Diyala Journal for Pure Science
International Conference for Pure Sciences (ICPS-2021)

Lateral Flow Immunoassay Based Colloidal Gold Nanoparticles for the Detection of Organophosphorous Pesticide (Propanoic) in Cow's Milk

Azhar M. Haleem[*1], Israa M. Haleem[2] and Ruaa H. Abass[3]

[1]Environment Research Center, University of Technology, Baghdad, Iraq
[2]Department of Animal Resources - College of Agricultural Engineering Science - Baghdad, Iraq
[3]Collage of Dentistry - University of Al-Farahidi - Baghdad, Iraq

[*]amhjanabi74@gmail.com

Received: 20 March 2021 **Accepted: 23 May 2021**

DOI: https://dx.doi.org/10.24237/djps.17.03.18-555-ICPS

Abstract

Simple, inexpensive, and high sensitive Lateral Flow Immunoassay (LFI) based colloidal gold nanoparticles antigen-coated as the label was designed for the fast determination of organophosphorus pesticide in cow's milk samples. The developed LFI detection strip consisted of four structural zones start from the sample pad, conjugate pad, detection zone, and finally absorbent pad. Immune-sensitive zone was synthesized and functionalized with Propanoic Hapten–OVA, Goat monoclonal-anti-Propanoic antibodies were produced. Propionic -cationized ovalbumin-HPR conjugates were fabricated and identified. The antibody optimal conditions were set and the influence of pH on the analytical properties of the antibody-based gold nanoparticles was also investigated. The detection limit of the LFI for Propanoic standard into milk samples were 0.5 and 100 ng/mL, respectively. The test development time for the LFI was less than 5 min, suitable for fast on-site measuring of Propanoic.

Keywords: Pesticides, lateral flow immuno-assay, organophosphorous, cow milk, gold nanoparticles.

Diyala Journal for Pure Science
International Conference for Pure Sciences (ICPS-2021)

Lateral Flow Immunoassay Based Colloidal Gold Nanoparticles for the Detection of Organophosphorous Pesticide (Propanoic) in Cow's Milk

Azhar M. Haleem, Israa M. Haleem and Ruaa H. Abass

الكشف عن بقايا المبيدات الفسفورية العضوية (بروبانويك) في حليب الابقار باستخدام تقنية التدفق المناعي المبنية على جزيئات الذهب النانوية

ازهار محمود حليم[1]، اسراء محمود حليم[2] و رؤى حبيب عباس[3]

[1]مركز بحوث البيئة - الجامعة التكنولوجية – بغداد – العراق
[2]قسم الانتاج الحيواني - كلية العلوم الزراعية - جامعة بغداد – بغداد – العراق
[3]كلية طب الاسنان – جامعة الفراهيدي – بغداد – العراق

الخلاصة

تعد تقنية التدفق المناعي المعتمدة على جسيمات الذهب النانوية الغروانية والمحملة بالمستضدات تقنية سهلة، عالية الحساسية وغير مكلفة للكشف عن بقايا المبيدات الفسفورية العضوية في حليب الابقار. يتكون شريط القياس المصمم لهذه المهمة من اربع مناطق بنائية الاولى هي منطقة تحميل العينة، منطقة الارتباط، منطقة الكشف واخيرا وسادة الامتصاص. تعد المنطقة الحساسة اهم منطقة في الشريط حيث تم تحميل (Propanoic Hapten-OVA)، حددت الظروف المثلى للقياس كتركيز الاجسام المضادة، ودراسة تاثير الاس الهيدروجيني على فعالية الشريط ، كان حد الكشف عن مبيد البروبايونيك في عينات الحليب يتراوح بين 0.5-100نانوغرام/ملليتراما وقت الكشف بلغ 5 دقائق ويعد هذا الفحص مناسبا جدا للكشف عن بقايامبيد البروبايونك في عينات الحليب موقعيا بدون الحاجة الى اجهزة تحليل معقدة.

الكلمات المفتاحية: فحص التدفق المناعي، مبيدات الفسفور العضوية، ذهب نانوي، حليب الابقار.

