

# **What is required to ensure a sustainable diagnostic capability for Emerging Plant Pests that threaten Australia?**

## **A Report prepared for Plant Health Australia (PHA)**

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## Executive Summary:

There is an emerging problem with diagnostic protocols for Emerging Plant Pests (EPPs) that have been commissioned in Australia, as over time they risk becoming outdated. We have conducted a small survey of diagnosticians who are actively involved in developing EPP diagnostic manuals and who also use these protocols to diagnose the presence or absence of EPPs in Australia. Where possible, the labor inputs required to conduct these activities were monitored and/or estimated in an attempt to determine a true cost in maintaining a diagnostic protocol. The aim of the survey was to identify activities that are required to

- a) ensure that the protocol is up to date and
- b) ready for operation

An average of 30 hours per year (\$2,100.00 at \$70.00/hr) was recorded for the three EPP protocols monitored over a 6 month period and 20-30 hours per year (\$1,400.00 - \$2,100.00) was the average required time inputs estimated from the survey to maintain an EPP diagnostic protocol. These figures represent the costs directly associated with the diagnostic protocol and do not include the costs associated with maintaining an accredited and functional laboratory.

Key findings from the survey include:

- Fifteen of the 19 protocols surveyed used PCR as part of the diagnostic protocol and the PCR protocols that were used on a regular basis, or, in a lab that routinely uses PCR, function more efficiently
- The availability of positive controls was a limiting factor for protocols that rely on ELISA or the use of semi-selective media for culturing.
- Taxonomy-based protocols require an on-going investment in staff training to ensure that staff are adequately trained to complete a diagnosis.
- The majority of diagnosticians could see the benefits of lab accreditation but were concerned about the costs required to implement the systems, and more importantly, the hidden costs associated with maintaining an accreditation level.
- All the diagnosticians were in favor of ring tests as this helps maintain a diagnostic capability for an EPP and it ensures that different labs can use and follow the protocol correctly.
- Workshops are an effective way to validate and ratify a diagnostic protocol at the national level.
- Active surveillance and the submission of samples to a diagnostic lab for testing were seen by most diagnosticians as an effective way to train staff and ensure that the protocol was ready for use.
- There is a concern that it is difficult to make improvements to the protocol in the absence of related research programs.
- Job security was a significant issue for most labs as it is critical to retain experienced staff that can use and follow the diagnostic protocol at the required level of competency. It was evident that level of support for trained staff varied greatly between organizations.

The data presented in this report reflects the views of the diagnosticians surveyed and provides critical baseline data from diagnostic laboratories from around Australia on the annual resources required to maintain a diagnostic manual for an EPP. These costs need to be recognized nationally in order to maintain a quality and sustainable diagnostic capacity for the detection of EPPs.

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# 1. Introduction

Diagnostic science is vital for the identification of exotic plant pests and diseases. Accurate, sensitive and timely tests are required to detect and manage exotic incursions, and to demonstrate area freedom where there is a prerequisite to export trade. The Australian Quarantine and Inspection Service (AQIS) also needs accurate pest and disease records to protect Australian agricultural industries from exotic pests and diseases and to enable Australian produce to trade internationally. Diagnostic services play a crucial role in maintaining and keeping these records up to date. The importance of plant disease diagnostics cannot be underestimated.

A report commissioned by Plant Health Australia in 2002 (Moran and Muirhead, 2002) found that funding was a key restraint for all the diagnostic providers surveyed and that there was little quality control to ensure the delivery of standardized techniques and methods such as those used in veterinary laboratories in Australia. They also reported that documented procedures for identifying emerging plant pests (EPPs) are generally not available and many laboratories had limited capabilities to identify exotic pests and plant pathogens. This situation has changed in recent years with the commissioning of a number of projects by both Plant Health Australia and the Office of the Chief Plant Protection Officer to generate diagnostic protocols for key exotic plant pests.

In the European Union the European and Mediterranean Plant Protection Organization (EPPO) has diagnostic protocols for regulated pests that are intended to be used by National Plant Protection Organizations to detect and identify the regulated pests of the EPPO and/or European Union lists. This process began in 1998 and the objective of this program is to develop an internationally agreed diagnostic protocol for each regulated pest.

A similar process began in the mid 1990's in Australia with the commissioning of the fire blight contingency plan (Merriman, 2002). This document contained a chapter dedicated to the diagnosis of *Erwinia amylovora* and involved the importation of appropriate positive controls for both PCR and culturing on semi-selective media. This document formed the basis of the diagnostic response during the 1997 incursion of *E. amylovora* in the Melbourne Royal Botanic Gardens (Jock *et al.*, 2000) and was invaluable in providing a quick and timely response in May 1997.

As a general rule, the diagnostic protocols recommend a particular means of detection or identification which is considered to have advantages (of reliability, ease of use etc.) over other methods. The protocols provide all the information necessary for a named pest to be detected and positively identified by a general expert (i.e. an entomologist, mycologist, virologist, bacteriologist etc.) who is not necessarily a specialist on the organism or its taxonomic group. Each protocol begins with some short general information on the pest (its appearance, relationship with other organisms, host range, effects on host, geographical distribution and its identity) and then, gives details on the detection, identification, comparison with similar species, and requirements for a positive diagnosis.

A flaw in the interpretation of test results for the *E. amylovora* diagnostic protocol was exposed during the national surveys for fire blight in the late 1990's (Rodoni *et al.*, 1999) and as such a validation process for EPP protocols under local conditions is considered mandatory in Australia.

There is an emerging problem with diagnostic protocols for EPPs that have been commissioned in Australia, as over time they risk becoming outdated. There are components of a diagnostic manual that require revision on an on-going basis. We have produced a small survey for diagnosticians that are actively involved in developing EPP diagnostic manuals and who also use these protocols to test “suspect” samples in Australia.

The aim of the survey is to identify activities that are required to

- a) ensure that the protocol is up to date and
- b) ready for operation.

We have requested that the survey participants make an estimate of the time (hours/year) it takes to complete these functions so that we can identify the financial costs required (on an annual basis) to maintain an EPP diagnostic manual.

The objective of the report is to firstly demonstrate what resources are required, on an on-going basis, to maintain existing diagnostic protocols that are established and validated. Maintenance of diagnostic manuals for fire blight, plum pox virus and maize dwarf mosaic virus will be trialed over a 6 month period and the inputs in labor (both expert and technical) and consumables required to ensure that the protocol remains valid will be documented in detail. Diagnosticians from across Australia that have prepared manuals for insect pests, fungi, bacteria and viroids were consulted to ensure that the needs/requirements for these manuals are also considered.

## **2. Maintenance of a diagnostic protocol – 6 month trial period**

Maintenance of manuals for plum pox virus, fire blight and maize dwarf mosaic virus were trialed over a 6 month period and the inputs in labor (both expert and technical) and consumables required to ensure that the protocol remains valid were documented.

The time spent upgrading and reviewing the 3 diagnostic manuals was accurately recorded and costed, together with the activities required to maintain a “ready-to-go” diagnostic capability.

