Activity 1121.1 LIMS and 1212.1 Laboratory RA Testing Report

September, 2023

Submitted to:

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About the project

Funded by Global Affairs Canada, the SAFEGRO project seeks to enhance the Vietnamese people's access to safe and competitive agri-food products, to improve the well-being of consumers and other agri-food actors. Alinea International implements the SAFEGRO project with the University of Guelph, focusing on Ha Noi and Ho Chi Minh City value chains.

Food safety is a significant public health concern. Recent research indicates that Vietnamese consumers do not trust food safety practices and enforcement at informal markets where they buy most food. Some Vietnam's commodity exports also suffers due to a lack of compliance with international standards.

SAFEGRO project works with national and municipal governments to modernise food safety capacity among regulators, thousands of smallholder farmers, cooperatives, processors, retailers and consumers along specific meat and vegetable value chains in Ha Noi and Ho Chi Minh city. SAFEGRO supports Vietnam's Ministry of Agriculture and Rural Development, Ministry of Health and Ministry of Industry and Trade jointly.

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LIST OF ACRONYMS AND ABBREVIATIONS

AAFC	Agriculture and Agri-food Canada
ACFS	Bureau of Agricultural Products and Food Standards (Thailand)
ACIAR	Australian Centre for International Agricultural Research
ADB	Asian Development Bank
AHD	Animal Health Department (MARD)
ALVA	Luxembourg Veterinary and Food Administration
APEC	Asia Pacific Economic Cooperation
AQSIQ	General Administration of Quality Supervision, Inspection and Quarantine (China)
ASEAN	Association of Southeast Asian Nations
AWP	Annual Work Plan
BRC	Global Standard for Food Safety
BoA	Bureau of Accreditation
CBT	Competency-Based Training
CC	Climate Change
CD	Capacity Development
CDC	Centre for Disease Control (USA)
CEA	Canadian Executing Agency
CFDA	China Food and Drug Administration
CFIA	Canadian Food Inspection Agency
CFSA	China National Food Safety Risk Assessment
CPO	Certification Program Owner
CSA	Climate Smart Agriculture
CSO	Civil Society Organizations
DAH	Department of Animal Health
DARD	Department of Agriculture and Rural Development
DFS	Department of Food Safety
DLP	Department of Livestock Production
DOIT	Department of Industry and Trade
EC	European Commission
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency (USA)
EU	European Union
FAO	Food and Agriculture Organization
FBO	Food Business Operations
FCSA	Food Control System Assessment Tool (FAO/WHO)
FDA	Food and Drug Administration (US)
FS	Food Safety
FSIS	Food Safety Inspection Services (USDA)
FSL	Food Safety Law
FSMA	Food Safety Management Authority (Vietnam)
FSMA	Food Safety Modernization Act (USA)

FSMB	Food Safety Management Board
FSRM	Food Safety Risk Management
FSSC	Food Safety Systems Certification
FSTWG	Food Safety Technical Working Group
GAC	Global Affairs Canada
GAP	Good Agricultural Practices
GACC	General Administration of Customs China
GBVCAs	Gender-Based Value Chain Assessments
GFSI	Global Food Safety Index
GFSI	Global Food Safety Initiative
GoC	Government of Canada
GoV	Government of Vietnam
HACCP	Hazard Analysis Critical Control Points
HC	Health Canada
HCMC	Ho Chi Minh City
HFPC	Health and Family Planning Commission (China)
HR	Human Resources
IAF	International Accreditation Forum
ICD	International Cooperation Department (MARD)
IFS	International Food Standard
ILO	International Labour Organization
ISO	International Standards Organization
IPPC	International Plant Protection Convention
IPSARD	Institute of Policy and Strategy for Agriculture and Rural Development
KFDS	Korean Ministry of Food and Drug Safety
LIMS	Laboratory Information Management System
LPD	Livestock Production Department (MARD)
MARA	Ministry of Agriculture and Rural Affairs (China)
MARD	Ministry of Agriculture and Rural Development
MFSA	Mauritius Food Standards Agency
MOA	Ministry of Agriculture (China)
MOC	Ministry of Cooperatives (Thailand)
MOF	Ministry of Finance
MOH	Ministry of Health
MOIT	Ministry of Industry and Trade
MOPH	Ministry of Public Health (Thailand)
MOST	Ministry of Science and Technology
MOU	Memorandum of Understanding
MRL	Maximum Residue Limit
NAEC	National Agriculture Extension Centre
NAFIQAD	National Agro-Forestry-Fisheries Quality Assurance Department
NGO	Non-Governmental Organization
NOP	National Organics Program
ODA	Official Development Assistance

OECD	Organization for Economic Cooperation and Development
OMAFRA	Ontario Ministry of Agriculture, Food and Rural Affairs (Canada)
OOG	Office of the Government
PHAC	Public Health Agency of Canada
PPD	Plant Protection Department (MARD)
PSC	Project Steering Committee
PPC	Provincial People's Committee
QCVN	National Technical Regulation
QCDP	Local Technical Regulation
RA	Risk Assessment
RASFF	Rapid Alert System for Food and Feed (EU)
RM	Risk Management
SAFEGRO	Safe Food for Growth Project
SAIC	State Administration of Industry and Commerce (China)
SAMR	State Administration of Market Regulation (China)
SEDEX	Supplier Ethical Data Exchange
SFDA	State Food and Drug Administration (China)
SFCA	Safe Food for Canadians Act
SFCR	Safe Food for Canadians Regulations
SME	Small and Medium Enterprise
SMETA	Sedex Members Ethical Trade Audit
SPS	Sanitary and Phytosanitary
TA	Technical Assistance
TCCS	Base National Standard
TCVN	National Standard
ToT	Training of Trainers
TPR	Trade Policy Review
UoG	University of Guelph
UK	United Kingdom
UN	United Nations
USDA	United States Department of Agriculture
VC	Value Chain
VFA	Vietnamese Food Agency
VFIORP	Vietnam Foodborne Illness Outbreak Response Protocol
VietGAP	Vietnamese Good Agricultural Practices
VNCC	Vietnam National Codex Committee
VND	Vietnamese Dong
vTPA	Voluntary Third-Party Assurance Program
WB	World Bank
WGs	Working Groups
WHO	World Health Organization
WOAH	World Organization for Animal Health (former OIE)
WTO	World Trade Organization

1. Activity 1121.1: Development of a national LIMS

Component 1: Enabling Environment.

Outcome 1120: Improved food safety control capacity of national and sub-national governments to support Vietnam's risk-based food safety management system.

Output 1121: TA provided for establishing a National Laboratory Information Management System (LIMS)/ Administration of an inter-ministerial network of food safety laboratories.

Activity 1121.1: Development of a national LIMS.

1.1. Summary

The Safe Food for Growth (SAFEGRO) technical assistance (TA) project will support the Government of Vietnam to ensure better access to safe and competitive agri-food products to improve the well-being of consumers as well as other beneficiaries (including poor farmers) in Vietnam. The SAFEGRO technical team will provide leading Canadian and international expertise to develop an internationally recognised risk-based food safety management system adapted to Vietnam, including technical advice from the Canadian Food Inspection Agency (CFIA), the CEA (Alinea and the University of Guelph (UoG)) and other leading food safety organizations. The project started in 2021 and will last five years.

The project's design, with three linked and complementary components, will ensure activities are built on a solid food safety risk analysis framework of 'science informing policy' with robust risk assessments to inform risk management and guide more effective risk communication. The project goal or outcome is to improve the well-being of consumers and agri-food sector actors, including poor farmers in Vietnam. The three project intermediate outcomes appropriately reflect the logical interdependent "Components" of a modernized national risk-based food safety management system. The project will support a technical and regulatory "Enabling Environment" (Component 1) for safe food to be "Supplied" (Component 2) through vibrant agri-food value chains. This will drive market "Demand" (Component 3) for safer food through improved performance of national and sub-national governments enforcing domestic food safety while meeting international standards. The most sustainable SAFEGRO results will be widely disseminated, scaled, and shared regionally so that Vietnam will be recognised for its food safety leadership.

The government of Vietnam has committed to promoting gender equality by making it a cross-cutting issue in all political, economic, cultural, and social spheres. Therefore, within the scope of the SAFEGRO project, gender equality shall be integrated across all activities in the TORs to ensure: (i) collection of gender-disaggregated data for reporting and MEL; (ii) gender-sensitivity languages, photos, images, messages, etc.; (iii) equal participation in decision-making and benefit of women and men from activities and services; (iv) gender analysis before any intervention, decisions, or outlining plans and; (v) GE mainstreaming shall be discussed at all meetings and consultations with government counterparts, regardless of the topics, technical or otherwise.

1.2. Scope of Work

Effective food safety enforcement requires strong support from a laboratory network to test inspection samples for non-compliance and support ongoing monitoring and evaluation for domestic and international trade. Central to the establishment of a national laboratory network is the development of a national and integrated LIMS for effective management, storage and reporting of all sample test data. The scope of work for development of a LIMS includes:

- Conducting a LIMS Needs Assessment
- Determination of equipment and software requirements to support a LIMS
- Development of a Customised LIMS for food safety laboratories
- Development of a National LIMS

The International Training, Education and Laboratory Specialist working with the National Laboratory STCs and the International LIMS Expert will support development of a National LIMS for the partner ministries beginning with a LIMS needs assessment and a virtual workshop to discuss the assessment findings.

A customised LIMS will be developed to permit sharing of food testing data within the inter-ministerial network of food safety laboratories and the designated national reference laboratory. Once the customised LIMS for the food safety labs has been designed, a modification process will begin to adapt the system to a laboratory network for inter-ministerial Administration.

Upon completion of this activity, the inter-ministerial network of food safety laboratories will be able to develop an understanding of the technical and logistical requirements for a national LIMS.

1.3. Methodology (LIMS)

The International Training, Education and Laboratory Specialist will work with the International Regulation/Policy Specialist, International Food Safety Specialist, Local Regulatory/Policy Specialist and Local Food Safety Specialist and the assigned STCs. The primary method(s) that will be used include:

- Conduct comprehensive interviews with crucial laboratory management and staff
- Document roles and responsibilities of crucial laboratory management and staff
- Document issues/risks/concerns and barriers to integration of a LIMS
- Define LIMS requirements based on input from all LIMS stakeholders
- Work with laboratory and IT personnel to document the existing client and server software/hardware environment, including the network infrastructure

LIMS Technical Requirements

1. Sample Registration (log-in and receipt)

Describe all the different sources from where samples are received (indicating the % contribution from each source).