Lateral Flow Immunoassay Based Colloidal Gold Nanoparticles for the Detection of Organophosphorous Pesticide (Propanoic) in Cow's Milk

Azhar M. Haleem, Israa M. Haleem and Ruaa H. Abass

Introduction

The lateral flow immunoassay (LFI) is a paper-based technique for the determination and measurement of particular substances within a complex mixture, by putting the sample at a test tool and the outcomes will be displayed within 5–30 minutes [1]. This method is distinguished by low costs, ease of production, rapid and portable, and popular applications in biomedicine, agriculture, food, and environmental sciences [2]. Now a day the interest has been growing in diagnostic with various test lines allowing the quick self-examination of multiple analytes present in samples [3].

Such assays (potentially a private LFIA) should be simple to work without the use of laboratory examination or individuals qualified in chemical analyses [4]. LFIAs are very good applicants as they are inexpensive to produce, easy to perform, and widely trusted by users [5]. Pesticides need high valid and accurate methods to detect their levels in food, soil, and water like GC or HPLC with mass spectrometry that are high-cost equipment, required a well-trained technician, a significant time for sample preparation, weakness to operate under field conditions [6] .

Many techniques and methods was developed to determine pesticides' residuals like chemical and biosensors, immunochemical methods, spectroscopic techniques [7-9].

The lateral flow immunoassay (LFI) has widely applied in pesticides detection. The immune test based on the adhesive of an antigen particle (pesticide) to distinct antibodies, the conducted test may be only using one antibody or multiple using by many antibodies [10, 11]. The determination of pesticide residues in milk and dairy products is an important issue in assuring the safety of milk. The residues of organophosphorus pesticides (OPP) insist on the environment for long times and access to the food chain causes the accumulation of these compounds in the tissue of plants and animals down to the human being by their food and water causes serious health problems because of their high toxicity. Many studies recorded OPP residues exceeding established maximum residue limits (MRL), Melgar results recorded that the number of total samples including the detectable concentration of OPP residues was 8.67 % in unpasteurized milk [12].

While Salas and his colleagues found that 39.6% of the pasteurized milk specimens included detectable concentrations of OPP residues, eight specimens included residuals passing established maximum residue limits (MRL) [13].

The main objective of this study is to develop fast and sensitive immunosorbent assay based on gold nanoparticles using monoclonal antibody for determination of organophosphorus pesticide (Propanoic).

Materials and Methods

Chemicals and reagents

All test strip materials were acquired from (Fisher, Germany), colloidal gold nanoparticles (50 nm), Bovine Serum Albumin (BSA), and Ovalbumin (OVA) were provided from Sigma Aldrich (St. Louis, MO, USA). Goat anti-mouse IgG was supplied from College of Medicine Al-Nahrine University (Baghdad, Iraq), while monoclonal antibody for Propanoic has been prepared in University of Technology, Environmental Research Center, Baghdad Iraq, as it was explained previously (Lan et al., 2019).

Construction of colloidal gold-mAb

The conjugation between colloidal gold nanoparticles and monoclonal antibodies to form colloidal gold-mAb was conducted according to [14]. The pH of gold nanoparticles solution set to (7.2) with 1 M NaOH, then the monoclonal antibodies suspension was wisely dropped with continues moving to reach a final concentration of antibodies (2) µg mL^{-1}, the formed mixture has been incubated at 37ºC for 30 min, then we added (1)% BSA with continues mixing for 30 min tell to obtain a homogeneous mixture, after that the solution was centrifuged for 15 min at 10000rbm, the precipitate pellet washed several times with phosphate buffer saline (PBS) supplied with 1% BSA, and the final pellet suspended with 1mL of washing solution and saved at 4°C.

Colloidal gold-strips preparation

Figure 1 shows the components of the strip which composed of four structural regions a sample pad, conjugate pad, nitrocellulose membrane and absorbent pad respectively. On the outer surface of the sensor zone (nitrocellulose membrane) we fixed the PRO-OVA and goat anti-mouse IgG by excelsior esp60 syringe pump as two functional lines, control line and the test line, while on the outer surface of the conjugate pad we fixed the colloidal gold-mAb using Air Jet Quanti3000 dispenser. The prepared strips were then collected and stored at clean dark container at 4 °C.

