamine extraction method from fish developed in this work.

with recoveries in the range of 82 – 102 % (Table 3). When the fish samples were analysed with the microplate ELAA, histamine was successfully detected and the recoveries achieved were 81 – 120 % regardless of the type of fish (tuna or sardines in tomato sauce). Considering the permitted levels of histamine in fish products (30 – 400 ppm) according to international legislation [8], the developed ELAA is particularly suitable for screening fish samples and detecting histamine at the lowest permitted levels (approximately 30 – 50 ppm) with good accuracy and recovery. We previously demonstrated the compatibility of the full-length H2 aptamer with detection of histamine in fish in a label-free AuNP-based assay [24]. This work further highlights the robustness of this aptamer, in its original full-length or its truncated format, for histamine detection in complex samples such as crude fish extracts.

3.2.2. *Detection of histamine with the aptamer-based lateral flow assays (LFA)*

Having established the assay principle on microtitre plates using the L2 aptamer and the CCP partially complementary DNA probe, the next step was to transfer this format to a lateral flow assay. There are very few reports in the literature detailing the use of aptamers for small molecule detection using LFAs [25]. These are normally based on a competitive format [57] as it is extremely challenging to form a sandwich with two aptamers due to limited binding sites on the target molecule. The possibility of using split aptamers in a sandwich format has been pursued but is quite difficult and costly [19,59]. In this work, the LFA design was based on the immobilization of the CCP on the nitrocellulose membrane to construct the test line (Figs. 1B and 1C). This CCP probe was aminated at its 5'-end to facilitate covalent UV crosslinking with the nitrocellulose membrane, thus eliminating the need for a protein anchor [21]. Biotinylated BSA was used to construct the control line and a streptavidin-based gold nanoparticle conjugate was used as the reporter for the biotinylated aptamer.

A streptavidin-HRP conjugate was immobilized on gold nanoparticles, to facilitate signal enhancement when used in combination with a HRP chromogenic substrate. This SA-HRP@AuNP reporter conjugate was characterized by UV-Vis spectrometry, zeta potential measurements and transmission electron microscopy (TEM). The UV-Vis absorption spectra of the bare AuNPs (before conjugation) showed a peak a 526 nm, which is consistent with the size of the nanoparticles (40 nm), while a red-shift from 526 nm to 531 nm was observed after conjugation (Fig. S6A). The peak at approximately 275 nm also confirmed the presence of protein on the surface of the AuNPs. Regarding the zeta potential measurements, bare AuNPs exhibited an average zeta potential of −46.7 ± 1.1 mV, which is in agreement with previous data [24]. When the surface of the AuNPs was coated with SA-HRP, the zeta potential of the particles increased to −33.2 ± 3.6 mV, indicating that the negative forces of the gold nanoparticles were partially neutralized by the protein on their surface (Fig. S6B) [32]. Finally, Transmission Electron Microscopy (TEM) was used to evaluate the shape and size of the particles. As can be seen in Fig. S6C, bare AuNPs were well-dispersed and spherical with a uniform diameter of 41 ± 1.3 nm, whilst the SA-HRP@AuNPs conjugate exhibited a slightly increased diameter (42 ± 2.9 nm) and a tendency to agglomerate due to the protein interaction, which has also been observed for antibody-gold nanoparticle conjugates [54].

A key parameter that can influence the performance of the assay is the composition of the running buffer. Based on previous reports on aptamer-based LFAs for the detection of small molecules [29,59], different buffers were compared in this work: a running buffer composed of PBST and supplemented with sucrose and PEG-20000 (R.B.), PBS, PBST and borate buffer (B.B.). As can be seen in Fig. S7A, the sucrose/PEG-containing running buffer (R.B.) was the best option since it resulted in a higher signal difference at the test line in the presence and absence of histamine (~ 60 % signal inhibition). On the other hand, limiting concentrations of the biotinylated L2 aptamer are required to allow for naked eye detection of changes in the intensity of the test line in the presence of low amounts of histamine. Considering this, different concentrations of L2-biot aptamer (0, 2.5, 5 and 10 nM) were initially used to spot on the strips and as can be seen in Fig. S7B, the highest intensities were achieved when at least 5 nM of L2-biot aptamer were used in the absence of histamine. However, in the presence of histamine, the use of 10 nM of the aptamer resulted in a false-negative result, indicating that the aptamer was in excess. In the case of 5 nM of the aptamer, approximately 50 % of signal inhibition was observed in the presence of histamine and this concentration was thus chosen for the final LFA design.

As the final concentration of the L2-biot aptamer was very low, the

Table 3
Quantification of histamine in the fish samples by reverse-phase high-performance liquid chromatography (RP-HPLC) and the developed enzyme-linked aptamer assay (ELAA). * MV ± SD, n = 4; ** MV ± SD, n = 2.

FAPAS QC material			**RP-HPLC**		**ELAA**	
Sample ID	**Canned fish type**	**Histamine (ppm)**	**Histamine (ppm)***	**Recovery (%)**	**Histamine (ppm)****	**Recovery (%)**
T27206QC	Tuna	37.7	30.8 ± 1.7	81.7	30.6 ± 1.1	81.2
T27243QC	Tuna	128	123.0 ± 4.7	96.1	123.7 ± 31.3	96.6
T27234QC	Sardines in tomato sauce	160	150.3 ± 4.4	93.9	191.8 ± 30.6	119.9
T27211QC	Sardines in tomato sauce	223	228.2 ± 11.2	102.3	202.7 ± 36.8	90.9

