www.sensorsportal.com

Sensors & Transducers

Published by IFSA Publishing, S. L., 2019

http://www.sensorsportal.com

A Lateral Flow Immunoassay Kit for the Detection of Tropical House Dust Mite *Blomia Tropicalis*

[1] Mark Angelo V. NGU, [2] Jose H. BERGANTIN, [2] John Donnie A. RAMOS

[1] The Graduate School, University of Santo Tomas, España Boulevard, Manila, 1015, Philippines

[2] College of Science, University of Santo Tomas, España Boulevard, Manila, 1015, Philippines

[1] Tel.: +639175182564, fax: +6329268633

[1] E-mail: markangelo.ngu.gs@ust.edu.ph

Received: 20 March 2019 /Accepted: 22 April 2019 /Published: 30 April 2019

Abstract: IgE-mediated allergic reactions such as asthma, atopic dermatitis and allergic rhinitis are triggered by allergens such as those from the house dust mite *Blomia tropicalis* (Bt). Detection of allergens is crucial in the management and prevention of allergic diseases. The study aimed to provide a proof of concept for a sandwich-format lateral flow immunoassay (LFIA) kit for the detection of Bt-specific proteins using gold nanoparticle-labeled polyclonal antibodies. Anti-Bt polyclonal antibodies produced from rabbits were purified using Protein A chromatography, and were labeled with gold nanoparticles (AuNP) synthesized using the citrate reduction method. These were then dispensed on glass fiber pad that serves as the conjugate pad. Test and control lines were established through the immobilization on nitrocellulose membrane pad of the unlabeled antibodies and anti-rabbit IgG respectively. The LFIA kit was constructed from the assembly of cellulose fiber pad as the sample pad, conjugate pad, and the nitrocellulose membrane pad. The anti-Bt polyclonal antibody concentration was 0.99 mg/mL upon purification and pre-concentration. Gold nanoparticles with 21.2 ± 1.0 nm diameter as observed under transmission electron microscope, were used for antibody conjugation at an optimal pH of 8.5 (borate buffer) and an optimal ratio of 10 µL 50 µg/mL antibody:100 µL AuNP. Optimal conditions such as color intensity and fastest migration time were observed with the treatment of 0.05 % Tween20 and 10 % sucrose in the conjugate pads; 5 % bovine serum albumin (BSA) and 0.05 % Tween20 in the sample pads, and 1 % BSA in the test pads. The limit of detection of the Bt LFIA is 0.054 µg/mL. This study provides a prototype for a low-cost, rapid, and equipment-free detection of *Blomia tropicalis*-specific proteins.

Keywords: *Blomia tropicalis*, Lateral flow immunoassay, Polyclonal antibodies, Allergy, Optimization.

1. Introduction

Allergy is now a major public health concern worldwide [1]. An alarming increase in the global prevalence of allergies has been recorded in both developed and developing countries [2]. In the Philippines, a 10.3 % change of prevalence of asthma per year was noted in children aged 13-14 [1]. In addition, a 20 % prevalence of allergic rhinitis was observed in mid-aged Filipino patients [3]. Allergy is an immunoglobulin E (IgE)-mediated disease caused by allergens that are ubiquitous in nature including those found in pollen, animal dander, fungi, insects, and house dust mites (HDMs). Tagged as aeroallergens, HDM allergens are easily dispersed into the air, causing a variety of allergic diseases [4]. *Blomia tropicalis* (Bt) is a species of HDM that is a prevalent source of allergens in tropical countries such as the Philippines [5–7]. Up to 90 % of Filipino allergic patients are sensitized to allergens from Bt [5].

Although there are allergy treatment options such as specific allergen immunotherapy and the use of antihistamines, detection of allergen in the environment is still important for allergy prevention through assessment of the effectivity of control measures [8-9].

Environmental allergen detection is important in the proper management of allergy. Although there are ELISA-based methods for dust mite allergen detection such as *Dermatophagoides pterynissinus, Dermatophagoides farinae,* and *Blomia tropicalis*, such methods require complex and expensive procedures and equipment. To our knowledge, there are no reports on Bt allergen detection assays that are available. According to the World Health Organization (WHO), an ideal diagnostic kit should follow the ASSURED criteria: affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users [10]. As such, the lateral flow immunoassay (LFIA) format fits to the ASSURED criteria as it is a paper-based assay that uses labeled antibodies to detect analytes. LFIA is a one-step detection process where results may be observed in minutes, making it advantageous over common immunoassays. However, due to its relatively higher limit of detection, the use of LFIA kits remains elusive.