Determination of the Lateral Flow Immunoassay performance

The optical cut off value was used for qualitative determination for the LFI strip, optical cut off can be described as the lowest concentration of substrate that the color of the test line decays [15, 16] while the quantitative examination was conducted by measuring the optical densities (OD) of the control line and test line by using the reader of the strip. The Propanoic standard solution was prepared by adding the stock solution to PBS (pH 7.2), with final concentrations (0, 0.5, 1, 10, 20, 50, and 100) ng/mL The standard solutions of the Propanoic were prepared at different concentrations with a range from 0–100 ng/mL to evaluate the specificity of the LFI strip test which was evaluated according to the color on the test line of each test strip. To show the eligibility of the current examination by comparison with conventional methods, completely random samples were analyzed for Propanoic residues using the LFI, ELISA, and GC-MS.

Diyala Journal for Pure Science
International Conference for Pure Sciences (ICPS-2021)

Lateral Flow Immunoassay Based Colloidal Gold Nanoparticles for the Detection of Organophosphorous Pesticide (Propanoic) in Cow's Milk

Azhar M. Haleem, Israa M. Haleem and Ruaa H. Abass

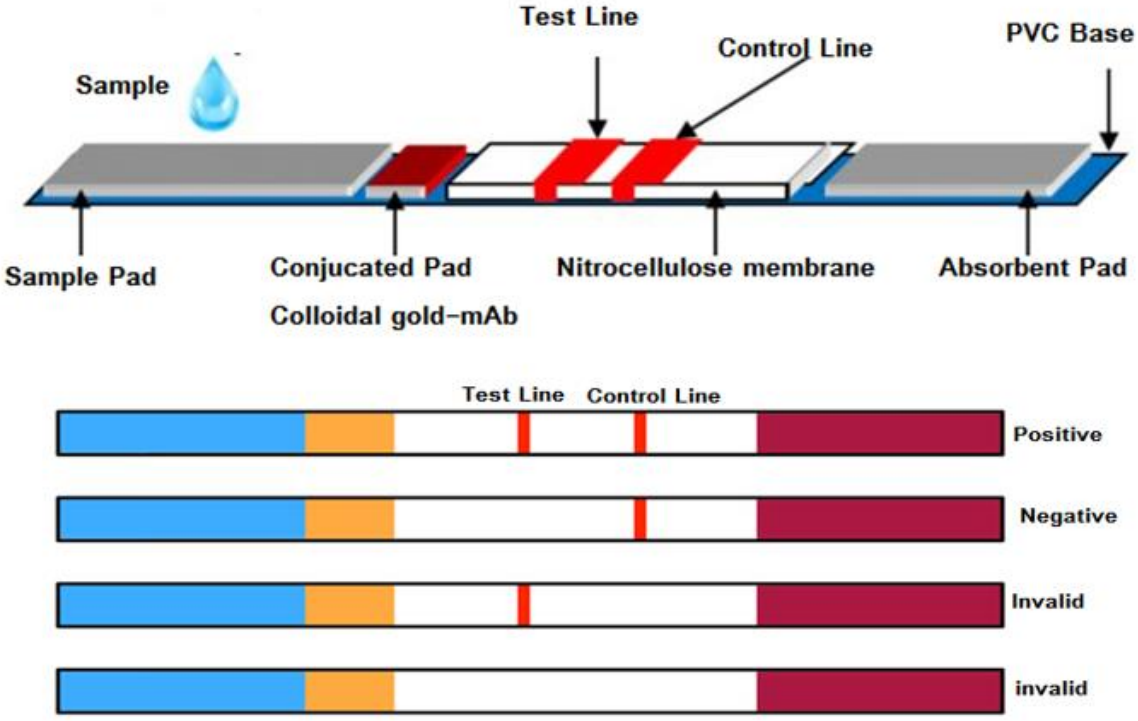

Figure 1: Colloidal gold-strip with four functional zone

Result and Discussions

The layout of the colloidal gold-labeled mAb sensor

Gold nanoparticles present proper visual, electronic, and magnetic properties for the construction of biosensors. In the current study, colloidal gold particles were obtained ready-made with a particle size reach to 50 nm and the highest absorbance at a wavelength of 519 nm while the colloidal gold- mAb have peak at 530nm because of the conjugated of the antibodies with the colloidal cold nanoparticles changing the particle size and causes peak shifted, these outcomes verified that the anti-Propanoic mAbs were strongly joined with the surface of gold nanoparticles. one ml of (0.5-2.5) µg/mL of Anti- Propanoic mAb solutions in serial tubes added to it 1mL of 10% sodium chloride to adjusted their pH range from 6.5–8.5 and shake well for 3 min, then the absorption of each sample was measured at wavelengths from 520-

580nm, 7.2 was the optimum pH of the nanoglod-mAb , at this pH value, 1.25 µg/mL of anti-Propanoic mAb was confirmed to be the lowest concentration for fixing colloidal gold solution.