amount of the reporter moiety should also be low to avoid non-specific signals from excess nanoparticles. However, under these conditions, the overall signal intensity was low (Fig. S7C, strip 1). Different strategies have been reported in the literature for signal enhancement and potential increased test sensitivity. Choi and colleagues explored the use of dual gold nanoparticle conjugates for the detection of troponin I, achieving a detection limit of 0.01 ng/mL [7]. An alternative approach for improved sensitivity involved the implementation of a test-zone pre-enrichment strategy, as demonstrated by Zang and co-workers, improving the LOD by 10 – 100-fold as compared to a conventional LFA setup [56]. The use of an enzyme chromogenic substrate such as TMB or metal-enhanced DAB in combination with particles bearing the HRP enzyme has also been reported [40]. Herein, we first evaluated the use of dual nanoparticles for signal enhancement. A streptavidin-HRP conjugate immobilized on small AuNPs (15 nm) and blocked with BSA was combined with larger AuNPs (40 nm) conjugated to an anti-BSA IgG antibody. As can be observed in Fig. S7C, the dual nanoparticle approach did lead to a general increase of signal intensity but non-specific signals and/or excess of nanoparticles hindered the detection of histamine since there was no signal change when histamine was present. However, the use of DAB as a substrate did slightly increase the line intensities thus facilitating visual inspection of the strips and evaluation of the results, albeit without affecting the LOD (Fig. S7D).

3.3. *Performance of the aptamer-based lateral flow assay for the detection of histamine in fish*

Once all the parameters were optimized, the lateral flow assay was used for the detection of histamine in fish using the design shown in Fig. 1B. A calibration curve was first constructed using histamine standard solutions prepared in buffer in the range of 1 – 2000 nM, equivalent to 0.01 – 11.1 ppm (mg/kg), considering the extraction of 100 mg of fish with 5 mL of water. As can be seen in Fig. 4A, when histamine was not present in the solution, the intensity of the test line was highest since all the L2-biot aptamer was available to hybridise with the CCP immobilized on the strip. When the concentration of histamine in the solution increased, the L2-biot aptamer preferentially bound histamine due to its high binding affinity and less aptamer was available to hybridise with the CCP, resulting in decreased test line intensity. At 8 nM (0.05 ppm) of histamine, there was a clear decrease of the intensity of the test line compared to the blank control, therefore this can be considered as the visual LOD (vLOD) of the test. After quantification of the intensity of the test lines using the ImageJ software, an LOD of 22.829 nM (0.127 ppm) and IC50 of 70.060 nM (0.389 ppm) were calculated.

The assay was then validated using quality control fish samples from FAPAS. Histamine extraction from these samples was performed as detailed earlier. For comparison, the commercially available HistaSure$^{\mathrm{TM}}$ fish antibody-based rapid test kit was used and the extracts were diluted according to the manufacturer's instructions and analysed in parallel with the aptamer-based LFA. The aptamer-based LFA successfully detected histamine in the fish extracts, as can be seen from the lower intensities of the test lines as compared to the blank control (Fig. 4B). The results obtained with the aptamer-based assay and the antibody-based commercial kit were very similar with the highest test line intensity observed for the sample with the lowest histamine content (37.7 ppm) and the lowest for the sample with the highest amount of histamine (223 ppm). Furthermore, test lines with similar intensities were observed for the two samples containing similar levels of histamine but in different matrices (tuna with 128 ppm histamine and sardines in tomato sauce with 160 ppm histamine), demonstrating that the assay is compatible with complex sample matrices and that the simple and rapid extraction procedure can be reliably used. Overall, the aptamer-based lateral flow assay developed in this work is more sustainable, stable and cost-effective than the antibody-based commercial kits, with an estimated cost of less than 1€ per strip when produced on a laboratory scale.

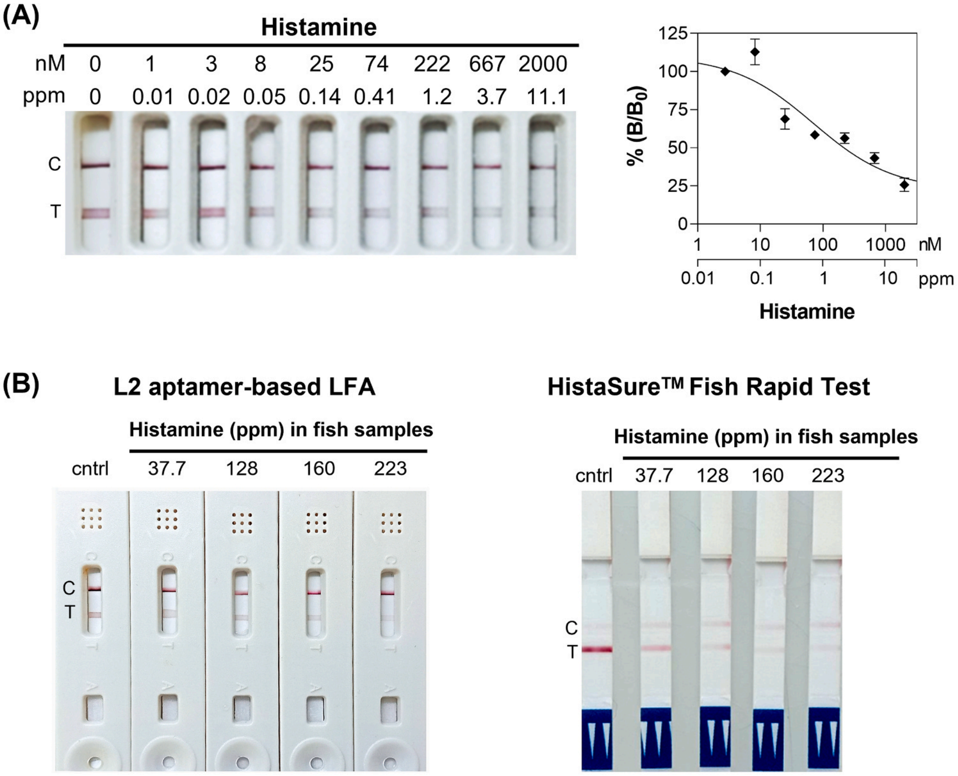

Fig. 4. L2 aptamer-based LFA for detection of histamine in fish. (A) Calibration curve with histamine standards prepared in buffer. (B) Application of the aptamer LFA (left panel) and the commercial HistaSure$^{\mathrm{TM}}$ fish rapid test (right panel) to the analysis of the FAPAS QC fish samples with histamine levels of 37.7 ppm (tuna; T27206QC), 128 ppm (tuna; T27243QC), 160 ppm (sardines in tomato sauce; T27234QC) and 223 ppm (sardines in tomato sauce; T27211QC).