Due to its physico-chemical properties, gold nanoparticles (AuNP) have been used in diagnostics research and development. The use of AuNP is advatangeous due to its high surface-to-volume ratio (signal enhancer) [11], high surface energy [12], facility of electron transfer between redox proteins and electrode surfaces [13], good biological compatibility and catalytic properties [14-15]. Gold nanoparticles are used to increase the availability to catalyze detection reaction, thus, they are used as labels for proteins such as antibodies or carriers of enzymes[12]. Gold nanoparticles have been used in various diagnostics to increase the sensitivity of LFIA [15].

In this study, we describe the first AuNP-labeled sandwich format LFIA kit for the Bt allergen detection in the environment. The study was intended to provide a proof of concept for an LFIA for Bt allergens, and as such, the LFIA kit was tested on standard solutions of Bt allergens with limited testing on real dust samples.

2. Materials and Methods

2.1. Bt Extract Preparation

Lyophilized *B. tropicalis* dust mites were purchased from the Siriraj Dust Mite Center (Thailand). Dust mite allergens were homogenized using liquid nitrogen and extracted mechanically with a mortar and pestle using Phosphate Buffered Saline (PBS) with SIGMAFAST EDTA-free protease inhibitor tablets {4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF), 2 mM; Phosphoramidon disodium salt, 1 mM; Bestatin hydrochloride, 130 mM; N-(trans-Epoxysuccinyl)-L-leucine 4-guanidinobutylamide] (E-64), 14 mM; Leupeptin, 1 mM; Aprotinin, 0.2 mM; and Pepstatin A, 10 mM} (Sigma, USA) at 4°C. Bradford assay was done to determine the protein concentration of the extract [16].

2.2. Production and Purification of Anti-Bt Polyclonal Antibodies

Anti-Bt polyclonal antibodies were developed in five rabbits (*Oryctolagus cunniculus*). A two (2) mL volume of 4.4 mg/mL Bt protein extract was mixed with 2 mL of adjuvant, and was subcutaneously injected at 10 sites, ranging from the dorso-lumbar to the ventrothoracic and abdominal regions of each rabbit. Booster doses of the Bt extract with the complete Freund´s adjuvant (Sigma, USA) were injected in rabbits on the 2nd week, and Bt extracts with incomplete Freund´s adjuvant (Sigma, USA) on the 4th and 6th weeks. Blood samples with a volume of 0.5 mL were extracted weekly for antibody titer determination through indirect ELISA [17]. On the 8th week, approximately ten (10) mL of blood were extracted. Amicon Ultra-15 Centrifugal Filter Units (Merck, USA) were used to pre-concentrate the antibodies. Purification of anti-Bt polyclonal antibodies was performed through protein A sepharose (Sigma, USA) affinity chromatography and then confirmed using SDS-PAGE as previously described [18]. Indirect ELISA was done to determine the reactivity and specificity of the purified anti-Bt polyclonal antibodies [17].

2.3. Synthesis and Characterization of AuNPs

Gold nanoparticles (AuNP) were synthesized as previously described [19]. Fifty (50) mL of chloroauric acid ($HAuCl_4 \cdot 3H_2O$) was heated to boiling with constant stirring. Five (5) mL of trisodium citrate (Sigma, USA) was then added quickly to the solution. The solution was cooled down to room temperature for 15 minutes with constant stirring. Amber colored bottles were used for AuNP storage at 4°C in the dark. Transmission electron microscopy (TEM) (FEI Tecnai G2 20 S-TWINScanning TEM, Holland) and UV-Vis spectroscopy (Perkin-Elmer, USA) were performed for AuNP characterization.

2.4. AuNP-Polyclonal Antibody Conjugation

For antibody conjugation, borate buffer (pH 8.5) was used to adjust the pH of AuNP. Antibodies were dispensed with a volume of 100 µL into a 1.2 mL portion of AuNP. This solution was then shaken for 20 minutes at 650 rpm using a digital shaker (IKA, USA). A volume of 100 µL of 1 % bovine serum

albumin (BSA) (Sigma, USA) was added as the blocking buffer with thorough shaking as above. The solution was then centrifuged for 20 minutes at 4°C at 14,000 rpm. Conjugation buffer which consists of borate buffer, sucrose and Tween®20 (Sigma, USA) was then used to resuspend the AuNP-antibody conjugate pellet.