Estimate sample volumes/year and attempt to estimate the average number of tests performed on each sample. Try to distinguish between tests and analytical results.

Provide an indication of how samples submitted to the laboratory are identified e.g. by a unique number, or a combination of parameters e.g. sample batch number and date received etc. Is there a

requirement for the proposed LIMS to automatically generate a sample identifying number with barcodes?

Describe, if appropriate, how lists of tests (groups/profiles of tests) are currently assigned to samples submitted for testing. What criteria are used to select one profile of tests versus another?

Describe in general terms any other information which accompanies samples when they arrive in the laboratory which must also be entered into the proposed LIMS during sample registration.

Determine if there is a requirement for samples to be registered in the LIMS before they are actually sampled.

2. Labels

Describe if any labels that are to be automatically (or manually) generated by the LIMS. Describe what information should be present on the labels. Include any barcode labeling requirements.

3. Worklists

Provide details of what requirements there are for the LIMS generating worklists (screen or paper-based lists of samples awaiting a particular analysis) or worksheets (screen or paper-based forms indicating the work outstanding on a particular sample).

4. Acquiring Analytical Results

A) Keyboard (manual) entry: Provide brief details of how test results are generally recorded e.g. by sample (completion of all test results for a particular sample before moving to the next sample) or by test (completion of all samples for one test before moving onto the next test). Or would a spreadsheet style entry be preferable?

Indicate what checks are (should) be performed on results as they are recorded e.g. checking against limits. Give examples of some typical limit checks.

B) Automatic (on-line) entry: Provide details (name/model only) of instruments to be connected on line and indicate what interfaces are available. Indicate which are to be connected during the SAFEGRO project and which are to be connected in a later phase.

5. Validation and Approval of Data

Provide details of how test data is validated (reviewed for accuracy e.g. by a senior analyst) and how test data is approved (accepted for release outside the laboratory e.g. by the Laboratory Supervisor).

Indicate what facilities are required to submit samples for re-test and for rejecting sample data.

6. Reporting

Indicate what reports must be generated on demand and/or automatically. Include examples if you wish but please keep in mind that the proposed LIMS should replace current laboratory reports functionally, but cosmetically they may have to be designed slightly differently.

Be aware that cosmetic improvements to reports can substantially enhance departmental image. Specify whether reports are also to be delivered automatically or on demand to external clients via email or fax.

Indicate the times of the day the various reports are required to be generated.

7. Statistics and General Calculations

Provide details of what the LIMS will be required to provide in terms of statistics and calculations. Indicate if computerized statistics packages already exist on-site to which a LIMS interface must be provided.

8. Graphics

Indicate what graphical displays of data are envisaged. Indicate if a computerized graphics package already exists on-site to which a LIMS interface must be provided.

9. Communications

In addition to any communication requirement which may have been mentioned in #7 and #8, indicate what other communication requirements are necessary. Should individual lab LIMS be networked and provide summary anonymized data to a national information management system?

Please indicate which requirements can be implemented at a later stage.

10. Security

Provide details of the classes of access security and confidentiality envisaged for the proposed LIMS, i.e. what information the different classes of personnel (laboratory, managerial and computer department) should be allowed to access.

11. Archiving

Indicate how long it is envisaged sample/test data must be accessible.

Detail any classes of sample information which should remain on-line for longer periods. Make sure vendors can indeed archive and retrieve data into the system – this is not always the
CASE and should not be assumed.

12. Tables of Reference Information

If possible, provide details of any tables of reference information which may need to be incorporated into the LIMS. For example, will the LIMS will be required to hold a reference table of analytical tests, products, suppliers/customers, instruments and users?

1.4. Laboratory Information Management System (LIMS)- Findings

Seven laboratories were assessed for implementation of an integrated Laboratory Information Management System. In Hanoi, these laboratories included The National Institute for Food Control (NIFC) and the National Institute of Nutrition (NIN) under the Ministry of Health (MOH), and Plant Protection Department pesticide Lab North (PPD) and NAFIQUAD 1 (Hai Phong) under the Ministry of Agriculture and Rural Development (MARD). In Ho Chi Minh City, the laboratories included CASE, NAFIQUAD 4 (MARD), and Quatest 3 (MOST). Only two of the seven laboratories (NIFC and CASE) have a functional LIMS. CASE developed its LIMS in-house, while NIFC used an outside company (Nanosoft) to build their LIMS.

Based on the LIMS needs assessment, and following discussions with NIFC and CASE, it was determined that the following aspects of the design of a national LIMS will need to be considered:

- Interoperability with existing LIMS systems
- Ability to share existing data and connections to equipment
- Standardization of laboratory report nomenclature
- LIMS design architecture
- (Software, Server storage and hardware compatibility)
- System finance, operations, and maintenance
- Specialized consultation

Since the NIFC LIMS was developed externally by Nanosoft, it is proposed that this system be replicated within at least one of the other laboratories that have been assessed. Doing so will enable at least two laboratories to be linked on a pilot basis (NIFC and the labs in which the LIMS will be installed). In addition, the CASE LIMS will need to be assessed to determine if the LIMS can be connected to the NIFC LIMS. To determine this, a hardware and operating systems overview will need to be conducted. The overview will indicate the preferred hardware platform and operating system(s), which will allow a determination of the potential for communication to other computer systems (LIMS) such as the NIFC LIMS. A database overview will also be conducted to determine which types of data would be shared amongst different laboratories.

Our analysis of LIMS systems at NIFC and CASE highlighted the fact that laboratory equipment used for analysis of samples are not directly connected to the LIMS, which means that all testing results must be accessed manually. One of the recommendations from the Report on the proposal for strengthening the national reference laboratories system for food safety in Vietnam (Contract No. HAN-2021-07) that was conducted by Vietnam, The Netherlands and the World Bank in 2021, was a requirement for government laboratories in Vietnam to expand the application of information technology (software) to digitize original data and organize Proficiency Testing (PTs) and provide reference materials. SAFEGRO will facilitate this by working with NIFC and CASE to connect selected laboratory equipment directly to their LIMS.

Nanosoft is currently conducting an analysis of laboratory equipment at NIFC to determine which equipment can be directly connected to the LIMS. Some of the equipment (Table 1) that SAFEGRO will purchase for NIFC will also be capable of direct connection to their LIMS.

2. Activity 1212.1: Risk Assessment Sampling in Value Chains

Component 2:	Supply Side.
Outcome 1210:	Strengthened capacity of poor farmers and other actors, particularly women, along selected value chains to supply safe agri-food products, taking gender equality and environmental sustainability considerations into account.
Output 1212:	TA provided to relevant authorities and other actors in agri-food trade, distribution and transport, mainly wholesale and retail markets, to improve hygiene and safety conditions.
Activity 1212.1:	Conduct RA survey in value chains, wholesale and retail markets, shopping malls, and supermarkets of selected value chains.

2.1. Summary

The Safe Food for Growth (SAFEGRO) technical assistance (TA) project will support the Government of Vietnam to ensure better access to safe and competitive agri-food products to improve the well-being of consumers as well as other beneficiaries (including poor farmers) in Vietnam. The SAFEGRO technical team will provide leading Canadian and international expertise to develop an internationally recognised risk-based food safety management system adapted to Vietnam, including technical advice from the Canadian Food Inspection Agency (CFIA), the CEA (Alinea and the University of Guelph (UoG) and other leading food safety organizations. The project started in 2021 and will last five years.

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2.2. Scope of Work

In this activity, technical assistance will be provided to MARD, MOH and MOIT municipalities to undertake food safety risk assessments in selected wholesale and geographically linked retail markets to develop a risk-based set of interventions to mitigate critical food safety risks, improving hygiene and safety at wholesale and retail markets. The risk assessment team and government inspectors will be trained to collect data and sample, collate data, test samples, and carry out analysis and reporting.

National expertise will be available to help market managers and government leaders make evidence-based food safety risk management decisions to guide SAFEGRO market interventions and potential for scaling. The objective is to ensure that Vietnamese market managers and government leaders:

- Understand the analytical approach to identify market hazards and exposure risk assessment
- Describe effective methods and procedures for controlling food safety risks
- Appreciate resources required to develop risk-based decision-making
- Develop risk management and risk communication plans

2.3. Methodology Risk Assessment Sampling

To identify the laboratories that will conduct the risk assessment sampling of the selected value chains, we conducted field visits of all laboratories for observation and interview. The laboratories that were visited in Hanoi included The National Institute for Food Control (NIFC) and the National Institute of Nutrition (NIN) under the Ministry of Health (MOH), and Plant Protection Department pesticide Lab North (PPD) and NAFIQUAD 1 (Hai Phong) under the Ministry of Agriculture and Rural Development (MARD). In Ho Chi Minh City, the visited laboratories included CASE, NAFIQUAD 4 (MARD), and Quatest 3 (MOST). During the laboratory visits, discussions were held with the laboratories to identify the technical requirements for microbiological, pesticide and heavy metal analysis.

Questions were asked regarding conducting sampling, and microbiological and chemical analysis of the selected fresh produce and meat samples at different nodes of the selected value chain(s), including traditional and modern distribution channels.