Several sensors have been reported in the literature during the last years for histamine detection in fish. A competitive LFA employing an antibody conjugated to gold nanoparticles instead of an aptamer was demonstrated by Zeng et al. [54] with a vLOD of 0.25 ppm, achieving a calculated LOD of 0.01 ppm for detection in fish. An AuNPs@FeP-chitosan oligosaccharide nanozyme-based colorimetric aptasensor was developed with an LOD of 1.89 nM, which was applied to salmon and shrimp analysis using microneedle patches for sampling [48]. Different chemosensors have also been developed, including a portable MIP-based electrochemical sensor shown to detect histamine in the range of 100 – 500 ppm in seafood [43]; a dual-mode colorimetric and fluorescent sensor based on N-doped carbon dots, dipicolinic acid and o-phenylenediamine with micromolar LODs (6.96 – 10.11 μM) for analysis of raw fish samples [55]; and a fluorescent "turn-on" amine sensor employing cationic C-C single bond covalent organic frameworks with a histamine LOD of 58 μM used to assess freshness of fish [14]. Finally, a combined MIP-aptamer electrochemical biosensor employing gold nanoparticles and carbon nanotubes was reported by Mahmoud et al. [27], with an LOD of 0.11 – 0.15 nM in buffer, and was applied to the analysis of canned tuna samples spiked with 10 – 40 ppm of histamine. The antibody competitive LFA [54] has similar performance to the one reported herein in terms of sensitivity and test time, requiring only a few simple steps to perform, but is more expensive and relies on animal hosts for antibody production. The other sensors described above are less sensitive, require complex fabrication processes and rely on longer analysis with multiple steps.

3.4. *Assay specificity and stability*

The specificity of the lateral flow assay was evaluated using a variety of small molecules, which could potentially interfere with histamine detection. Biogenic amines that can be found in fish along with histamine (spermidine, cadaverine, tryptamine and tyramine) were analysed. The histamine precursor histidine, as well as methylhistamine and dopamine, which can also be present in biological fluids [22] were also tested. As can be seen in Fig. 5, no cross-reactivity was observed for any of the compounds tested, further confirming the suitability of the assay for histamine detection in diverse samples. The storage stability of the LFA test was finally evaluated with an Arrhenius accelerated thermal stability study as detailed in the Supplementary Material. A shelf life of approximately one year at 22°C was predicted under the storage conditions tested since no significant difference in the signal ratio in the presence and absence of histamine (% B/B0) was observed in the course of 13 days storage at 55°C (Fig. S8). This long shelf life of the test can potentially be attributed to the inherent stability of DNA, which is one of the advantages this test offers over antibody-based equivalent ones.

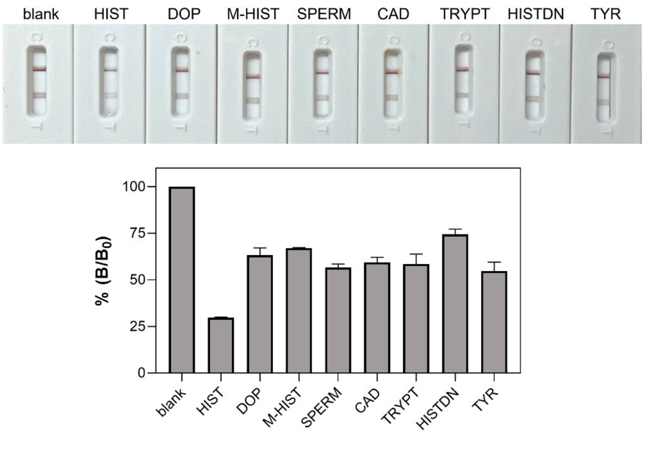

Fig. 5. Specificity of the lateral flow assay (LFA). HIST: histamine; DOP: dopamine; M-HIST: methylhistamine; SPERM: spermidine; CAD: cadaverine; TRYPT: tryptamine; HISTDN: histidine; TYR: tyramine.

3.5. *Analysis of biological fluids*

To broaden the utility of the developed assay, we investigated its applicability to the detection of histamine in blood with potential application for the rapid detection of allergic reaction. In the case of blood analysis, the basal levels of histamine in plasma are approximately 2.7 – 3.6 nM (0.3 – 0.4 ng/mL) [4,17]. During an anaphylactic episode, there is a swift and notable surge in histamine concentrations in blood and plasma, typically escalating within the first 10 minutes of the onset of symptoms [10]. These elevated levels return to baseline within approximately 30–60 min. Consequently, the sensor must not only exhibit high sensitivity and specificity but also operate with exceptional speed to rapidly detect histamine. The strip design used for the analysis of blood is shown in Fig. 1C where a blood separator sample pad was incorporated, and all the other components were identical to the LFA devices developed for detection in fish. A calibration curve was first constructed in the presence of fingerprick blood from a healthy subject by spiking histamine in the range of 3–2000 nM to evaluate the performance of the LFA under these conditions. The blood matrix did not affect the assay performance, with similar test line signal patterns observed in the absence (Fig. 4A) and presence of blood (Fig. 6A) for the same range of histamine concentrations. After plotting the intensity of the test lines against the logarithm concentrations of histamine [% (B/B$_0$)] and fitting the data using a sigmoidal 4PL model, an IC50 of 23.590 nM and LOD of 8.368 nM were calculated (Fig. 6A).

