Australian Government
Australian Centre for International Agricultural Research

Final report

Small research and development activity

project	**Optimisation of the Single Drop Genomics assay to detect food pathogens**
project number	HORT/2014/027
date published	26 May 2016
prepared by	Jimmy Botella
co-authors/ contributors/ collaborators	
approved by	Eric Huttner, RPM Crop Improvement and Management
final report number	FR2016-13
ISBN	978-1-925436-45-7
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

Contents

1 Acknowledgments

We wish to acknowledge the invaluable help provided by Dr Ben Stoddard in facilitating our contacts and meetings in Cambodia. Dr Suzie Newman also helped with the establishment of the first contacts in Cambodia and Lao PDR.

2 Executive summary

On-site, quick and low-cost pathogen detection is the holy grail of disease diagnostics. Our research team has developed a comprehensive, field-ready technology for on-site pathogenic nucleic acid detection named Single-Drop Genomics (SDG). SDG combines a simple yet accurate on-site sampling process with a robust isothermal amplification and a novel naked-eye evaluation providing Yes/No results.

In this project we have optimised the SDG technology to detect food borne pathogens. We have also performed extensive consultations with multiple stakeholders in Cambodia and Laos PDR about the possible applications of the technology in both countries. SDG has been identified by local officials as being a potential ‘game changer’ capable of delivering strong benefits for different agricultural sectors.

3 Introduction

Nucleic acid point-of-care (POC) bioassays that can be performed on-site with minimal equipment, rapidly and at low cost are in high demand (Yager et al. 2006). Classical techniques and methods for detecting disease DNA/RNA biomarkers include the polymerase chain reaction (PCR) and ligase chain reaction (LCR) (Barany, 1991). However, these methods require a thermal cycler to achieve rapid DNA detection and hence are not suitable for field or on-site applications. Nonetheless, recent developments in isothermal DNA amplification methods may potentially overcome this limitation (Craw & Balachandran, 2012). For instance, new approaches such as the Recombinase Polymerase Amplification (RPA) (Piepemburg et al., 2006), Helicase Dependent Amplification (HDA) (Vincent et al., 2004) and Loop-mediated isothermal amplification (LAMP) (Notomi et al., 2000) combined with lateral flow strips and portable fluorometers (Rohrman and Richards-Kortum, 2012; Lutz, S. et al., 2010; Mahalanabis et al., 2010), have been developed to enable POC detection of pathogenic DNA. However such readout methods whilst convenient are still dependent on the use of relatively sophisticated equipment and may still present financial and technical obstacles for worldwide adoption. A field-ready comprehensive assay incorporating the complete process from on-site sample preparation to results display is still elusive.

Agriculture is one area that can benefit from the use of low cost on-site assays. The agricultural industry is a major contributor to the world economy (~USD$1500 billion per year) (Agrios, 2005). However, crop diseases are a major concern in agriculturally reliant economies, especially in developing countries, with yearly losses of USD$220 billion. Disease identification is traditionally done by visual examination of symptoms requiring experienced plant pathologists and in many cases is very subjective. Sensitive diagnostic methods for disease identification include enzyme-linked immunosorbent assays (ELISAs) (Kokoskova & Janse, 2009), immunoblots (Wright & Morton, 1989), immunofluorescent tests (Janse & Kokoskova, 2009) and various iterations of PCR based assays (Price et al., 2010). However, all these detection methods require expensive and sophisticated equipment and can only be performed in specialized laboratories by well-trained technicians. Early detection is the ideal method to control disease outbreaks before it spreads to neighbouring fields but lack of on-site detection delays the deployment of disease control strategies resulting in huge crop losses. Similarly, early detection of livestock pathogens is essential to avoid the spread of diseases, especially in modern farms using intensive production methods. The usefulness of POC methods non-dependent on sophisticated technological requirements can also be extended to the detection of human pathogens in vegetables. Hence, there is a tangible need to develop new cheap, sensitive and reproducible disease diagnostic technologies that can be applied in the field without the need to access sophisticated laboratory equipment and highly trained personnel.

We have recently developed a field-ready, on-site comprehensive assay for pathogen detection from a single drop of extract i.e. Single-Drop Genomics (SDG), in under 90 minutes with minimal equipment (Wee et al. 2015). We demonstrated that SGD can accurately detect various plant pathogens in *Arabidopsis thaliana* and commercial crops such as bananas. Finally, to demonstrate the versatility of this approach, we proved its efficacy in detecting a wide range of pathogens in species from several kingdoms including plant and cattle viruses, *E. coli*-laced water samples, HIV infected cells, malaria infected blood, influenza virus infected cells and tuberculosis bacteria. SDG has major advantages over any other existing assays, (1) it can be performed on-site (no need to transport samples to specialized labs); (2) does not need power supply; (3) requires only minimal equipment; (4) it is very fast (under 90 min to perform the assay); (5) it is cheap (< $2 USD); (6) it needs no refrigeration (kits can be stored at room temperature); and (7) it does not need highly trained personnel.

