Hort Innovation

Final Report

Field-based testing for fall armyworm, Spodoptera frugiperda

Project leader:

Dr Mark Blacket

Delivery partner:

Agriculture Victoria Research Division

Project code:

MT19014

Project:

Field-based testing for fall armyworm, Spodoptera frugiperda (MT19014)

Disclaimer:

Funding statement:

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Contents

Public summary

In early 2020, a new plant pest fall armyworm (FAW), Spodoptera frugiperda, was detected across northern Australia. FAW is a high priority plant pest of many plant industries, capable of causing damage to hundreds of different types of plants, including horticultural commodities. FAW is likely to now be present year-round in warmer regions of northern Australia, with annual seasonal migrations of moths, moving thousands of kilometers, to southern parts of Australia.

FAW are extremely difficult to tell apart from other caterpillars and moths already present in Australia. To address this several new DNA-based tests have recently been developed for the identification of FAW using Loop Mediated Amplification (LAMP) technology. LAMP tests are rapid, highly accurate tests which are particularly suitable for use in the field, employing simple user-friendly protocols and portable devices to identify FAW in under an hour.

Agriculture Victoria previously developed a LAMP test for FAW and are currently conducting a one-year Hort Innovation funded project to validate FAW LAMP testing for in-field use in Australia.

This project involved several activities, including:

1. Directly comparing three existing FAW LAMP tests to assess the performance of each against a broad panel of moth species, to determine how effectively each can detect FAW.
2. Conducting a workshop to directly trial the new DNA test in the field and demonstrate to growers and agronomists the LAMP identification process.
3. Assess the advantages and disadvantages of using LAMP technology for the identification of FAW, to determine which factors may hinder this technology being widely deployed and used by industry.

The overall aim of this project was to validate the in-field tools available for monitoring the movement of FAW through providing simple standardized protocols for use of a standard national rapid assay for the detection of FAW.

Keywords

Fall armyworm (FAW), *Spodoptera frugiperda*; plant pest; LAMP assay; infield diagnostics

Introduction

Fall armyworm (FAW), *Spodoptera frugiperda*, was recently detected across northern Australia, after initially being found in the Torres Strait Islands and far north Queensland. FAW is a high priority plant pest of many plant industries, including horticultural commodities. Distribution modelling indicates that FAW will likely be able to persist year-round in northern Australia, with populations migrating south and building up over the warmer months (Maino *et al.* 2021).

The Department of Agriculture, Water and the Environment (DAWE) recently funded the successful development of a rapid molecular test, using LAMP technology, to be applied in the field for early detection and identification of this pest, i.e. FAW LAMP 'Phase I' project (Agarwal *et al.* 2022).

The current project conducted 'Phase II' of the FAW LAMP research including:

- Facilitating the rapid identification of FAW in regional Australia using LAMP technology;
- Increased awareness of rapid, in-field FAW LAMP testing in regional Australia, especially northern Australia;
- Improved knowledge of technological requirements in regional Australia to allow the rollout of LAMP technology for FAW detection and surveillance.

The objectives of the project align with the National Plant Biosecurity Diagnostic Strategy in promoting and validating a LAMP assay for fall armyworm for rapid, field-based identification. The intended outcome of this project is to foster national collaboration in monitoring the movement of fall armyworm through the provision of a rapid and accurate diagnostic assay with standardized protocols for use standard, national, rapid assay.

Methodology

LAMP infield Validation

Three LAMP assays have recently been published for the detection of FAW: Congdon *et al.* (2021), Agarwal *et al.* (2022), and Osabutey *et al.* (2022). All three published FAW LAMP assays were tested in this project to validate the assays and make recommendations for infield use. To achieve this, we:

• Compared all three assays against the same panel of DNA from identified Noctuidae moth species (*sensu* Agarwal *et al.* 2022), Appendix I.

• Designed a synthetic gBlock for each assay to act as a positive control for each LAMP assay, Appendix I. Gene Fragment gBlocks are targeted synthetic oligonucleotide dsDNA which can be used as standards in qPCR reactions (Conte *et al.* 2018). A gBlock fragment as a standard provides comparable sensitivity, reliability, and assay performance to a purified amplicon standard.

Demonstration of infield LAMP

A demonstration of the infield LAMP assay (Agarwal *et al.* 2022) was conducted in May 2022, in Lindenow Gippsland Victoria. Attended by local growers and agronomists, who collected suspect FAW caterpillar samples for LAMP testing. Caterpillars were processed for DNA extraction, and LAMP assay identification using infield compatible protocols (Appendix V and VI).

Simple Gap Analysis

Impediments to use of current LAMP technology using current technology were examined. Participant surveys from the FAW LAMP infield demonstration were conducted to provide grower / agronomist feedback on the use of this technology (Appendix III).

Results and discussion

LAMP infield Validation

Two new gBlock sequences were designed for Osabutey and Congdon's FAW LAMP assays (Appendix I). A comparison of each of the FAW LAMP assays showed that the most rapid and reliable assays were Agarwal *et al.* 2022 and Osabutey *et al.* 2021, with these assays showing strong rapid amplification and differentiation of the gBlock anneal derivative peaks (Table 1, Figures 1, 2, 3), as well as very few non-target species amplifying (Appendix I). The Agarwal *et al.* (2022) was chosen as the assay to use for developing infield compatible protocols, and for demonstrating FAW LAMP in the field.

Table 1. Comparison of gBlock results for three recently published FAW LAMP assays.

	Agarwal *et al.* 2022		**Osabutey *et al.* 2022**		**Congdon *et al.* 2021**	
	1:6:3, 0.4 µM		**1:6:3, 0.4 µM**		**1:4, 0.4 µM**	
gBlock dilution (copies/µL)	**Time (min)**	**Temp (°C)**	**Time (min)**	**Temp (°C)**	**Time (min)**	**Temp (°C)**
10^8	7.50	80.5	7.00	83.6	11.50	83.0
10^7	9.75	81.0	8.25	84.0	13.50	83.1
10^6	10.00	80.9	9.50	83.9	16.00	83.1
10^5	13.75	80.7	10.50	83.9	18.00	83.0
10^4	15.25	80.6	13.75	83.8	20.00	83.0
10^3	13.00	80.8	11.00	84.0	16.50	83.0
Neg						
S. frugiperda DNA	8.75	77.9	11.75	82.3	8.75	83.3