With the aim of using the assay for the rapid detection of elevated histamine levels in blood, a dual-strip LFA cassette was employed (Fig. 6B). Considering that the developed LFA is a "signal-off" type, one strip serves as the control to establish the baseline signal in the absence of histamine, whilst the second strip is used for the analysis of blood. The presence of elevated histamine levels in the sample would thus be indicated by the signal decrease at the test line of the second strip as compared to the control strip. The test was first performed in the absence of histamine and no major difference in the intensities of the test lines between the two strips was observed when buffer or blood were analysed, again demonstrating no matrix effect from the blood (Fig. S9A). Having established the suitability of buffer as the negative control, fingerprick blood samples from ten healthy subjects of different individuals was spiked with a high concentration of histamine (200 nM)

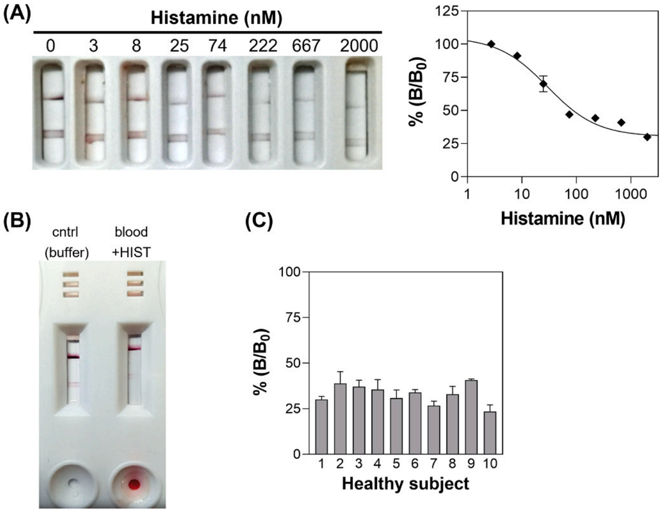

Fig. 6. Lateral flow assay (LFA) for histamine detection in biological fluids. (A) Calibration curve in the presence of fingerprick blood. (B) Use of dual cassette for detection of histamine spiked in blood using the strip with buffer as the negative control. (C) Detection of histamine spiked in fingerprick blood from healthy subjects.

to reflect a severe anaphylactic episode [4]. The volunteers were 70 % women and 30 % men in the age groups of 31–40 (60 %) and 41–50 (40 %), of European (60 %), Asian (20 %), North American (10 %) and African (10 %) origin (Fig. S9B). As can be seen in Fig. 6C and Fig. S9C, histamine was successfully detected in the blood of all subjects and more than a 60 % signal decrease was observed in all cases when histamine was present, facilitating a clearly discernible signal intensity in the event of an allergic reaction. The implementation of this dual-strip LFA represents a significant advancement in allergy diagnosis, enabling the detection of an allergic reaction with just one drop of fingerprick blood in less than 10 minutes, allowing for rapid management and administration of appropriate treatment.

There are very few examples in the literature describing histamine sensors for blood analysis mainly due to the complexity of the matrix and the transient nature of histamine in this biological fluid. A MIP-aptamer electrochemical biosensor previously reported by Mahmoud et al. [27] was applied to the detection of histamine spiked in human plasma in the range of 5 – 30 nM. Another electrochemical chemosensor using the histamine-selective cucurbit[6]uril ionophore was demonstrated with an LOD of 300 nM in buffer and > 97 % recoveries when histamine was spiked in serum at > 1 μM [34]. Finally, Alqahtani et al. [2] developed a chemosensor relying on the chemical transformation of histamine to a fluorescent derivative able to detect histamine spiked in plasma in the range of 1 – 200 ng/mL. In all these examples, blood was not analysed directly but rather after extensive pre-treatment steps requiring laboratory infrastructure and longer overall analysis time. In the aptamer LFA described in this work, fingerprick blood was directly analysed without any prior treatment, further highlighting the advantages of this test and its potential in allergy management.

3.6. Advantages, limitations and other potential applications

Histamine is classified as an acute toxic substance, irritant and a health hazard [37]. It can be produced by different bacterial species and is also present in the body as a metabolite of the amino acid histidine with a variety of biological functions [17]. It is thus evident that rapid and sensitive detection of histamine is essential to ensure food safety and public health. Current instrumental analytical methods like liquid chromatography coupled with mass spectrometry (LC-MS), the gold standard for histamine detection, rely on expensive equipment, complex sample pre-treatment and trained personnel. Whilst significant technological advances have been made towards miniaturization and development of portable LC-MS systems capable of analysing crude samples to eliminate complex pre-treatment requirements, there are still many challenges to be addressed for on-site analysis [42]. Alternative antibody-based assays can be very sensitive and are easier to perform, but they often suffer from specificity issues, mainly due to the limited binding sites and high structural similarity with other small molecules. Moreover, with the continuous increase in antibody production cost [5], antibody-based assays are becoming incompatible with routine, low-cost analysis.

In this work, we demonstrated the development of DNA aptamer-based lateral flow assays for facile and quick detection of histamine. These tests are easy to perform, requiring very few simple steps, rapid and with low overall cost compared to antibody-based ones partially because of the low production cost of synthetic DNA.