2.5. Pad Treatment and Assembly

Cellulose fiber (Merck, USA), glass fiber (Merck, USA) and nitrocellulose membrane (Merck, USA) were used as sample, conjugate and test pads respectively. Sample pads were treated with the sample buffer which consists of 10 mM PBS (pH 7.4), BSA and Tween®20. Conjugation pads were treated with conjugation buffer as mentioned previously. Anti-Bt polyclonal antibodies and anti-rabbit IgG control (Sigma, USA) were dispensed on test pads using a 10 μL pipettor. Blocking of the test pads was done using 1 % BSA and subsequent washing was done using a wash buffer (10 mM PBS (pH 7.4) + 0.04 g of SDS). An untreated cellulose fiber pad was used as the adsorbent pad. Assembly of LFIA kit was facilitated using the adhesive plastic backing card (Merck, USA) with an overlap of around 0.2 cm (Fig. 1). The pads were then cut using a guillotine cutter.

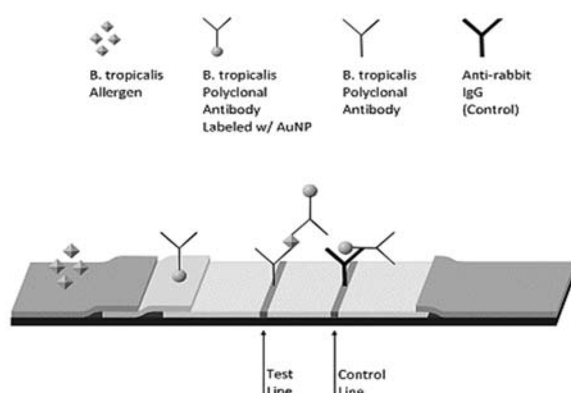

Fig. 1. Diagram of Lateral Flow Immunoassay for *B. tropicalis*.

2.6. LFIA Kit Optimization

The effect of pH (5.5 to 10.0) on the AuNP-antibody conjugation was determined using 2-(N-morpholino) ethanesulfonic acid (MES) (Sigma, USA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Sigma, USA), and borate buffers. Concentrations of anti-Bt antibodies ranging from 12.5 to 100 μg/mL were used in determining the optimum antibody concentration required for conjugation. Univariate optimization was then done on conjugate pads which include treatment with various concentrations of Tween®20 (0.01 % to 0.05 %). The sucrose concentration was likewise optimized, with range from 5 % to 15 %, and the use of 10 % trehalose (Sigma, USA), and 5 % sucrose-trehalose mixture was also evaluated.

Sample pad treatment was also optimized where different concentrations of Tween®20 (0.01 % to 0.07 %) and BSA (1 % to 7 %) were used. The concentration of ovalbumin (1% to 5%) (Sigma, USA) as an alternative blocking agent was also optimized. For the test pad, BSA concentration (0.5 % to 1 %) was optimized. Treatment period of BSA was also optimized in the process (3 to 10 minutes). Optimization of AuNP diameter used in conjugation was also done using the lab-synthesized AuNP, and commercial 40 nm and 60 nm AuNP (Sigma, USA).

Various concentrations of Bt extracts in PBS were dispensed on the assembled LFIA kit with a volume of 80 μL. A red dot or line indicated a positive result, and these were analyzed using ImageJ 1.50. For the computation of the limit of detection, photographs of the Bt LFIA strips were captured using a smartphone (iPhone 6, Apple, USA) in a lab-assembled light box [20]. The images were analyzed using ImageJ 1.50. Testing was done in triplicate samples.

The limit of detection of the LFIA kit was computed as three times the standard deviation of eleven (11) blank readings divided by the slope of the calibration curve.

2.7. LFIA Application to House Dust Samples

House dust samples were also tested for the presence of Bt allergens using the assembled LFIA kit. Dust samples from a house in the city of Manila, Philippines were collected using a conventional vacuum cleaner. An amount of 0.1 g of dust samples were then mixed with 10 mL of PBS-T (pH 7.4) buffer. This mixture was then filtered using Whatman® 0.5 μm syringe filter (GE Healthcare™, USA). Five drops from the filtered samples were then applied to the LFIA kit, and the results were compared with indirect ELISA that was done in parallel [17].

3. Results and Discussion

The dust mite allergen extraction yielded 4.4 mg/mL of Bt total proteins from a starting material of 1 g of Bt mites. In the final step of antibody production and purification, pre-concentration of antibodies through centrifugal filter units (Merck, USA) yielded 0.99 mg/mL anti-Bt polyclonal antibodies. Indirect ELISA showed high reactivity and specificity of the purified Bt polyclonal antibodies to Bt protein extract.