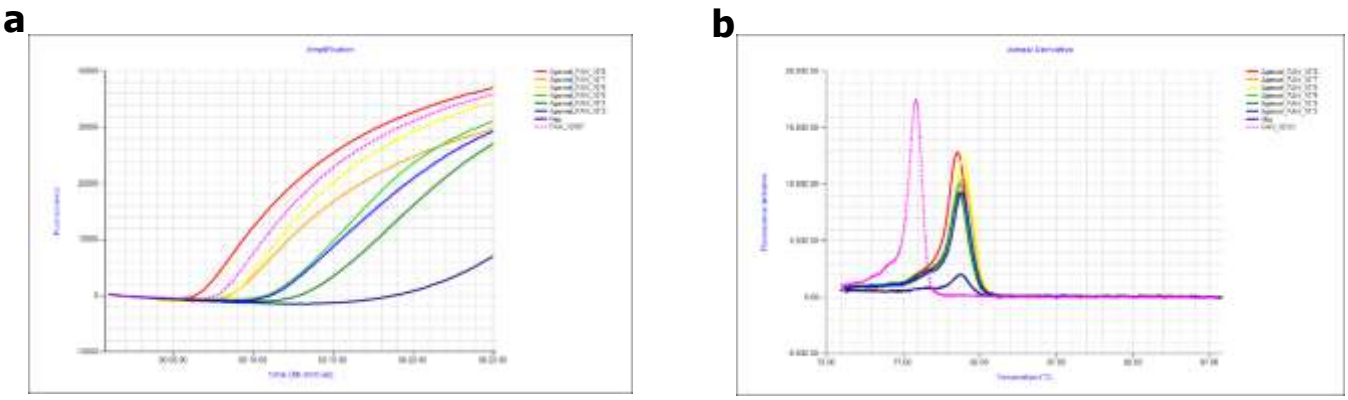

Figure 1. FAW LAMP assay using published protocols (Agarwal *et al.,* 2022). **a)** amplification profile of gBlock dilution series ranging from 1 x 10^8 copies/µL to 1 x 10^3 copies/µL and *S. frugiperda* (VAITC 10707) DNA. **b)** Anneal derivative of LAMP amplicons showing two peaks, 78 °C for FAW DNA (pink) and 81 °C for gBlock DNA.

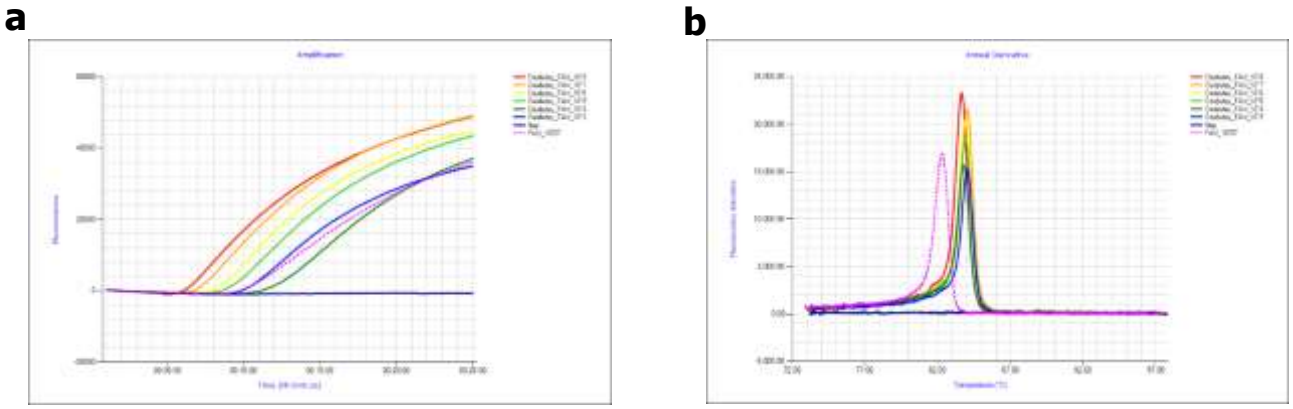

Figure 2. FAW LAMP assay using published primers (Osabutey *et al.,* 2022) and optimized primer ratio as 1:6:3, 0.4 µM. **a)** amplification profile of gBlock dilution series ranging from 1 x 10^8 copies/µL to 1 x 10^3 copies/µL and *S. frugiperda* (VAITC 10707) DNA. **b)** Anneal derivative of LAMP amplicons showing two peaks, 82.3 °C for FAW DNA (pink) and 84 °C for gBlock DNA.

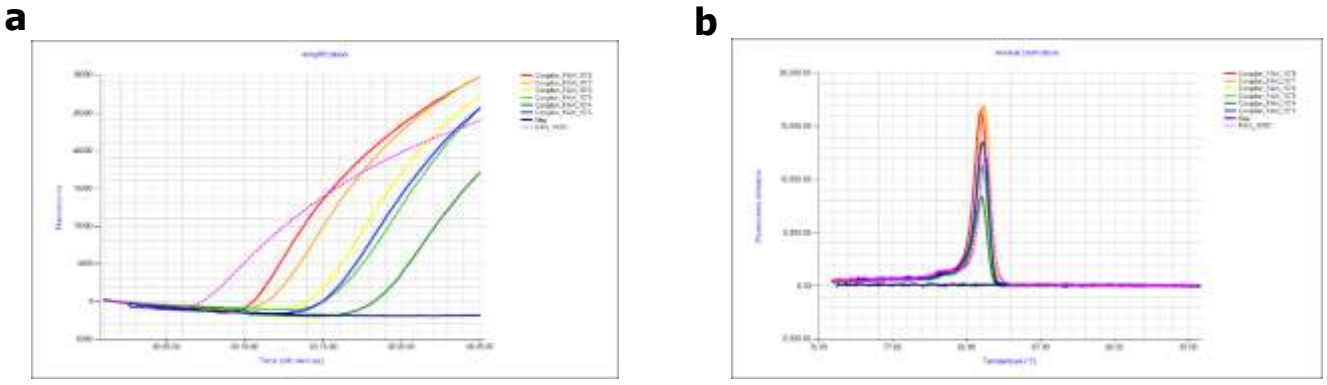

Figure 3. FAW LAMP assay using published protocols (Congdon *et al.,* 2021). **a)** amplification profile of gBlock dilution series ranging from 1 x 10^8 copies/µL to 1 x 10^3 copies/µL and *S. frugiperda* (VAITC 10707) DNA. **b)** Anneal derivative of

LAMP amplicons showing two overlapping peaks, 83.1 ^{o}C for FAW DNA (pink) and 83.3 ^{o}C for gBlock DNA.

Demonstration of infield LAMP

A demonstration of the infield LAMP assay (Agarwal *et al.* 2022) was conducted in May 2022, in Lindenow Gippsland Victoria (Figure 4). This demonstration workshop was attended by six local growers and agronomists from Lindenow, Bairnsdale and Orbost, who brought along suspect FAW caterpillar samples for LAMP testing.

Caterpillars were processed for DNA extraction, with live insects placed briefly in hot water to euthanize them, prior to DNA extraction being performed using an infield compatible protocol (Appendix V). Two LAMP runs were performed on the twelve samples provided, with the results (Appendix II, attached) matching the participants prior identifications of FAW or non-target species. The whole identification process took less than one hour to complete.

After the demonstration the attendees completed survey forms to provide feedback on the use of LAMP for diagnostics (Appendix III, attached).