Even though the focus of this work was food safety and allergy diagnosis and management in a timely manner, the LFAs described herein could find potential applications in other areas as well, such as the prevention of consumer fraud, especially in the case of expensive fish such bluefin (red) tuna which are susceptible to fraudulent practices driven by the high demand and elevated cost [38]. Testing for histamine build-up in fresh fish during prolonged storage could be implemented in fish markets for routine monitoring as well as for supply chain control to ensure fish freshness and combat fraudulent practices. Due to its high solubility, histamine is readily dissolved in water and can contribute to aquatic environment contamination. Depending on environmental conditions such as temperature, pH and salinity, naturally occurring histamine-producing bacteria can thrive and rapidly metabolize histidine, naturally present in organic matter (e.g. from aquatic plants or dead fish), into histamine through enzymatic decarboxylation and subsequently release it into the water [15]. The histamine LFA tests could therefore be used for monitoring environmental waters to ensure public health and minimize disruptions of aquatic and terrestrial ecosystems. The potential rise of histamine levels in food processing and aquaculture waste is also of concern to public health since protein-rich waste or fermentation products can lead to increased histamine production. The risks associated with exposure to harmful levels of histamine could thus be reduced by employing systematic monitoring practices to prevent accumulation. The LFA tests described herein could also be used to monitor histamine levels as an indicator of bacterial activity and potential presence of other harmful compounds, thus preventing further contamination of water bodies and soil and improving waste management strategies.

4. Conclusions

Aptamer-based assays for the detection of histamine in fish and human fingerprick blood were demonstrated in this work. The H2 aptamer previously reported by our group was first truncated to eliminate non-essential bases and the shortest variant with the highest binding affinity was chosen for assay development. A short partially complementary DNA probe was then designed to facilitate detection. In the absence of histamine, the biotinylated truncated aptamer hybridises with the complementary probe immobilised on microtitre plates or nitrocellulose membrane and colour is produced by streptavidin conjugated to horseradish peroxidase or gold nanoparticles, respectively. When histamine is present in the sample though, it is preferentially bound by the aptamer in solution leading to signal loss due to decreased availability of free aptamer for hybridisation with the probe. A simple procedure requiring minimum infrastructure was developed for efficient extraction of histamine from fish and was validated by liquid chromatography. The fish extracts were analysed with the microplate aptamer assay and the recoveries achieved were comparable to liquid chromatography. The same fish extracts were also successfully analysed with the LFA which was shown to exhibit excellent analytical performance in buffer (LOD of 22.829 nM (0.127 ppm) and IC50 of 70.060 nM (0.389 ppm)) with an analysis time of less than 10 minutes. Furthermore, by simply incorporating a suitable sample pad into the LFA design it was possible to analyse whole blood with an LOD of 8.368 nM and IC50 of 23.590 nM and detect elevated levels of histamine associated with allergic shock in less than 10 minutes. The developed assays exhibited very high specificity as no interference from other biogenic amines or related molecules was observed and long storage stability of approximately 1 year at 22°C. They can be valuable tools for the rapid screening of foodstuffs to prevent intoxication or for analysing biological fluids for the rapid detection of an allergic reaction and administration of appropriate treatment. Future work will focus on further simplifying the test and reducing operator steps by incorporating the gold nanoparticles and the aptamer in the conjugate pad. Further improving visual detection will also be considered by using alternative labels (e.g. coloured latex or carbon nanoparticles) for enhanced contrast and colour intensity and by combining the LFA with smartphone image analysis to eliminate subjective interpretation and facilitate semi-quantification.

Environmental implications

The rapid detection of histamine is critical as the ingestion of histamine-rich foods or deficiencies in histamine-degrading enzymes can lead to histamine accumulation and produce intolerance, which is associated with adverse health effects mimicking an allergic reaction with symptoms ranging from mild to even severe and potentially fatal anaphylaxis. In this work, easy-to-use and cost-effective lateral flow assays were developed for the rapid and accurate aptamer-based detection of histamine in fish and fingerprick blood samples.

CRediT authorship contribution statement

Mairal-Lerga Teresa: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Redondo M.Carmen Bermudo:** Validation, Formal analysis, Data curation. **Skouridou Vasso:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization. **Jauset-Rubio Miriam:** Conceptualization. **O' Sullivan Ciara:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors state that they have nothing to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.138540.

Data availability

Data will be made available on request.

References

[1] Ahmed, S., Ansari, A., Li, Z., Mazumdar, H., Siddiqui, M.A., Khan, A., Ranjan, P., Kaushik, A., Vinu, A., Kumar, P., 2025. Point-of-care health diagnostics and food quality monitoring by molecularly imprinted polymers-based histamine sensors. Adv Sens Res 4, 2400132. https://doi.org/10.1002/adsr.202400132.

[2] Alqahtani, A., Alqahtani, T., Abdelazim, A.H., 2024. Development of fluorescence chemo sensor for selective histamine determination in spiked human plasma samples. Spectrochim Acta A Mol Biomol Spectr 308, 123711. https://doi.org/10.1016/j.saa.2023.123711.

[3] Ameri, M., Daryanavard, S.M., 2023. Experimental design application for measuring histamine in tuna fish samples by phenyl isothiocyanate derivation method using ultra-performance liquid chromatography. J Chromatogr Sci 62, 570–579. https://doi.org/10.1093/chromsci/bmad060.

[4] Boehm, T., Reiter, B., Ristl, R., Petroczi, K., Sperr, W., Stimpfl, T., Valent, P., Jilma, B., 2019. Massive release of the histamine-degrading enzyme diamine oxidase during severe anaphylaxis in mastocytosis patients. Allergy 74, 583–593. https://doi.org/10.1111/all.13663.

[5] Chen, C., Garcia, Z., Chen, D., Liu, H., Trelstad, P., 2025. Cost and supply considerations for antibody therapeutics. mABS 17, 2451789. https://doi.org/10.1080/19420862.2025.2451789.

