ISSN 1978-0524 (print) | 2528-6374 (online)

# Food safety and quality training: the use of a lateral flow immunochromatographic strip test for rapid detection of pesticide residue on leafy vegetables

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Received 25 July 2023; accepted 29 October 2023; published 6 November 2023

#### ABSTRACT

Ensuring the safety and quality of leafy vegetables, especially by controlling any traces of pesticides, is crucial for maintaining human health. Exposure to significant amounts of pesticide residue over a prolonged period of time might result in acute poisoning or long-term adverse health effects. This international community service program aimed to expand the vocational knowledge and of the people of Timor-Leste in using lateral immunochromatographic (LFI) strip test for rapid detection of pesticide residue on green leafy vegetables. The whole activity was organized by UAD in partnership with PCIM Timor-Leste and the An-Nur Islamic Foundation. The present program included several main activities, including preparation, internal coordination, external consolidation, technology needs assessment (TNA), program implementation, vocational knowledge transfer, food safety and quality training, monitoring, and evaluation. The participants' knowledge of food safety and food hygiene increased significantly from 52% prior to training to 96% after the training. More than 90% of participants had an improvement in their awareness about food safety standards, hygiene and sanitation practices, and the need to be cautious while selecting raw food items, particularly green leafy vegetables. Moreover, the ability to detect pesticide contamination in vegetables using LFI technology improved by 84%, rising from just 8% before training to 92% after training. All participants expressed their interest in the future continuation of this training program provided by UAD in Timor-Leste.



# **KEYWORDS**

Food safety Food quality Lateral flow immunoassay Pesticide contamination Leafy vegetables



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#### 1. Introduction

The use of pesticides and other chemical agents in agriculture and food production has been frequent and continuous, with the aim of controlling insects and diseases. However, this practice has led to many threats to the environment and public health, as well as the continued existence of pathogens [1]–[3]. Pesticide exposure to significant amounts of residue observed in green leafy vegetables and fruits over a long period of time may result in acute poisoning or long-term adverse effects on human health, possibly increasing the risk of developing various types of cancer, Alzheimer's disease, Parkinson's disease, oxidative stress, endometriosis, and reproductive disorders [4]-[6]. Vegetables have a crucial role in the nutritional composition for human that is advisable to consume them in their fresh condition, without complex processing, since they possess significant nutritional value and are rich in minerals, vitamins, fibers, and antioxidants [7], [8]. On the contrary, vegetables pose one of the main routes through which pesticides reach humans, five times the rate of exposure observed in other mediums like air and water. To guarantee the safety of food consumption, specifically vegetables, it is imperative that pesticide residues do not surpass the maximum residual limits (MRLs) [9]. Therefore, stakeholders involved in green leafy vegetable supply chains must make synergetic efforts to ensure the sustainable use of synthetic pesticides to reduce the risk of pesticide contamination in food items.

The United Nations Food and Agriculture Organization (FAO) has released an investigation specifying that the global agricultural usage of pesticides in 2021 amounted to 3.54 million metric tons of

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active ingredients (Mt), representing a 4% rise compared to 2020, an 11% increase over the last decade, and a two-times higher since 1990 [10]. Even though the United States, Europe, and most countries banned dichlorodiphenyltrichloroethane (DDT) and organochlorine (OCs) in the early 1970s due to ecotoxicity and environmental persistence, they were still produced until now [11]–[13]. Moreover, other groups, including organophosphates (OPs), synthetic pyrethroids, and carbamates, and also new groups, such as diamides, avermectins, spinosyns, and neonicotinoids, were perpetually introduced in agriculture due to their low persistence and high effectiveness [14]. After the crop harvest, however, it is critical to assure food safety and quality throughout the supply chain [15]. Contamination, inappropriate handling, the overuse of synthetic chemicals, and insufficient storage and transportation are possible at every phase of the food supply chain and may all result in foodborne diseases, increasing risks, and threatening customer trust [16]–[18].