A successful synthesis of AuNPs is indicated by a wine-red solution using the citrate reduction method. The UV-vis spectroscopy gave a λ_{max} of 520 nm, and TEM results gave a size of 21.2 ± 1.0 nm of AuNP (Fig. 2), indicating successful synthesis of the AuNPs.

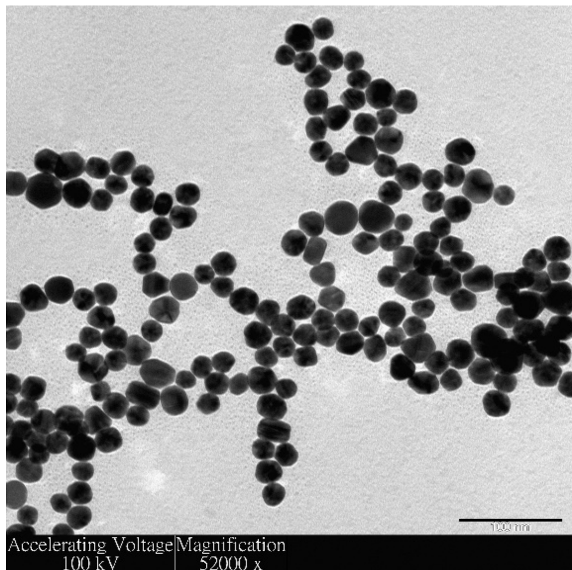

Fig. 2. TEM micrograph of the synthesized AuNP.

The optimal pH for the conjugation of anti-Bt antibody to the AuNP is 8.5 (borate buffer) while the optimal polyclonal antibody-to-AuNP ratio is 10 µL 50 µg/mL antibody: 100 µL AuNP. Optimal conditions are summarized in Table 1. Sample pad optimal conditions are 5 % BSA, in 5 % Tween®20. The flow of fluid was achieved for 2 minutes. Both BSA and Tween®20 resulted in slower migration rates (> 5 mins.) at lower concentrations. The sample pad detaches from the adhesive plastic backing at higher concentration.

Table 1. LFIA Optimal Parameters of Sample, Conjugate and Test Pads.

Parameters	Sample Pad	Conjugate Pad	Test Pad
Tween20 (w/v)	0.05 %	0.05 %	
BSA (w/v)	5 %		1 %
Sucrose (w/v)		10 %	
Time of Incubation			5 mins.

Ideal conditions were observed at 0.05 % Tween®20 and 10 % sucrose for the conjugate pad. Lesser concentrations of Tween®20 resulted in weak reactions (Fig. 3B). The use of other sugars such as trehalose or sucrose-trehalose mixture yielded smudged results (Fig. 3A). Ideal conditions for the test pads include 1 % BSA and 3-minute treatment time (Fig. 3C). Weak reactions were observed at longer period of treatment. The use of synthesized ~20 nm AuNP gave the ideal results, while these were not observed at larger diameters of AuNPs. There is also a decreasing trend in the mean red color intensity versus the concentration of Bt allergen concentrations. (Fig. 4). The limit of detection of the LFIA kit for Bt proteins is 0.054 µg/mL.

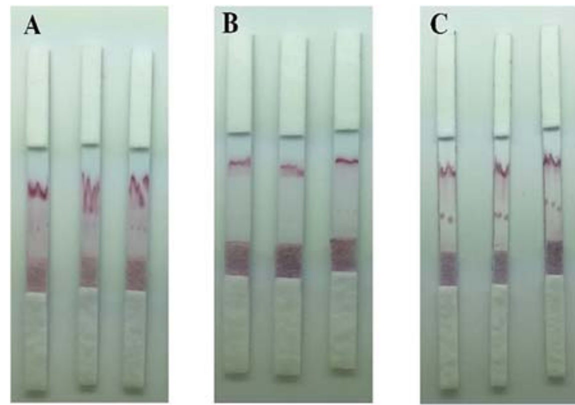

Fig. 3. Developed LFIA kit for *B. tropicalis* detection showing smudged results (A); weak results (B) and ideal color formation (C).

Characterized house dust mite allergens range from enzymes to structural proteins. Allergens from *B. tropicalis* include more than 20 IgE-binding proteins such as Blo t 1 (cysteine protease), Blo t 3 (trypsin), Blo t 4 (α-amylase), Blo t 6 (chymotrypsin), Blo t 9 (serine protease), Blo t 10 (tropomyosin), Blo t 11 (paramyosin), Blo t 13 (fatty-acid binding protein) and other uncharactized proteins [18, 21–23]. When an atopic individual is exposed to such allergens, i twill result to elevated production of allergen-specific immunoglobulin epsilon (IgE) which will trigger Th2-mediated allergic responses [24–26].