[6] Chen, C., Zhang, Y., Wang, X., Qiao, X., Waterhouse, G.I.N., Xu, Z., 2024. A core-satellite self-assembled SERS aptasensor containing a "biological-silent region" Raman tag for the accurate and ultrasensitive detection of histamine. Food Sci Hum Wellness 13, 1029–1039. https://doi.org/10.26599/FSHW.2022.9250089.

[7] Choi, D.H., Lee, S.K., Oh, Y.K., Bae, B.W., Lee, S.D., Kim, S., Shin, Y.B., Kim, M.G., 2010. A dual gold nanoparticle conjugate-based lateral flow assay (LFA) method for the analysis of troponin I. Biosens Bioelectron 25, 1999–2002. https://doi.org/10.1016/j.bios.2010.01.019.

[8] Debeer, J., Bell, J.W., Nolte, F., Arcieri, J., Correa, G., 2021. Histamine limits by country: A survey and review. J Food Prot 84, 1610–1628. https://doi.org/10.4315/JFP-21-129.

[10] Dribin, T.E., Schnadower, D., Wang, J., Camargo, C.A., Michelson, K.A., Shaker, M., Rudders, S.A., Vyles, D., et al., 2022. Anaphylaxis knowledge gaps and future research priorities: a consensus report. J Allergy Clin Immunol 149, 999–1009. https://doi.org/10.1016/j.jaci.2021.07.035.

[11] Duan, N., Li, C., Zhu, X., Qi, S., Wang, Z., Wu, S., 2022. Simultaneous coupled with Separate SELEX for heterocyclic biogenic amine-specific aptamers screening and their application in establishment of an effective aptasensor. Sens Actuators B Chem 352, 130985. https://doi.org/10.1016/j.snb.2021.130985.

[12] Dwidar, M., Seike, Y., Kobori, S., Whitaker, C., Matsuura, T., Yokobayashi, Y., 2019. Programmable artificial cells using histamine-responsive synthetic riboswitch. J Am Chem Soc 141, 11103–11114. https://doi.org/10.1021/jacs.9b03300.

[13] Ellington, A.D., Szostak, J.W., 1990. In vitro selection of RNA molecules that bind specific ligands. Nature 346, 818–822. https://doi.org/10.1038/346818a0.

[14] Feng, X.N., Liu, X.Y., Cao, D.X., Zhou, Y.J., Cui, Y.X., Kong, D.M., 2025. "Turn-on" mode fluorescence detection of amines based on a cationic covalent organic framework linked with C−. C Single Bond J Hazard Mater 489, 137617. https://doi.org/10.1016/j.jhazmat.2025.137617.

[15] Frith, A., Hayes-Mims, M., Carmichael, R., Bjornsdottir-Butler, K., 2023. Effects of environmental and water quality variables on histamine-producing bacteria concentration and species in the Northern Gulf of Mexico. Microbiol Spectr 11, e0472022. https://doi.org/10.1128/spectrum.04720-22.

[16] Fu, H.J., Su, R., Luo, L., Chen, Z.J., Sørensen, T.J., Hildebrandt, N., Xu, Z.L., 2022. Rapid and wash-free time-gated FRET histamine assays using antibodies and aptamers. ACS Sens 7, 1113–1121. https://doi.org/10.1021/acssensors.2c00085.

[17] Heidarzadeh-Asl, S., Maurer, M., Kiani, A., Atiakshin, D., Stahl Skov, P., Elieh-Ali-Komi, D., 2024. Novel insights on the biology and immunologic effects of histamine: a road map for allergists and mast cell biologists. J Allergy Clin Immunol S0091-6749(24)02415-1. https://doi.org/10.1016/j.jaci.2024.12.1081.

[18] Jauset Rubio, M., Svobodová, M., Mairal, T., Schubert, T., Künne, S., Mayer, G., O'Sullivan, C.K., 2016. β-Conglutin dual aptamers binding distinct aptatopes. Anal Bioanal Chem 408, 875–884. https://doi.org/10.1007/s00216-015-9179-z.

[19] Jing, L., Xie, C.Y., Li, Q.Q., Yao, H.F., Yang, M.Q., Li, H., Xia, F., Li, S.G., 2022. A sandwich-type lateral flow strip using a split, single aptamer for point-of-care detection of cocaine. J Anal Test 6, 120–128. https://doi.org/10.1007/s41664-022-00228-w.

[20] John Ho, L.S., Fogel, R., Limson, J.L., 2020. Generation and screening of histamine-specific aptamers for application in a novel impedimetric aptamer-based sensor. Talanta 208, 120474. https://doi.org/10.1016/j.talanta.2019.120474.

[21] Justo, C.A.C., Skouridou, V., Cools, P., Mulinganya, G., Ibañez-Escribano, A., O'Sullivan, C.K., 2025. Biotin/avidin-free sándwich aptamer-based lateral flow assay (ALFA) for the diagnosis of Trichomonas vaginalis. Sens Diagn 4, 216–228. https://doi.org/10.1039/D4SD00342J.

[22] Knežević, S., Ognjanović, M., Nedić, N., Mariano, J.F.M.L., Milanović, Z., Petković, B., Antić, B., Djurić, S.V., Stanković, D., 2020. A single drop histamine sensor based on AuNPs/MnO2 modified screen-printed electrode. Microchem J 155, 104778. https://doi.org/10.1016/j.microc.2020.104778.

[23] Kypr, J., Kejnovska, I., Renciuk, D., Vorlickova, M., 2009. Circular dichroism and conformational polymorphism of DNA. Nucleic Acids Res 37, 1713–1725. https://doi.org/10.1093/nar/gkp026.

[24] Lerga, T.M., Skouridou, V., Bermudo, M.C., Bashammakh, A.S., El-Shahawi, M.S., Alyoubi, A.O., O'Sullivan, C.K., 2020. Gold nanoparticle aptamer assay for the determination of histamine in foodstuffs. Microchim Acta 187, 452. https://doi.org/10.1007/s00604-020-04414-4.