These questions included the following for microbiological analysis:

- Data Management and Analysis Systems: a functioning Laboratory Information Management System (LIM) to manage samples, testing protocols, and results
- The amount of Laboratory Space and Design including: adequate space for sample processing, testing, and equipment; segregation of different testing areas to prevent cross-contamination; adequate lighting and temperature control to maintain a controlled environment; microbiological safety cabinets (Biosafety Cabinets) used to analyze foodborne pathogens
- Incubators: used to cultivate and grow microorganisms at controlled temperatures.
- Microbiological Media and Culture Supplies: Different types of agar media for growing and isolating specific types of microorganisms; sterile petri dishes, test tubes, pipettes, and other lab supplies
- Analytical Instruments: PCR machines for DNA-based detection and identification of microorganisms
- Positive and negative control samples for validating testing methods
- Qualified microbiologists and technicians trained in food safety and testing methods
- Adherence to standard operating procedures (SOPs) and good laboratory practices (GLP)

In collaboration with the laboratories, the technical requirements for pesticide and heavy metal analysis were determined through a series of questions including the following:

- Documentation and Reporting: laboratory information management system (LIMS) for sample tracking, data management, and reporting
- Facility and Infrastructure: adequate space for sample storage, preparation, analysis, and equipment
- Controlled environmental conditions (temperature, humidity, lighting) to prevent degradation of samples
- Equipment: high-performance liquid chromatography (HPLC) systems for separating and analyzing pesticide compounds; gas chromatography (GC) systems for volatile compound analysis; mass spectrometers (MS) for accurate identification and quantification of pesticide residues; spectrophotometers for UV-Vis analysis; liquid and solid phase extraction systems for sample preparation; analytical balances for precise weighing of samples and reagents
- Consumables and Supplies: high-quality analytical standards and reference materials for calibration and validation; quality control samples (blank, spike, duplicate) for method

validation and accuracy assessment; reagents and chemicals required for sample preparation and analysis.

- Accreditation and Compliance: compliance with relevant regulatory standards (ISO 17025) for accurate and reliable testing
- Participation in proficiency testing programs to ensure the laboratory's proficiency in pesticide analysis.
- Detailed standard operating procedures (SOPs) for each analytical method

2.4. Decisions for laboratory use

A food microbiology testing laboratory requires a range of technical equipment and facilities to effectively analyze and assess the microbial content and safety of food products. The specific requirements can vary based on the scope of testing, the types of samples being analyzed, and the regulatory standards the laboratory needs to adhere to. Similarly, an analytical testing laboratory (a lab that conducts tests for pesticides, heavy metals, and other chemical contaminants) requires specific equipment, facilities, and personnel expertise to ensure accurate and reliable testing of pesticides and related compounds. **A major requirement for any laboratory conducting microbiological and chemical analysis of samples is a functioning Laboratory Information Management System (LIM) to manage samples, testing protocols, and results.**

With the exception of a functional LIMS system, all laboratories that were visited were determined to have exceptional infrastructure and had previously conducted microbiological and/or chemical analysis of the selected value chains.

The laboratories to be used for the risk assessment project in Hanoi are:

- National Institute for Food Control (NIFC)
- National Institute of Nutrition (NIN)
- Plant protection Department pesticide Lab North (PPD)
- NAFIQUAD 1 (Hai Phong)

The only laboratory in Hanoi with a functioning LIMS system is the National Institute for Food Control (NIFC). Since this is a strict requirement, it was decided the initial sampling of each value chain would be conducted by NIFC. The NIFC would also conduct the microbiological sampling of each value chain. Sub samples of each value chain would be produced by NIFC and shipped to the other laboratories for analysis as follows:

- National Institute of Nutrition (NIN): Antimicrobial resistance analysis (in collaboration with FAO)
- Plant protection Department pesticide Lab North (PPD): Pesticide analysis
- NAFIQUAD 1 (Hai Phong): Heavy metal analysis

The Laboratories to be used for the risk assessment project in HCMC are:

- CASE
- NAFIQUAD 4
- Quatest 3

The only laboratory in HCMC with a functioning LIMS system is CASE. Therefore, as with NIFC in Hanoi, it was decided the initial sampling of each value chain would be conducted by CASE, which would also conduct the microbiological sampling of each value chain. Sub samples of each value chain would be produced by CASE and shipped to the other laboratories for analysis as follows:

- NAFIQUAD 4: Pesticide analysis
- Quatest 3: Heavy metal analysis

Our analysis and conclusions agree with the Report on the proposal for strengthening the national reference laboratories system for food safety in Vietnam (Contract No. HAN-2021-07) that was conducted by Vietnam, The Netherlands and the World Bank in 2021. The conclusion of the report was that the testing laboratory selected as the national reference testing laboratory for food safety for a specific target group must meet all requirements/criteria of the national reference testing laboratory of food safety. The report indicated that all testing laboratories meet over 60% of the requirements of the National reference testing laboratory for food safety of the registered target group. All the laboratories surveyed and evaluated have the potential to develop into the national reference testing laboratory for food safety of the registration field.

The Report recommended that the following laboratories as potential laboratories to become the national reference testing laboratory for food safety in order of priority for each field of area as follows:

Table 1: Potential testing laboratories for food se by criteria

No	Criteria/Group of criteria	Laboratory/Organization
1	Veterinary drug residues	1. NIFC 2. QUATEST 3 3. NAFIQAD 4
2	Pesticides residues	1. NIFC 2. QUATEST 3 3. CASE 4. NAFIQAD 4 5. NPCTC
3	Mycotoxins and Phytotoxins	1. NIFC 2. QUATEST 3 3. NAFIQAD 4
4	Heavy metals and minerals	1. NIFC 2. QUATEST 3 3. NAFIQAD 4
5	Microorganism	1. NIFC 2. QUATEST3 3. NAFIQAD 4 4. CASE

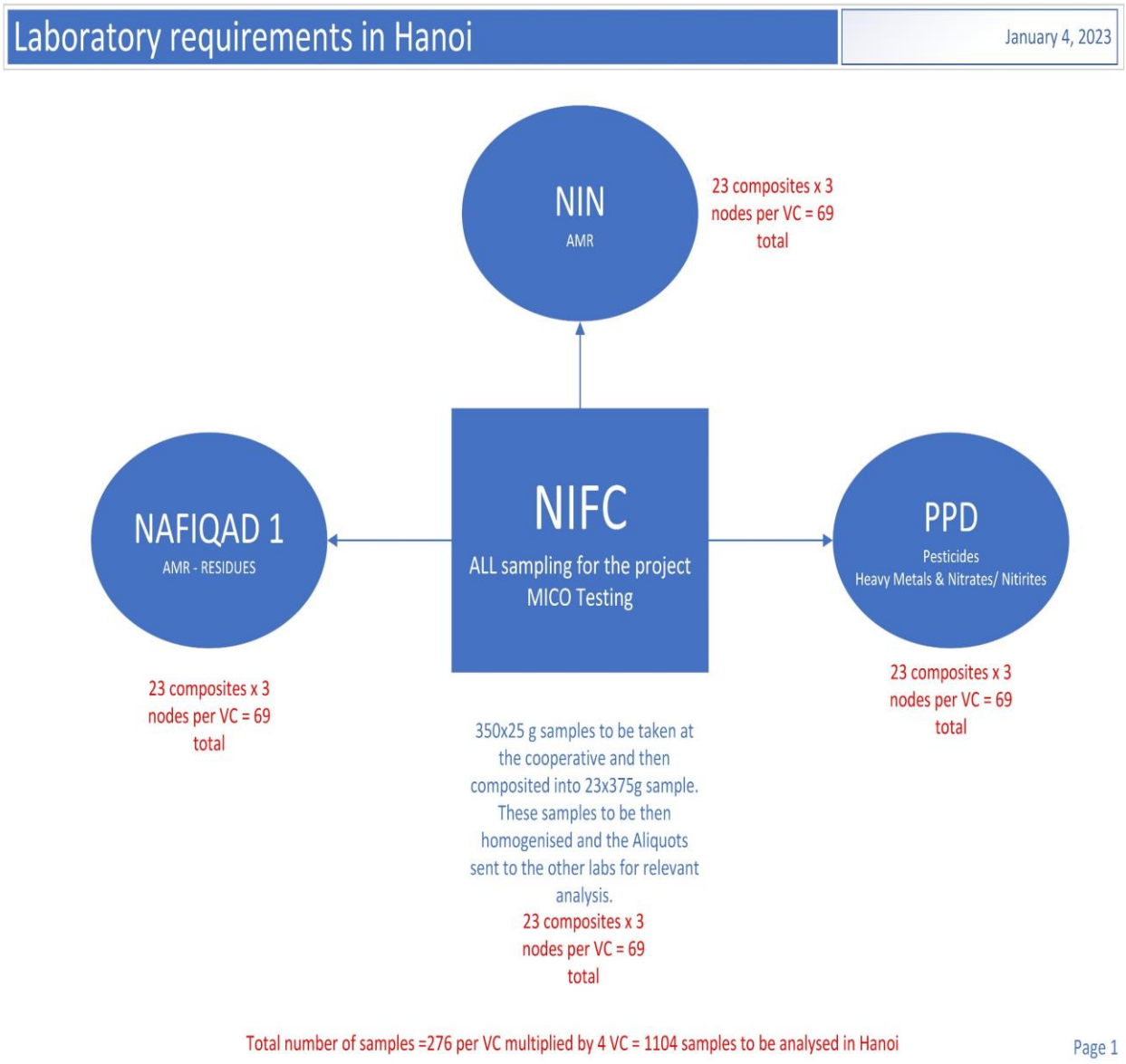


Figure 1: Laboratories to be used in Hanoi. The number of samples to be analyzed at each node (Farmer/Coop, wet market, retail) in the value chain is indicated in red.

