In the field with LAMP

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ABSTRACT

Effective biosecurity is underpinned by rapid detection of pathogens within an evidence-based testing framework, and supported by a quality management system. Loop-mediated isothermal amplification (LAMP) is a molecular diagnostics platform that detects the genome of a pathogen. The LAMP enzyme is more resistant to inhibitors, so the pathogen nucleic acid (DNA or RNA) does not need to be purified, enabling detection directly from the sample. This makes LAMP different from other molecular tests, and gives it a robustness that allows LAMP to be used in resource-limited settings, be field-deployable and used as a point-of-care tool. Agriculture Victoria, with the support of colleagues from the Asia–Pacific region including Timor-Leste and Bhutan, have been developing and verifying LAMP assays for foot-and-mouth disease virus (FMDV) and African swine fever virus (ASFV). These two pathogens affect food production systems and animals of cultural importance in the region and – if detected in Australia – within our borders. We are developing an advanced quality management system to support the adoption and implementation of this emerging technology. Specifically, our research is determining the best in-field sample type for FMDV and ASFV, accruing verification data obtained through ongoing quality control and virtual communication, and establishing a proficiency testing program to assess the reagents and operators. The establishment of a sample and data management framework will support confirmatory testing for these significant pathogens, as required.

I am a microbiologist. Our laboratory at Agriculture Victoria develops tools to support biosecurity. Biosecurity is underpinned by the rapid detection of a pathogen.

Loop-mediated isothermal amplification (LAMP) detects the genome of a pathogen. It is adaptable, it uses very specific primers, and it can detect the [genome] of an RNA virus or of a DNA virus or of a pest. It is dynamic, in that we can use it to test different sample types, whether that be clinical material from an animal, or an environmental sample – such as from swabbing the floor of an abattoir, or even from a rope that pigs have played around with (Figure 1). LAMP is fast in detecting the pathogen. In some cases we have made detections within approximately 20 minutes of taking the sample. Agriculture Victoria is working across the state of Victoria to implement and develop LAMP assays to support biosecurity outcomes.

One of the nice things about LAMP is that you can often do the test without actually extracting the nucleic acid from the sample. This makes it quite

This record has been prepared from a transcript and the slides of the presentation.

Figure 1. LAMP assays are rapid, adaptable and dynamic, usable with varied pathogen genomes and different sample types in a range of environments.

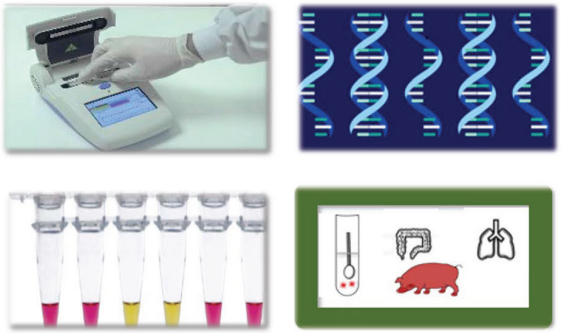

Figure 2. Scientific rigour and assay validation principles provide the foundation of the LAMP assays. Nucleic acid extraction is not required. The assay is optimised for each virus and sample combination. It can be applied where resources are limited, and in the field and as a point-of-care tool.

different from a lot of other types of molecular assays, and it means you do not need additional pieces of scientific equipment to extract nucleic acid. Although the test needs to be optimised for each virus and sample type, this robustness in the enzyme means that the assay can be deployed in settings where resources are limited. To perform and interpret the test, you can take a small machine – which you could hold in your hand – into the field. The assay readout can be adapted to enable detection of positive samples by colorimetric method – and in fact you do not even need the machine when using this detection method; you just need boiling water down to about coffee temperature to stimulate the reaction (Figure 2).

We have been using assay-validation principles to provide a foundation of these LAMP assays for biosecurity outcomes. In this talk I mention two of the pathogens we have targeted and some of the work we have been doing.