The details of the activities during the 6 month period are listed below.

### **2.1 Plum Pox Virus: *Brendan Rodoni (Sept 06 – April 07)***

Sharka, caused by plum pox potyvirus (PPV), was first reported in 1915 in Bulgarian plums and is currently one of the most serious diseases of *Prunus* (peach, apricot, nectarine, plum, sweet and sour cherry, almond, and wild and ornamental types). Australia is currently free of Sharka disease. This disease is recognized as the most serious disease threat to the stone fruit industry in Australia, which has an annual farm gate value of 200 million Australian dollars.

In 1992, Plum pox symptoms were first detected in Chile in an experimental orchard and this virus is now considered to be widespread in Chile. This disease had been kept out of North America by very strict quarantine regulations until October 1999 when it was detected in Adams county Pennsylvania (USA) and in June 2000 was confirmed in Canada.

In the period from September 2006 until April 2007, the following issues relating to PPV and the diagnostic protocol required some attention:

- Determine whether the PPV protocol would detect a new strain of PPV reported in Canada
- Validate the primers used in the PPV protocol as part of the requirement to screen 2 samples submitted for testing.
- Held discussions with Industry and stakeholders regarding the issue of fruit as a pathway for transmission
- Participation in the development of a global protocol for the detection of PPV
- Validate a new batch of antisera that arrived as part of a new PPV ELISA kit

#### **2.1.1 Determine whether the PPV protocol would detect a new strain of PPV reported in Canada**

A new and distinct strain of PPV (PPV-W) was reported from Canada in 2003-04. PPV-W was detected in plums showing no symptoms on fruits and only mild symptoms on leaves. The nucleotide sequence has been published and analysis of this data indicates that the primer pairs that are recommended in the PPV diagnostic protocol will amplify this strain. The suggested restriction enzyme digest for identification of the PPV strain will not work and it will be a requirement to sequence the PCR product as a first priority. Evidence from Europe indicates that the PPV-specific antisera in the REAL ELISA kit will detect this strain of PPV. **(3 hours)**

### **2.1.2 Validate the primers used in the PPV protocol as part of the requirement to screen 2 samples submitted for testing.**

Two samples were submitted to our diagnostic lab by Biosecurity Victoria for screening for PPV. The primer set were validated against healthy prunus sap and a positive control consisting of the PPV PCR product cloned in to a pUC-based vector and used at a dilution of 1/10,000. (4 hours)

### **2.1.3 Held discussions with Industry and stakeholders regarding the issue of fruit as a pathway for transmission**

Although not directly related to the diagnostic protocol for the detection of PPV, the diagnostician was asked by DAFF to participate in a “Plum Pox discussion” as part of a risk assessment for the importation of fruit into Australia. Emergence of new strains, detection of PPV and fruit as a pathway were the main topics discussed. (15 hours)

### **2.1.4 Participation in the development of a global protocol for the detection of PPV**

A global protocol for the detection of plum pox virus (PPV) is being developed and there is an opportunity for our involvement in a “ring test” program to ensure labs around the world can detect this virus. We received a PPV ELISA detection kit in October and is now ready for use in our lab. We will continue with our involvement in this project and maintain email contact with the PPV protocol organizers. (Total hours 10 hrs)

### **2.1.5. Validate a new batch of antisera that arrived as part of a new ELISA kit**

As part of our involvement in the development of global protocol for the detection of PPV we received a new ELISA kit from Spain (REAL antisera). We currently do not have any PPV positive material that we can use as a positive control and therefore it is difficult to validate the kit in a meaningful way.

The protocol in the kit has not changed in any significant way since the PPV protocol was developed in 2004 and so there is no validation steps required to modify the protocol. (4 hours)

## **2.2 Fire blight: Brendan Rodoni (July 06 – Dec 06)**

Fire blight is a bacterial disease of pome fruit that affects many plants in the family Rosaceae and is caused by the bacterium *Erwinia amylovora* (Ea). Previously undetected in Australia, the disease is a major threat to the Australian apple and pear industries. It is anticipated that the Goulburn Valley cannery intakes would drop 65% in a serious epidemic of fire blight.

### **2.2.1 July 06: A report of a virulent strain of *E. amylovora* that does not have the pEA29 plasmid – diagnostic implications**

We found a paper in the literature (published April 2006) that reported the presence of a virulent strain of *E. amylovora* that does not have the pEA29 plasmid. This does have implications for our diagnostic protocol as our recommended PCR protocol targets a region of the pEA29 plasmid. We immediately validated other chromosomal based PCR protocols for the detection of *E. amylovora* and we plan to use these primer sets, together with the globally preferred pEA29 based PCR primers, when testing samples. **(15 hours)**

### **2.2.2 Preparation of semi-selective Media**

We made up two batches of media (August and November) in preparation for samples submitted to our diagnostic service. **(8 hours)**

### **2.2.3 Organize immature Pear Slices for initial pathogenicity assays (October, 2006)**

Annually we arrange for 200-400 immature pear fruitlets to be stored in a controlled atmosphere chamber and use as a pathogenicity assay if we want to screen colonies of interest. **(3 hours)**

## **2.3 Maize dwarf mosaic virus (MDMV): Andrew Geering (July 06 – Dec 06)**

Maize dwarf mosaic virus (MDMV) is a member of potyvirus genus and probably occurs in every country in the world where corn is grown with the main exception of Australia. MDMV has the potential to spread to all regions in Australia where maize and sorghum is cultivated. The entry potential of MDMV into Australia is most likely via the importation of infected seed and it is anticipated that establishment and spread potential of this virus in Australia is high. MDMV is regarded as being one of the most important pathogens of sorghum and corn in the USA and the economic impact of MDMV on the maize and sorghum industries in Australia would be significant.

During the 6 month period from July 06 to December 06 we did not spend any time on the MDMV protocol. In fact we have not spent any time on this protocol since the completion of the diagnostic manual in 2004. It is likely that the ELISA kit is passed it's used-by date. **(0 hrs)**

## **2.4 Summary of costs associated with maintaining a diagnostic protocol**

A total of 21 hours over the 6 month period was devoted directly to maintaining the PPV protocol and a further 15 hours was required to attend a 2 day workshop on PPV related issues. For fire blight a total of 26 hours over the 6 month period was required to maintain the fire blight diagnostic protocol. For MDMV however, zero hours were dedicated to this manual over the 6 month period. Based on the data from these three protocols the average hours required to maintain a protocol for a six month period is approximately 15 hours, or 30 hours per year. Conservatively it costs approximately \$70.00/hr to employ the experienced scientists that are responsible for maintaining a protocol. The actual costs associated with maintaining a diagnostic protocol for an EPP is estimated at \$2,100.00 per annum.



### 3. Survey results: EPP-specific questions

Diagnosticians from across Australia that have prepared manuals for exotic pest insects, fungi, bacteria, viruses and viroids were asked a series of questions via the phone or in person. The survey questionnaire used for the interviews is provided in Appendix 1.