The implementation of food safety and quality control starts at the on-farm level and continues throughout the whole supply chain [17]. Hence, it is essential for farmers and food producers to adhere to both international and national regulations and standards dealing with food risks in order to minimize food losses and protect both their consumers and themselves from potential food hazards [16], [18]. Moreover, the maximum levels of pesticides residue detected in agricultural products and the safety and quality of fresh food from plant origin in Indonesia are ruled by the Indonesian National Standard (SNI) 7313:2008 and the Decree of the Indonesian Minister of Agriculture (KEMENTAN RI) No. 53/PERMENTAN/KR.040/12/2018, respectively [19], [20]. As with Indonesia, the Timor-Leste government believes that establishing a robust national food control system will be crucial in protecting consumer health, ensuring the safety of food consumption, and promoting fair trade practices, ultimately leading to sustainable human and economic development [21], [22]. Consequently, the World Health Organization (WHO) Country Office has been promoting Timor Leste's participation in the Codex Alimentarius Commission since 2012 [22].

Nowadays, Timor-Leste is a recently established independent nation that has a limited amount of technological and human resources. The local people demands the government's obligation to address the significant problem of food security and malnutrition by ensuring the provision of safe, high-quality, and nourishing food to the people in compliance with Codex standards, guidelines, and recommendations [22], [23]. The local food suppliers in Timor-Leste possess limited food safety training, and the establishment of food safety, hygiene, and quality monitoring system is almost absent. There is a significant prevalence of chronic malnutrition, stunting, under-nutrition, food poisoning, and unsafe food consumption in Timor-Leste [21]. In the lack of food safety standards, regulation, and a national food control scheme, the country has become a vulnerable entity for low-quality food and food items. Currently, the primary obstacles are formulating a suitable legislative structure, creating national food safety regulations, fostering cooperation across several sectors to ensure food safety, and implementing a risk and laboratory-based inspection program as an integral component of the food control planning in national level [21]-[23]. Therefore, technical assistance is necessary to improve food safety and quality knowledge, raise public awareness, and protect consumer rights [22]. This may include providing fundamental food safety and hygiene training to hoteliers, food processors, food establishments, and other food handlers, as well as implementing a nationwide public awareness initiative on food safety.

In this regard, Universitas Ahmad Dahlan (UAD), in collaboration with the Special Branch of Muhammadiyah (PCIM) Timor-Leste, facilitated food safety training in Timor-Leste through the International Community Service program from July until November 2023. The main objective of this international community service program in Timor-Leste was to improve the knowledge and skills of local people on how to use the LFI strip test kit to quickly detect pesticide residue on green leafy vegetables in order to improve food safety status. The researchers developed a LFI strip test kit for rapid, easy-to-use, cost-effective, and sensitive on-site detection of OCs and OPs residues in fruits and vegetables. The implementation of food safety training by UAD, in accordance with Codex standards, provided Timor-Leste people the opportunity to promote public health, food safety, and quality, as well as food industry competitiveness in the future. A strong partnership with all relevant stakeholders is crucial for a successful and sustainable strategy in Timor-Leste, enabling the achievement of the government's national goals.

#### 2. Method

This international community service program was conducted in Kampung Alor, Dili, and Timor-Leste (8.50° LS, 125.55° BT) from July until August 2023 and was attended by 25 local participants from various places nearby. The participants consist of 8 males and 17 females, with an age range of 18 to 55

ISSN 1978-0524 (print) | 2528-6374 (online)

years old. This place is included in the sub-district of Dom Aleixo, Dili City, where in the last five years the total population averaged around 3,531 people with an area of 0.49 km². Materials used in this community service were leafy vegetables, including lettuce, Chinese cabbage, spinach, Pak choi, and Bok choi, hot mineral water, lateral flow immunochromatographic strip test kit for rapid detection of OPs, OCs, pyrethroid, carbamate, and dithiocarbamate pesticide residue (product code: PAL-NH1002-10) developed by PT Purnama Laboratory (Jakarta, Indonesia) with an initial validation test performed by Vocational Program of Foodservice Industry (BISMA UAD). While the rapid test kit used in this test containing fresh lateral flow immunochromatographic strips, extract liquid, 15 ml sterile polypropylene centrifuge test tubes with blue screw cap conical bottom, and clean pipettes. The equipment employed in this work were scissors, knife, chopping board, plastic gloves, tweezers, paper cup, and book note.