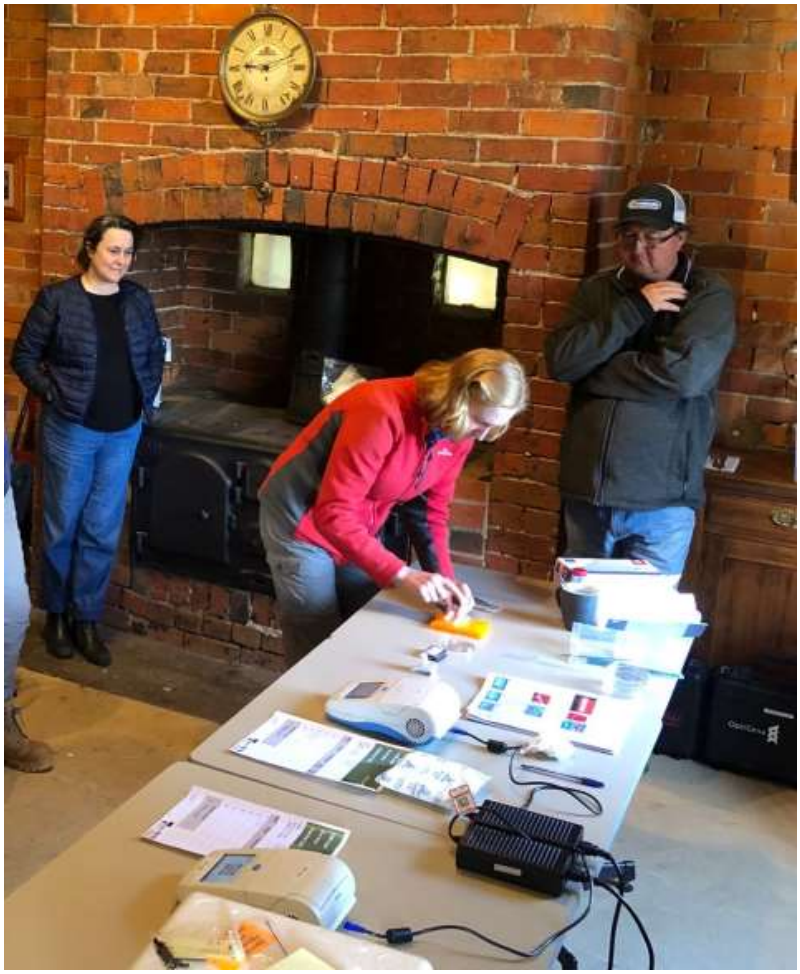

Figure 4. FAW LAMP demonstration workshop conducted in Lindenow, Gippsland Victoria, May 2022. Local growers and agronomists successfully tested suspect FAW caterpillars using the LAMP assay, with identification results produced in less than one hour. (For further details of image, see Appendix at end of report).

Simple Gap Analysis

The advantages and disadvantages of current LAMP technology are provided in Table 2 below.

- The greatest impediments to use of current LAMP technology appears to be both the cost and accessibility of current technology (Table 2, Appendix III).
- Availability of the technology locally, with provision of commercially available kits would improve uptake of this technology in the short-term.
- Longer-term development of simple to use, cheap testing kits based on this technology would be useful for even larger uptake of these assays for infield diagnostics.

Table 2. Advantages and disadvantages of current LAMP technology

	Advantages	**Disadvantages**
LAMP testing conducted infield	Rapid turnaround time (<1 hour) for diagnostics	Relies on availability of specialized equipment, tests and trained staff.
LAMP testing conducted in GenieIII units	High quality testing results, with real-time amplification and anneal derivative results, available within <1hour.	Requires expensive equipment (GenieIII) and commercial reagents, limiting the availability of these assays being used.
LAMP assays conducted using commercially available kits	Non-reliance on specialist laboratory staff to prepare reagents for use in the field. Also, production of quality-controlled reagents will ensure greater confidence in the reliability of test results.	Requires a commercial provider to undertake production of commercial kits, which relies on there being a suitable level of demand for kits.
LAMP assays conducted using simpler technology, e.g. colorimetric LAMP.	These tests are cheaper to run as they do not require specialized equipment, such as the GenieIII.	Assays take longer to provide results, and do not provide secondary confirmation of positive results (i.e. there is no anneal derivative). Assays are also not currently optimised for infield use, which would require development of suitable infield protocols.
Future development of LAMP based tests, with user-friendly, simple to use technology	Would enable greater accessibility of assays, allowing a greater amount of testing to be conducted by non-specialists. Providing rapid species identification for control and management of pests.	Requires research to develop simpler approaches to be developed, which could be produced commercially.

Outputs

Table 3. Output summary

Output	Description	Detail
Laboratory SOP and Infield compatible workflow for FAW LAMP	Standardized infield compatible protocols for FAW LAMP	Appendices V and VI.

Outcomes

This project: (i) validated standard protocols for in-field detection of FAW using LAMP; (ii) improved industry, grower, and biosecurity knowledge of the detection of FAW in the field using LAMP technology; (iii) identified gaps which may impede roll-out of this new technology nationally.

The project demonstrated the use of infield FAW LAMP directly to growers and agronomists who regularly have to identify FAW morphologically. The infield demonstration engaged local growers and obtained feedback through a post workshop survey (Appendix III). An industry article has been drafted and is being prepared for publication in the Melon News (Appendix IV). An engagement event (workshop) was conducted to directly demonstrate the FAW LAMP infield testing approach to growers / agronomists.

Monitoring and evaluation

Table 5. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
1. To what extent has the project achieved its expected outcomes?	This project: (i) validated standard protocols for in-field detection of FAW using LAMP; (ii) improved industry, grower, and biosecurity knowledge of the detection of FAW in the field using LAMP technology; (iii) identified gaps which may impede roll-out of this new technology nationally.	Future demonstrations of LAMP technology would be useful for other regions, industries Development of commercially available kits would assist with uptake of assays.
2. How relevant was the project to the needs of intended beneficiaries?	The project demonstrated the use of infield FAW LAMP directly to growers and agronomists who regularly have to identify FAW morphologically.	
3. How well have intended beneficiaries been engaged in the project?	The infield demonstration engaged local growers and obtained feedback through a post workshop survey (Appendix III). An industry article has been drafted and is being prepared for publication	Future demonstrations of LAMP technology would be useful for other regions, industries.

	in the *Melon News* (Appendix IV).	
4. To what extent were engagement processes appropriate to the target audience/s of the project?	An engagement event (workshop) was conducted to directly demonstrate the FAW LAMP infield testing approach to growers / agronomists.	
5. What efforts did the project make to improve efficiency? processes appropriate to the target audience/s of the project?	The original planned fieldwork was greatly reduced due to Covid restrictions, which affected both interstate and within state travel. An alternative activity, directly testing the three available FAW LAMP assays in the laboratory (Appendix I) was conducted, instead of extensive field testing of the FAW LAMP assay.	

Recommendations

A practical application of this project is the provision of the laboratory SOP and infield compatible workflow (Appendices V and VI), that provide a national, rapid assay for accurate detection of FAW using standardized protocols.

The demonstrations of the FAW LAMP assay in the current project provided useful feedback regarding the potential roll out of this technology. It would be valuable to conduct future workshops / demonstrations in other geographic regions and industries to provide a greater level of awareness and uptake of this technology.

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Intellectual property

'No project IP or commercialization to report'

Acknowledgements

We thank Greg Chandler (Hort Innovation) for assisting with implementing and managing this project.

We also thank Bonnie Dawson (Food & Fiber Gippsland) and Alexandra Keith (Bulmer Farms) for their help with organising and running the demonstration workshop in Gippsland.

Appendices

Appendix II (2-pages, attached).

FAW LAMP run results (x 12 samples) from the demonstration in Gippsland, May 2022.

Appendix III (6-pages, attached).

Grower and agronomist survey sheets (x 6 attendees) following the FAW LAMP demonstration in Gippsland, May 2022.

Appendix IV

Draft article prepared for Melons News in consultation with Courtney Archer, Communications Officer Melons Australia.