For assuring more mAb molecules stability and joining with the gold nanoparticle, 2 µg/mL of anti- Propanoic mAb (increasing 60% of mAb based on 1.25 µg/mL) was used for the sensors.

Optimization of the LFI

Generally, the analytical performance of the LFI is significantly affected by several parameters, such as the coating antigen, probes amounts, sensitivity of the antibody, and nitrocellulose membrane, as well as the secondary antibody concentration (goat anti-mouse IgG), in addition to immunoreagent volume, and blocking buffer. The current study concentrates on estimating the influence of the blocking buffer, coating antigen, and immunoreagent amount.

Currently, the influence of the blocking solution on the sensor speed of release, the reaction time of the test strip, and the background color, the impacts of immobilization concentration and the integration rate of coating antigen (pMm–BSA) were adjusted using the crossed tests to increase the sensitivity of the LFI. The test was adjusted with negative (0 ng/mL) and positive samples (100 ng/mL of Propanoic) using various laboratory conditions.

When the compatibility rate of coating antigen (pMm–BSA) for the test line was 16:1, the positive and negative color differences were more apparent. While the straight form of the T-line was more identical. Also notified that the color intensity of negative and positive difference of the T-line was the most obvious, on test line 3 (T3) the color intensity was most obvious when the coating concentration was 20 µg/mL.

Consequently, the optimal concentration and integration rate of pMm–BSA on the T-line was 0.5 mg/mL and 16:1, sequentially.

The immunoreagent concentrations of LFI were evaluated as the previous method. Lastly, the optimum mixture of 6 µL/cm of colloidal gold-labeled mAb probe, 0.5 µL/cm of pMm–BSA

(0.3 mg/mL), and 0.5 µL/cm of antimouse IgG (0.5 mg/mL) were distributed on the test strip. According to these typical conditions, the LFI showed a unique T-line and high sensitivity.

The characteristics of the LFI were assessed by measuring Propanoic standards. The Propanoic standards were diluted with 1 M PBS (pH 7.2) at concentrations of 0.5–100 ng/mL. As shown in figure 3, when the concentration of Propanoic was 10 ng/ mL, the color intensity of the T-line was alike or weaker than that of the negative sample. When the concentration of Propanoic was more than 10 ng/mL, the red line disappeared and only the control line designated the red line. Hence, the optimal cut-off value of the LFI for Propanoic was 10 ng/mL.

To verify LFI process for the quantitative measurement of Propanoic, the optical density (OD) of the test and control lines were examined by a mobile strip reader. According to these experimental conditions, the Propanoic standard curve for the quantitative determination was created by plotting OD test/OD control × 100% against the concentration.

As shown in figure 3, the standard curve showed a significant linear range from 0.5 ng/mL to 50 ng/mL, with an adequate correlation coefficient ($R^2 = 0.9911$).

Accuracy of the LFI

For more evaluation of the accuracy and efficiency of the fabricate LFI, ten samples of fresh local milk were collected from markets, all samples were fortified with different concentrations of Propanoic pesticides (5, 10, and 50) µg/mL. The quantitative recovery was calculated based on the appearance and development of color in the test strip. Table 1 summarized the recoveries of pesticide that ranged from 82.12±3.1 to 94.19±3.6 these outcomes showed that the prepared test strip could be adequately used to the detection of Propanoic residue milk samples.

The comparison of LFI with both ELISA and HPLC-MS for ten fresh local milk supported with (5, 20, and 50) µg/mL of Propanoic pesticide were shown in figure 4.

The analysis of variance showed no significant differences between the three detection modes at the 5% probability level. there was a good correlation between LFI and ELISA (0.142857),

and (0.5) between LFI and HPLC-MS, from results can be noted there are no significant differences in the quantitative

measurement as compared to applied methods, which indicated that prepared LFI strips were good for the detection of Propanoic in milk samples.