[25] Li, X., Sun, F., Chang, R., Xie, Z., Peng, C., Zhang, Y., Wang, Z., Liu, G., 2023. A versatile strategy of designing gold nanoparticle-cDNA nanoprobes in developing aptamer-based lateral flow assay for small-molecule detection. Langmuir 39, 8690–8697. https://doi.org/10.1021/acs.langmuir.3c00645.

[26] Li, Y.F., Lin, Z.Z., Hong, C.Y., Huang, Z.Y., 2021. Histamine detection in fish samples based on indirect competitive ELISA method using iron-cobalt co-doped carbon dots labeled histamine antibody. Food Chem 345, 128812. https://doi.org/10.1016/j.foodchem.2020.128812.

[27] Mahmoud, A.M., Alkahtani, S.A., Alyami, B.A., El-Wekil, M.M., 2020. Dual-recognition molecularly imprinted aptasensor based on gold nanoparticles decorated carboxylated carbon nanotubes for highly selective and sensitive determination of histamine in different matrices. Anal Chim Acta 1133, 58–65. https://doi.org/10.1016/j.aca.2020.08.001.

[28] Mairal Lerga, T., Jauset-Rubio, M., Skouridou, V., Bashammakh, A.S., El-Shahawi, M.S., Alyoubi, A.O., O'Sullivan, C.K., 2019. High affinity aptamer for the detection of the biogenic amine histamine. Anal Chem 91, 7104–7111. https://doi.org/10.1021/acs.analchem.9b00075.

[29] Mermiga, E., Pagkali, V., Kokkinos, C., Economou, A., 2023. An aptamer-based lateral flow biosensor for low-cost, rapid and instrument-free detection of ochratoxin A in food samples. Molecules 28, 8135. https://doi.org/10.3390/molecules28248135.

[30] Moyano, A., Salvador, M., Martínez-García, J.C., Socoliuc, V., Vékás, L., Peddis, D., Alvarez, M.A., Fernández, M., Rivas, M., Blanco-López, M.C., 2019. Magnetic immunochromatographic test for histamine detection in wine. Anal Bioanal Chem 411, 6615–6624. https://doi.org/10.1007/s00216-019-02031-6.

[31] Nelis, M., Decraecker, L., Boeckxstaens, G., Augustijns, P., Cabooter, D., 2020. Development of a HILIC-MS/MS method for the quantification of histamine and its main metabolites in human urine samples. Talanta 220, 121328. https://doi.org/10.1016/j.talanta.2020.121328.

[32] Okyem, S., Awotunde, O., Ogunlusi, T., Riley, M.B., Driskell, J.D., 2021. High-affinity points of interaction on antibody allow synthesis of stable and highly functional antibody-gold nanoparticle conjugates. Bioconjugate Chem 32, 1753–1762. https://doi.org/10.1021/acs.bioconjchem.1c00261.

[33] O'Sullivan, C.K., Mairal, T., Jauset-Rubio, M., Svobodova, M., Skouridou, V., Esposito, V., Virgilio, A., Galeone, A., 2021. Aptamers against the β-conglutin allergen: Insights into the behavior of the shortest multimeric (intra)molecular

DNA G-quadruplex. Int J Mol Sci 22, 1150. https://doi.org/10.3390/ijms22031150.

[34] Pereira, A.R., Araujo, A.N., Montenegro, M.C.B.S.M., Gomes Amrim, C.M.P., 2020. A simpler potentiometric method for histamine assessment in blood sera. Anal Bioanal Chem 412, 3629–3637. https://doi.org/10.1007/s00216-020-02597-6.

[35] Poli, C., Laurichesse, M., Rostan, O., Rossille, D., Jeannin, P., Drouet, M., Renier, G., Chevailler, A., Tarte, K., Bendavid, C., Beauvillain, C., Amé-Thomas, P., 2016. Comparison of two enzymatic immunoassays, high resolution mass spectrometry method and radioimmunoassay for the quantification of human plasma histamine. J Pharm Biomed Anal 118, 307–314. https://doi.org/10.1016/j.jpba.2015.11.001.

[36] Prante, M., Segal, E., Scheper, T., Bahnemann, J., Walter, J., 2020. Aptasensors for point-of-care detection of small molecules. Biosensors 10, 108. https://doi.org/10.3390/bios10090108.

[37] PubChem, 2025. ⟨https://pubchem.ncbi.nlm.nih.gov/compound/774#datasheet=LCSS⟩ (accessed 1 April 2025).

[38] Sáez-Hernández, R., Antela, K.U., Mauri-Aucejo, A.R., Morales-Rubio, A., Luque, M.J., Cervera, M.L., 2023. A fast and non-invasive imaging procedure to fight red tuna fraud. LWT 186, 115231. https://doi.org/10.1016/j.lwt.2023.115231.

[39] Slavkovic, S., Johnson, P.E., 2018. Isothermal titration calorimetry studies of aptamer-small molecule inter-actions: practicalities and pitfalls. Aptamers 2, 45–51.

[40] Tripathi, P., Kumar, A., Sachan, M., Gupta, S., Nara, S., 2020. Aptamer-gold nanozyme based competitive lateral flow assay for rapid detection of CA125 in human serum. Biosens Bioelectron 165, 112368. https://doi.org/10.1016/j.bios.2020.112368.

[41] Tuerk, C., Gold, L., 1990. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249, 505–510. https://doi.org/10.1126/science.2200121.

[42] Vargas Medina, D.A., Vasconcelos Soares Maciel, E., de Toffoli, A.L., Lanças, F.M., 2020. Achievements on modern instrumentation for miniaturized liquid chromatography coupled to mass spectrometry. Trends Anal Chem 128, 115910. https://doi.org/10.1016/j.trac.2020.115910.