This project was designed to test the efficiency of the SDG technology in detecting food pathogens.

4 Collection of pathogen samples and optimisation of growth conditions

All four targeted pathogens (*Salmonella enteritidis*, *Escerichia coli 0157:H7, Listeria monocytogenes* and *Campylobacter jejuni*) were obtained and culturing conditions established. Genomic DNA was extracted from at least three independent cultures for all four pathogens. Sequencing of amplicons generated from all four pathogens confirmed the identity of each pathogen.

5 Identification of genomic sequences suitable for pathogen detection

Through a combination of literature search and bioinformatics analysis we identified genomic regions for each of the four pathogens. For every pathogen we selected at least two regions to target. In the case of *E. coli* O157:H7 we designed primers against the gene responsible for the O157 antigen (RFBE) as well as the *STX1* and *STX2* genes responsible for the production of the Shiga toxin. The invA gene was selected for Salmonella despite not being specific for *S. enteritidis* but exist in all Salmonella species. This decision was made as the invA gene is a frequently used common target gene for Salmonella and we wanted the ability to detect other dangerous *Salmonella* species. However, we have also targeted two other genomic regions that are unique to *S. enteritidis* so that we have the flexibility to specifically target *S. entertidis* or all *Salmonella* species.

6 Development of LAMP assays with purified pathogen DNA

The original SDG assay was developed using isothermal recombinase polymerase amplification (RPA) as the amplification method although it was also equally effective with other amplification methods such as polymerase chain reaction (PCR). In the initial stages of our project we observed that different batches of enzymes provided by the manufacturer of the RPA reagents produced inconsistent results. After consulting the manufacturer we discovered that they periodically change the reagents in the enzyme mix and these changes affected the reliability of the flocculation reaction used as a key step in the SDG technology. For this reason we decided to change the isothermal amplification technique and adopt the loop-mediated isothermal amplification (LAMP) as the choice for our project. LAMP formulation lacks ‘contaminating’ chemicals that can interfere with the SDG readings. A minimum of two sets of primers were designed against every one of the targeted regions and compared against all other known genes in the Genbank database to ensure that they are specific for the intended pathogen species. All of the primer combinations were tested and the best performing primer sets from these assays were chosen for future development of the SDG tests.

7 Sampling methodology optimization

The first step in the detection system is the sampling and enrichment of extracts for 12-24 hours to comply with international gold standards. Two important issues were optimized in this step, the possibility of cross-contamination between samples during the maceration

stage and the risks to the operator caused by the pathogen cultures after the enrichment step. The handling of plant material could lead to cross-contamination of samples and therefore false positives in the assays. After trialling several options we have developed a standard operating procedure in which samples are collected and placed in individual 50 ml falcon tubes containing 4-6 metal ball bearings together with the growth media for each of the pathogens. Vigorous shaking of the tubes provides the means to macerate the tissue and release any contamination into the media. This method minimises cross-contamination and was fully tested in the field in Cambodia, where negative samples were detected immediately after positive ones proving the absence of cross-contamination between samples.

The enrichment step is a requirement of the international 'gold standard' operating procedures adopted for the detection of food pathogens. As a result of such enrichment step, the final culture contains a high concentration of pathogenic bacteria that poses a high risk of exposure for laboratory operators. This risk can be minimized with the use of proper Standard Operating Procedures (SOPs) and protective equipment in the laboratory. Nevertheless, our objective was to develop a methodology for the poorly resourced laboratories in developing countries. We have now established a method by which tubes containing the incubation media (directly obtained from the maceration step) are boiled before opening thus destroying the pathogens and eliminating the health risks for operators. The boiled media can be directly added to the amplification reaction thus further simplifying the handling requirements.

Our developed sampling/enrichment step is now all performed in one single tube and no manipulation is required while the pathogens are alive. In essence, 1-2 grams of sample is added to a falcon (50 ml) tube containing 4-6 metal ball bearings and the culture media. Once the sample has been loaded to the tube, it is capped and shaken for 30 seconds by hand. The tissue is then allowed to incubate at the required temperature for 12-16 hours and immediately boiled for 30 minutes. A sample from this solution is then loaded into the isothermal amplification reaction.