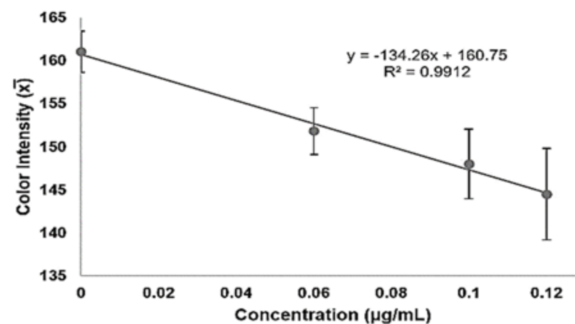

Fig. 4. Calibration curve for Bt proteins.

Until now, detection of allergens from dust mites remains elusive. An LFIA kit for dust mite has been reported, but this is for the detection of HDM species not seen in the tropics [27]. *B. tropicalis* is one of the species of HDMs ubiquitously prevalent in the tropics, especially the Philippines, thus, the development of this LFIA kit for Bt detection.

Low concentration of anti-Bt polyclonal antibodies was observed in the rabbit sera. Concentration of IgE in sera produced from immunized animals varies from 50-200 µg/mL, while the total immunoglobulin concentration is between 5-20 mg/mL [28]. Ideally, IgE antibodies are used for the LFIA kits, however, production of this type of antibody will only yield ~0.05 % of the total immunoglobulin even with

priming the immune system of the immunized animal. As such, immunoglobulin G (IgG) was used in this case as this is the most predominant type of antibody produced polyclonally [28]. Polyclonal antibodies are more stable over a range of pH and salt concentration in comparison to monoclonal antibodies [28]. Monoclonal antibodies are directed to highly specific epitopes while polyclonal antibodies are produced by multiple B-cell clones targeting multiple epitopes in an antigen [28]. As several allergens are present in a house dust mite, the use of polyclonal antibodies is ideal for detection of such allergens. Polyclonal antibody production in animals often involves IgG production which are commonly used in immunoassays.

Lateral flow immunoassay is based on the binding of allergen to the polyclonal antibody conjugated to AuNPs and immobilized antibody. As the fluid flows, the allergen in the sample migrates from the sample pad to the conjugate pad that contains the polyclonal antibody-AuNP conjugate (Fig. 1). The allergen-antibody conjugate complex will then be captured by the immobilized antibody as well as the anti-rabbit IgG which serves as the control that captures excess antibody-AuNP conjugate in the test pad, giving red color lines as a result.

The conjugation process allows the antibodies to be adsorbed on the AuNP surfaces. This is achieved with the adjustment of pH to 8.5 in an alkaline solution; a pH level slightly above the isoelectric point of the ligand (antibody) [29–32]. This conjugation was then followed by mixing, centrifugation, and suspension of conjugates to blocking agents and buffers [30–32]. This conjugation process shows the antibodies being adsorbed on the gold nanoparticles surfaces mainly due to the covalent or soft acid-soft-base interaction of sulfur residues in the antibodies with gold [33].

To ensure the smooth flow of the sample and the conjugate, surfactants were used in the LFIA. With high concentrations of surfactants, the surface tension between the sample pad and the adhesive backing will be lessened that will lead to pad detachment from the backing. This same principle also applies to BSA with increasing concentrations. Slower migration of fluid was observed at lower concentrations of the surfactant. Denaturation of proteins, as in this case, antibodies, will occur at higher concentration of detergents, such as surfactants.

As an important process, optimization of the blocking of antibodies was done. Lesser allergens will bind to antibodies with high concentrations of the blocking agent that will lead to weak reactions (Fig. 3B). This has been observed in test pads treated with BSA for more than 5 minutes. Other blocking agents such as ovalbumin were also used, but it may not have been able to block the anitbodies effectively, causing smudged results (Fig. 3A).

To enhance the lateral flow of fluids in LFIA kits, sugars are used [34]. Sucrose is the most commonly used sugar in such experiments. Smudged results were observed in the treatment of conjugate pads with trehalose and sucrose-trehalose mixture. As trehalose is more viscous than sucrose, this may have resulted to the smudged results [35].