The implementation of this international community service program is carried out in several sequential stages (Fig. 1), starting with coordination for activity preparation, one-time counseling and practice, monitoring of activities once a month, and evaluation of program implementation. A UAD team coordination meeting with Mr. Ipolito Soares was carried out via a Zoom online meeting held on July 3, 2023, and then continued with an internal team coordination meeting at campus. Furthermore, the UAD performed the on-site hands-on vocational training, which was conducted offline at the An-Nur school, Dili, on August 20, 2023, regarding general principles of food safety practices education, and continued with the tryout of using LFI to test pesticide residues on various leafy vegetables. Then, on August 21, 2023, the first activity evaluation was done. On November 15, 2023, online training on food safety, halal, and quality was carried out, followed by measuring the knowledge capacity of the community after receiving training using a validated questionnaire. This capacity building is seen in cognitive, affective, and psychomotor aspects, which will support program sustainability. The Institute of Research and Community Service (LPPM), UAD, is continuously monitoring this program.

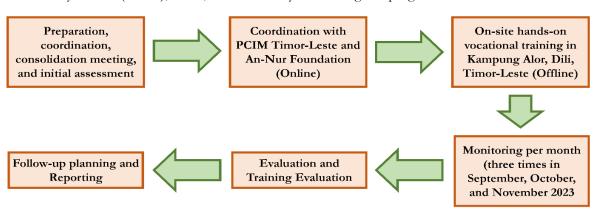


Fig. 1. The flow chart of program implementation method

### 3. Results and Discussion

### 3.1. Community Overview

Kampung Alor is divided into several parts, including Anin Fuic, Rai Lacan, and Hamahon, which are involved in a hundred-year-long history of Islamic development in Timor-Leste. According to Timor-Leste's Islamic history, Syaikh Abdullah Afif, an Arabian trader who arrived, carried on business, and eventually settled in Dili around 1514, spread the Islamic faith in this area before Umar Muhdlar did so in 1678. After around one hundred and fifty years, in the early 19th century, many Islamic traders from the Dutch East Indies (Indonesia) came and settled in Dili. Most of them came from Kupang, Makassar, Adonara, Solor, Bima, Buton, and the Maluku Islands. However, native Timor-Leste residents still make up the majority of Kampung Alor's population, with Indonesian, Arabian, and Chinese immigrants coming in second, third, and forth. In this area, Islamic religion is the second-biggest religion group after Christianity, and interestingly, the majority of local people are able to speak two or more languages, including Tetum, Portuguese, Malay, and Bahasa Indonesia. In 2023, Muhammadiyah, an international Islamic foundation originating from Indonesia, was officially established in Timor-Leste based on Muhammadiyah Central Board Decree No. 197/KEP/I.0/D/2023, and the first selected Chairman of the PCIM Timor-Leste was Mr. Ipolito Soares [24]. In this program, UAD collaborated with PCIM Timor-Leste and An-Nur Foundation led by Mr. Anwar da Costa Fig. 2.