First, foot-and-mouth disease (FMD) – a severe, highly contagious viral disease of livestock that disrupts regional and international trade in animals and animal products, causing significant economic impact. Australia estimates that a small FMD outbreak, even if controlled in 3 months, could cost around AUD 7.1 billion; a large 12-month outbreak would cost AUD 16 billion.

Figure 3. Application of an internal positive control in Bhutan for foot-and-mouth disease virus (FMDV) LAMP:

- independent verification of sample quality;
- confirmation of clinical FMDV cases

Statistical analysis confirmed this new RT-LAMP-FMDV test as fit-for-purpose as a herd diagnostic tool, with diagnostic specificity >99% and sensitivity 79% on unextracted field samples (oral swabs).
Source: Bath *et al.* 2020.

We took an established assay for foot-and-mouth disease for LAMP, and we modified it so that we could remove the nucleic-acid-extraction test step. We also developed an internal positive control that could be added to each of the samples, to assess for any inhibitors of the assay in the sample. That removed the chance of having false negatives – which is especially vital for a very important disease.

A team then travelled to Bhutan and independently verified this method (Figure 3). They could confirm clinical cases of foot-and-mouth disease in the field, using oral swabs. Statistical analysis included in Bath *et al*. (2020) demonstrated this was fit-for-purpose as a herd diagnostic test on unextracted oral swabs for FMD.

Within this state, Victoria, we are working with the Office of the Chief Veterinary Officer to train field veterinarians on how to use this LAMP assay. Policy is being worked through to enable this point-of-care testing for FMD by Agriculture Victoria, and meanwhile we have developed a system for training our vets, and giving them very easy, practical, low equipment test kits. In addition to having positive and negative quality controls and an internal quality control, we are also developing proficiency testing panels that can be sent to operators to develop confidence in the use of the assay in the field. Full implementation is currently in development.

Recently we have been working on a second assay, this time for African swine fever virus (ASF), which causes 80–100% mortality in pigs. The virus was detected in Timor-Leste in September 2019. As Agriculture Victoria Research already had a LAMP assay that was being developed in the laboratory, we sent a team to Timor-Leste to work with our colleagues there to assess the diagnostic performance of this assay (Mee *et al*. 2020; Phillips *et al*. 2021). The assay supported a whole-country-prevalence survey of ASF (436 samples, 48 villages), and the work was all completed in Timor by our Timorese colleagues that we had trained. It is important to note that we also are biobanking those positive samples, to send back to the National Reference Laboratory in Australia, and we are providing ongoing test support. That happened just before the COVID

pandemic, and although our team has not been able to go back to Timor-Leste the systems that were put in place, including the provision of reagents and communication, have enabled us to provide ongoing support.

This robust quality platform that has been established can be applied to other targets. Some recent work on a similar LAMP assay for khapra beetle, which is in Papua New Guinea, was recently published by Agriculture Victoria Research in December 2021 (Rako *et al*. 2021) and an assay is also being developed for fall armyworm.

References

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Dr Stacey Lynch is a Senior Research Scientist, Agriculture Victoria Research, based at AgriBio, the Centre for AgriBioscience. Her primary focus is to deliver applied scientific outcomes through viral diagnostics and pathogen surveillance. Specific diagnostic and surveillance activities

include the development and implementation of in-field molecular methods for African swine fever virus and foot-and-mouth disease virus, and surveillance activities of avian influenza in wild birds and pathogens in mosquitoes (such as Ross River virus, Murray Valley encephalitis virus and *Mycobacterium ulcerans*). Key areas of research use genomic tools to enhance traditional significant animal investigations and surveillance programs.

Stacey regularly provides lectures in a range of undergraduate and post-graduate courses through the Faculty of Veterinary and Agricultural Sciences, and has co-supervised a number of research students from La Trobe University (School of Applied Systems Biology), The University of Melbourne, The University of Liverpool, and the International Livestock and Research Institute, Ethiopia.