The manual dispensing of antibodies using a pipettor instead of a regent dispenser system is one of the limitations of this study which may have resulted in the variations of mean red values and a higher limit of detection. At best, this has somehow provided a semi-quantitative method for detection of envionmental Bt allergens

The LFIA kit we developed exhibited a LoD of 54 ng/mL. The LoD of our LFIA kit gave a comparable value with other available LFIA kits for allergen detection (Table 2). For the real sample analysis, the mean red value obtained with the dust sample extracts was 156.8 (n=2). This value translates to an interpolated solution concentration of 0.02973 µg/mL and a 2.973 µg/g concentration in the dust sample.

To the best of our knowledge, this LFIA kit is the first attempt at detection of Bt dust mites that dwell in tropical environments which can be used for allergy prevention and environment monitoring.

Table 2. List of available LFIA for allergen detection.

Allergen	Antibodies	LoD	Reference
Fungal α-amylase (Asp o 21)	pAb/mAb	1 ng/mL	[36]
Fungal α-amylase	pAb	0.32 ng/mL	[37]
Mouse urinary protein (Mus m 1)	pAb	31 pg/mL	[38]
Rat urinary protein (Rat n 1)	mAb	31 pg/mL	[38]
Shrimp protein extract (tropomyosin)	pAb/mAb	25 µg/mL	[39]
Blomia tropicalis extract	pAb	54 ng/mL	this study

While ELISA remains the gold standard in both allergy detection and monitoring, the method still requires equipment and trained personnel in order for it to be used. The advent of assays that fall under the "ASSURED" classification are in demand today as the global demand and market potential is increasing through time [10]. With this, our LFIA kit would be contributory in meeting such needs.

4. Conclusion

This study has provided a proof of principle for a simple one-step LFIA kit for the detection of *B. tropicalis*. This kit will be useful in Bt-specific protein detection for allergen sensitization avoidance. In addition, this paves the way for a low-cost

diagnostics deliverable to end-users. This study opens the way to future LFIA applications for quick detection of analytes both in the environment and in medical diagnostics.

Acknowledgements

This research was funded by the Department of Science and Technology, Philippine Council for Health Research and Development (DOST-PCHRD). The authors would like to thank Prof. Arben Merkoçi, Ph.D. and Mr. Daniel Quesada-González, Ph.D. of Catalan Institute of Nanoscience and Nanotechnology (ICN2), Barcelona for sharing their expertise in LFIA kit development. The researchers also thank Ms. Mary Denneth R. Fuentes, RMT, MSMT for her technical assistance in the polyclonal antibody development in this project, and to Mr. Chan Y. F. Frankie of Electron Microscope Unit, the University of Hong Kong for the TEM services.

Ethical approval: Clearance and permits from the University of Santo Tomas Research Center for the Natural and Applied Sciences (UST-RCNAS) Institutional Animal Care and Use Committee (IACUC) were secured (UST IACUC code no.: RC2015-1460202).

Conflict of interest: No conflict of interest to declare.

References

[1]. The Global Asthma Report 2014 http://globalasthmareport.org/burden/burden.php (accessed Jun 19, 2018).

[2]. Soriano J. B., Abajobir A. A., Abate K. H., Abera S. F., Agrawal A., Ahmed M. B., Aichour A. N., Aichour I., Aichour M. T. E., Alam K., *et al.*, Global, Regional, and National Deaths, Prevalence, Disability-Adjusted Life Years, and Years Lived with Disability for Chronic Obstructive Pulmonary Disease and Asthma, 1990–2015: A Systematic Analysis for the Global Burden of Disease Study 2015, *Lancet Respir. Med.*, Vol. 5, Issue 9, 2017, pp. 691–706.

[3]. Abong J. M., Kwong S. L., Alava H. D. A., Castor M. A., de Leon J. C., Prevalence of Allergic Rhinitis in Filipino Adults Based on the National Nutrition and Health Survey 2008, *Asia Pac. Allergy*, Vol. 2, Issue 2, 2012, pp. 129-135.

[4]. Tupker R. A., De Monchy J. G. R., Coenraads P. J., Homan A., Van Der Meer J. B., Induction of Atopic Dermatitis by Inhalation of House Dust Mite, *J. Allergy Clin. Immunol.,* Vol. 97, Issue 5, 1996, pp. 1064–1070.

[5]. Ramos J. D., Paul M., Castillo S., Del Rosario M., Ann M., Gapay S., Go T. P., Kamantigue E. G., Allergenicity and Cross-Reactivity of 3 House Dust Mite Species Among Filipino Allergic Patients, *Philipp. J. Sci.,* Vol. 136, Issue 2, 2007, pp. 139–146.