8 SDG pathogen assays in contaminated plant samples

Food contamination with either of the selected pathogens is extremely rare in Australia, therefore we tested the complete assay method on vegetables bought in the supermarket that had been artificially spiked with pathogen by spraying diluted pathogen solutions onto their surface. Our SDG assay consistently detected contaminated samples with an easy to see flocculation while negative controls failed to develop precipitate. Following the initial optimization of the SDG assay we proceeded to perform a number of blind and double blind assays with satisfactory results.

9 Multiplex assay

We developed a single multiplex SDG assay able to detect samples contaminated with either *E. coli* or *S. enteritidis* by combining two sets of primers into a single tube. The presence of *E. coli* or *S. enteritidis*, individually or in combination consistently produced the flocculation display. This SDG assay is therefore able to detect the two most common food pathogens in one single test. Although the test cannot identify the specific pathogen contained in the food, it can easily mark the food as 'unhealthy' reducing the cost of detecting contamination. Those samples identified as contaminated can then be further analysed to determine the nature of the contaminant.

10 In country consultations on the uses and potential of the SDG technology

During the course of the project, I visited Cambodia and Laos PDR for consultations. I had a number of meetings where I described the technology to a number of stakeholders in different government departments followed by extensive discussions about possible applications of the SDG technology in both countries. The meetings included medium and high level officials at different ministries as well as officers involved in 'day-to-day' laboratory tasks. The level of interest in the technology was overwhelming and a number of applications were identified as priorities in each country.

In Cambodia I had meetings at the Royal University of Agriculture (RUA); the General Directorate of Agriculture (GDA) and the Cambodian Agricultural Research and Development Institute (CARDI). In Laos I had meetings at the National University of Lao; Department of Livestock and Fisheries (Ministry of Agriculture and Forestry); Ministry of Health; Plant Quarantine Division (Ministry of Agriculture and Forestry); and the Department of Agriculture, Plant Protection Center (Ministry of Agriculture and Forestry).

During my visit I also arranged for practical demonstrations to be performed as an 'end of project' in country activity.

11 In country demonstrations of the SDG technology and sample survey

We chose the most common food contaminant (*E. coli*) to perform our in-country demonstrations (Cambodia and Lao PDR) as well as the analysis of approximately 100 vegetable samples in Cambodia. All the reagents necessary for the demonstrations as well as the survey were carried in our luggage at room temperature from Australia.

In Lao PDR we performed a demonstration at the quarantine division headquarters; attended by the Director General of Quarantine. In this meeting we first explained the principles of the technology in a PowerPoint presentation followed by an in-depth discussion about the possible applications of the technology for quarantine purposes and bilateral trade. We performed assays of several samples containing pathogenic DNA in the same meeting room while the discussion was taking place. In this way, the participants had the opportunity to see firsthand the extremely simple nature of the assays as well as the optical naked eye reading. Due to the level of interest that the technology had arisen, we were also asked to perform an additional demonstration at the National Food Laboratory. This demonstration was carried out using pathogenic DNA in the presence of the laboratory personnel that routinely perform these assays using more expensive and complicated kits. After the assays had been completed, the head of the laboratory asked us to leave some of the assay reagents that were intended for the survey in Cambodia behind so they could start using SDG immediately at their laboratory. We had to respectfully decline the request as we had not performed proper training of the laboratory personnel.

In Cambodia we performed the SDG demonstration using real samples from farms and markets. We first performed a sampling trip with the invaluable help of the GDA, who provided two officers to guide us to different farms and markets. We collected samples from several farms as well as a vegetable stall in a rural setting and three markets; two of which were in the outskirts of Phnom Penh and one was located in the city.

A total of 100 samples were analysed for the presence of *E. coli*. The incidence of contamination was far higher than expected with 33% of all samples testing positive. Samples from farms showed a 30% contamination rate with *E. coli* while samples from

markets showing 35% contamination. A single sample was taken from a small poodle of water in a farm field showed no contamination.

We observed numerous animals (cattle, dogs, etc.) roaming free in the farm fields.

Analysis of the results for market samples shows a very clear correlation between the origin of the samples and the levels of contamination. Samples obtained from rural environments such as a roadside vegetable stall in the countryside showed an extremely high contamination rate (56% of the samples). Samples from a rural market in a small village showed contamination in 45% of the analysed vegetable samples. This market showed very little hygienic conditions, meat and vegetable stalls were positioned side by side, no refrigeration was used for the meat or the vegetables. Even though stalls had individual roofs and were covered from the direct sun exposure, the heat was very intense and vegetables looked withered with no indication that they had been washed or sprayed with fresh water. The floor of the market was packed dirt with numerous puddles. In open contrast, when we visited a market in the city, only one out of sixteen samples showed *E. coli* contamination (6%). This market had a cement floor that would allow for periodical cleaning or at least hosing of the floor. In addition, vegetables in this market looked fresher and we observed that they appeared wet, probably as a result of having been washed or at least sprayed with water.