Identification of Fall Armyworm through in-field DNA testing

In early 2020, a new plant pest fall armyworm (FAW), *Spodoptera frugiperda*, was detected across northern Australia. FAW is a high priority plant pest of many plant industries, capable of causing damage to hundreds of different types of plants, including horticultural commodities. FAW is likely to now be present year-round in warmer regions of northern Australia, with annual seasonal migrations of moths, moving thousands of kilometers, to southern parts of Australia.

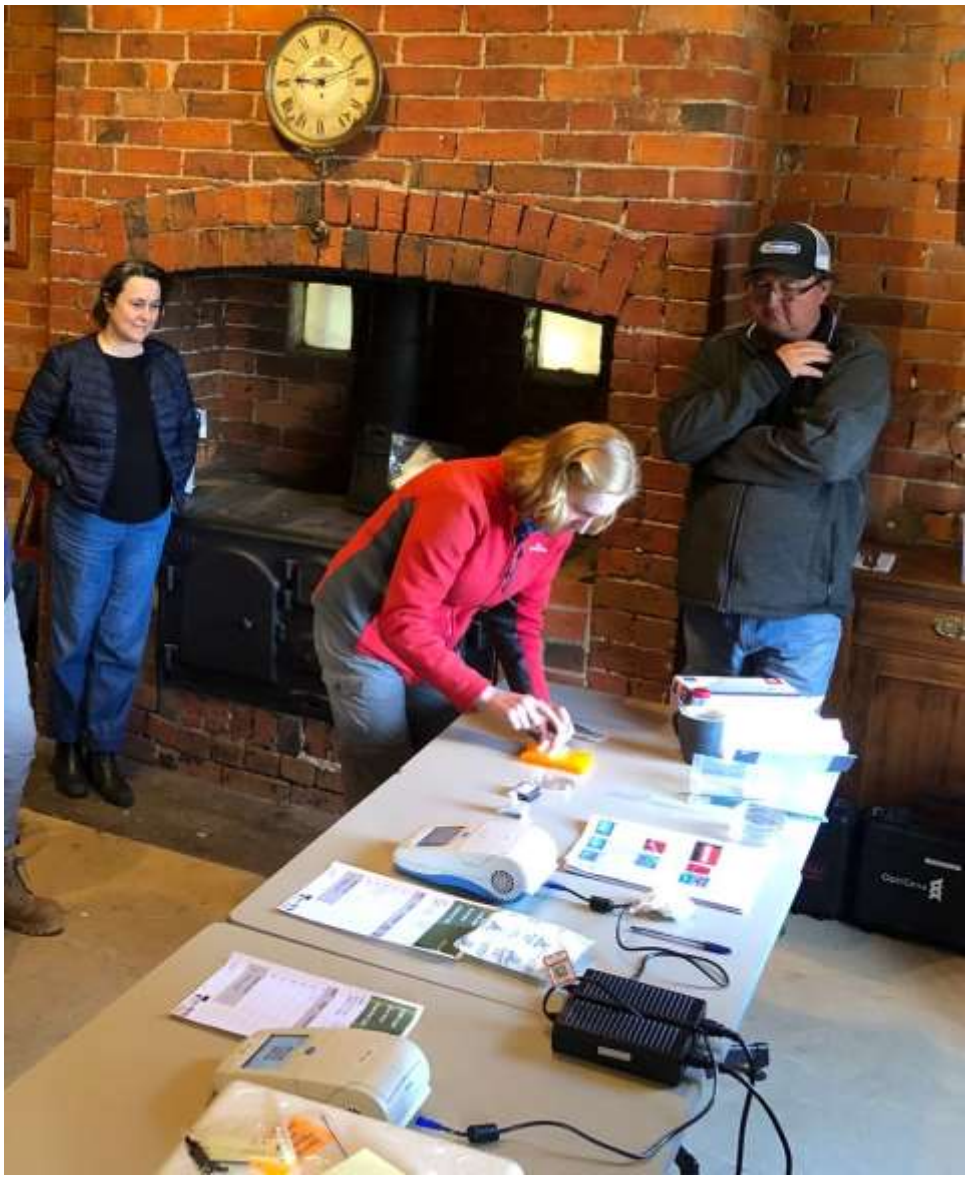
FAW are extremely difficult to tell apart from other caterpillars and moths already present in Australia. To address this several new DNA-based tests have recently been developed for the identification of FAW using Loop Mediated Amplification (LAMP) technology. LAMP tests are rapid, highly accurate tests which are particularly suitable for use in the field, employing simple user-friendly protocols and portable devices to identify FAW in under an hour.

Agriculture Victoria previously developed a LAMP test for FAW and are currently conducting a one-year Hort Innovation funded project to validate FAW LAMP testing for in-field use in Australia. This project involved several activities, including:

- Directly comparing three existing FAW LAMP tests to assess the performance of each against a broad panel of moth species, to determine how effectively each can detect FAW.
- Conducting a workshop (image below) to directly trial the new DNA test in the field and demonstrate to growers and agronomists the LAMP identification process.
- Assess the advantages and disadvantages of using LAMP technology for the identification of FAW, to determine which factors may hinder this technology being widely deployed and used by industry.

The overall aim of this project was to validate the in-field tools available for monitoring the movement of FAW through providing simple standardized protocols for use of a standard national rapid assay for the detection of FAW.

This project was funded by Hort Innovation using the melon, nursery, sweet potato and vegetable industry research and development levies and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.



(Above image provided for draft article).

Legend: ***FAW LAMP demonstration workshop conducted in Lindenow, Gippsland Victoria, May 2022. Local growers and agronomists successfully tested suspect FAW caterpillars using the LAMP assay, with identification results produced in less than one hour.***

[Photographer: Agriculture Victoria (Mark Blacket); Pictured (L-R): Isabel Valenzuela (AgVic), Alexandra Keith (Bulmer Farms), Mick Cox (Riviera Farms); Date: 17th May 2022]

Appendix V. Infield compatible workflow for performing FAW LAMP assay (single page, below).

FAW LAMP assay

DNA EXTRACTION (QuickExtract, 6-well strips)

1. Pipette 50 µL of QuickExtract solution into each well

2. Transfer one FAW sample to each well using single-use toothpicks (x 6). Discard used toothpicks

3. Close lids, place in LAMP machine, close machine lid

4. Select "QuickExtract DNA Extract" and press START



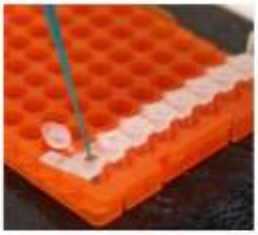
LAMP ASSAY (Master Mix, 8-well strips – KEEP COLD)

5. Transfer DNA into each corresponding 6 wells of strip using a single-use 1 µL loop (or pipette)

6. 7th well NEG (Do not open), 8th well POS gBlock

7. Close lids, place in LAMP machine, close machine lid

8. Select "FAW LAMP" program and press START




RESULTS

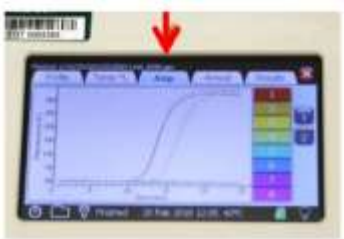
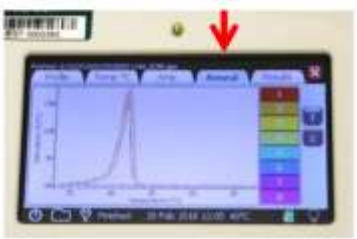
9. Amplification – POSITIVE (Note TIME)

10. No amplification – NEGATIVE (Flat line)

11. Peak – POSITIVE (Note TEMPERATURE, expect peak at ~78.5 °C)

12. No peak – NEGATIVE

13. gBLOCK – POSITIVE AMP + PEAK (expect peak at ~81 °C)


Appendix VI (9-pages, attached). Laboratory SOP for preparing the FAW LAMP assay.