The questionnaire was designed to determine what diagnostic tests are used in the diagnostic protocol with an emphasis on what aspects of each diagnostic test required ongoing maintenance, how much time is required on keeping in touch with the literature and other experts overseas and what ongoing training needs are required.

Some additional issues that relate directly to the upkeep and proficiency of a diagnostic protocol and which were included in the survey were:

- proficiency testing of laboratories to conduct diagnostic protocols for EPPs
- the role of active surveillance in maintaining a diagnostic capability
- technical training of staff
- maintenance and, where appropriate, upgrade of lab standards and accreditation levels
- import permits for test controls

A series of generic questions relating to the maintenance of a diagnostic laboratory that can conduct a diagnostic protocol for the detection of an EPP when required, was also included as part of the survey and the results from these questions are presented in Section 4 of this report.

The diagnosticians that were interviewed and the EPPs that the protocol is targeting are listed in Table 1. A copy of the survey responses for each EPP is provided in Appendix 2. Following is a summary of the answers for each question of the survey.

#### ***3.1 PCR as the diagnostic test for EPP detection***

Fifteen of the nineteen EPP protocols used PCR to detect the EPP. In most instances the primers and positive controls were checked only when the protocol was used to screen for the presence of the EPP on a specimen submitted for testing. At least two of the protocols therefore have not checked the validity of the primers or positive controls since the protocol was developed and validated as part of a research contract (MDMV, Lentil anthracnose). Several diagnosticians would like to check their controls and primers at least on an annual basis (**approx 6-8 hours/yr**). The general consensus was that primers and controls should be validated a least twice a year.

The issue of validating the primers and positive controls was not major for the protocols that are used on a regular basis either to process samples submitted for testing (i.e. PSTVd) or for protocols that are used for post entry quarantine (i.e. PEBV).

**Table 1: The EPPs, corresponding diagnostician, the date the protocol was completed, the tests used and number of tests conducted per year.**

EPP	Diagnostician	Date protocol completed	Diagnostic tests used	No. of samples per year
Asian Citrus Physllid ( <i>Diaphorina citri</i> )	Mali Malipatil (Vic DPI)	2007	PCR Morph/Taxonomy	2
Queensland Fruit fly ( <i>Bactrocera sp.</i> - <i>Bactrocera tryoni</i> )	Peter Gillespi (NSW DPI)	2005	PCR Morph/Taxonomy (NATA protocol)	3-4
Potato leaf miner ( <i>Liriomyza cicerina</i> , <i>Liriomyza trifolii</i> )	Mali Malipatil (Vic DPI)	2006	PCR Morph/Taxonomy	2
<i>Fusarium oxysporum</i> form sp. <i>ciceris</i> (Chickpea wilt)	James Cunnington (Vic DPI)	2007	PCR	3
<i>Colletotrichum truncatum</i> – Lentil Anthracnose	Kurt Lindbeck (Vic DPI)	2005	PCR Morph/Taxonomy	0
<i>Phytophthora ramorum</i>	James Cunnington (Vic DPI)	2006	PCR	1-2
<i>Tilletia indica</i> (Karnal bunt)	Gordon Murray (NSW DPI)	2003	PCR Morph/Taxonomy	1-5
Pierces Disease ( <i>Xyella fastidiosa</i> )	Jo Luck (Vic DPI)	2004	PCR Culturing	4-6
<i>Erwinia amylovora</i> (fire blight)	Brendan Rodoni (Vic DPI)	2002	PCR Culturing	6
Citrus Canker	Deb Hailstones (NSW DPI)	2006	PCR Culturing Leaf inoculations	1-2
Citrus greening	Deb Hailstones (NSW DPI)	2006	PCR	2
Maize Dwarf mosaic virus (MDMV)	Andrew Geering (Qld DPI)	2004	RT-PCR ELISA	1-2
Pea early browning virus (PEBV)	Angela Freeman (Vic DPI)	2007	RT-PCR	40-50
Broad bean stain virus (BBSV) Broad bean true mosaic virus (BBTMV) Broad bean mottle virus (BBMV)	Angela Freeman (Vic DPI)	2007	ELISA TBIA	40-50
Pea Enation mosaic Virus (PEMV)	Angela Freeman (Vic DPI)	2007	ELISA TBIA	40-50
Plum pox virus (PPV)	Brendan Rodoni (Vic DPI)	2004	RT-PCR ELISA	2
Red clover vein mosaic virus (RCVMV)	Angela Freeman (Vic DPI)	2007	PCR (Commercial kit)	40-50
Potato spindle tuber viroid (PSTVd)	Deb Hailstones (NSW DPI)	2004	RT-PCR	20-30

The PCR protocol for the Queensland fruit fly is NATA accredited and states that DNA must be extracted from “positive” Queensland Fruit Fly each time the PCR protocol is conducted. This level of control ensures the validity of the nucleic acid extraction and the primers. The PSTVd protocol also has a reliable supply of PSTVd infected tissue due to the biological assay that screens for pathogenicity; infected tissue from this assay is harvested and stored for use as a positive control. The PEBV protocol has a continuous supply of infected tissue as the

diagnostician has an AQIS permit to import infected tissue and propagate fresh PEBV-infected plants in their PEQ glasshouse. Most other protocols relied on past extracts as positive controls and used internal host PCR protocols or “house keepers” to validate the extraction process and primers. Although scientifically sound, most diagnosticians would prefer to have a constant source of infected tissue.

Of interest is the PCR protocol for RCVMV as this protocol has been purchased from a commercial company in much the same way as an ELISA kit. A limitation of this test is that the diagnostician does not know what region of the RCVMV genome the primers amplify and relies completely on the validation of the test by the commercial company.

In most instances the diagnosticians were not concerned or frustrated by the emergence of new reagents that could possibly improve the PCR protocol. In some instances quantitative PCR protocols had been published for detection of the EPP (i.e. *P. ramorum*, Lentil anthracnose) but most diagnosticians still believed that the conventional PCR protocol that they were using was sufficient. To introduce the qPCR in to the protocol would require a survey of plant samples selected randomly in Australia as part of a survey to screen for false negative reactions and to validate the qPCR protocol against a selection of positive isolates/extracts, if present. Many of the PCR protocols were highly regarded globally and at the leading edge of diagnostic development for that EPP (Queensland Fruit Fly, PEBV, Lentil anthracnose).

Since the first time that the fire blight protocol was used in 1997, new and superior Taq polymerase enzymes have been produced and over the years this has resulted in a decrease in the concentration of primers from those recommended in the original paper for the recommended fire blight primer set. This validation step was introduced into the current protocol over time and was conducted with other PCR protocols used in the lab for endemic pathogens. This highlighted the advantage of the diagnostic lab doing PCR diagnostics on a regular basis.

Significant improvements to a diagnostic protocol are usually as a result of a related research project (i.e. Citrus Canker, Citrus Greening) and it is the time and consumables provided by the research project that allows for the modifications to be made.

Additional issues that related to the PCR protocols involved costs associated with PCR consumables. Surprisingly some labs were not supported for or had not budgeted sufficiently to allow for purchase/re-purchase of lab consumables.