12 Conclusions and recommendations

We obtained very clear instructions from ACIAR at the start of the project to

(1) Demonstrate whether the SDG technology could be reliably applied in a resource-poor environment;

(2) Investigate the best uses for the technology in Cambodia and Lao PDR (not necessarily limited to food safety); and

(3) Evaluate the level of interest in the technology at the national level in Cambodia and Lao PDR.

12.1 Conclusions

1.- The SDG technology is ideally suited for developing country environments and can be successfully deployed.

2.- There is a very high incidence of contamination with dangerous food pathogens in vegetables (at least in Cambodia).

3.- The contamination pattern is very revealing showing much higher incidence of contamination in rural areas.

4.- There is an extraordinary level of interest in adopting the SDG technology with a strong desire to ‘take ownership’ of future developments and implementation.

5.- After ample consultation and discussions with local officials there were three major initial areas in which the SDG technology would make an immediate and important impact:

a) Animal husbandry: Use of SDG as an early warning system for infectious and contagious diseases in farm animals. In addition, it was pointed out the use of SDG to monitor and contain disease outbreaks.

b) Plant quarantine and bilateral trade: Disease control at border crossings.

c) Human health: Initial (fast and cheap) screening for food safety in urban and rural markets with the vision to create a national plan to improve the sanitary education of stall holders.

12.2 Recommendations

1.- Given the positive results from the SRA and the extraordinary level of interest on the SDG technology we recommend the development of a full size ACIAR project with Lao PDR as one of the participants.

2.- According to the requests made by the local researchers and government officials we believe that the project should involve several ACIAR Research Programs to cover the topics outlined in “Conclusion #5” above.

3.- The project should include a very strong component on capacity building (to ensure continuation into the future).

4.- The PNG government has expressed a strong interest in the SDG technology and is in the process of committing internal government funds for the immediate start of a project in 2016 to deal with the threat of the Bogia Coconut Syndrome (BCS) to the Asia Pacific coconut germplasm collection. We believe that given this kind of commitment, PNG should be seriously considered for any future projects.

13 References

13.1 References cited in report

Agrios, G.N. Plant pathology, Edn. 5th. (Elsevier Academic Press, Amsterdam ; Boston; 2005).

Barany, F. The ligase chain reaction in a PCR world. *PCR Methods Appl* **1**, 5-16 (1991).

Craw, P. & Balachandran, W. Isothermal nucleic acid amplification technologies for point-of-care diagnostics: a critical review. *Lab on a Chip* **12**, 2469-2486 (2012).

Janse, J.D. & Kokoskova, B. Indirect immunofluorescence microscopy for the detection and identification of plant pathogenic bacteria (in particular for Ralstonla solanacearum). *Methods Mol Biol* **508**, 89-99 (2009).

Kokoskova, B. & Janse, J.D. Enzyme-linked immunosorbent assay for the detection and identification of plant pathogenic bacteria (in particular for Erwinia amylovora and Clavibacter michiganensis subsp. sepedonicus). *Methods Mol Biol* **508**, 75-87 (2009).

Lutz, S. et al. Microfluidic lab-on-a-foil for nucleic acid analysis based on isothermal recombinase polymerase amplification (RPA). *Lab on a Chip* **10**, 887-893 (2010).

Mahalanabis, M., Do, J., ALMuayad, H., Zhang, J.Y. & Klapperich, C.M. An integrated disposable device for DNA extraction and helicase dependent amplification. *Biomedical Microdevices* **12**, 353-359 (2010).

Notomi, T. et al. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* **28** (2000).

Piepenburg, O., Williams, C.H., Stemple, D.L. & Armes, N.A. DNA detection using recombination proteins. *Plos Biology* **4**, 1115-1121 (2006).

Price, J.A., Smith, J., Simmons, A., Fellers, J. & Rush, C.M. Multiplex real-time RT-PCR for detection of Wheat streak mosaic virus and Triticum mosaic virus. *J Virol Methods* **165**, 198-201 (2010).

Rohrman, B.A. & Richards-Kortum, R.R. A paper and plastic device for performing recombinase polymerase amplification of HIV DNA. *Lab on a Chip* **12**, 3082-3088 (2012).