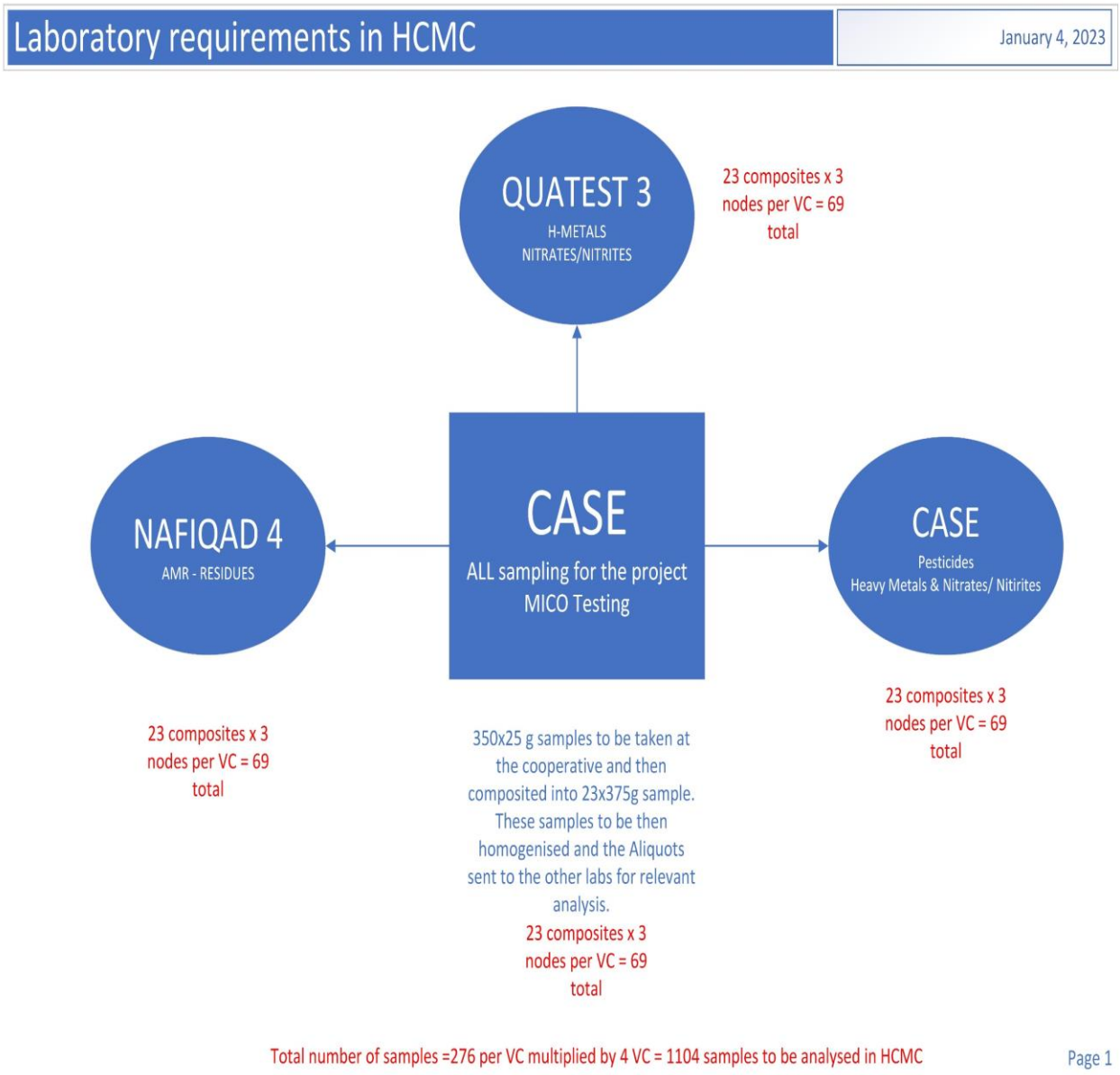


Figure 2: Laboratories to be used in HCMC. The number of samples to be analyzed at each node (Farmer/Coop, wet market, retail) in the value chain is indicated in red.

3. Initial locations for value chain sampling

3.1. Information for the COOP and Value Chain to be sampled

The following information was requested from each COOP to make sure that the selected value chains are being produced and in enough quantities (i.e. there are enough farmers growing each value chain).

Name of CO-OP:

Number of growers in the CO-OP	
List of crops in the CO-OP	
Which crops will be harvested between May 2023 and July 2023	
Approximation of KG per week/crop for May through to July.	
Which wholesale market do they supply?	

1. List of the growers for each establishment / Cooperative (We need this to set up coding internally for the traceability of the laboratory samples).
2. A List of associated crops linked to the growers (i.e. Mr X grows A, B,C- Mr Y grows D,E,F etc. You can redesign the table to best suit)

Crop	Grower A?	Grower B?	Grower C?	Grower D?	Grower E?	Grower F?	Grower G?	Grower H?	Grower I?
Morning Glory									
Mustards Greens									
Cucumber									

3. We can organise the sampling at what time of day the product is typically sent to the Coop. (Approximately, please tick all the appropriate time frames.)

Morning Glory

6am -8am	8am to 10am	10am to 12pm	12pm to 2pm	2pm to 4pm	4pm to 6pm	Other time?

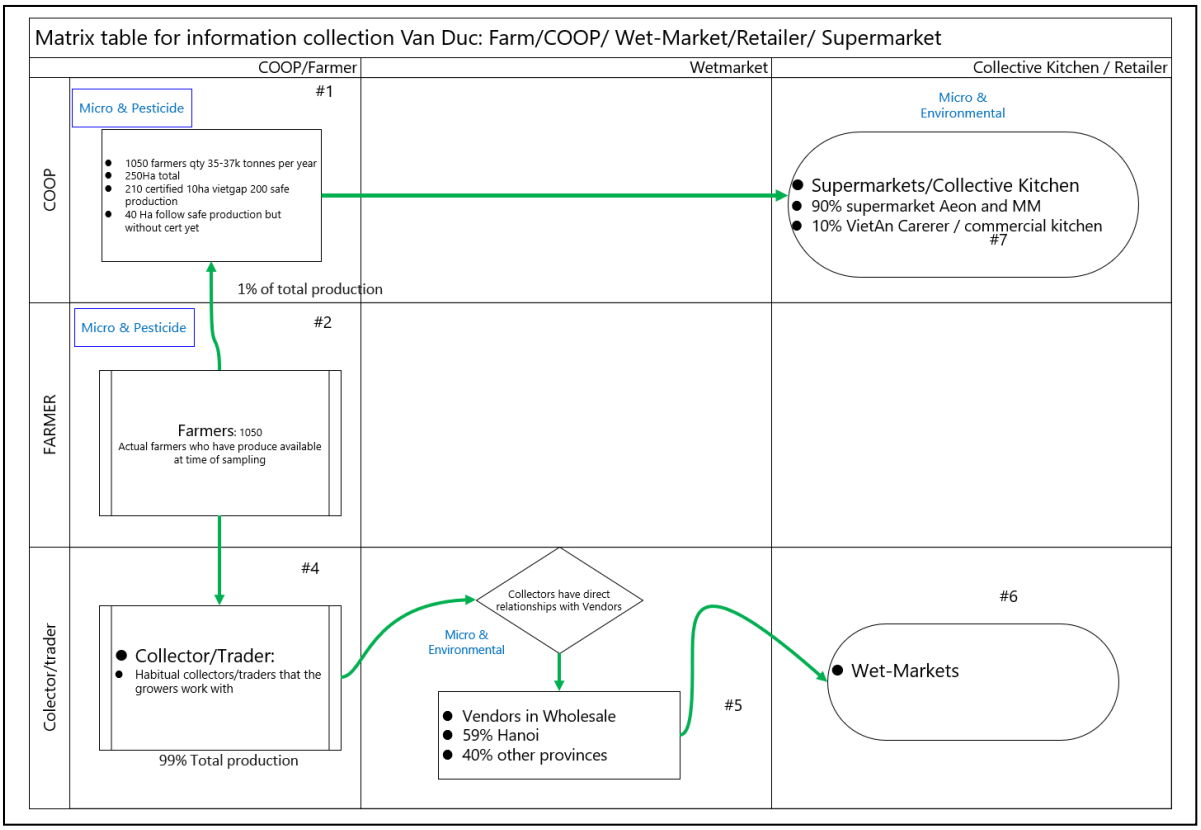
Mustard Greens

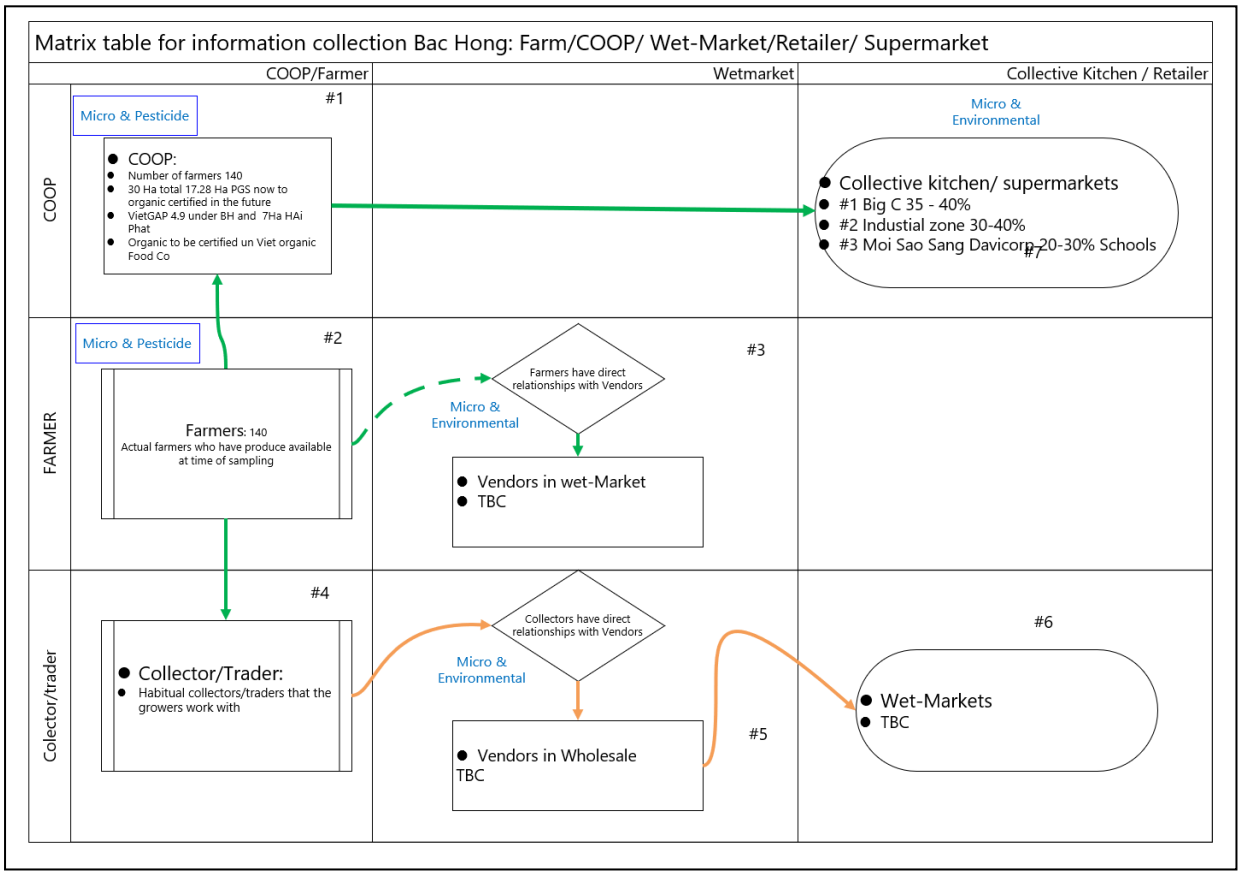
6am -8am	8am to 10am	10am to 12pm	12pm to 2pm	2pm to 4pm	4pm to 6pm	Other time?