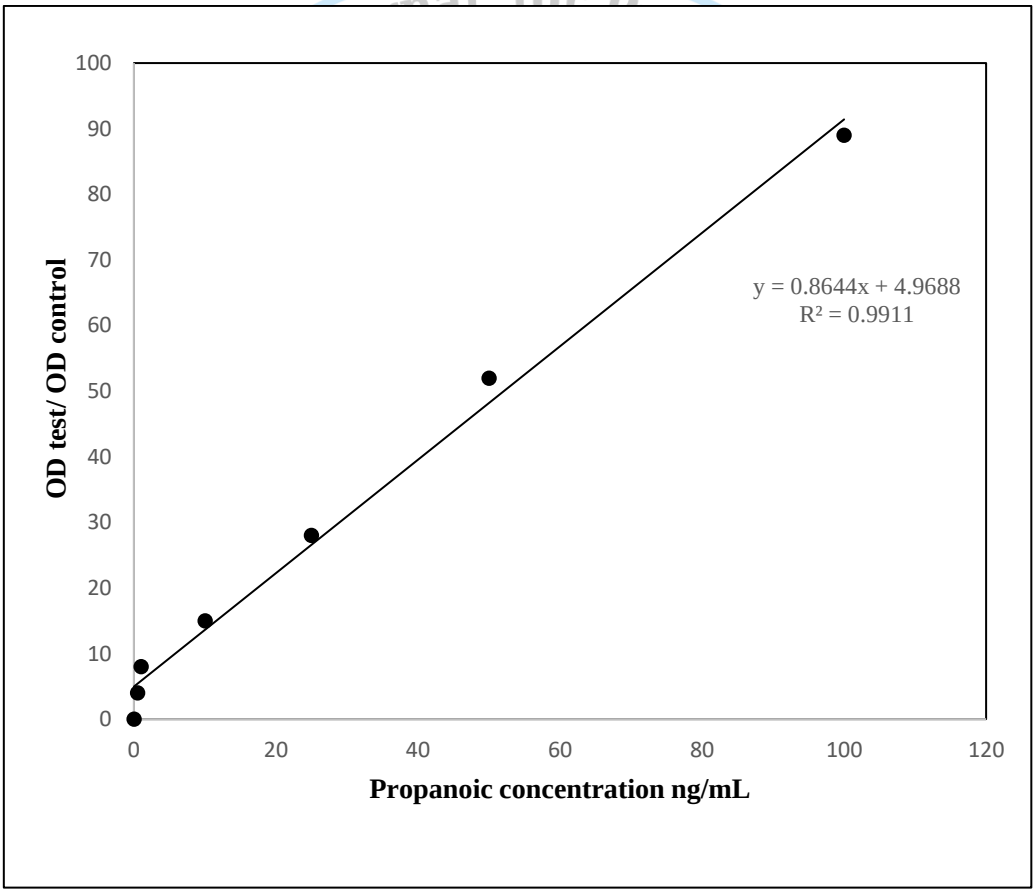

Figure 2: The standard curve for Propanoic is represented by the concentration vs. the relative absorbance value that calculated from dividing the absorbance of the test by the absorbance of the conductance at 100%.

Diyala Journal for Pure Science
International Conference for Pure Sciences (ICPS-2021)

Lateral Flow Immunoassay Based Colloidal Gold Nanoparticles for the Detection of Organophosphorous Pesticide (Propanoic) in Cow's Milk

Azhar M. Haleem, Israa M. Haleem and Ruaa H. Abass

Table 1: Recovery rate of propanoic from milk samples by prepared strip

Samples	Concentration of Added Propanoic(mg/kg)	Recovery rate (%)
S1	5	88.30±2.12
	10	91.20±1.2
	50	92.21±1.5
S2	5	82.45±2.3
	10	85.16±2.8
	50	91.13±3.4
S3	5	93.11±3.1
	10	95.87±2.3
	50	82.12±3.1
S4	5	86.12±2.7
	10	89.34±1.2
	50	92.43±1.3
S5	5	86.73±2.7
	10	94.18±3.6
	50	91.34±2.4
S6	5	86.24±2.2
	10	92.12±2.1
	50	94.19±3.6
S7	5	91.23±3.2
	10	92.81±2.4
	50	89.66±2.6
S8	5	88.17±2.8
	10	91.23±1.3
	50	92.88±1.9
S9	5	87.62±1.6
	10	92.12±2.4
	50	85.61±3.7
S10	5	87.91±2.8
	10	91.70±1.9
	50	93.83±1.6

Each number refer mean± standard deviation for three replicates
Recovery rate= measured concentration/added concentrations) × 100%