Vincent, M., Xu, Y. & Kong, H.M. Helicase-dependent isothermal DNA amplification. *Embo Reports* **5**, 795-800 (2004).

Wee EJH, Lau HY, Botella JR, Trau M. Re-purposing bridging flocculation for on-site, rapid, qualitative DNA detection in resource-poor settings. *Chemical Communications* **51**, 5828-31 (2015).

Wright, S.F. & Morton, J.B. Detection of vesicular-arbuscular mycorrhizal fungus colonization of roots by using a dot-immunoblot assay. *Appl Environ Microbiol* **55**, 761-763 (1989).

Yager, P. et al. Microfluidic diagnostic technologies for global public health. *Nature* **442**, 412-418 (2006).

13.2 List of publications produced by project

n/a

14 Appendixes

14.1 Appendix 1: Letter of support from the Director General - Department of Livestock and Fisheries (Lao PDR).

Lao People's Democratic Republic
Peace Independence Democracy Unity Prosperity

Ministry of Agriculture and Forestry
Department of Livestock and Fisheries

Ref. No. 0574 /DLF

Vientiane Capital, Date 31 MAR 2015

To: Dr Jimmy Botella
Professor of Plant Biotechnology
Chair, Research Committee
School of Agriculture and Food Sciences
University of Queensland
Brisbane, Queensland 4072, Australia
Phone +61-7 33651128 Email j.botella@uq.edu.au

Dear Professor Botella,

Thank you for the presentation and discussion about the new SDG technology. After consultation with my colleagues I am convinced that the technology can have very important applications for our country. Our Department is in charge and monitor of animal and fish production in LAO PDR with considering of free pathogen in the animal as well as animal product before it reached to consumer because Food safety is a big issue in our country. Having a rapid and cheap test for food pathogens would benefit consumers in my country and we could make good use of it. Animal diseases cause big loses to our agricultural sector and containment is extremely important. The development of SDG for infectious and contagious diseases will provide us with a valuable tool to contain the spread of diseases by diagnosing them early in the farms and therefore avoid big loses for farmers. Diseases such as avian flu, foot and mouth disease, Porcine Respiratory Reproductive Syndrome spread rapidly and early intervention is paramount to contain them. I can also see the use of this technology in border crossings as well as slaughter houses.

I want to convey my full support for your future ACIAR proposal and would like my department to participate as a partner in a future ACIAR project. We have a collection of isolates for the important diseases and food pathogens in LAO PDR and we want to use those isolates to produce SDG diagnostics specifically designed for our needs.

Unlike many other projects that have a limited life, I can envisage that this kind of technology will be self-sustainable and not limited to the duration of the project. This is important for the long term impact to our economy and we would like to take charge of the development and deployment and therefore ensure that the benefits last for a long time.

Please convey our support to the ACIAR authorities and assure them that we will provide official support for this endeavor.

Sincerely,

Dr. Bounkhouang KHAMBOUNHEUANG
Director General
Department of Livestock and Fisheries

Department of Livestock and Fisheries, Ban Sithan Neua, Souphnouvong Road, Sikhottabong District
Vientiane Capital, Lao PDR Telephone: +856-21-215141; Fax: +856-21-215242, email: laodlf@gmail.com

14.2 Appendix 2: Letter of support from the Director General - Department of Agriculture

Lao People's Democratic Republic
Peace Independence Democracy Unity Prosperity

Ministry of agriculture and Forestry
Department of Agriculture

No. 0672 /DOA
Vientiane, date: 09 APR 2015

Dear Professor Jimmy Botella
School of Agriculture and Food Science
Universiy of Queensland, Brisbane, Qld 4072, Australia
Tel: 61-7-3365 1128, Fax: 61-7-3365 1699
Email: j.botella@uq.edu.au
www.uq.edu.au

Subject: Supporting Letter on the new Single Drop Genomics (SDG) technology

Reference to meeting dated 18 March 2015 at the Department of Agriculture; we would like to express our sincere thanks to your presentation on the new technology Single Drop Genomics (SDG) which will be of high value to our country. Lao PDR has numerous border crossings with countries such as Thailand, Cambodia and Vietnam and the introduction of new pests and diseases are a constant threat to our agricultural sector. The introduction of SDG, tailored to our needs, could be a very valuable tool for our border inspectors providing a fast and easy to use method to prevent dangerous pathogens from entering our country. We addition SDG could benefit our domestic agricultural sector in many rural areas where we lack plant pathology expertise but the incidence of diseases is high. A quick diagnostic can provide farmers with the correct treatment for their crops, saving them money, avoiding unnecessary pesticide use and increasing their yield.