[43] Venkatesh, S., Yeung, C.C., Li, T., Li, S.C., Sun, Q.J., Li, L.Y., Li, J.H., Lam, M.H.W., Roy, V.A.L., 2021. Portable molecularly imprinted polymer-based platform for detection of histamine in aqueous solutions. J Hazard Mater 410, 124609. https://doi.org/10.1016/j.jhazmat.2020.124609.

[44] Visciano, P., Schirone, M., Paparella, A., 2020. An overview of histamine and other biogenic amines in fish and fish products. Foods 9, 1795. https://doi.org/10.3390/foods9121795.

[45] Wang, B., Jiang, H., Tang, R., Tan, Y., Xia, X., Zhang, X., 2023. Construction of histamine aptamer sensor based on Au NPs nanozyme for ultrasensitive SERS detection of histamine. J Food Comp Anal 120, 105337. https://doi.org/10.1016/j.jfca.2023.105337.

[46] Wang, L., Yao, L., Ma, Q., Mao, Y., Qu, H., Zheng, L., 2023. Investigation on small molecule-aptamer dissociation equilibria based on antisense displacement probe. Food Sci Hum Wellness 12, 1257–1264. https://doi.org/10.1016/j.fshw.2022.10.008.

[47] Wang, T., Chen, L., Chikkanna, A., Chen, S., Brusius, I., Sbuh, N., Veedu, R.N., 2021. Development of nucleic acid aptamer-based lateral flow assays: a robust platform for cost-effective point-of-care diagnosis. Theranostics 11, 5174–5196. https://doi.org/10.7150/thno.56471.

[48] Wang, W., Feng, R., Wei, K., Xu, J., Dong, W., Li, J., Sun, J., Wang, S., Mao, X., 2025. An integrated colorimetric biosensing platform containing microneedle patches and aptasensor for histamine monitoring in seafood. J Hazard Mater 489, 137536. https://doi.org/10.1016/j.jhazmat.2025.137536.

[49] Wang, X., Yang, F., Deng, C., Zhang, Y., Yang, X., Chen, X., Huang, Y., Ye, H., Zhong, J., Wang, Z., 2022. A dual-mode method based on aptamer recognition and time-resolved fluorescence resonance energy transfer for histamine detection in fish. Molecules 27, 8711. https://doi.org/10.3390/molecules27248711.

[50] Wu, G., Ding, Z., Dou, X., Chen, Z., Xie, J., 2024. Recognition and detection of histamine in foods using aptamer modified fluorescence polymer dots sensors. Spectrochim Acta A Mol Biomol Spectr 317, 124452. https://doi.org/10.1016/j.saa.2024.124452.

[51] Xu, X., Liu, X., Qiu, Y., Song, Y., Zhou, X., Ding, Y., 2024. Colorimetric aptasensor based on Fe3O4@MOF@Pt nanozymes for ultrasensitive detection of histamine in hish. Food Control 166, 110702. https://doi.org/10.1016/j.foodcont.2024.110702.

[52] Xu, Y., Lu, Y., Yu, J., Liu, W., Jing, G., Li, W., Liu, W., 2023. Determination of seven biogenic amines in tuna with high-performance liquid chromatography coupled to electrospray ionization ion mobility spectrometry. Food Anal Methods 16, 909–917. https://doi.org/10.1007/s12161-023-02455-y.

[53] Yu, H., Alkhamis, O., Canoura, J., Liu, Y., Xiao, Y., 2021. Advances and challenges in small-molecule DNA aptamer isolation, characterization, and sensor development. Angew Chem Int Ed Engl 60, 16800–16823. https://doi.org/10.1002/anie.202008663.

[54] Zeng, L., Xu, X., Guo, L., Wang, Z., Ding, H., Song, S., Xu, L., Kuang, H., Liu, L., Xu, C., 2021. An immunochromatographic sensor for ultrasensitive and direct detection of histamine in fish. J Hazard Mater 419, 126533. https://doi.org/10.1016/j.jhazmat.2021.126533.

[55] Zhang, X., Wang, J., Hasan, E., Sun, X., Asif, M., Aziz, A., Lu, W., Dong, C., Shuang, S., 2024. Bridging biological and food monitoring: A colorimetric and fluorescent dual-mode sensor based on N-doped carbon dots for detection of pH and histamine. J Hazard Mater 470, 134271. https://doi.org/10.1016/j.jhazmat.2024.134271.

[56] Zhang, Y., Liu, X., Wang, L., Yang, H., Zhang, X., Zhu, C., Wang, W., Yan, L., Li, B., 2020. Improvement in detection limit for lateral flow assay of biomacromolecules by test-zone pre-enrichment. Sci Rep 10, 9604. https://doi.org/10.1038/s41598-020-66456-1.

[57] Zhao, Z., Wang, H., Zhai, W., Feng, X., Fan, X., Chen, A., Wang, M., 2020. A lateral flow strip based on a truncated aptamer-complementary strand for detection of type-B aflatoxins in nuts and dried figs. Toxins 12, 136. https://doi.org/10.3390/toxins12020136.

[58] Zhou, J., Wang, J., Huang, X., Xia, L., Tao, H., Wu, Y., 2024. Coral-like silver citrate as robust laccase mimetics in a novel colorimetric aptasensor for sensitive and highly selective detection of histamine in food. Food Control 165, 110645. https://doi.org/10.1016/j.foodcont.2024.110645.

[59] Zhu, C., Zhao, Y., Yan, M., Huang, Y., Yan, J., Bai, W., Chen, A., 2016. A sandwich dipstick assay for ATP detection based on split aptamer fragments. Anal Bioanal Chem 408, 4151–4158. https://doi.org/10.1007/s00216-016-9506-z.