[6]. Albano P., Ramos J. D., Association of House Dust Mithe-Specific IgE with Asthma Control, Medications and Household Pets., *Asia Pac. Allergy,* Vol. 1, Issue 3, 2011, pp. 145-151.

[7]. Catanghal R. A. M., Paller V. G. V., Mite Fauna and Mite Antigen Detection in House Dust Found in Residential Areas in Los Baños, Laguna, Philippines, *Southeast Asian J. Trop. Med. and Public Health,* Vol. 43, Issue 5, 2012, pp. 1114–1121.

[8]. Mahdy A. M., Webster N. R., Histamine and Antihistamines, *Anaesth. Intensive Care Med.,* Vol. 18, Issue 4, 2017, pp. 210–215.

[9]. Larsen J. N., Broge L., Jacobi H., Allergy Immunotherapy: The Future of Allergy Treatment, *Drug Discov. Today,* Vol. 21, Issue 1, 2016, pp. 26–37.

[10]. Peeling R. W., Holmes K. K., Mabey D., Ronald A., Rapid Tests for Sexually Transmitted Infections (STIs): The Way Forward, *Sex. Transm. Infect.,* Vol. 82, Issue (suppl_5), 2006, pp. v1–v6.

[11]. Pérez-López B., Merkoçi A., Nanoparticles for the Development of Improved (Bio)Sensing Systems, *Analytical and Bioanalytical Chemistry*, Vol. 399, Issue 4, 2011, pp. 1577–1590.

[12]. Heddle J., Gold Nanoparticle-Biological Molecule Interactions and Catalysis, *Catalysts,* Vol. 3, Issue 3, 2013, pp. 683–708.

[13]. Larguinho M., Baptista P. V., Gold and Silver Nanoparticles for Clinical Diagnostics - From Genomics to Proteomics, *J. Proteomics,* Vol. 75, Issue 10, 2012, pp. 2811–2823.

[14]. Chandra P., SinghJ., Singh, A., Srivastava A., Goyal R. N., Shim Y. B., Gold Nanoparticles and Nanocomposites in Clinical Diagnostics Using Electrochemical Methods, *J. Nanoparticles,* Vol. 2013, 2013, pp. 1–12.

[15]. Quesada-González D., Merkoçi A., Nanomaterial-Based Devices for Point-of-Care Diagnostic Applications, *Chem. Soc. Rev.,* Vol. 47, Issue 13, 2018, pp. 4697-4709.

[16]. Olu M., Alamu E. A., Protein Modification during Germination of Sorghum Bicolor, *Res. J. Pharm. Biol. Chem. Sci.,* Vol. 4, Issue 4, 2013, pp. 1044–1055.

[17]. Ramos J. D. A., Teo A. S. M., Ou K. L., Tsai L. C., Lee B. W., Cheong N., Chua K. Y., Comparative Allergenicity Studies of Native and Recombinant Blomia Tropicalis Paramyosin (Blo t 11), *J. Allergy Clin. Immunol,* Vol. 58, Issue 5, 2003, pp. 412–419.

[18]. Ramos J. D. A., Immunoglobulin E Binding Activity of Recombinant and Native Blo t 11 Allergens, *Acta Manilana,* Vol. 55, 2007, pp. 1–8.

[19]. Parolo C., De la Escosura-Muñiz A., Merkoçi A., Enhanced Lateral Flow Immunoassay Using Gold Nanoparticles Loaded with Enzymes, *Biosens. Bioelectron.,* Vol. 40, Issue 1, 2013, pp. 412–416.

[20]. Salcedo A. R. M., Sevilla F. B., Colorimetric Determination of Mercury Vapor Using Smartphone Camera-Based Imaging, *Instrum. Sci. Technol.,* Vol. 46, Issue 4, 2018, pp. 450–462.

[21]. Yan Chua K., CheongN., Kuo, I.-C., Wah Lee B., Cheng Yi F., Huang C.-H., Nyin Liew L., The Blomia Tropicalis Allergens, *Protein Pept. Lett.,* Vol. 14, Issue 4, 2007, pp. 325–333.

[22]. Bukantz S. C., Lockey R. F., Bousquet J., Allergens and Allergen Immunotherapy, Second Edition, *CRC Press, Taylor & Francis Group*, 1998.

[23]. Gao Y. F., Wang D. Y., Ong T. C., Tay S. L., Yap K. H., Chew F. T., Identification and Characterization of a Novel Allergen from Blomia Tropicalis: Blo t 21, *J. Allergy Clin. Immunol.,* Vol. 120, Issue 1, 2007, pp. 105–112.