Diyala Journal for Pure Science
International Conference for Pure Sciences (ICPS-2021)

Lateral Flow Immunoassay Based Colloidal Gold Nanoparticles for the Detection of Organophosphorous Pesticide (Propanoic) in Cow's Milk

Azhar M. Haleem, Israa M. Haleem and Ruaa H. Abass

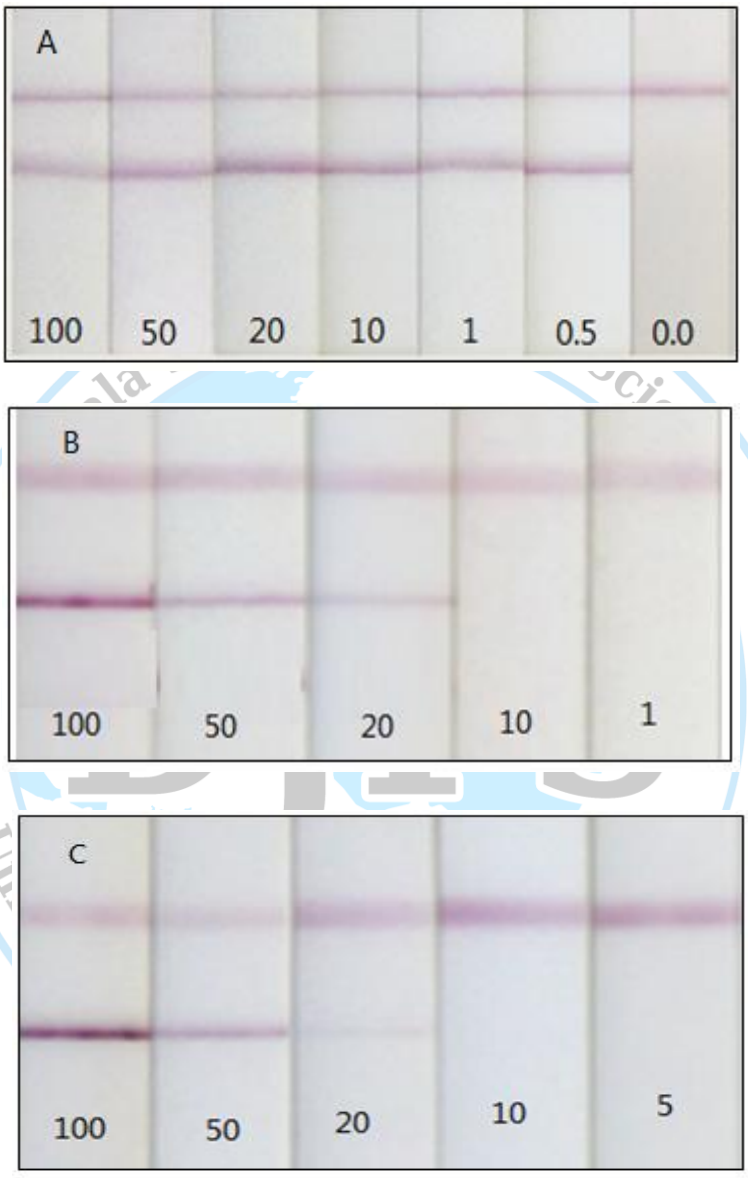

Figure 3: Color development by LFIs test strip at 5 minutes by Propionic pesticide at milk samples (A) at pH 7.2, (B) at pH 6, (C) at pH 8, concentrations (0, 0.5, 1, 5, 10, 20, 50, and 100)

Lateral Flow Immunoassay Based Colloidal Gold Nanoparticles for the Detection of Organophosphorous Pesticide (Propanoic) in Cow's Milk