Department of Agriculture, as a National Plant Protection Organization of Lao PDR, would like to take this good opportunity want to convey our full support for future ACIAR project. We would like to participate in both the development and the execution of the project. It is our opinion that the legacy of the project will be important if we can train our own personnel in Australia as discussed.

In this regard, it would be very much appreciated if you will consider further closely communicate with our Plant Quarantine Division as following contact address:

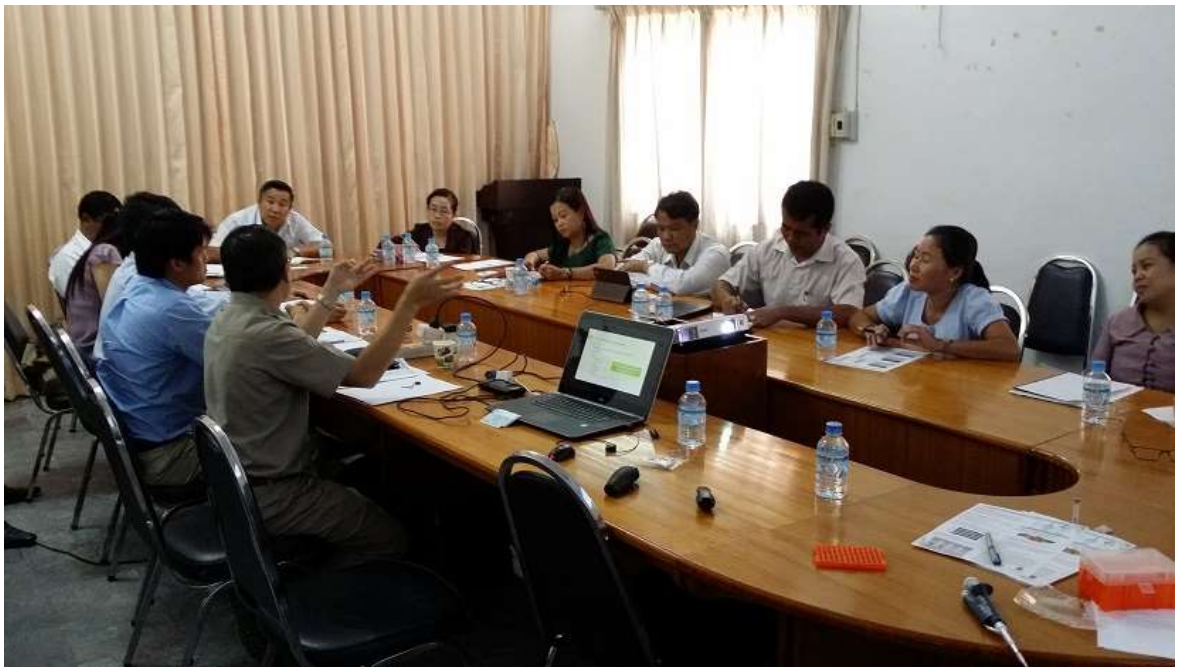
Plant Quarantine Division,
Department of Agriculture, Ministry of Agriculture and Forestry
Lane Xang Avenue, Patuxay Square, P.O. BOX 811, Vientiane Lao PDR.
Tel/Fax:(856 21) 452649;
e-mail: suinthavong@yahoo.com, souliya_ss@yahoo.com, thatsanaly@yahoo.com

Yours Sincerely,

Monthathip Chanphengxay
Director-General

Lane Xang Avenue, Patuxay Square, P.O. BOX 811, Vientiane Lao PDR.
Tel: (856 21) 412350, Fax: (856 21) 452649 ;