SOP for LAMP detection of Fall armyworm (FAW), *Spodoptera frugiperda*

Agriculture Victoria Research
May 2022
Issue No. 1

Authors:

Issue No.	Date of Issue	Amendment Details
01	30/5/2022	Created by Arati Agarwal & Mark Blacket (Version 01)
01	30/5/2022	Reviewed by Arati Agarwal & Mark Blacket

Project Number: MT19014 (CON-002093)

Department of Jobs, Precincts and Regions
1 Spring Street Melbourne Victoria 3000
Telephone (03) 9651 9999

FAW LAMP Survey, May 2022

Locality Lindenow East Gippsland

1. What is your current role? (e.g. Diagnostician, Biosecurity Officer, Policy Advisor, Surveillance, Industry Representative, Education, Grower, Agronomist)

   Grower / Agro

2. What experience do you have with FAW? In which crops?

   Minimal experience in FAW. Have only learnt about it over past 6 months as we grow Corn.

3. LAMP assays have been developed for a range of pests, including:
   FAW, Queensland fruit fly, phylloxera, Varroa mite, BMSB, Khapra Beetle.
   Would rapid diagnostic tests for some of these pests be useful to you? Which pests?

   FAW

4. Would it be useful to have commercially produced LAMP kits available for any of these pests?

   Yes. Would be alot more beneficial than sending the pest away for testing.

5. In your opinion, what are the main <u>advantages</u> of LAMP technology?

   Instant diagnosis

6. In your opinion, what are the main <u>disadvantages</u> of LAMP technology?

7. Where do you see this technology being used? If yes, how would you use this technology?

   Agronomists ect being able to easily / quickly identify pest and make decisions on sprays ect.

8. What do you think the barriers are to adoption of this technology? Specifically in your field?

   Knowledge and training involved in order to use this technology.

FAW LAMP Survey, May 2022

Locality Bairnsdale

1. What is your current role? (e.g. Diagnostician, Biosecurity Officer, Policy Advisor, Surveillance, Industry Representative, Education, Grower, Agronomist)

   Agronomist.

2. What experience do you have with FAW? In which crops?

   some experience in sweet corn.

3. LAMP assays have been developed for a range of pests, including:
   FAW, Queensland fruit fly, phylloxera, Varroa mite, BMSB, Khapra Beetle.
   Would rapid diagnostic tests for some of these pests be useful to you? Which pests?

   Yes, FAW.

4. Would it be useful to have commercially produced LAMP kits available for any of these pests?

   Yes.

5. In your opinion, what are the main <u>advantages</u> of LAMP technology?

   Quick, accurate identification of important pests.

6. In your opinion, what are the main <u>disadvantages</u> of LAMP technology?

   - cost
   - accessibility

7. Where do you see this technology being used? If yes, how would you use this technology?

   Area wide indentification of pest presence. I would use it to adjust spray programs appropriately.

8. What do you think the barriers are to adoption of this technology? Specifically in your field?

   - cost.

FAW LAMP Survey, May 2022

Locality Orbost

1. What is your current role? (e.g. Diagnostician, Biosecurity Officer, Policy Advisor, Surveillance, Industry Representative, Education, Grower, Agronomist)

   Production Manager / Agronomist

2. What experience do you have with FAW? In which crops?

   Solely sweet corn, maize & popcorn. In QLD & Vic.

3. LAMP assays have been developed for a range of pests, including:
   FAW, Queensland fruit fly, phylloxera, Varroa mite, BMSB, Khapra Beetle.
   Would rapid diagnostic tests for some of these pests be useful to you? Which pests?

   Yes, FAW.

4. Would it be useful to have commercially produced LAMP kits available for any of these pests?

   Yes.

5. In your opinion, what are the main <u>advantages</u> of LAMP technology?

   Efficiency - time saving & cost saving if the machine was locally available.

6. In your opinion, what are the main <u>disadvantages</u> of LAMP technology?

   Cost of the machines

7. Where do you see this technology being used? If yes, how would you use this technology?

   If there was a machine at the DPI or Ag Vic office & agronomists could bring in samples for testing.

8. What do you think the barriers are to adoption of this technology? Specifically in your field?

   Having the equipment readily & locally available.

FAW LAMP Survey, May 2022

Locality Lindenow VIC

1. What is your current role? (e.g. Diagnostician, Biosecurity Officer, Policy Advisor, Surveillance, Industry Representative, Education, Grower, Agronomist)

   Agronomist

2. What experience do you have with FAW? In which crops?

   Corn. over Summer

3. LAMP assays have been developed for a range of pests, including:
   FAW, Queensland fruit fly, phylloxera, Varroa mite, BMSB, Khapra Beetle.
   Would rapid diagnostic tests for some of these pests be useful to you? Which pests?

   Probably not viable - work off usual diagnostics

4. Would it be useful to have commercially produced LAMP kits available for any of these pests?

   potentially

5. In your opinion, what are the main <u>advantages</u> of LAMP technology?

   identify between similar looking species

6. In your opinion, what are the main <u>disadvantages</u> of LAMP technology?

   expensive, Many variables

7. Where do you see this technology being used? If yes, how would you use this technology?

   —

8. What do you think the barriers are to adoption of this technology? Specifically in your field?

   —

FAW LAMP Survey, May 2022

Locality Lindenow

1. What is your current role? (e.g. Diagnostician, Biosecurity Officer, Policy Advisor, Surveillance, Industry Representative, Education, Grower, Agronomist)

   Agronomist

2. What experience do you have with FAW? In which crops?

   Been seeing FAW for last two years in sweetcorn

3. LAMP assays have been developed for a range of pests, including:
   FAW, Queensland fruit fly, phylloxera, Varroa mite, BMSB, Khapra Beetle.
   Would rapid diagnostic tests for some of these pests be useful to you? Which pests?

   Yes - FAW

4. Would it be useful to have commercially produced LAMP kits available for any of these pests?

   Yes.

5. In your opinion, what are the main <u>advantages</u> of LAMP technology?

   Quick diagnosis so you know what you are dealing with.

6. In your opinion, what are the main <u>disadvantages</u> of LAMP technology?

   Expense of the machine

7. Where do you see this technology being used? If yes, how would you use this technology?

   Yes if available locally.

8. What do you think the barriers are to adoption of this technology? Specifically in your field?

   Cost of machine.

FAW LAMP Survey, May 2022

Locality Lindenow

1. What is your current role? (e.g. Diagnostician, Biosecurity Officer, Policy Advisor, Surveillance, Industry Representative, Education, Grower, Agronomist)

   Agronomist.

2. What experience do you have with FAW? In which crops?

   FAW has been present during the last 2 summers. Found only in sweet corn on our farm so far.