Azhar M. Haleem, Israa M. Haleem and Ruaa H. Abass

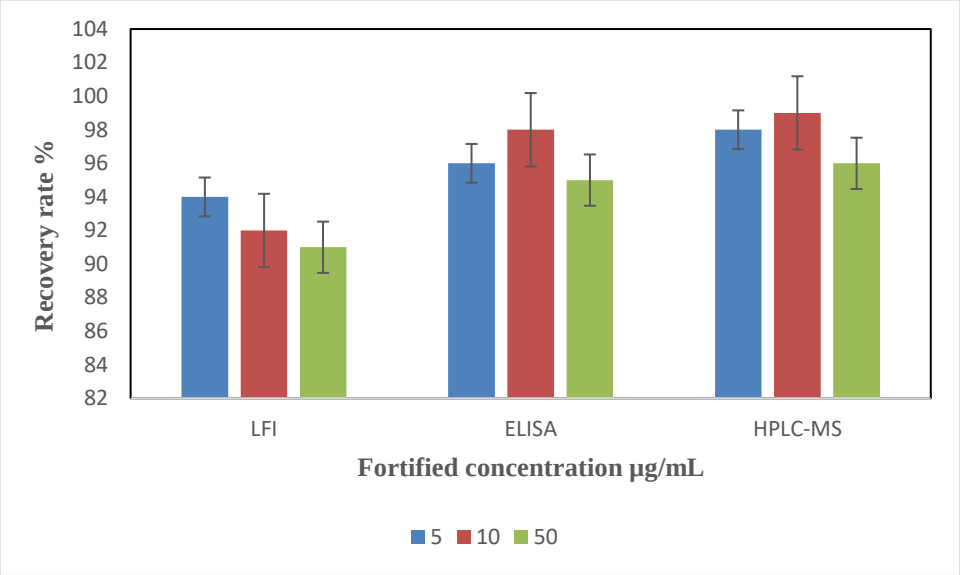

Figure 4: Comparison between the recovery rate (RR %) of Propanoic pesticide from fresh milk samples by LFI, ELISA, and HPLC-MS

Conclusions

Lateral Flow Immunoassay was prepared and examined using the colloidal gold-labeled mAb sensors, these probes were designed for qualitative and quantitative Propanoic determination in fresh milk samples, and the fabricated tool was simple, fast, and accurate as comparison to the ELISA and HPLC-MS with acceptable readings. The detection limit of the LFI for Propanoic standard into milk samples were 0.5 and 100 ng/mL, respectively

References

1. B. O'Farrell, Topics in Companion Animal Medicine, 30(4), 139-147(2015)
2. M. Sajid, A.N. Kawde, M. Daud, A literature review. Journal of Saudi Chemical Society, 19(6), 689-705(2015)
3. S. Song, N. Liu, Z. Zhao, E.N. Ediage, S. Wu, C. Sun, S. De Saeger, A. Wu, Analytical chemistry, 86(10), 4995-5001(2014)

4. Y. Sun, J. Yang, S. Yang, Q. Sang, M. Teng, Q. Li, R. Deng, L. Feng, X. Hu, G. Zhang, Rsc Advances, 8(17), 9580-9586(2018).
5. Y. Liang, Y. Liu, J.F. Zhu, M.T. Fan, X.J. Liu, Chinese journal of analytical chemistry, 36(5), 647-652(2008)
6. Z.L. Xu, Q. Wang, H.T. Lei, S.A. Eremin, Y.D. Shen, H. Wang, Analytica chimica acta, 708(1-2), 123-129(2011)
7. P. Rengasamy, A. Kantharimuthu, M. Balaraman, S. Sivanesan, African Journal of Food Science, 10(3), 41-47(2016)
8. E.M. Shaker, E.E. Elsharkawy, Environmental toxicology and pharmacology, 39(1), 433-440(2015)
9. P.B. Gautam, R. Sharma, K. Lata, Y.S. Rajput, B. Mann, Journal of food science and technology, 54(13), pp.4213-4219(2017)
10. strip for detection of soymilk in milk. J Food Sci Technol. 54(13):4213–4219.Y. Ivanov, T. Godjevargova, Dairy & Veterinary Sciences Journal,11(3), 1-5(2019)
11. A. El-Ansary, L. Faddah, Nanotechnology, science and applications, 3, 65-76(2010).
12. M. Melgar, M. Santaeufemia, M.A. Garcia, Journal of Environmental Science and Health Part B, 45(7), 595-600(2010)
13. J. Salas, M. González, M. Noa, N. Pérez, G. Díaz Journal of agricultural and food chemistry, 51(15), 4468-4471(2003)
14. T. Le, P. Yan, J. Xu, Y. Hao, Food Chemistry, 138(2-3), 1610–1615(2013)
15. D. Kong, Z. Xie, L Liu, S. Song, H. Kuang, C. Xu, Food and Agricultural Immunology, 28(3), 414–426(2017)
16. Y. Xie, L. Zhang, T. Le, Food and Agricultural Immunology, 28(3), 403–413(2017)