Cucumbers

6am -8am	8am to 10am	10am to 12pm	12pm to 2pm	2pm to 4pm	4pm to 6pm	Other time?

Based on the answers to the above questions the first two value chains to be sampled will be at Van Duc and Bac Hong. The information for each location is included in the following matrix tables.





3.2. Collection and analysis of samples

Sampling will be conducted in each value chain based on an estimated prevalence of microbiological and chemical contamination of 1%. This is a standard approach that is used internationally when the true contamination level is unknown. Based on a contamination level of 1%, a total of 350 samples will need to be obtained from each node of each value chain. Therefore a total of 1,050 samples will need to be collected from each value chain. This is a very large number of samples to be collected, and would be difficult to analyze in a timely fashion. To reduce the amount of samples to be analyzed, the samples will be combined (composited) as follows:

- 350 samples of 25 g each will be taken at each node in the value chain. Groups of 15 samples will be composited into a single sample. This will result in 23 (350/15 = 23) composited samples at each node of the value chain. Therefore, for each value chain, there will be 69 (23 samples at each node x 3 nodes) samples to analyze.
- Once the samples have been composited, they will need to be homogenized. This will require a large homogenizer. To facilitate this, SAFEGRO will purchase one large homogenizer for NIFC and one for CASE.
- Due to requirements by the laboratories to analyze samples for pesticides and heavy metals separately, these samples will be taken separately and sent to PPD, NAFIQUAD 1 (Hanoi), NAFIQUAD 3 and Quatest 3 (HCMC) directly. A list of pesticides that will be analyzed (those that

are banned in Vietnam) is included in Annex A, B and C. The standard sampling protocols for pesticides is included in Annex D and E.

- The standard operating procedure for sample collection and microbiological analysis of representative value chain (cucumbers) is included in Annex F.
- The standard operating procedure for pesticide analysis of representative value chain (cucumbers) is included in Annex G.

3.3. Identification and labelling of the samples

For each value chain, each sample that is collected will be labelled as follows:

Labelling Requirement for the samples

Safegro Ref	Conglomerate Number	YEAR	MONTH	PHC	PUC	SAMPLE DATE	SUBMISSION DATE	TESTING DATE	SAMPLE TYPE	Alphanumeric number	Barcode
	01	23	04	01	01	21	21	22	29	01230401012121229	*01230401012121229

Figure 3: Label Requirement for the Samples

- Each grower will be given a unique ID number.
- Each Production Unit Code (PUC) will be given a unique number (If applicable)
- Each Packhouse Code (PHC) (COOP) will be given a Unique number.
- Sample TYPE
  - 01 MICRO *E. coli*
  - 02 MICRO *Salmonella*
  - 10 Pesticides Multi Residue
  - 11 Pesticides Dithiocarbamates
  - 20 Heavy Metals
  - 30 Nitrates
  - 40 Nitrites
- The code will be inputted under each criterion, and a barcode produced.
- This will then be printed into a standard label format and stuck to the conglomerate sample bag.
- This will then be inputted into the Lab system on arrival.

NB: This can be done manually or via Barcode software and a label printer.

Conglomerate to FARMER NUMBER 01 to 40 (Farmer number allocated from 01-40)
Sample numbers 01 to 30
Sample type 01 or 02
01= Micro; 02=Chemical

3.4. Requirement for laboratory equipment

The large number of samples that are required to statically assess each value chain is untenable for the laboratories to analyze in a timely fashion. To reduce the workload, individual samples will be combined into fewer larger samples prior to analysis as described above. This will require the use of large

equipment (large volume homogenizers) to process the samples. None of the laboratories in Vietnam (government or commercial) have the required equipment. Therefore, it is proposed that SAFEGRO purchase and import two large value homogenizers for donation to NIFC and CASE. In addition to the homogenizers, SAFEGRO will also purchase and donate automated Petrifilm readers to facilitate quantitative analysis of microbiological samples, as well as Adenosine Triphosphate hygiene monitoring equipment to assess environmental hygiene at wet markets and retail markets (Table 1). In addition to facilitating the sampling of the value chains, this equipment will also increase the future capacity of each laboratory to analyze samples in the future.

The Report on the proposal for strengthening the national reference laboratories system for food safety in Vietnam (Contract No. HAN-2021-07) highlighted the need to update equipment in the laboratories that were assessed. For example, the Report indicated that NIFC should have a plan to invest in replacing old equipment with too long operating time. Similarly, for CASE, the Report recommended that CASE should consider additional facilities and equipment, which would include invest in replacing old equipment that has a long operating time.

Therefore, SAFEGRO's donation of equipment to CASE and NIFC will address some of the recommendations of the Report on the proposal for strengthening the national reference laboratories system for food safety in Vietnam and will help each lab obtain infrastructure required for a national reference laboratory. Additional laboratory equipment for rapid analysis of microbiological samples (automated Petrifilm readers) and for environmental (hygiene monitoring) will be purchased domestically by SAFEGRO for donation to NIFC, CASE and additional laboratories to increase and modernize their lab infrastructure.

Table 2: Equipment to be donated to government laboratories

Equipment	Quantity	Description	Labs to receive	Comments	
Stomacher and associated equipment/supplies (Bag racks, stomacher bags)	2	Homogenizer for consolidating vegetable samples	NIFC/CASE	Imported	
Petrifilm reader	2	Micro pathogen sampling	NIFC/CASE	Local procurement	
ATP monitoring devices		Environmental monitoring	NIFC/CASE/NAFIQD QPM (Haiphong)/ Kim Quan Retail Market	Local procurement	

Annex 1. MARD Circular 19-2022. List Of Pesticides Permitted for Use and Banned From Use

MARD -------	SOCIALIST REPUBLIC OF VIETNAM Independence – Freedom – Happiness ----------
No.: 19/2022/TT-BNNTPTN	*Hanoi, 22/12/2022*

CIRCULAR
On Amendment of the List of Pesticides Approved for Use, and the List of Pesticides Banned from Use in Vietnam
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Pursuant to the Law on Plant Protection and Quarantine dated 25/11/2013.
Pursuant to the GoV Decree 15/2017 dated 17/2/2017 regarding regulations on functions, mandates, jurisdictions and organizational structure of Ministry of Agriculture and Rural Development
At the proposal of General Director of Department of Plant Protection
The Minister of MARD hereby issues the Circular regarding the List of pesticides approved for use and the List of pesticides banned from use in Vietnam.
Article 1: To issue, as an attachment to this Circular:

1. *The list of pesticides approved for use in Vietnam (see attached the Annex 1), include:*

(a) Use in agriculture.

- Insecticides: 689 active ingredients with 1670 trade names
- Fungicides: 651 active ingredients with 1492 trade names
- Herbicides: 256 active ingredients with 765 trade names
- Rodenticides: 8 active ingredients with 37 trade names
- Growth stimulators: 58 active ingredients with 172 trade names
- Insect attractants: 8 active ingredients with 8 trade names
- Snail pesticides: 31 active ingredients with 152 trade names
- Adjuvants (spreaders): 5 active ingredients with 6 trade names

(b) Termiticides: 14 active ingredients with 21 trade names
(c) Preservatives for forest products: 7 active ingredients with 8 trade names
(d) Warehouse disinfectants: 3 active ingredients with 9 trade names
(e) Products used for golf courses:
✓ Insecticides: 2 active ingredients with 2 trade names.
✓ Herbicides: 1 active ingredient with 1 trade name.
✓ Growth regulators: 1 active ingredient with 1 trade name.
(f) Use for seed treatment:
✓ Insecticides: 10 active ingredients with 16 trade names.
✓ Fungicides: 13 active ingredients with 13 trade names.
(g) Post-harvest preservatives for agri-products:
✓ 1 active ingredient with 1 trade name.

2. *The List of Pesticides Banned from Use (see attached the Annex 2), include:*
   (a) Insecticides and preservatives for forest products: 23 active ingredients.
   (b) Fungicides: 6 active ingredients.
   (c) Rodenticides: 1 active ingredient.
   (d) Herbicides: 1 active ingredient.

3. *The HS Codes for the pesticides allowed for use and banned from use in Vietnam shall comply with Sections 22 and 23 of Appendix I under MARD Circular 11/2021/TT-BNNPTNT dated 20/9/2021 regarding issuance of HS Codes for the list of goods under the jurisdiction of MARD and the list of merchandises subjected to specialized inspection of agriculture sector. In CASE there is a revised legal documents regarding HS codes is issued, such legal document shall be applied.*

Article 2: Effective

1. This Circular takes effect from 16/1/2023.
2. This Circular supersedes the Circular 19/2021/TT-BNNPTNT dated 28/12/2021.

Article 3: Enforcement

1. The General Director of the Department of Plant Protection; Heads of relevant Units under MARD, Directors of DARDs, and concerned organizations and individuals, are liable to implement this Circular.
2. Any difficulty that may arise over the course of enforcement, the concerned organizations and individuals can reflect the CASE to the MARD (via Dept. of Plant Protection) for consideration and solution.