### **3.2 ELISA as the diagnostic test for EPP detection**

Only 6 of the protocols used ELISA as a test for EPP detection and all of these targeted plant viruses (MDMV, PPV, BBSV, BBMV, BBTMV and PEMV).

Both the MDMV and PPV ELISA protocols are essentially for incursion response and do not generally get used for one-off specimens submitted for testing. As a result the antisera can sit idle for one or more years and be out-of-date when it is time for use. For MDMV the ELISA kits have not been replaced since the completion of the protocol in 2004. For PPV a new kit was supplied to the lab as part of the EPPO-global diagnostic protocol in 2006. However the original PPV ELISA kit had not been replaced since 2004. Of concern for the PPV protocol is the absence of a positive control for the ELISA kit. There is no import permit to allow the importation of PPV infected plant tissue into Australia. The original diagnostic protocol used

expressed PPV coat protein as a positive control – this supply has since run out. The replacement cost for these ELISA kits is approximately \$500.00 each and should be mandatory every second year.

In contrast the ELISA protocols for the legume viruses operate smoothly as they are required for the routine and on-going testing of pulse lines introduced into Australia through a Post Entry Quarantine (PEQ) facility. In addition the diagnostician has appropriate import permits that allow the import of virus infected material into the country on a needs basis. This material can be inoculated onto host plants in the PEQ glasshouse and ensures a fresh and ongoing supply of virus infected material that can be used for positive controls. These two factors are important for maintenance of an ELISA protocol for the detection of an EPP.

The ELISA protocols should be checked and validated 6 monthly (**12 hours/annum**). This is the case for the legume viruses as this lab conduct routine testing, but for MDMV and PPV this does not occur.

The tissue blot immunoassay (TBIA) is used in combination with ELISA to detect the legume viruses. The ELISA test is used to screen pooled samples and the TBIA is used to screen individual plants.

### **3.3 Fungal and Bacterial cultures as the diagnostic test for EPP detection**

Seven of the diagnostic protocols surveyed used some form of bacterial or fungal isolation on to artificial media and for *F. oxysporum*, *C. truncatum*, *T. indica* and *X. citri* non-selective media is used. For *P. ramorum* the media is selective for *Phytophthora* species and endemic *Phytophthora* species are used as a positive control. All these media are easily prepared and commonly used within the diagnostic labs and preparation of these media on a regular basis is therefore not required for these EPP protocols.

However, both *X. fastidiosa* and *E. amylovora* have a requirement for semi-selective media for culture. Both the *X. fastidiosa* and *E. amylovora* media is usually made up as samples arrive for testing. This is not ideal and these media should be made up every 4-6 months (**4 days/yr**). There is a requirement for a viable positive control to ensure the semi-selective media are working correctly. There is no virulent strain of *X. fastidiosa* in Australia and to accommodate this issue the semi-selective media prepared in Australia was taken to the USA and used to isolate virulent *X. fastidiosa*. Unfortunately this validation step only validates the procedure. A nonpathogenic attenuated strain of *E. amylovora* is available for use to validate each batch of semi-selective media.

### **3.4 Taxonomy/Morphology as the diagnostic test for EPP detection**

Five of the EPP protocols (Asian citrus Psyllid, Queensland Fruit Fly, Potato leaf miner, Lentil Anthracnose and Karnal Bunt) used taxonomic or morphological properties for identification. Taxonomic properties are a key component of the protocols for insects and both diagnosticians emphasized the importance of the maintenance of reference specimens (**6 hrs/yr**), keeping up to date with the latest taxonomic developments in the literature (**20 hrs/yr**) and training staff. As part of the NATA accreditation for Queensland Fruit Fly protocol, two internal and one external lab tests are conducted each year and involve the correct identification of a range of fruit fly species (**20 hours/year**).

The taxonomic skills for accurately identifying *Colletotrichum truncatum* is standard. The accurate identification of *Tilletia* spores for the karnal bunt protocol requires significant technical skills for the germination of the spores as many spores are cracked etc via the handling process. It is critical to have experienced staff to maximize the chances of germinating viable spores if present.

### **3.5 Has the protocol been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?**

For most of the EPPs surveyed the protocols were current and did not require further work. Indeed some of the protocols were world class and have been adopted by other countries (Queensland Fruit Fly, Lentil Anthracnose). For the legume viruses the ELISA test used is the accepted standard test globally. However, the diagnostician responsible for these protocols can see the advantage of developing molecular tests for the detection of the viruses.

Australian scientists are conducting research to improve the Karnal Bunt protocol by developing techniques to extract DNA directly from *Tilletia* spores. New strains of PPV have been reported in recent years but there are reports in the literature that the current PCR and ELISA tests will detect these strains.

For fire blight on the other hand, virulent strains of *E. amylovora* have been reported that do not contain the pEA29 plasmid. The most widely used PCR protocol for the detection of this pathogen targets this plasmid. Additional chromosomal based PCR protocols need to be added to the fire blight diagnostic manual. A QPCR protocol has recently been published for the detection of *P. ramorum* and ideally this protocol should be evaluated and validated under Australian conditions. The Pierce's disease protocol is still relevant but has not been reviewed and updated since it was published 4 years ago.

Most diagnosticians expressed frustration at not having the time and resources to make these small amendments to the EPP protocols. Currently, there is no mechanism to do this. Generally speaking the incorporation of improvements relies on the outcomes of related research projects to identify and validate the techniques. If changes were required, at least 1-2 weeks of work would need to be invested. Improvements could be made as part of the on-going PEQ-AQIS project. It was generally thought that there were minimal opportunities to update and validate new procedures into existing protocols and this was seen as a weakness that will increase with time.

### **3.6 Are you or your organization doing active surveillance for the EPP?**

Active surveillance was a reality for only two of the EPPs (Queensland Fruit Fly - Tri State trapping and AQIS at ports of entry; Karnal Bunt in WA). National surveys for citrus canker have just been completed and the fire blight national surveys were completed in 1999/2000.

The Diagnosticians interviewed could see several advantages in surveying for EPPs. A summary of their comments include:

- Surveys for EPPs such as *P. ramorum* that has such a wide host and which impacts on several industries, would be extremely useful
- Survey samples submitted for testing help to maintain a diagnostic capability. It is a good dry run for an incursion and it helps to keep staff trained, and importantly, to train new members of staff.

- Active surveillance is a great way to ensure your diagnostic protocol works and that you have staff that can do the test competently.
- Active surveillance programs provide evidence for the absence of the pathogen. It is worthwhile to survey for EPPs to confirm that these pests are “known not to occur”. As it stands the status of many of our EPPs is “not known to occur”.
- Active surveillance is a good idea but, I think there are higher priorities. It is more a matter of “who pays” for the surveillance. It is an expensive exercise to both government and industry

### **3.7 How often do you keep in touch with the literature?/Do you stay in touch with experts internationally?**

The time required by each diagnostician to keep up to date with the literature and to keep in touch with colleagues internationally varied greatly depending on the EPP (See Table 2 for details). One diagnostician found it difficult to find time to keep in touch with the literature and colleagues and spends zero hours per year due to other work commitments. Most diagnosticians kept in touch with the literature on a regular basis ranging from weekly to monthly with an average time allocation of approximately **4-26 hours per year**.