14.3 Appendix 3: Consultation with government officials and food safety survey.

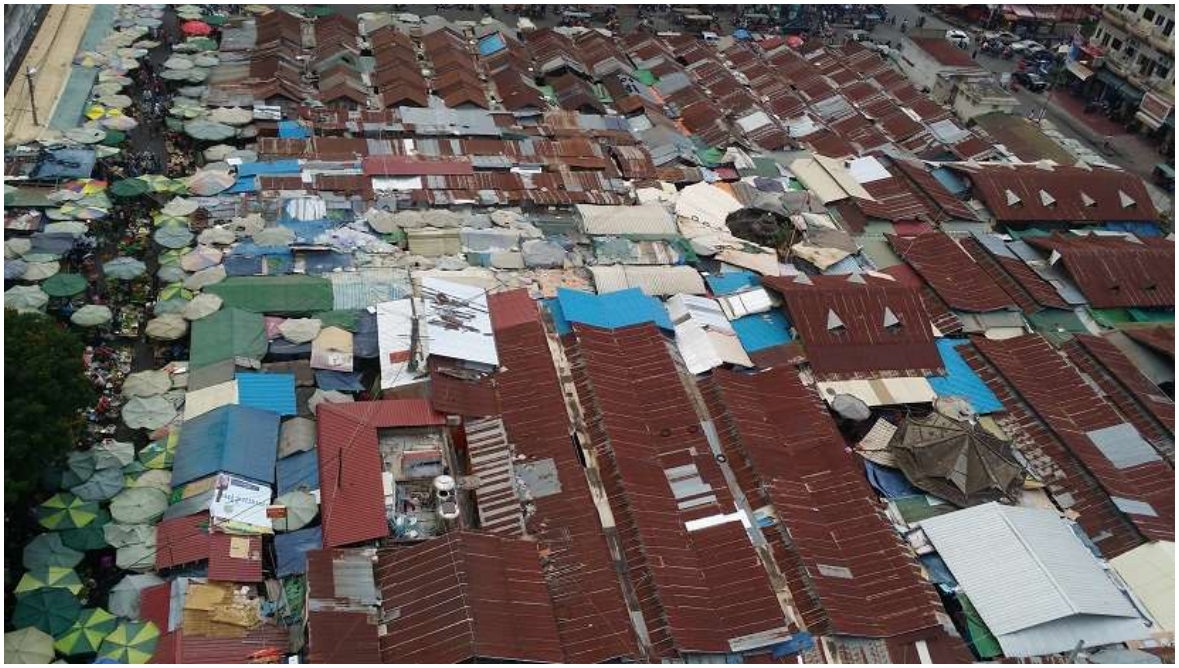


Meeting with the Lao PDR Director General of Quarantine. The SDG assays were performed during the discussion (bottom corner).

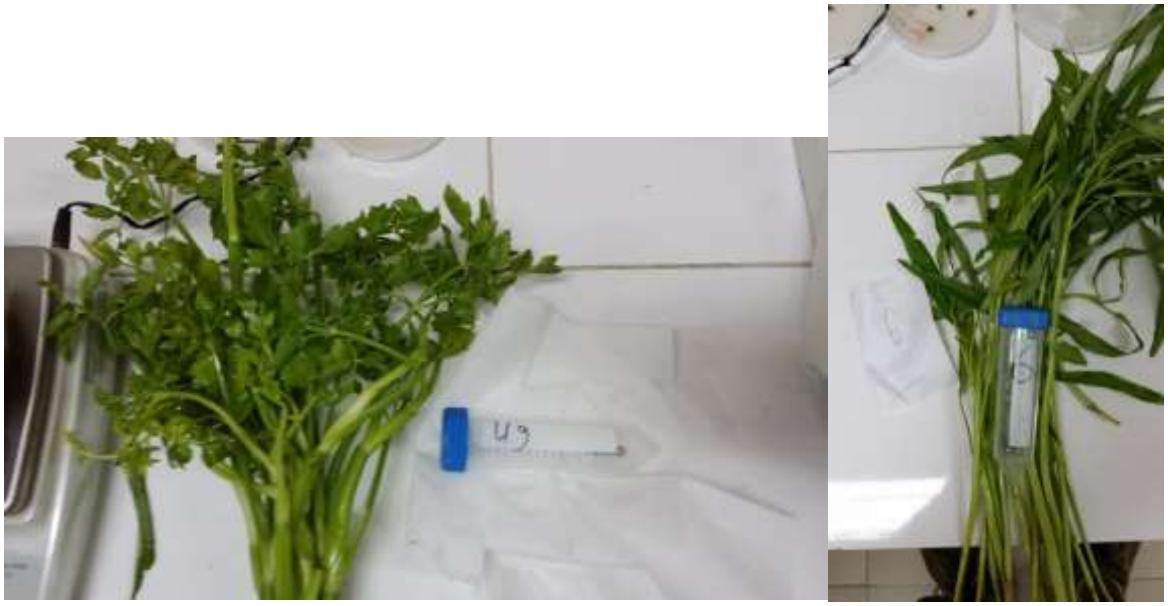
Sample collection at a farm site.

Sample collection at a market.

Sample collection at a market.



Aerial view of a food market.



Samples at the laboratory before assay.

Performing assays at the GDA laboratory (Phnom Penh).