[24]. Heijink I. H., Van Oosterhout A. J. M., Strategies for Targeting T-Cells in Allergic Diseases and Asthma, *Pharmacol. Ther.,* Vol. 112, Issue 2, 2006, pp. 489–500.

[25]. Holgate S. T., Polosa R., Treatment Strategies for Allergy and Asthma, *Nat. Rev. Immunol.,* Vol. 8, Issue 3, 2008, pp. 218–230.

[26]. Shakib F., Ghaemmaghami A. M., Sewell H. F., The Molecular Basis of Allergenicity, *Trends in Immunology*, Vol. 29, Issue 12, 2008, pp 633–642.

[27]. Tsay A., Williams L., Mitchell E. B., Chapman M. D., A Rapid Test for Detection of Mite Allergens in Homes, *Clin. Exp. Allergy,* Vol. 32, Issue 11, 2002, pp. 1596–1601.

[28]. Lipman N. S., Jackson L. R., Trudel L. J., Weis-Garcia F., Monoclonal Versus Polyclonal Antibodies: Distinguishing Characteristics, Applications, and Information Resources, *ILAR J.,* Vol. 46, Issue 3, 2005, pp. 258–268.

[29]. Chun P., Colloidal Gold and Other Labels for Lateral Flow Immunoassays, *Lateral Flow Immunoassay*, 2009.

[30]. Guo Y. R., Liu S. Y., Gui W. J., Zhu G. N., Gold Immunochromatographic Assay for Simultaneous Detection of Carbofuran and Triazophos in Water Samples, *Anal. Biochem.,* Vol. 389, Issue 1, 2009, pp. 32–39.

[31]. Chiao D. J., Shyu R. H., Hu C. S., Chiang H. Y., Tang S. S., Colloidal Gold-Based Immunochromatographic Assay for Detection of Botulinum Neurotoxin Type B, *J Chromatogr B Anal. Technol Biomed Life Sci,* Vol. 809, Issue 1, 2004, pp. 37–41.

[32]. Wu W., Yu L., Fang Z., Lie P., Zeng L., A Lateral Flow Biosensor for the Detection of Human Pluripotent Stem Cells, *Anal. Biochem.,* Vol. 436, Issue 2, 2013, pp. 160–164.

[33]. Miessler G. L., Fischer P. J., Tarr D. A., Macrae C. F., Bruno I. J., Chisholm J. A., Edgington P. R., Mccabe P., Pidcock E., Rodriguez-Monge L., *et al*., Inorganic Chemistry, *Pearson Education, Inc.,* Vol. 41, 2008.

[34]. Mansfield M. A., Nitrocellulose Membranes for Lateral Flow Immunoassays: A Technical Treatise, in Lateral Flow Immunoassay, *Humana Press: Totowa, NJ*, 2009, pp. 1–19.

[35]. Galmarini M. V., Baeza R., Sanchez V., Zamora M. C., Chirife J., Comparison of the Viscosity of Trehalose and Sucrose Solutions at Various Temperatures: Effect of Guar Gum Addition, *LWT - Food Sci. Technol.,* Vol. 44, Issue 1, 2011, pp. 186–190.

[36]. Bogdanovic J., Koets M., Sander I., Wouters I., Meijster T., Heederik D., van Amerongen A., Doekes G., Rapid Detection of Fungal α-Amylase in the Work Environment with a Lateral Flow Immunoassay, *J. Allergy Clin. Immunol.,* Vol. 118, Issue 5, 2006, pp. 1157–1163.

[37]. Koets M., Sander I., Bogdanovic J., Doekes G., Van. Amerongen A., A Rapid Lateral Flow Immunoassay for the Detection of Fungal Alpha-Amylase at the Workplace, *J. Environ. Monit.,* Vol. 8, Issue 9, 2006, pp. 942–946.

[38]. Koets M., Renström A., Zahradnik E., Bogdanovic J., Wouters I. M., Van Amerongen A., Rapid One-Step Assays for on-Site Monitoring of Mouse and Rat Urinary Allergens, *J. Environ. Monit.,* Vol. 13, Issue 12, 2011, pp. 3475–3480.

[39]. Koizumi D., Shirota K., Akita R., Oda H., Akiyama H., Development and Validation of a Lateral Flow Assay for the Detection of Crustacean Protein in Processed Foods, *Food Chem.,* Vol. 150, 2014, pp. 348–352.

___

Published by International Frequency Sensor Association (IFSA) Publishing, S. L., 2019 (http://www.sensorsportal.com).