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(a). UAD team in front of An-Nur Moque, Dili, Timor-Leste

(b). UAD team and supreme leader (*Imam*), of An-Nur Moque, Dili, Timor-Leste

Fig. 2. UAD collaborated

## 3.2. Technology Overview

LFIs are analytical sensors designed to detect one or more analytes by coupling labels (nanoparticles, liposomes, enzymes, etc.) with bioreceptors (antigens, antibodies, aptamers, etc.) [25], [26]. This coupling generates a signal (colorimetric, chemiluminescent, or chemiluminescent) that provides either quantitative or qualitative information regarding the target analyte. The standard LFIs equipment comprises four distinct parts each composed of a unique material, including absorbent pad, nitrocellulose (NC) membrane, conjugated pad, detection membrane, sample pad, test line (T), control line (C), and plastic backing (Fig. 3 (a) and Fig. 3 (b)) [25]. The sample pad is filled with the solution to be analyzed during the test, then the subjected sample passes through the strip via capillary action. After the sample ends up at the conjugated pad, which contains an immobilized detection receptor for biomolecule components conjugated with the analyte, a label, and conjugate engage in an interaction that results in the formation of an analyte-conjugate complex, which subsequently passes to the detection membrane (Fig. 3 (b)). The membrane is typically composed of a porous substance such as fiberglass or nitrocellulose. After that, a capture bioreceptor is situated to form a line known as the test line, which serves as a zone of capture for the analyte or complex [26]. By observing the test line, one can visually or analytically determine the quantitative, semi-quantitative, qualitative, or result of the test. Subsequent to the test line is the control line, which contains an additional immobilized capture bioreceptor utilized to validate the assay's functionality. In addition to providing an additional diffusion force to facilitate the complete movement of the sample along the strip, the absorbent pad functions as a refuse reservoir to diminish the visual indication signal and impede reoccurrence of the sample (Fig. 3 (c)) [25].

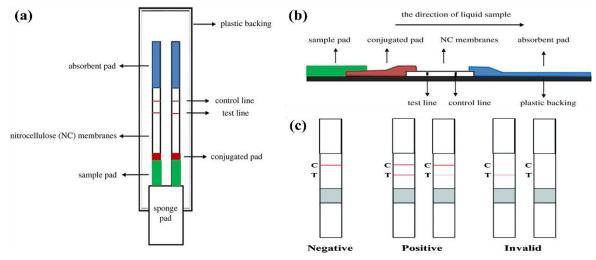


Fig. 3. (a) Schematic diagram of the LFI. (b) The visual results determination in test procedure

## 3.3. Preparation, coordination, consolidation meeting, and initial assessment

The main objective of the preparation, coordination, and consolidation phase is to provide the UAD community service team with the necessary skills and knowledge to fully take advantage on the educational benefits that come with engaging in the training session in Timor-Leste. During this phase, the team

ISSN 1978-0524 (print) | 2528-6374 (online)

engaged in technical preparatory sessions with PCIM Timor-Leste and established learning objectives. The main idea of engaging with local people in the early stages of the community service process is to actively collaborate with the community and enhance leadership capability, aspirations, skills, and confidence to learn and implement the knowledge provided in the food safety and hygiene training. In this training, the UAD team prepared two activities: not only vocational hands-on training on food safety and hygiene but also motorcycle service (Fig. 4).

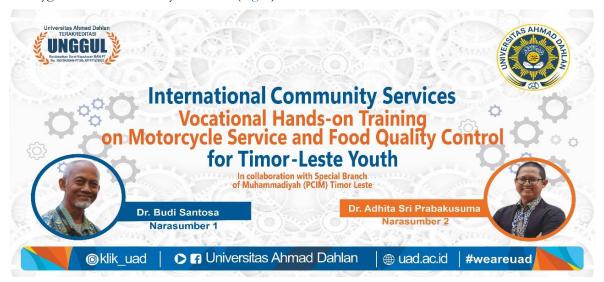


Fig. 4. Backdrop banner of international community services: Vocational hands-on training on food quality control and motorcycle service

## 3.4. On-site Hands-on Training Implementation

### 3.4.1. Specimen Extraction

Prior to use, the leafy vegetable specimens were subjected to a temperature range of 15–35 °C. The procedure involved cutting the material into small pieces (0.1–0.5 grams) using a clean knife, then grinding it using a mortar and pestle and transferring it into a specimen collection tube (Fig. 5. (a)). Next, a total of 2–2.5 ml of warm boiled water was added to the plastic centrifuge tube (Fig. 5 (b)), closed tightly, and shaken vigorously for 15–30 seconds. Place the tube on a table and allow the particles to settle. The upper layer of the liquid can then be used for testing. The specimens should be kept at a temperature range of 2–8 °C within a time frame of 24 hours.