Five diagnosticians did not keep in touch with experts/peers for a range of reasons. One diagnostician is the “second lab” in Australia that conducts the test and is happy to leave the literature reviews etc to the laboratory responsible for generating the diagnostic protocol. Of the remaining diagnosticians there was no common reason why this was not required and it may simply be that a review of the literature is sufficient to stay in touch with developments for that EPP. At the other end of the scale significant time (100 hrs) is spent by the diagnostician responsible for the Queensland Fruit Fly protocol liaising with colleagues mainly to collect specimens of fruit flies as an ongoing and critical validation process for this protocol. The diagnostician responsible for the fire blight protocol finds an effective way of keeping up to speed with trends and maintaining networks with fire blight experts is to attend the ISHS International workshop on fire blight which is held every three years. Most diagnosticians kept in touch with experts on a regular and/or needs basis with an average time allocation of **1-26 hrs per year**.

### **3.8 Is there a second laboratory in Australia that has the capacity to verify the diagnosis of the EPP?**

There is no second lab in Australia that has been trained to use the diagnostic protocol for 11 of the 19 EPPs reviewed in this survey. Six of these protocols are associated with the exotic viruses affecting pulses and these protocols have just been completed this year. Additionally several of these protocols are ELISA-based and not technically demanding if a lab has the right facilities and quarantine permits to maintain the positive controls required to conduct these tests. For the potato leaf minor several labs can identify the basic morphological features but are not able to conduct the technically demanding tissue dissections required to complete the identification. For *P. ramorum*, there is more than one protocol in place in Australia for the detection of this pathogen. For *X. fastidiosa*, a commercial diagnostic lab does offer a test to industry and it is not known if this test is similar to that recommended in the Pierce’s disease protocol. The lack of a second laboratory was considered a weakness in the preparedness for an incursion of these EPPs.

There are two labs that are trained to use the protocols for Karnal Bunt, fire blight, PSTVd, Citrus Canker and Citrus Greening and in most cases has been the result of funded workshops.

One diagnostician identified the problem of training 2 new staff in the last five years, which can be a costly process. There are a number of people from around Australia who are trained in the identification of fruit flies as a result of the regular 2-day training workshops offered by the leading lab. However no other labs in Australia are formally involved in inter-lab ring tests as they are not yet at the NATA accreditation level. A second lab has a capability to use the diagnostic protocol for lentil anthracnose. However this lab is based within a university and is not interested in testing industry material that is associated with routine diagnostics.

Every diagnostician recognized the importance of training and in most instances a **2 day workshop** is required to train skilled pathologists and entomologists on how to use a diagnostic manual for an EPP. Several diagnosticians identified additional points of interest from these training exercises and include:

- A requirement for re-training after 5 years just to re-fresh the skills and identify any additions to the protocol
- The importance of follow-up consultation as part of the training procedures
- Financial support for attendance at the workshop and for the purchase of consumables required to run a protocol
- Positive feedback from workshop participants, who are mostly experienced pathologists and entomologists usually results in improvements to the diagnostic manual
- For insect identification, on the job training is the best form of training for the taxonomic properties of insects

### **3.9 Time inputs associated with maintaining a diagnostic protocol for an EPP**

An important goal of this project was to identify the time and costs associated with maintaining a diagnostic protocol. These costs are in most instances not currently funded and we are reliant on the diagnostician to take on these responsibilities in addition to their normal workloads. This has already resulted in no time commitments for one of the EPP protocols surveyed in this study (Table 2).

The ongoing costs are associated with remaining up to date in the literature, maintaining networks with experts who are familiar with the EPP, updating the protocols, training staff and replacing outdated consumables that are specifically associated with the tests (i.e. ELISA kits). A summary of the time inputs and consumables required for each EPP is provided in table 2.

The diagnosticians frequently commented that they would like “more time” to do certain tasks better. This was particularly evident with some of the EPP protocols that are not frequently used (MDMV, Lentil Anthracnose) as no work had been dedicated to maintaining these protocols since they were initially commissioned for development and usually involved checking the validity of positive controls, primers and antisera etc. These time estimates are included in the “ideal” column of Table 2. Several diagnosticians simply requested more time to review the literature in a more professional manner than is currently the case.

The ongoing maintenance of the insect EPP protocols was considerably higher than the other EPPs and was mainly due to the requirement to maintain specimen collections and replenish dwindling supplies of important insect species that are required to support the EPP protocol. Two of the three protocols for exotic bacteria (*X. fastidiosa* and *E. amylovora*) require the use of semi-selective media that needs to be prepared on a regular basis. Time is required to prepare this media to ensure that the lab is ready-to-go when samples are received for testing.

When ELISA or PCR protocols were used in labs that are not routinely doing ELISA tests on that virus, or, the PCR facility was not routinely doing PCR, there were ongoing consumables associated with replacing out-dated enzymes and ELISA kits.

**Table 2: Time inputs identified by EPP Diagnosticians that are required on an ongoing basis to maintain a diagnostic protocol for the detection of an EPP.**

EPP	Time inputs (hrs/year)				
	Literature	Contact experts/peers	“other” time inputs	Ideal	Total
Asian Citrus Psyllid	6	6	6(specimens for taxonomy) \$500.00 (PCR consumables)		<b>18</b> <b>[\$500.00]<sup>1</sup></b>
Queensland Fruit fly	12	100 (specimens) 10 (workshop)	20 (staff training)		<b>142</b>
Potato leaf miner	6	6	30 hrs (staff training)	6 (PCR)	<b>42 (6)<sup>2</sup></b>
Chickpea wilt	12	No		8 (PCR)	<b>12 (8)</b>
Lentil Anthracnose	10	5			<b>15</b>
<i>Phytophthora ramorum</i>	4	4		8 (PCR)	<b>8 (8)</b>
Karnal bunt	26	26			<b>(52)</b>
Pierces Disease	0 (52 – ideal)	No		30 (media prep)	<b>0 (82)</b>
Fire blight	6	24			<b>30</b>
Citrus Canker	3	1			<b>4</b>
Citrus greening	1	1			<b>2</b>
MDMV	10	No	\$500,00 (ELISA)	12 (PCR)	<b>10 (12)</b> <b>[\$500.00]</b>
PEBV	6	No			<b>6</b>
BBSV BBTMV BBMV	20	10	20 hr/yr (ELISA)		<b>50</b>
PEMV	7	3	20 hr/yr (ELISA)		<b>30</b>
PPV	6	24			<b>30</b>
RCVMV	5	4			<b>9</b>
PSTVd	No(rely on other)	No (rely on other)	2 hr/yr (PCR)		<b>2</b>

1. The figures in [ ] are the routine costs of consumables required to maintain the protocol

2. The figures in ( ) are the ideal time (hrs) required to complete a task to a suitable standard.

The least number of hours required to maintain a protocol on annual basis was 2 hours (for Citrus Greening) and the most hours required was 142 hours per annum for Queensland fruit Fly. The average number of hours for the 19 EPPs surveyed was 20 hours, and including the “ideal” hours was 30.5 hours. Conservatively it costs approximately \$70.00/hr to employ the experienced scientists that are responsible for maintaining a protocol. The costs therefore to maintain a protocol is somewhere between \$1,400.00 and 2,100.00 per annum, per EPP diagnostic protocol.