ON BEHALF OF MINISTER
VICE MINISTER

Signed and Stamped
Phung Duc Tien

Sent to:
- OOG.
- Line ministries and sectors
- MOJ (DOLDR)
- PPCs, DARD
- GDVC
- MARD: Minister and VMs, departments
- Filed at VT, BVTV

MARD

SOCIALIST REPUBLIC OF VIETNAM
Independence – Freedom – Happiness

ANNEX II

LIST OF PESTICIDES BANNED FROM USE IN VIETNAM

(Issued as an attachment to the MARD Circular 19/2022/TT-BNNPTNT)

#	Common name of active ingredient/pesticide
Insecticides, and preservatives for forest products	
1	Aldrin
2	BHC, Lindane
3	Cadmium compound (Cd)
4	Carbofuran
5	Chlordane
6	Chlordimeform
7	DDT
8	Dieldrin
9	Endosulfan
10	Endrin
11	Heptachlor
12	Isobenzen
13	Isodrin
14	Lead (Pb)
15	Methamidophos
16	Methyl Parathion
17	Monocrotophos
18	Parathion Ethyl
19	Sodium Pentachlorophenate monohydrate
20	Pentachlorophenol
21	Phosphamidon
22	Polychlorocamphene
23	Trichlorfon (Chlorophos)
Fungicides	
1	Arsenic (As)
2	Captan
3	Captafol
4	Hexachlorobenzene
5	Mercury (Hg)
6	Selenium (Se)
Rodenticides	
1	Talium compond
Herbicides	
1	2.4.5. T

Annex 2. List of Pesticides Banned from Use. EN

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Independence – Freedom – Happiness

ANNEX II

LIST OF PESTICIDES BANNED FROM USE IN VIETNAM

(Issued as an attachment to the MARD Circular 19/2022/TT-BNNPTNT)

#	Common name of active ingredient/pesticide
Insecticides, and preservatives for forest products	
1	Aldrin
2	BHC, Lindane
3	Cadmium compound (Cd)
4	Carbofuran
5	Chlordane
6	Chlordimeform
7	DDT
8	Dieldrin
9	Endosulfan
10	Endrin
11	Heptachlor
12	Isobenzen
13	Isodrin
14	Lead (Pb)

15	Methamidophos
16	Methyl Parathion
17	Monocrotophos
18	Parathion Ethyl
19	Sodium Pentachlorophenate monohydrate
20	Pentachlorophenol
21	Phosphamidon
22	Polychlorocamphene
23	Trichlorfon (Chlorophos)
Fungicides	
1	Arsenic (As)
2	Captan
3	Captafol
4	Hexachlorobenzene
5	Mercury (Hg)
6	Selenium (Se)
Rodenticides	
1	Talium compond
Herbicides	
1	2.4.5. T

Annex 3. List of Pesticides Permitted for Use

11.1. Annex 1. List of Pesticides Permitted for Use.pdf

Annex 4. CACGL-33-1999-Recommended-Methods-of-Sampling-for-Pesticide-Residues-for-the-Determination-of-Compl.

CACGL-33-1999-Recommended-Methods-of-Sampling-for-Pesticide-Residues-for-the-Determination-of-Compl.pdf

TCVN11923_2017_Ky thuat lay mau phan tich vi sinh trong TP và TACN.

TCVN11923_2017_Ky thuat lay mau phan tich vi sih trong TP và TACN.pdf

Annex 5: STANDARD OPERATING PROCEDURE FOR ENUMERATION OF BACTERIA IN FOOD PRODUCTS AND FOOD INGREDIENTS USING PETRIFILM COUNT PLATES AND THE MOST PROBABLE NUMBER TECHNIQUE

1. APPLICATION

This method is applicable for the enumeration of aerobic bacteria, *E. coli*/coliform bacteria, and *Salmonella*, in food products.

2. PRINCIPLE

Petrifilm plates are a ready-to-use product developed by the 3M Company, St. Paul, MN. Media are coated onto films, so that traditional media preparation is unnecessary, and both labour and time savings are realized. Aerobic bacteria can be enumerated on the Petrifilm Aerobic Count (AC) plates. *E. coli* and coliform bacteria can be enumerated on Petrifilm *E. coli*/Coliform Count Plates. *Salmonella* bacteria can be enumerated on Petrifilm *Salmonella* Express Plates.

The method uses bacterial culture plates containing dry medium and a cold-water-soluble gelling agent. One ml samples are added directly to the plates. Pressure, when applied to the plastic spreader placed on the overlay film, spreads the sample over 20 cm^2.

The gelling agent is allowed to solidify, and the plates are then incubated and counted. Petrifilm plates contain modified Standard Methods nutrients; a cold-water-soluble gelling agent; and an indicator which causes the colonies to develop a pigment allowing for easier enumeration. Plastic spreading devices are provided with the Petrifilm plates. The concave side is designed to spread the sample over the growth area.

3. MATERIALS AND SPECIAL EQUIPMENT (need to renumber)

1) Petrifilm Aerobic Count plates (500 total) (AOAC 990.12), Petrifilm *E. coli*/Coliform Count Plates (500 total) (AOAC 991.14), Petrifilm *Salmonella* Express Plates (500 total) (AOAC 2014.01). **Note: Extra Petrifilm have been included for wastage.**

A. Petrifilm plates. Store at/or below 8 °C

B. Plastic spreading device

C. Petrifilm Package insert, including instructions for use.

2) Buffered peptone water (150 liters)

3) Lactose broth (7 liters)

4) Tween 80

3) 69 ounce Whirl-Pak bags (750 bags)

4) Incubators capable of maintaining 35°C +/- 2°C

5) colony counter and/or magnified illuminator

6) Coolers for sample collection

7) Disposable gloves

8) Sterile tubes for dilutions and MPN analysis

9) Garbage bags for collection of waste in the COOP/ Wet Market etc.

10) Miscellaneous laboratory equipment (pipette tips, vortexer, etc.)

4. COLLECTION OF SAMPLES

Sample collection for microbiological analysis

The vegetable to be enumerated is Dutch cucumbers. Each individual sample will consist of 1 Dutch cucumber. Sampling will be conducted as follows:

A. There are 12 farmers. Thirty samples will be collected from each farmer for a total of 360 samples. Each sample (consisting of 1 Dutch cucumber) will be placed in an individual Whirl-Pak bag. Each bag will be labelled (see section 4).

B. Each sample will be placed in a cooler with ice packs and following the conclusion of sampling, the coolers will be immediately transported to the laboratory and stored in a refrigerator until microbial testing begins.

Sample collection for chemical analysis

Each individual sample will consist of 1 Dutch cucumber. Sampling will be conducted as follows:

A. There are 12 farmers. Thirty samples will be collected from each farmer for a total of 360 samples. Each sample (consisting of 1 Dutch cucumber) will be placed in an individual Whirl-Pak bag. Each bag will be labelled with a barcode (see section 4).

B. Each sample will be placed in a cooler and following the conclusion of sampling, the coolers will be immediately transported to the laboratory and stored in a refrigerator.

C. The samples for chemical analysis will be shipped to the PPD laboratory.

5. Labelling Requirement for the samples

Safegro Ref	Farmer Number	Sample Number	YEAR	MONTH	PHC	PUC	SAMPLE DATE	TESTING DATE	SAMPLE TYPE	Alphanumeric number	Barcode
	03	01	23	04	02	03	21	22	01	03012304020321220 1	

Each sample will be labelled with a barcode consisting of the following information:

A. Farmer number 01 to 40 (Farmer number allocated from 01-40)

B. Sample numbers 01 to 30

C. Sample type 01 or 02

D. 01= Microbiological testing

E. 02=Chemical testing

6. PROCEDURE

Carry out the test in accordance with the following instructions:

6.1 Handling of Food Sample

6.1.1. Prior to analysis, keep samples refrigerated (2-8 °C).

6.1.2. Analyze samples as soon as possible after their receipt in the laboratory.

6.2 Preparation for Analysis

6.2.1. Have sterile buffered peptone water ready. Disinfect the surface of the work area.

6.2.2. Place the Petrifilm plate on a flat surface. Mark identifying sample information on the Petrifilm.

6.3 Preparation of Sample

6.3.1. Add 400 ml of buffered peptone water and 400 µl of Tween 80 to each sample bag that will be tested for microbial analysis.

6.3.2. Seal the bags and mix vigorously by shaking for 20 up and down strokes and 20 side by side strokes to ensure that the buffered peptone water and Tween 80 solution adequately washes all surfaces of the cucumbers.

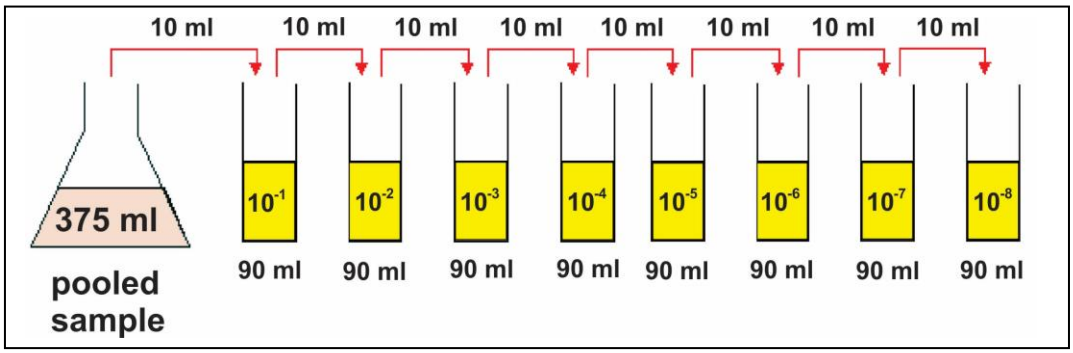
6.3.3. Store the samples at 4°C for 18-24 hours.

6.3.4. Thirty samples were collected from each farmer. To pool the samples, separate the 30 samples from each farmer into 2 groups of 15 samples each. For the first group of 15 samples, remove 25 ml of buffered peptone water from each sample and place into a sterile container. This will result in a final volume of 375 ml. Repeat this process for the second group of 15 samples. Do this for each farmer. There will now be two pooled samples (each consisting of 375 ml) for each farmer (24 total samples).