3. LAMP assays have been developed for a range of pests, including:
   FAW, Queensland fruit fly, phylloxera, Varroa mite, BMSB, Khapra Beetle.
   Would rapid diagnostic tests for some of these pests be useful to you? Which pests?

   No, unless fruit fly comes to our region.

4. Would it be useful to have commercially produced LAMP kits available for any of these pests?

   Potentially if a pest like FAW developed chemical resistance as then you could check ~~about~~ that it is FAW before selecting sprays.

5. In your opinion, what are the main <u>advantages</u> of LAMP technology?

   Rapid diagnosis of species with a fairly simple process.

6. In your opinion, what are the main <u>disadvantages</u> of LAMP technology?

   ?

7. Where do you see this technology being used? If yes, how would you use this technology?

   Mainly for diagnostics for samples which are hard to identify under a microscope.

8. What do you think the barriers are to adoption of this technology? Specifically in your field?

   Cost. In my field, ~~practicality~~ it is not too hard to tell FAW visually.

FAW LAMP

Run sheet

Gippsland, 2022

AGRICULTURE VICTORIA

Date: 17/5/22
Genie III number: GEN 1388
LAMP run number: 1388 – 0955

Well	Sample	Participant	Amp (Time)	Anneal (Melt)
1	Orbost 7/4/22		7.2	77.7
2	"		7.6	78.1
3	"		7.3	78.2
4	"		7.5	77.9
5	"		7.3	77.9
6	"		7.2	77.9
7	Negative control	-		
8	Positive control	+	7.3	80.8

FAW LAMP

Run sheet

Gippsland, 2022

AGRICULTURE VICTORIA

Date: 17/5/22

Genie III number: 1385

LAMP run number: 1385-0090

Well	Sample	Participant	Amp (Time)	Anneal (Melt)
1	Lindenow 16/5/22 (S)	M. Trish.	7:3	77.7
2	" (M)			78.0
3	" (L)			
4	Lindenow 13/4/22 Heliothis			
5	" FAW	(S)	19.1	78.1
6	" "	(M)	17.4	78.1
7	Negative control	-		
8	Positive control	+	8.5	80.6

SOP for LAMP detection of Fall armyworm (FAW), *Spodoptera frugiperda*

Agriculture Victoria Research
May 2022
Issue No. 1

Authors:

Issue No.	Date of Issue	Amendment Details
01	30/5/2022	Created by Arati Agarwal & Mark Blacket (Version 01)
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Project Number: MT19014 (CON-002093)

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1. SCOPE AND APPLICATION

This procedure describes the specific detection of nucleic acids from Fall armyworm, *Spodoptera frugiperda,* using loop-mediated isothermal amplification (LAMP) assays for applications in the laboratory and field. Total nucleic acids from sample lysates, pure DNA extracts, or fresh field samples can be tested.

2. PRINCIPLE

This SOP presents the protocol for a LAMP assay that allows the rapid and sensitive detection of the Fall armyworm (FAW), *Spodoptera frugiperda*, in the laboratory and field using six primers and an isothermal polymerase with strand displacing activity, in targeting the mitochondrial 16S locus. Two inner primers (FIP and BIP) and two outer primers (F3 and B3), interact to amplify products which are single-stranded loops, allowing secondary primer binding (forming stem-loop structures) without the need for lengthy thermocycling (Notomi *et al.,* 2000). The addition of loop primers (FL and BL) can facilitate a faster reaction by binding to loops that are of the incorrect orientation to bind to FIP and BIP (Nagamine *et al.,* 2002). Detection of positive results are via either single channel fluorescence measurement (Genie II) or two-colour fluorescence excitation (Genie III) (OptiGene, UK). The Genie III is a small portable device that can deliver results of species detections in real-time in the laboratory and field.

gBlocks® Gene Fragment is a targeted synthetic oligonucleotide dsDNA which can be used as standards in qPCR reactions (Conte et al., 2018). A gBlock fragment as a standard provides comparable sensitivity, reliability, and assay performance to a purified amplicon standard. Here we have developed a gBlock fragment to be used as synthetic positive control in LAMP assays.

3. LABORATORY PERSONAL PROTECTIVE EQUIPMENT

3.1. Laboratory coat

3.2. Nitrile, latex or vinyl gloves

3.3. Closed-in shoes and safety glasses

4. REAGENTS

4.1. Ultrapure water (Invitrogen, Australia) Appendix B)

4.2. TE buffer, pH 8.0 (Invitrogen, Australia) (Appendix C)

4.3 QuickExtract DNA extraction solution 50 mL 1.0 (Epicentre, USA) ordered from Astral Scientific (Appendix D)

4.4. Isothermal Master Mix, OG-ISO-DR004 (OptiGene, UK) stored at 4^{o}C until opened (Appendix E)

4.5. Samples for testing

4.6. Primers as per Table 1 below ordered from Sigma and stored at -20^{o}C

4.7. FAW LAMP gBlock sequence (positive control) ordered from Integrated DNA Technologies (Iowa, USA), see below

Table 1. Primer sequences used to target *Spodoptera frugiperda* COI gene (Agarwal et al.,2022)

Primer	Sequence 5'-3'	Target species	Gene
FAW_F3	ATGATACTTACTATGTAGTTGCTCATTTC	*Spodoptera frugiperda*	COI
FAW_B3	TTCATGAAATATAAGAATCAGGATAATCA		
FAW_FIP	GGATTTAAAGATAATCCAGTAAATAATGG**CAYTATGTTTTATGAATA GGAGCTG**		
FAW_BIP	CCTTATWTATTAAAAATTCAATTTTTTATTATATTTAT**CCGAGGTATAC CTGCTAAYCCTAAA**		
FAW_FL	CARTGAATAAATCCHCCTAAAATAGC		
FAW_BL	GGAGTAAATTTAACTTTYTTCCCA		

Note: Bold, underlined sequence region indicates F2 and B2 primer regions

5. *S. FRUGIPERDA* gBlock

Positive control is a gene block fragment with the following details:

Organism Name = *Spodoptera frugiperda*, COI mitochondrial gene

gBlock Name = FAW_gBlock 252 bp

gBlock Sequence

5'_
cccATGATACTTACTATGTAGTTGCTCATTTCgggCACTATGTTTTATCAATAGGAGCTGcccGCTATTTTAGGTGGATTTAT
TCACTGgggCCATTATTTACTGGATTATCTTTAAATCCgggCCTTATATATTAAAAATTCAATTTTTTATTATATTTATCcccG
GAGTAAATTTAACTTTCTTCCCAgggTTTAGGATTAGCAGGTATACCTCGcccTGATTATCCTGATTCTTATATTTCATGAAc
cc -3'

Ordered from Integrated DNA Technologies (Iowa, USA): *S. frugiperda* double stranded DNA gBlock Gene Fragment 250 ng. Prepare as described below and use 10^6 copies/reaction.

Calculating copy number:

It is usually necessary to dilute the resuspended gBlock Gene Fragment to a specific copy number/µL.