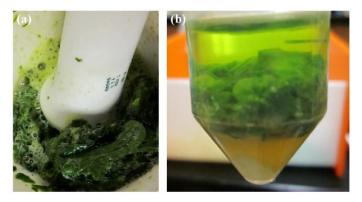


Fig. 5. (a) Sample preparation. (b) Immersing prepared sample in a warm water

## 3.5. Rapid Pesticide Residue Test

In order to conduct the pesticide residue test rapidly, the trainer instructed the participants to add the extraction liquid, incubate 3 minutes at room temperature at 20–30oC, and immerse the LFI test strip into the liquid portion of the sample (Fig. 6). The participants were advised to ensure that the strip was dipped in the correct direction, with a hand sign indicating upward motion, and to not submerge it beyond the colored line marked by arrow signs. The test strip was to be kept in the liquid for 5–10 seconds to

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ensure proper soaking. Afterward, the participants were instructed to place the test strip on a clean, flat surface, and refrain from touching or moving it for 6 minutes at 20–30oC. Finally, they were to read the test result (Fig. 6).

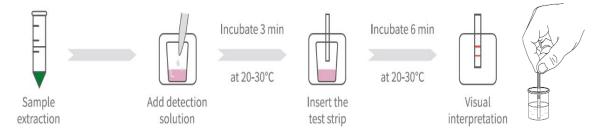


Fig. 6. Procedure to perform rapid pesticide residue test using LFI strip

## 3.6. Learning Process

Participants attentively and seriously listened to the trainer's theoretical presentation for a duration of 60 minutes. Participants were familiarized with the contents of the LFI test kit as well as the protocols for its use and safekeeping. The trainer responded right away to the active questioning and concerns raised by the participants (Fig. 7). The trainer conducted simulations and direct practice by inviting three volunteer participants to do autonomous testing. Subsequently, the trainer offers an evaluation of the participants' executed practice (Fig. 7). The participants showed great enthusiasm during the training session, which continued until its completion. Subsequently, the trainer inquired about the participants' feedback and opinions following their involvement in the food safety and hygiene training. Participants expressed a strong desire for the implementation of more trainings on the same topic in Timor-Leste.



Fig. 7. Vocational hands-on food safety and hygiene training activities

Improving community development through the delivery of community services utilizes pedagogical methods to facilitate collaborative approaches to societal transformation and progress by fostering competencies, skills, practical experiences, and self-confidence, enhancing comprehension of societal demands, public issues, and advocating for a comprehensive approach to empowerment. Education facilitated by means of community action might include creative activities such as observation, role-playing, and engaging in practice while getting constructive evaluation. Individuals engage in learning with the intention of actively participating in the collective objective, therefore assuming the shared burden of effecting social transformation, in this case linked to food safety and hygiene awareness that focus on the quality of living, well-being, and public health. Forty percent of the samples found traces of organochlorine and organophosphate pesticides, according to the test results. The results of the tests suggested that vegetable products in Kampung Alor, Dili, require further investigation with regard to protecting food safety in order to prevent the presence of pesticide residues that could have adverse effects on human health. Increasing social knowledge before and after training as show in Table 1.