These samples must be labelled as follows:

Safegro Ref	Farmer Number	Sample Number	YEAR	MONTH	PHC	PUC	SAMPLE DATE	TESTING DATE	SAMPLE TYPE	Alphanumeric number	Barcode
	03	01	23	04	02	03	21	22	02	01230402032122	
		02			02				02	02230402032122	
	04	01	23	04	02	04	21	22	02	01230402042122	
		02			02				02	02230402042122	
	07	01	23	04	02	07	21	22	02	01230402072122	
		02			02				02	02230402072122	
	11	01	23	04	02	11	21	22	02	01230402112122	
		02			02				02	02230402112122	
	12	01	23	04	02	12	21	22	02	01230402122122	
		02			02				02	02230402122122	
	16	01	23	04	02	16	21	22	02	01230402162122	
		02			02				02	02230402162122	
	21	01	23	04	02	21	21	22	02	01230402212122	
		02			02				02	02230402212122	
	28	01	23	04	02	28	21	22	02	01230402282122	
		02			02				02	02230402282122	
	32	01	23	04	02	32	21	22	02	01230402322122	
		02			02				02	02230402322122	
	33	01	23	04	02	33	21	22	02	01230402332122	
		02			02				02	02230402332122	
	37	01	23	04	02	37	21	22	02	01230402372122	
		02			02				02	02230402372122	
	40	01	23	04	02	40	21	22	02	01230402402122	
		02			02				02	02230402402122	

6.3.5. for each pooled sample, make eight, 10-fold serial dilutions using buffered peptone water, by removing 10 ml from each pooled sample, and adding it to 90 mls of buffered peptone water in a sterile bottle. Mix vigorously. This will be the first 10-fold dilution. Next, remove 10 ml from the first 10-fold dilution, and add it to 90 mls of buffered peptone water in a sterile bottle. Mix vigorously. This is the second 10-fold dilution. Repeat this process until eight, 10-fold dilutions have been made. This process is demonstrated in the figure below:



6.4 Petrifilm Inoculation and Incubation

6.4.1. All pooled samples will be tested using Petrifilm Aerobic Count plates, Petrifilm *E. coli*/Coliform Count Plates, and Petrifilm *Salmonella* Express Plates. Lift the top film and carefully inoculate 1 ml of pooled sample or diluted sample to the center of bottom film. For each pooled sample and dilution, use two Petrifilm plates to produce replicate plates. A pipette or 1000 µl pipettor or a can be used for sample addition.

6.4.2. Drop the Petrifilm cover onto sample.

6.4.3. Distribute sample evenly using a downward pressure on the center of the plastic spreader, concave side down. Do not slide the spreader across the film. Leave plate undisturbed for at least 1 minute to permit the gel to solidify.

6.4.4. Return unused plates to foil pouch. Seal pouch by folding and taping the open end. Store resealed foil pouch in a cool dry place. Use plates within one month after opening pouch. Exposure of Petrifilm plates to temperatures above 25 °C and/or humidities >50% RH can affect the performance of the plates. Do not use plates that show orange or brown discoloration. Expiration date and lot number are noted on each package of Petrifilm plates. The lot number is also noted on individual test films.

6.4.5. Incubate plates with the clear side up in stacks not exceeding 20 units. Incubate at 35°C +/- 2°C for 48 ± 3 hours. Examine for bacteria.

6.5 Reading Petrifilm Results

6.5.1. Count plates promptly after incubation period. If impossible to count at once, store plates in the freezer. This should be avoided as a routine practice.

6.5.2. Use a standard colony counter for counting purposes. A magnified-illuminator may also be used to facilitate counting.

6.5.3. The circular growth area is approximately 20 cm^2. Estimates can be made on plates containing more than 250 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine total count per plate.

6.5.4. Calculate the number of colonies per ml or g of sample from the number of colonies obtained in plates chosen at dilution levels which give a statistically significant result.

6.5.5. When counting colonies on duplicate plates of consecutive dilutions, compute the mean number of colonies for each dilution before determining average bacterial count.

6.5.6. To isolate colonies for further identification, lift the top film and pick the colony from the gel.

6.6 Interpretation of Results

6.6.1. Count all dots regardless of size or intensity as colonies. The presence of very high concentrations of colonies on the plates will cause the entire growth area to become red or pink; record results as "too numerous to count" (TNTC). Occasionally, on overcrowded plates, the center may lack visible colonies but many small colonies will be seen on the edges. When this occurs, record results as TNTC; further dilution of the sample is required.

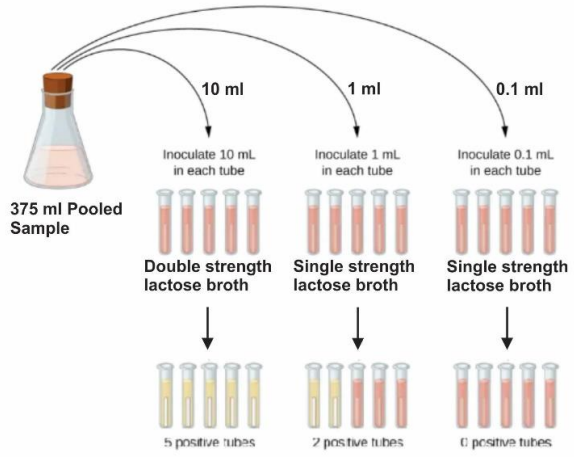
6.6.2. Some organisms can liquefy the gel, allowing them to spread out and obscure the presence of other colonies. If a liquefier interferes with counting, an estimated count should be made by counting the unaffected areas.

6.7 Most Probable Number analysis for *Salmonella*

6.7.1. Make 5 tubes of double strength lactose broth and 10 tubes of single strength lactose broth for each pooled sample (24 pooled samples in total) to be tested.

6.7.2. For each pooled sample, use a sterile pipette to add 10 ml to 5 tubes containing 10 ml double strength lactose broth.

6.7.3. For each pooled sample, add 1 ml to 5 tubes containing 10 ml single strength lactose broth and 0.1 ml to the remaining 5 tubes containing 10 ml single strength lactose broth.



6.7.4. Incubate all tubes at 35°C +/- 2°C for 48 ± 3 hours.

6.7.5. All tubes must be tested for the presence of microbial growth by optical density at 600 nm. A commercially available real-time polymerase chain reaction test will be used to confirm the presence of *Salmonella* in each tube that shows growth by optical density.

6.7.6. Each tube with a positive *Salmonella* RT-PCR result shall be scored as a positive.

6.7.6. Compare the number of tubes giving a positive reaction to a standard MPN chart (see below) and record the number of bacteria present in it. For example, a sample that shows a result of 3–2–1 (3 × 10 ml positive, 2 × 1 ml positive, 1 × 0.1 ml positive) will result in an MPN value of 17, meaning that the sample contains an estimated 17 *Salmonella* per 100 ml.

MPN chart for 5 tubes each at 0.1, 0.01, and 0.001 g or ml inocula, the MPNs and 95 percent confidence intervals.

Pos. Tubes				Conf. lim.		Pos. tubes				Conf. lim.	
0.1	0.01	0.001	MPN/g	Low	High	0.1	0.01	0.001	MPN/g	Low	High
0	0	0	<>	–	6.8	4	0	2	21	6.8	40
0	0	1	1.8	0.09	6.8	4	0	3	25	9.8	70
0	1	0	1.8	0.09	6.9	4	1	0	17	6	40
0	1	1	3.6	0.7	10	4	1	1	21	6.8	42
0	2	0	3.7	0.7	10	4	1	2	26	9.8	70
0	2	1	5.5	1.8	15	4	1	3	31	10	70
0	3	0	5.6	1.8	15	4	2	0	22	6.8	50
1	0	0	2	0.1	10	4	2	1	26	9.8	70
1	0	1	4	0.7	10	4	2	2	32	10	70
1	0	2	6	1.8	15	4	2	3	38	14	100
1	1	0	4	0.7	12	4	3	0	27	9.9	70
1	1	1	6.1	1.8	15	4	3	1	33	10	70
1	1	2	8.1	3.4	22	4	3	2	39	14	100
1	2	0	6.1	1.8	15	4	4	0	34	14	100
1	2	1	8.2	3.4	22	4	4	1	40	14	100
1	3	0	8.3	3.4	22	4	4	2	47	15	120
1	3	1	10	3.5	22	4	5	0	41	14	100
1	4	0	11	3.5	22	4	5	1	48	15	120
2	0	0	4.5	0.79	15	5	0	0	23	6.8	70
2	0	1	6.8	1.8	15	5	0	1	31	10	70
2	0	2	9.1	3.4	22	5	0	2	43	14	100

Pos. Tubes				Conf. lim.		Pos. tubes				Conf. lim.	
0.1	0.01	0.001	MPN/g	Low	High	0.1	0.01	0.001	MPN/g	Low	High
2	1	0	6.8	1.8	17	5	0	3	58	22	150
2	1	1	9.2	3.4	22	5	1	0	33	10	100
2	1	2	12	4.1	26	5	1	1	46	14	120
2	2	0	9.3	3.4	22	5	1	2	63	22	150
2	2	1	12	4.1	26	5	1	3	84	34	220
2	2	2	14	5.9	36	5	2	0	49	15	150
2	3	0	12	4.1	26	5	2	1	70	22	170
2	3	1	14	5.9	36	5	2	2	94	34	230
2	4	0	15	5.9	36	5	2	3	120	36	250
3	0	0	7.8	2.1	22	5	2	4	150	58	400
3	0	1	11	3.5	23	5	3	0	79	22	220
3	0	2	13	5.6	35	5	3	1	110	34	250
3	1	0	11	3.5	26	5	3	2	140	52	400
3	1	1	14	5.6	36	5	3	3	180	70	400
3	1	2	17	6	36	5	3	4	210	70	400
3	2	0	14	5.7	36	5	4	0	130	36	400
3	2	1	17	6.8	40	5	4	1	170	58	400
3	2	2	20	6.8	40	5	4	2	220	70	440
3	3	0	17	6.8	40	5	4	3	280	100	710
3	3	1	21	6.8	40	5	4	4	350	100	710
3	3	2	24	9.8	70	5	4	5	430	150	1,100
3	4	0	21	6.8	40	5	5	0	240	70	710

Pos. Tubes				Conf. lim.		Pos. tubes				Conf. lim.	
0.1	0.01	0.001	MPN/g	Low	High	0.1	0.01	0.001	MPN/g	Low	High
3	4	1	24	9.8	70	5	5	1	350	100	1100
3	5	0	25	9.8	70	5	5	2	540	150	1700
4	0	0	13	4.1	35	5	5	3	920	220	2600
4	0	1	17	5.9	36	5	5	4	1600	400	4600
						5	5	5	>1600	700	–

Annex 6. STANDARD OPERATING PROCEDURE DETERMINATION OF PESTICIDES (INSECTICIDES, FUNGICIDES, HERBICIDES) IN FOOD PRODUCTS AND FOOD INGREDIENTS USING LC-MS AND GC-MS

1. APPLICATION

This method is applicable for identifying and quantifying pesticides (i.e., insecticides, fungicides, herbicides) in food products.