## **4. Survey results: General questions related to maintaining diagnostic protocols**

As part of the survey we asked a series of questions that related to the infrastructure and support required to maintain a diagnostic capability for the detection of an EPP. The questions asked were:

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- lab accreditation
- staffing
- other

*Q12: Do you think “ring tests” between Australian labs would be useful?*

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

*General comments:*

We have decided to record the answers to these questions anonymously as we see that knowing who made the comments makes no difference to the outcomes of this survey and in some instances the comments may be considered negative by certain organizations in Australia. We have tried to neutralize each answer so as not to reflect on any one organization as again this has little impact on this survey. The issues highlighted by each diagnostician however is significant and does identify the key areas of concern for diagnosticians that are responsible for screening field samples for the presence of EPPs. The survey results for each diagnostician interviewed are provided in Appendix 3.

### **4.1 What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?**

#### **4.1.1 Lab accreditation**

The majority of diagnosticians could see the benefits of accreditation. Lab accreditation is important and if established correctly can take enormous pressure off the lab diagnostician. There are more and more court cases in which diagnostic test results are submitted as evidence and industry want surety in diagnosis.

The down-side of accreditation such as NATA is the costs to implement the systems to support the accreditation and the increased on-going costs to maintain accreditation. Major concerns included the time and cost of establishing accreditation as well as the ongoing costs of maintaining an accreditation standard. To quote one diagnostician: “It is frustrating when our managers insist that our labs reach an accreditation level and do not provide adequate resources to maintain the desired level of operation”. One lab that is NATA accredited noted that 10% of the diagnostician’s staff time is dedicated to maintaining NATA standards. A second diagnostician estimated that to comply to a NATA-like system will add 30% to the time that it would normally take to conduct a test and to maintain the standard would cost around \$10,000.00 per year. Several organizations are looking at accreditation because they can see that some level of accreditation will be mandatory at a national level in the near future.

### 4.1.2 Staffing

Job security for staff is an issue. Retaining experienced staff is the most critical area in maintaining a diagnostic capability for EPPs. This is not only for staff that are experienced in the lab, but also field based staff who are familiar with the industry, the agronomy of crops and who have a strong understanding of the endemic pests and pathogens that affect the crop in the field. It is important that the diagnosticians and technicians are active and are using their tests/skills on a routine basis and that more than one person knows how to use the diagnostic protocol as they are a back-up if you are not present. This applies to some degree for the senior scientists but also for the junior scientists and technicians that actually do most of the lab work. The level of expertise and knowledge of these diagnosticians needs to be appropriately respected and valued. At least one person within the group should be employed on an on-going basis who has a broad experience in this field and who has experience in the interpretation of test results.

The level of financial support for this work varies greatly from one organization to the next. It is becoming increasingly difficult to maintain staff due to short term contracts. In several labs there are no funds to support relevant staff to work on or maintain the EPP protocol and there is no support staff to ensure that these teams are “lab ready” to conduct the EPP protocol.

### 4.1.3 Other

A range of issues were identified in addition to lab accreditation and staffing and include:

- Resources for a well run lab are often lacking. Time is often required to optimize procedures, to make a diagnostic test better or to increase the efficiency of lab procedures. To do this takes time (i.e. **40 hours** to optimize a new PCR protocol etc) but because this type of work is not seen as a priority money is not provided to support this work.
- For virus diagnostics PCR will continue to be the diagnostic test of choice, particularly as the current supply of antiserum runs out. It will become important for labs to not only have a solid PCR capability but also an ability to screen large numbers of samples using PCR.
- It is difficult to conduct pathogenicity assays for EPPs in many of the diagnostic facilities in Australia. Although not essential for many of the protocols, particularly as sequencing data of PCR products provides the ultimate information required to confirm a positive test result, pathogenicity assays are required in some instances.
- Resources for operating costs and consumables, particularly for PCR, is poorly funded in several organizations.
- Maintenance of reference collections and associated databases is also critical and funds are often limited to support this work.

## **4.2 Do you think “ring tests” between Australian labs would be useful?**

All the diagnosticians interviewed could see an advantage in being involved in “ring tests” for a number of reasons including:

- Helps maintain a diagnostic capability and is a great way to ensure that diagnostic labs can correctly use a protocol.
- Should be conducted between labs working on the EPP within Australia as well as labs from overseas. The overseas labs are important as we need to ensure that the diagnosis that we generate is accepted off-shore.

- Participation in global ring tests assures the acceptance of test results generated from the diagnostic protocols.
- Ring tests also foster contact between fellow “like-minded” scientists and fuels collaboration.

Most diagnosticians agreed that the ring tests need to be organized at a national level but all parties were concerned at the extra expenses this would place on a diagnostic network/system that is already under funded. One alternative was to have a “ring test” of a technique (i.e. PCR or ELISA) and send samples of a common pathogen (i.e. TSWV) to all the labs and compare the results between the labs. This would test the competence of each lab to do a “type” of test. The responsibility of organizing the ring tests could be rotated through the major labs.

It was seen to be important to have an independent body oversee the “ring tests”. Some of the bodies/organizations suggested as most appropriate were the National Diagnostic Network, OCCPO and SPHDS and the Plant Health Committee.

### ***4.3 Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?***

There were no problems with the importation of positive controls for several diagnosticians. Generally speaking insects can safely be imported into the country and the main difficulties with these EPPs is finding scientists overseas who have both access to appropriate specimens and who are also willing to go to the trouble of sending you the specimens. It was suggested on several occasions that it may be useful to offer to purchase these specimens from our international collaborators. In two instances, the diagnosticians traveled overseas and collected the positive controls from their collaborators in person.

Other diagnosticians sourced their positive controls from specimens that were identified during recent incursions and in several instances permits were available to propagate EPP-infected material in AQIS approved glasshouses.

However, several diagnosticians have found it difficult to import material and were frustrated by apparent variation and inconsistencies in AQIS import permits for both plant material and antiserum. AQIS has recently changed the rules and diagnosticians now need 1 permit for each species of dried specimens. This can be time consuming.

### **4.4 General comments:**

The majority of the “General Comments” involved the process of generating the diagnostic protocols. There was concern about a lack of consistency and quality between diagnostic protocols. Pest Risk Analysis is an important component of a diagnostic manual and several protocols have been generated that do not include this type of analysis and this can result in an inferior diagnostic capability. The standard of diagnostic protocols should be regulated at a federal level.

One diagnostician interviewed was disappointed that they had had no feedback from the funding body that supported the development of the protocol. They do not know how the protocol has been received, or if in fact it is the national protocol that would/should be used by other labs or if the funding body has circulated the protocol to third parties.