ISSN 1978-0524 (print) | 2528-6374 (online)

Table 1. Increasing social knowledge before and after training

Indicator -	Number of participants (in percentage)		
	Before Traininng	After Training	Difference
Knowing food safety and hygiene issues in Timor-Leste	40%	92%	52%
Understanding how to keep food safe and maintaining food hygiene	52%	96%	36%
Knowing food contaminants	36%	92%	56%
Knowing chemical hazards of pesticide residues and their effects to human health	32%	96%	64%
Able to determine the occurrence of			
pesticide contamination in vegetable and	8%	92%	84%
fruits using LFI technology			

According to Table 1, which was obtained by assessing the participants' learning outcomes by voting using "know" or "don't know" questions, it is obvious that all measures of training success have shown a very significant improvement when comparing the knowledge levels before and after the training. The indicator that had the largest growth was the ability to detect pesticide residue in vegetables and fruits using LFI technology. Prior to the training, only 8% of participants were informed of the use of LFI for rapid testing of pesticide residues. Interestingly, this percentage increased significantly 92% by the end of the training. Individuals who have prior knowledge of LFI technology have participated in government-led food safety and hygiene training. Regarding the indication pertaining to understanding food safety and maintaining food hygiene, over 50% of participants already had knowledge of this topic. However, their knowledge significantly improved to 96% after their participation in the training. The majority of participants, over 90%, observed an enhancement in their understanding of food safety standards, hygiene and sanitation practices, as well as the need of being careful while choosing raw food products, particularly green leafy vegetables. All participants absolutely affirmed that this training was very beneficial to their lives and expressed a desire for more training.

## 4. Conclusion

This international community service program, which is based on the research results and the use of applicable technology, was conducted in Kampung Alor, Dili, and Timor-Leste in July until August 2023. The study demonstrated that the community has experienced improved capability, engaged actively, and expressed a strong interest in ensuring the program's long-term viability. The community's knowledge and abilities in rapidly testing for pesticide residue contamination on different leafy vegetables have been expanded through this program. The test results indicated that 40% of the samples had traces of organophosphate and organochlorine pesticides. The test findings indicate that vegetable products in Kampung Alor, Dili, need further observation, specifically in ensuring food safety against pesticide residues that might adversely affect human health. The present implementation of this program has several limitations that require improvement in the future. These include expanding study duration, promoting hygiene and sanitation practices for green leafy vegetables, and assessing participants' comprehension levels before and after training. The community passionately requires the program to be continued through sustainable activities. To ensure the continued performance of this work, it is essential to provide various food safety and hygiene trainings, conduct extensive investigations on the food safety standards of different food types in Timor-Leste, and establish community groups that are knowledgeable about food safety.

### Acknowledgment

We express our sincere gratitude and appreciation to the international partners in Timor Leste and colleagues in Indonesia for their insightful support, guidance, and providing in-kind additional funding on this community service, particularly Mr. Ipolito Soares, the Chairman of the Special Branch of

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Muhammadiyah (PCIM) Timor Leste; Mr. Anwar da Costa, the Chairman of the An-Nur Islamic Foundation Timor Leste; Engr. Mariano Sabino Lopes, Deputy Prime Minister of Timor Leste; and Mr. Muhammad Sayuti, M.Pd., M.Ed., Ph.D., the Secretary of the Central Board of Muhammadiyah.

### **Declarations**

**Author contribution.** All authors made an equal contribution to the primary authorship of this work. The final manuscript was reviewed and approved by all authors.

**Funding statement.** This community service was financially supported by the Institute of Research and Community Service (LPPM) Universitas Ahmad Dahlan through International Community Service Program under Grant Number U.12/SPK-PkM-MONOTAHUN-15/LPPM-UAD/VII/2023 entitled "Vocational Training (Motorcycle Service and Food Quality Control) to Empower the Economic Potential of Muslim Youth in Timor Leste."

**Conflict of interest.** The authors declare that the study was performed without any commercial or financial interest that may be indicated as a possible conflict of interest.

**Additional information.** Additional information of this article is available for this paper and has been deposited to online repository mentioned in the supplementary material statement.

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## Supplementary Material

The online version contains supplementary material for this article (Progress Report of the International Community Service in Timor-Leste written in Bahasa Indonesia and presentation slides provided in English) can be accessed at https://doi.org/10.6084/m9.figshare.24542473.v1.