To calculate copy number:

500 ng = 3214 fmol (written on the tube)

(fmol) (1 x 10^{-15} mol/fmol) (Avogadro's number) = copy number/µL

(3214 fmol) (1 x 10^{-15} mol/fmol) (6.022 x 10^{23}) = **1.93 x 10^{12} copies/µL**

5.1 Add 193 µL of TE buffer, pH 8.0 (Invitrogen, Australia) to the tube with lyophilised gene fragment. Incubate at 50°C for 10 min and mix well. Spin down the tube in a centrifuge. This is the stock solution stored as **1 x 10^{10} copies/µL.**

5.2 **Prepare a dilution series:**

1. To obtain 10^8 copies add 2 µL of 10^{10} copies to 198 µL of TE buffer.
2. To obtain 10^6 copies add 2 µL of 10^8 copies to 198 µL of TE buffer.

6. EQUIPMENTS AND CONSUMABLES

6.1. Small volume pipettes (i.e., Gilson P2, P10, P20, P200) and sterile filtered tips to fit

6.2. Bench top centrifuge or zip spin

6.3. Sterile nuclease-free 1.5mL tubes and rack

6.4. Genie strip tubes and caps, 150µl (GeneWorks cat#: OPG-OP-0008-50) & rack

6.5. Genie III (8 sample capacity) portable real-time fluorometer (OptiGene, UK), GeneWorks

6.6. Computer with the latest Genie Explorer software V 2.0.7.11 (OptiGene, UK) free to download.

7. SAMPLING AND SAMPLES

Samples consist of non-destructive cell lysates, total nucleic acids extracted from psyllids, or fresh adults encountered in the field. The standard laboratory method for DNA extraction follows the QIAGEN DNeasy blood and tissue extraction kit. Other methods are also suitable provided that final DNA/RNA solution is EDTA-free.

8. PROCEDURE FOR PRIMER MASTER MIX PREPARATION

8.1. In the PCR clean room (or flow hood) prepare the Primer Master Mix (1:6:3, 0.4 µM) to a final volume of 100 µL as per the Table 2 below.

8.2. Dilute F3 and B3 stock primer only to 10 µM (make up 100 µL each of B3 and F3) (10 µL of primer + 90 µL of ultrapure water).

8.3. Add 62 µL of ultrapure water to primer mix (total 100 µL).

8.4. The primer mix can be made in 500 µL aliquots (as in Table 2, below x 5), then stored at -20^{o}C for short term storage, up to a month.

Table 2. Primer concentrations and volumes used in the 100 µL Primer Master Mix for LAMP assay

Reagent	Concentration (µM)	Recipe (µL)
FAW_F3	10	10
FAW_B3	10	10
FAW_FIP	100	6
FAW_BIP	100	6
FAW_FL	100	3
FAW_BL	100	3
H_2O	N/A	62

9. PROCEDURE FOR LAMP ASSAY MASTER MIX PREPARATION AND LAMP RUN

9.1. Determine the number of reactions to be performed allowing for positive control sample and a no template control (NTC). Add half an extra reaction and calculate the reagent volumes required to prepare the LAMP assay Master Mix (leaving out template).

9.2. In a clean (non-contaminated) area prepare a reaction master mix based on Table 3 below. Add each reagent in descending order to a 1.5 ml tube, leaving template out. Spin briefly.

Table 3. Single 25 µL reaction setup for both LAMP assays and one strip 8.5 wells mastermix setup (following Blacket *et al.* 2020)

Reagent	Per Sample (µL)	Mastermix (x 8.5) (µL)
Primer Master Mix	10	85.0
Isothermal Master Mix	14	119.0
Template DNA	1	-

9.3. Aliquot out 24 µL of master mix to each well of the Genie strip and move to the template bench (or store at -20°C for later use in the field).

9.4. Add 1 µl of template DNA using 10 µL pipette, closing each set of tubes as you go. Alternatively, if in the field can use 1 µl disposable loops, as this eliminates the need for pipettes.

9.5. The no template control (NTC) well (generally well number 7) should remain closed. 1 µl of 10^6 gBlock synthetic positive is added to the 8^{th} well.

9.6. Turn on the Genie III machine and insert the Genie strip in the machine. Make sure that the machine is being charged or run from a power source. Select or input the cycling parameters (as per Blacket et al 2020):

65°C for 25 min (isothermal)

98-73°C with 0.05°C/s ramping (annealing curve analysis)

Visualised in the Blue channel

9.7. Enter in the sample details, date and Genie serial and run number in your notebook and start the run. Total run time is estimated to be 35 min.

9.8. The Amplification curve, anneal derivative curve and results of LAMP amplification time and anneal derivative temperature can be seen and recorded from the Results table, on the Genie device.

9.9. At the completion of the run, results can be exported with an USB cable (supplied with the LAMP machine) for further analysis on the computer in the Genie Explorer software version V2.0.7.11. The run details are stored in the LOG file of the Genie machine. Results may also be recorded directly form the device by-hand.

10. TEST REPORT

The Genie Explorer software generates an experiment report in Blue channel that displays all the run details and the run profile (A), amplification curve & rate (B), and anneal curve and derivative peak (C).

(A) LAMP run details and run profile:

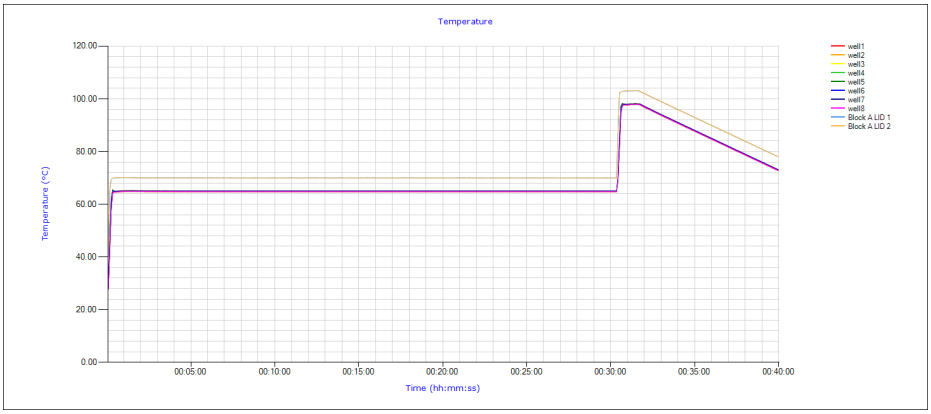
Genie III Profiles

QuickExtract (QE), 65°C 6 min, 98°C 2 min

FAW LAMP (see image below)

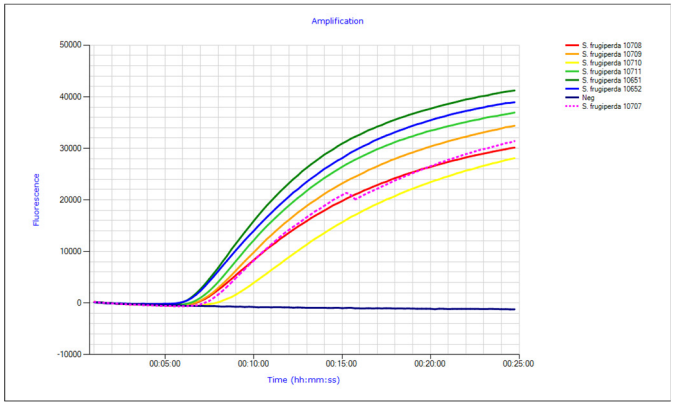
Amplification, 65°C for 25 min followed by,

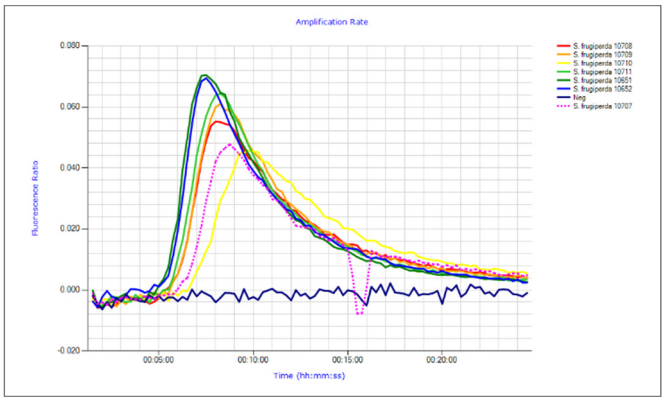
Annealing, 98°C to 73°C with ramping at 0.05°C/s.