14.4 Appendix 4: The food pathogen diagnostic protocol

1. Place 1g of vegetable leaf in a 50ml tube containing 3 ball bearings and 10ml growth media (media differs for each pathogen, see below for details)
2. Shake vigorously tubes for 1 min to ensure tissue is broken up
3. Place tube in incubator overnight
4. The next day, boil the sample for 30 minutes to ensure all pathogens are killed
5. Dilute culture 1 in 15 with water
6. Rehydrate freeze-dried LAMP reaction with 9ul 0.89M betaine
7. Add 1ul diluted microbial culture
8. Incubate at 63oC for 50 minutes in an incubator
9. Add 20ul of flocculation solution to each LAMP reaction (http://pubs.rsc.org/en/Content/ArticleLanding/2015/CC/C4CC10068A#!divAbstract)
10. Flick the tube for approximately 15 seconds to encourage flocculation
11. Hold tube up to light and allow flocculation to take place (approximately 10 seconds)
    a. Positive reaction: sample flocculates and settles on the bottom of the tube leaving the supernatant transparent
    b. Negative reaction: the entire sample remains turbid and non-transparent

Growth medium and conditions

E.coli O157:H7

Growth media: Buffered peptone

Growth conditions: 41.5oC

Salmonella enteritidis

Growth media: Buffered peptone

Growth conditions: 37oC

Listeria monocytogenes

Growth media: Half Fraser Broth

Growth conditions: 30oC

Campylobacter jejuni

Growth media: Preston broth

Growth conditions: 41.5oC in modified atmosphere using microaerophilic generators bags

Freeze dried LAMP reaction

Bst amplification solution for freeze drying

- 1.1U/ul of dialysed Bst 2.0 warmstart polymerase (NEB)
- 50mM Tris (pH 8.8)
- 25mM (NH4)2SO4
- 25mM KCl
- 20mM MgSO4
- 0.25% Triton x100
- 3.4mM dNTPs
- LAMP primer mix
    - 4.6uM FIP and BIP primers
    - 0.6uM B3 and F3 primers
    - 2.3uM LF and LB primers (if available)
- 5% Trehalose

Freeze drying protocol

- Aliquot the solution into individual tubes (3.49ul of the solution is the equivalent amount of reagents for a 10ul reaction)
- Freeze the solutions
- Freeze dry the solutions in a freeze drier at -80oC for at least 4 hours
- Store at -20oC until required

Primers found to reliably detect each pathogen

E.coli 0157:H7

Primers were designed to detect the wzy gene, which is an oligosaccharide repeat unit polymerase that is required for the O157 antigen.

wzy-1-F3	GTTCTGCTCCATACGTAGT
wzy-1-B3	GGTTAACAATAGAGCAAGTTGA
wzy-1-FIP	ACAGGGAATAAAGCATCAAGACTTATGATAAAAAATTTGCTCCCATG
wzy-1-BIP	AATTCCTTTTCTAACTCTGGTGTCGAAGTGTTCCATATGTTGTTTCT

Campylobacter jejuni

Primers were designed to detect the hipO gene that encodes a Hippuricase that is specific to C. jejuni.

hipO-F3	ATAGGCACATTTTTTCATTTCG
hipO-B3	GAAACTAGAAAGCTAACTGAAGA
hipO-FIP	GGTTGCAAAAGAATTATTTGGCGAACCTCACTTGCCATTAAAGGA
hipO-BIP	TCGTTATTCATAGTCACTGGTGCGTAAAGGTATTGCACAGGC

Salmonella enteritidis

Primers were designed to detect the host invasion gene invA that is required for infection by Salmonella.

invA-1-F3	CGGCCCGATTTTCTCTGG
invA-1-B3	CGGCAATAGCGTCACCTT
invA-1-FIP	GCGCAGCATCCGCATCAATAATATGGTATGCCCGGTAAACAG
invA-1-BIP	GAACGGCGAAGCGTACTGGACATCGCACCGTCAAAGGAA
invA-1-LF	ACCGGCCTTCAAATCGGCA
invA-1-LB	GGGAAAGCCAGCTTTACGG

Listeria monocytogenes

Primers were designed to detect the invasion associated protein p60 (iap) gene that is required for infection by Listeria monocytogenes.

iap-1-F3	GTACATCTGGCGCACAAT
iap-1-B3	GCCAACTAGATATTTACCCCAG
iap-1-FIP	CGCTACCATAGTCAAAGAACACTAACTAGCACTACAAGAATTTCTGA
iap-1-BIP	TTCTCACGTTGGTATCTACGTTGAGCCGTGGATGTTATCGT
iap-1-LF	ATCACCAGGTTTTGCTTGAGAT
iap-1-LB	GCGCAAGACAATGGCGT