It is not clear how to get your EPP diagnostic protocol accepted nationally and that SPHDS role should be to drive this process and advise/inform the PHC. Several diagnosticians have found it disappointing that there is no clear direction at the national level on what processes are involved to establish a protocol as the national diagnostic standard.

On a separate a concern was made about a lack of corporate memory within some federal bodies due to the high turnover of staff. There is a fear that new staff are not sure what work has been commissioned in past years and that they will commission work that has already been done.

## **5. Summary and Conclusions**

Based on actual time estimates to maintain a diagnostic protocol, the labor inputs by ten diagnosticians from across Australia who maintain 19 EPP protocols was estimated to be 20-30 hours per year for each diagnostic protocol.

The time inputs to maintain the diagnostic manuals for three EPPs (PPV, fire blight, MDMV) ranged from 0 to 26 hours for the 6 month period. A number of activities were documented and included predictions of the ability of the protocol to detect recently reported strains, re-ordering and validation of test reagents, the preparation of semi-selective media in readiness for testing field material, and for PPV, the involvement in the validation of a global diagnostic protocol. Based on an hourly rate of \$70.00/hr to employ an experienced scientist the costs associated with this time input are conservatively estimated at \$2,100.00 per year based on average 30 hours per year commitment to each protocol.

Ten diagnosticians were interviewed relating to diagnostic protocols for 19 separate EPPs. Fifteen of the 19 EPP protocols used PCR to detect the EPP and most labs do not routinely check primers and positive controls due to time restraints. Generally speaking the PCR protocols that were used on a regular basis appeared to function more efficiently than occasionally used PCR protocols and highlights the importance of active labs and protocols for optimum performance.

For ELISA-based protocols the availability of positive controls was a key issue and is dependant on the use of virus-infected tissue. Currently the ELISA protocols for both PPV and MDMV are not valid due to the absence of a positive control. In contrast the ELISA protocols for the legume viruses are functional as the positive controls can be propagated as part of a PEQ permit. A similar scenario was evident with the semi-selective media for *E. amylovora*, which uses a non-pathogenic strain of the fire blight pathogen as a positive control, compared to *X. fastidiosa*, which does not have a permit to allow the importation and culture of an isolate of the Pierce's Disease pathogen. For the protocols that use taxonomy as a diagnostic tool, on-going and regular training of staff was a critical and time consuming factor with up to 20 hours/year required to ensure staff are adequately skilled to complete a diagnosis.

Most diagnosticians were not concerned or frustrated by the emergence of new reagents that could possibly improve the PCR protocol. It was evident that most of the improvements that had been made to the protocols in recent years were the result of associated research projects. There was a concern however that once the research activities cease there will be no time for the diagnosticians to introduce and validate modifications to the protocols. Indeed this was the experience of several diagnosticians. Active surveillance and the submission of samples to a diagnostic lab for testing were seen by most diagnosticians as an effective way to train staff and ensure that a diagnostic protocol was operational and ready-to-go.

Most diagnosticians kept in touch with the literature on a regular basis with the time inputs ranging from 0-24 hrs/year, depending on the EPP. Not all the diagnosticians actively kept in touch with peers/experts for each EPP for a number of reasons. Only 8 of the 19 EPP protocols had more than one lab that was capable of using the diagnostic protocol to detect the EPP. Training workshops were generally considered as valuable exercises and necessary for a lab to obtain the required skills to use an EPP protocol. The workshops are an effective way to

validate or ratify the protocol at a national level and positive feedback from the workshop by experienced diagnosticians inevitably results in modifications to the protocol that make it more user friendly, relevant and accepted by peers. Finances to support the 1-2 day workshops were the major limiting factor.

Estimated labor inputs to maintain a diagnostic protocol for an EPP ranged from 2-142 hours per year, with average of 20 hours/yr spent maintaining activities such as staff training, literature reviews, maintaining networks with experts, updating protocols, replacing outdated consumables and validating controls. Almost without exception each diagnostician expressed a need for “more time” to spend on these vital activities and an average of 30.5 hours/year was the estimated ideal time allocation to maintain the protocols at an appropriate standard. Based on an arbitrary and conservative labor fee of \$70.00/hr, the costs required to maintain a protocol was estimated at between \$1,400.00 and \$2,100.00 per annum, for each EPP protocol.

The majority of the diagnosticians could see the benefits of lab accreditation but were concerned about the costs required to implement the systems, and more importantly, the hidden costs associated with maintaining an accreditation level. Lab environments can become extremely stressful if these systems are not adequately supported by individual organizations. Job security was a significant issue for most labs as it is critical to retain experienced staff that can use and follow the diagnostic protocol at the required level of competency. It was evident that level of support for trained staff varied greatly between organizations. All the diagnosticians were in favor of ring tests as this helps maintain a diagnostic capability for an EPP and it ensures that different labs can use and follow the protocol correctly. Ring tests were seen as way to foster contact between fellow “like-minded” scientists that inevitably fuel collaboration. The involvement of international labs was also seen as a significant opportunity to increase the benefits of ring tests.

The availability and access to positive controls was an issue. Some protocols are more dependant on positive controls than others and the diagnosticians interviewed expressed frustration at apparent inconsistencies in the provision of AQIS import permits between labs. AQIS has recently changed the rules and diagnosticians now need one permit for each species of dried specimen to be imported. Restrictions on access to positive controls will impact on the ability of diagnostic labs in Australia to conduct a valid diagnostic test for the detection of an EPP. There were some concerns about the lack of consistency and quality of the diagnostic protocols that have been generated in recent years – many lack a pest risk analysis. A standard format should be regulated at a national level.

A key part of developing an internationally outstanding plant health system for Australia is ensuring there are resources and the capability available to quickly and reliably identify potential incursions. There is also the need to ensure consistency and best practice in diagnostic procedures. Federal and state agencies within Australia are developing an appropriate and consistent approach to plant health management within and among organizations, jurisdictions and industries (Moran and Muirhead, 2002). At the same time plant disease diagnostic laboratories are currently going through a period of change in Australia. Government services are being cut back and staff is struggling to deliver efficient quality services and job security for staff is an ongoing problem. It is important that Australia retains excellent diagnostic services for plant pests and diseases for quarantine, trade and sustainable agricultural systems.

The data presented in this report provides critical baseline data from diagnostic laboratories from around Australia on the annual resources required to maintain a diagnostic manual for an EPP. These costs need to be recognized nationally by the plant health community if our goal is to maintain a quality and sustainable diagnostic capacity for the detection of EPPs that threaten the Australian agricultural industry.