(B) Amplification curve and amplification rate data from a LAMP experiment

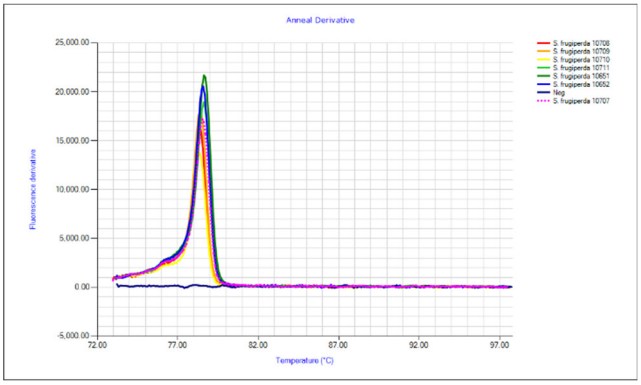
For the FAW LAMP assay amplification generally occurs within 10 minutes (see image below).

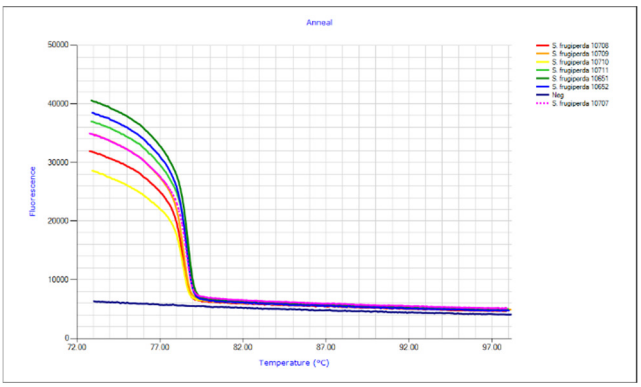




(C) Anneal curve and derivative peak data from a LAMP experiment

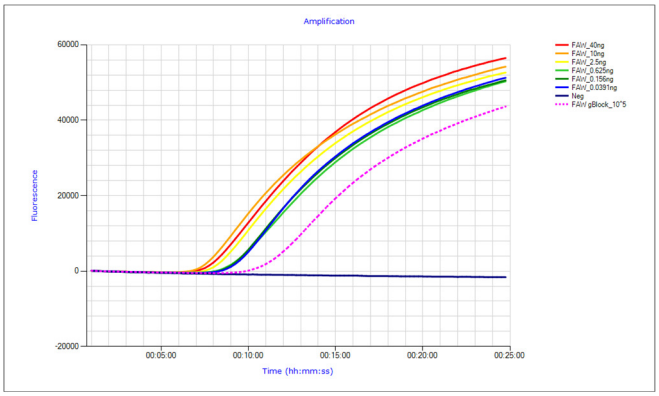
For the FAW LAMP assay the anneal derivative from FAW DNA is expected around ~78.5°C (see image below).

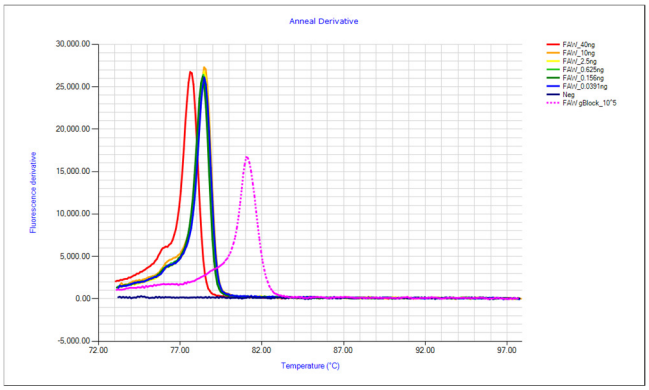




(D) Amplification curve and anneal derivative peak data from a FAW LAMP experiment using gBlock

For the FAW LAMP assay the anneal derivative peak for the gBlock occurs around ~81.0°C (10^5 pink peak), (see image below).





Note: Two anneal peaks are present: one for FAW DNA at 78.5°C, and a second peak for gBlock 10^5 at 81.0°C.

11. REFERENCES

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12. APPENDICES

Appendix A: Field Testing

EQUIPMENTS AND REAGENTS

Additional requirements for field testing: Esky, Kimwipes/paper towel, forceps, entomology pins/toothpicks. DNA extraction in the field is undertaken using the QuickExtract DNA extraction solution 1.0 (Epicentre, USA).

PROCEDURE FOR DNA EXTRACTION IN THE FIELD

QuickExtract extraction method:

12.1. Pipette 50 µl of the QuickExtract solution into each well of the Genie strip. This can be done in the laboratory and stored at -20°C for later use.
12.2. DNA was extracted from single (thoracic) legs removed from adult and late-instar larval Noctuidae specimens.
12.3. Use a "single-use" disposable toothpick to prick (or equivalent) the sample into the extraction solution, taking care to avoid cross contamination between each specimen. Add one specimen per well.
12.4. Incubate samples in the Genie machine and press the run button for a pre-set cycling profile, programmed at 65°C for 6 min, followed by 98°C for 2 min.
12.5. Incubate on ice for 1 min or store DNA at -20°C until required.

Appendix B
Ultrapure water (Invitrogen, Australia)

Once the bottle is opened pipette 1 ml into 1.5 mL sterile Eppendorf tubes and store at room temperature until required to avoid contamination. Label tubes.

Appendix C

TE buffer, pH 8.0 (Invitrogen, Australia)

Once the bottle is opened pipette 1 ml into 1.5 mL sterile Eppendorf tubes and store at room temperature until required to avoid contamination. Label tubes.

Appendix D

QuickExtract (QE) DNA extraction 50 mL solution 1.0 (Epicentre, USA), ordered from Astral Scientific, stored at -20°C.
Once the QE bottle is opened pipette 1 ml into 1.5 mL Eppendorf tubes and freeze at -20°C until required to avoid contamination.

Appendix E

Isothermal Master Mix (DR004) stored at 4°C. This comes with 2 bottles of lyophilized master mix and one bottle of rehydration buffer. Following the manufacturers protocol: Add 2.25 mL of buffer per bottle.

1. Allow to sit for 10 mins to resuspend.
2. Vortex briefly.
3. Aliquot 250 µL into 1.5 ml sterile Eppendorf tubes and store at -20°C until required. Use fresh gloves for touching this vial (no DNA and gBlock near it).
4. The Mastermix is light sensitive and should be kept in the aluminium packet which comes with this reagent.