2. PRINCIPLE

Approximately 900 active substances belonging to 100 classes are present in the formulation of commonly used pesticides. The most widely used are benzoylureas, carbamates, organophosphorous compounds, pyrethroids, sulfonylureas, and triazines**.** Their diverse chemical structures and physicochemical properties are better addressed by applying a multi-residue method for their determination and quantification. The capabilities of mass spectroscopy (MS) in combination with gas (GC) and liquid chromatography (LC) for the determination of chemical contaminants are well established and have been extensively applied to assess pesticides in fruits and vegetables. Using GC and LC allows for separating pesticides of different classes (e.g., different polarity and volatility) with high sensitivity. Identification and quantification using mass spectrometry allow for the accurate confirmation of results in a single run.

The sample preparation technique used for selective extraction and desorption of the target compounds is the most important step before chromatographic analysis. Hence, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction technique with acetonitrile and sodium chloride followed by purification steps will be applied.

The AOAC Official Method 2007.01 will be followed to extract and detect the pesticides. The limits of quantification of this methodology are <10ng/g.

3. MATERIALS AND EQUIPMENT

3.1. Reagents

- Magnesium sulfate, anhydrous.
- Acetic acid, ACS grade.
- Acetonitrile, HPLC grade.
- Toluene, HPLC grade
- Methanol, HPLC grade.
- Sodium acetate, anhydrous.
- PSA (Primary Secondary Amine) sorbent or dispersive-SPE tubes
- Formic acid, ACS grade.
- Water, HPLC grade - Millipore water (deionized distilled).
- Triphenylphosphate (TPP)
- Helium
- Nitrogen

3.2. Standards

- Pesticide standards.—High purity reference standards of the pesticide analytes, quality standards (spike solution), and internal standards (ISs)

  Standard solutions.— The concentrations of the calibration curve standards will be 5.0, 10, 25, 50, 100, 250, and 1000 ng/g, prepared in 1% acetic acid in MeCN MeCN as stipulated in the reference method.
- Blank — samples verified to be free of analytes above the detection limit.

Equivalent standards/solutions may be substituted upon consultation with SAFEGRO. Purity and counterions are to be taken into account when calculating standard concentrations. The stability time frame of the solution depends on the expiration date of the components used or the listed expiration date, whichever ends sooner.

3.3. Other Materials

- 69 ounces Whirl-Pak bags (750 bags)
- Disposable gloves
- 50 mL centrifuge tubes
- Sample cups – e.g., 4.5 oz specimen containers w/caps
- Miscellaneous laboratory consumables (pipette tips, centrifuge tubes, microcentrifuge tubes, etc.)

3.3. Equipment

- Coolers for sample collection
- Refrigerators
- Balance (sensitivity 0.01 g)
- Food processor & blender
- Centrifuge
- Oven
- Ion trap, quadrupole, time-of-flight (TOF), or other GC/MS instrument (equipped with electron impact ionization, autosampler, and automatic control/data collection).
  - Analytical column:(5%phenyl)-methylpolysiloxane (low bleed) analytical column (DB-5ms or equivalent).
  - Signal-to-noise ratio (S/N) of the quantitative ion for the pesticides >10.
- Triple quadrupole, ion trap, or other LC/MS/MS instrument (equipped with it is capable of electrospray ionization (ESI) in the positive mode, autosampler, and automatic control/data collection)
  - Analytical column: 3 mm particle size C18 column or equivalent
  - C18 guard column
  - Signal-to-noise ratio (S/N) of the quantitative ion for the pesticides >10.
- Freezer (-20°C)
- Miscellaneous laboratory equipment (micropipettes, vortexer, etc.)

4. COLLECTION OF SAMPLES

Each individual sample will consist of 1 Dutch cucumber. Sampling will be conducted as follows:

A. There are 12 farmers. Thirty samples will be collected from each farmer for a total of 360 samples. Each sample (consisting of 1 Dutch cucumber) will be placed in an individual Whirl-Pak bag. Each bag will be labelled with a barcode (see section 5).

B. Each sample will be placed in a cooler, and following the conclusion of sampling, the coolers will be immediately transported to the laboratory and stored in a refrigerator.

C. The samples for chemical analysis will be shipped to the PPD laboratory.

5. LABELLING REQUIREMENTS FOR THE SAMPLES

Safegro Ref	Farmer Number	Sample Number	YEAR	MONTH	PHC	PUC	SAMPLE DATE	TESTING DATE	SAMPLE TYPE	Alphanumeric number	Barcode
	03	01	23	04	02	03	21	22	01	03012304020321220 1	

Farmer number: 01 to 40 (Farmer number allocated from 01-40)
Sample numbers 01 to 36
Sample type 01 (Microbiological testing) or 02 (Chemical testing)

6. PROCEDURE

Carry out the test in accordance with the following instructions:

6.1 Handling of Food Sample

6.1.1. Prior to analysis, keep samples refrigerated (2-8 °C, preferably at 4 °C).

6.1.2. Analyze samples as soon as possible after their receipt in the laboratory and no later than 24 hs from arrival.

6.2 Preparation for Analysis

6.2.1. Have all reagents in stock.

6.2.2. Prepare stock solutions (e.g.,2000 ng/uL) of the standards in acetonitrile with 0.1% acetic acid. Store in dark vials in the freezer. Keep them for up to 1 month.

6.2.3. Prepare the solutions as specified in AOAC Official Method 2007.01.

6.2.4. Prepare the sealable cups containing 6.0 ± 0.3 g anhydrous magnesium sulfate + 1.5 ± 0.1 g anhydrous sodium acetate. Weight the reagents carefully.

6.2.5. Prepare the centrifuge tubes with 0.05 ± 0.01 g PSA sorbent + 0.15 ± 0.03 g anhydrous magnesium sulfate per mL of sample extract for dispersive-SPE cleanup.

6.3 Preparation of the Sample

6.3.1. Keep the samples refrigerated (at 2-8°C, preferably 4°C) until testing. Testing should be performed within 24 hours of receiving the samples.

6.3.2. Thirty samples will be collected from each farmer. To pool the samples, separate the 30 samples from each farmer into 2 groups of 15 samples each.

6.3.3. For the first group of 15 samples, cut a 2 cm (height) piece from each cucumber and place all the pieces in a container. Store the samples in the freezer before further processing to minimize losses.

6.3.4. Process the frozen pieces together using a food processor. Take 200 g of the comminuted sample and homogenize it using a blender. Repeat this process for the second group of 15 samples. Do this for each farmer. There will now be two pooled samples for each farmer (12 farmers x 2 pool samples each = 24 total samples).

The samples will be labeled as follows:

Safegro Ref	Farmer Number	Sample Number	YEAR	MONTH	PHC	PUC	SAMPLE DATE	TESTING DATE	SAMPLE TYPE	Alphanumeric number	Barcode
	03	01	23	04	02	03	21	22	02	0123040203212	
		02			02				02	0223040203212	
	04	01	23	04	02	04	21	22	02	0123040204212	
		02			02				02	0223040204212	
	07	01	23	04	02	07	21	22	02	0123040207212	
		02			02				02	0223040207212	
	11	01	23	04	02	11	21	22	02	0123040211212	
		02			02				02	0223040211212	
	12	01	23	04	02	12	21	22	02	0123040212212	
		02			02				02	0223040212212	
	16	01	23	04	02	16	21	22	02	0123040216212	
		02			02				02	0223040216212	
	21	01	23	04	02	21	21	22	02	0123040221212	
		02			02				02	0223040221212	
	28	01	23	04	02	28	21	22	02	0123040228212	
		02			02				02	0223040228212	
	32	01	23	04	02	32	21	22	02	0123040232212	
		02			02				02	0223040232212	
	33	01	23	04	02	33	21	22	02	0123040232212	
		02			02				02	0223040232212	
	37	01	23	04	02	37	21	22	02	0123040237212	
		02			02				02	0223040237212	
	40	01	23	04	02	40	21	22	02	0123040240212	
		02			02				02	0223040240212	

6.3.5. Label all vials and tubes that will be used in the method by applying the system indicated in 6.3.4.

6.3.6. Transfer 15 g of the homogenized sample into a centrifuge tube, add 15 ml of 1% sodium acetate in acetonitrile, the vial containing the pre-weighed anhydrous magnesium sulfate + 1.5 ± 0.1 g anhydrous sodium acetate, and 75uL of the solution of the internal standard. Seal the containers. Shake for 1 min and centrifuge as indicated in the reference method.

6.3.7. The container with the remaining sample will be sealed and stored in the freezer in CASE re-analysis is necessary.

6.3.8. Centrifuge the tubes at >1500 rcf for 1 min.

6.3.9. Transfer 1–2 mL of the acetonitrile extracts (supernatant) to the dispersive-pre-prepared SPE tubes with the PSA sorbent and magnesium sulfate.

6.3.10. Seal the tubes and mix for 30 s.

6.3.11. Centrifuge the dispersive-SPE tubes at >1500 rcf for 1 min.

6.3.12. For GC testing: transfer 0.5 ml of the extract to a GC vial and 50 uL of the TPP solution. Cap the vials, shake to mix. If a Large Volume Injection (LVI) is not available, the sample should be concentrated as indicated in the reference method.

6.3.13. For LC testing: Uncap the vails prepared in 6.3.12 and transfer 150 uL to vials for LC. Add formic acid solution in water to match the acid and organic solvent concentration of the initial LC mobile phase. Cap the vials and mix.

6.3.14. Prepare blanks, test mix, and calibration spiking standards as indicated in the reference method.

6.3.15. Conduct GC/MS and LC/MS/MS with the corresponding samples using conditions appropriate for the available equipment and in accordance with the reference method.

6.3.16. Quantify the concentration based on linear least squares calibration of analyte peak areas plotted versus analyte concentration.