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## **Appendix 1: Survey Questionnaire**

### **Background Information**

*EPP Name:*

*Date EPP protocol completed:*

### **Questions:**

Q1: *What diagnostic tests do you use for your EPP?*

Q2: *If PCR, how often do you check*

- Primers?*
- Positive and negative controls?*
- Have new reagents been released which would improve the performance of your test?*
  - If yes, what is required to incorporate these improvements into the protocol?*

*Other issues related to PCR.*

Q3: *If ELISA, how often do you*

- re-order antisera?*
- Check positive controls?*
- Have new reagents been released which would improve the performance of your test?*
  - If yes, what is required to incorporate these improvements into the protocol?*

*Other issues related to ELISA*

Q4: *If isolation and culturing is required, how often do you*

- prepare stock solutions for selective media?*
- Check controls (if present)?*

*Other issues related to fungal and bacterial isolations.*

Q5: *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*

Q6: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*

Q7: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*

- If yes, what is required to incorporate these changes into the existing manual?*

*How do you validate these techniques against the existing protocol?*



Q8: *Are you or your organization doing active surveillance for this EPP?*

- *-If yes, how often?*
- *If no do you think this would be worthwhile?*

Q9: *Do you stay in touch with experts internationally?*

- *If yes, how often?*

Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*

- *If yes, are they “up to speed” with the diagnostic protocol?*

*What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*

**Generic Questions:**

Q11: *What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- *lab accreditation*
- *staffing*
- *other*

Q12: *Do you think “ring tests” between Australian labs would be useful?*

Q13: *Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

*General comments: (Please add any other comments that you see are relevant to the maintenance of an EPP protocol).*

## Appendix 2: Survey responses to EPP specific questions (Q 1 – 10)

### 2.1: Asian Citrus Psyllid (*Diaphorina citri*)

#### Background Information

*Diagnostician:* Mali Malipatil

*EPP Name:* Asian Citrus Psyllid (*Diaphorina citri*)

*Date EPP protocol completed:* June 2007

*How many samples/yr do you screen for the EPP?*

2/yr

#### Questions:

Q1: *What diagnostic tests do you use for your EPP?*  
Morphology, PCR

Q2: *If PCR, how often do you check*

- *primers?*
- *Positive and negative controls?*

The primers and positive controls are checked at least once per year

- *Have new reagents been released which would improve the performance of your test?*

No

- *If yes, what is required to incorporate these improvements into the protocol?*

- *Other issues related to PCR.*

Costs for Consumables is an ongoing issue (value of approx \$500.00/yr)

Q3: N/A *If ELISA, how often do you*

- *re-order antisera?*
- *Check positive controls?*
- *Have new reagents been released which would improve the performance of your test?*
  - *If yes, what is required to incorporate these improvements into the protocol?*
- *other issues related to ELISA*

Q4: N/A *If isolation and culturing is required, how often do you*

- *prepare stock solutions for selective media?*
- *Check controls (if present)?*
- *Other issues related to fungal and bacterial isolations.*

- Q5 *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*  
 It is important to keep up with the literature, on a fortnightly basis,  
 (6 hrs/yr)  
 Maintenance of reference specimens is also a requirement
- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
 No
  - *If yes, what is required to incorporate these changes into the existing manual?*
  - *How do you validate these techniques against the existing protocol?*
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
 No, but AQIS does conduct surveys as this insect is the vector for Citrus Greening.  
 -*If yes, how often?*  
 - *If no do you think this would be worthwhile?*  
 It would be a good thing if Vic DPI did survey for this insect.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
 (6 hrs/yr)
- Q9: *Do you stay in touch with experts internationally?*  
 Yes, via email and the phone.  
 - *If yes, how often?*  
 Fortnightly  
 (6 hrs/yr)
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
 Not yet as this protocol is still under development  
 - *If yes, are they “up to speed” with the diagnostic protocol?*  
  
 - *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
 Morphology training is required and is approx 1 day/yr input for trained ongoing staff.  
 Molecular training is dependant on the individual.

## 2.2: Queensland Fruit fly (*Bactrocera* sp. - *Bactrocera tryoni* (Queensland fruit fly))

### Background Information

*Diagnostician:* Peter Gillespi (NSW DPI)

*EPP Name:* Fruit fly (*Bactrocera* sp. - *Bactrocera tryoni* (Queensland fruit fly))

*Date EPP protocol completed:* 2005

How many samples/yr do you screen for the EPP? 3-4 tests per year

### Questions:

Q1: *What diagnostic tests do you use for your EPP?*

Morphology/Taxonomy and PCR. This protocol is a NATA accredited protocol.

Q2: *If PCR, how often do you check*

- *primers?*
- *Positive and negative controls?*

The primers and positive controls are checked as the protocol is run on field samples. Part of the NATA protocol states that DNA must be extracted from “positive” fruit flies.

- *Have new reagents been released which would improve the performance of your test?*

The science/quality of the primers is sound and have been developed and validated as part of an international protocol.

- o *If yes, what is required to incorporate these improvements into the protocol?*

- *Other issues related to PCR.*

The PCR test is a very easy and straight forward protocol that is robust. The training course that is conducted for fruit fly diagnostics clearly demonstrates the robustness of the test on a regular basis.

Q3: N/A *If ELISA, how often do you*

- *re-order antisera?*
- *Check positive controls?*
- *Have new reagents been released which would improve the performance of your test?*
  - *If yes, what is required to incorporate these improvements into the protocol?*
- *other issues related to ELISA*

Q4: N/A *If isolation and culturing is required, how often do you*

- *prepare stock solutions for selective media?*
- *Check controls (if present)?*
- *Other issues related to fungal and bacterial isolations.*

Q5: *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*

Accurate identification of morphological features of the fruit flies is an important component of the fruit fly protocol. As part of the NATA accreditation we run two internal and one external lab tests each year and involves the correct

identification of a range of fruit fly species. The same fly species are distributed to all participants where possible.  
(20 hours/year)

- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
No, this diagnostic protocol is first class.
  - *If yes, what is required to incorporate these changes into the existing manual?*- *How do you validate these techniques against the existing protocol?*

- Q7: *Are you or your organization doing active surveillance for this EPP?*  
Yes, traps are set as part of the “Tri-State” agreement. The Federal government also conducts surveys at the points of entry.  
*-If yes, how often?*  
On an ongoing and regular basis  
*- If no do you think this would be worthwhile?*

- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
Subscribe to several relevant journals and review literature on a regular basis  
(12 hours/yr)

- Q9: *Do you stay in touch with experts internationally?*  
Yes  
*- If yes, how often?*  
Via email, and directly with colleagues who actively conduct research on fruit flies. We are always seeking specimens of new strains and species of fruit flies.  
(100 hrs/yr)

Attend a 3-4 day training course every 3 – 4 years. This type of training should be mandatory for all senior diagnosticians involved with fruit fly diagnostics. This course is currently run out of Queensland and the key organizer is approaching retirement. It is important that this continues after this person does retire.  
(10 hrs/yr)

- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
Yes.
  - *If yes, are they “up to speed” with the diagnostic protocol?*There are a number of people from around Australia who are trained in the identification of fruit flies as a result of our regular 2-day training workshop. However no other labs in Australia are formally involved in our inter-lab ring tests.
  - *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*The most appropriate way to get up to the NATA standard is to participate in the inter-lab ring testing process.

## 2.3: Potato leaf miner (*Liriomyza cicerina*, *Liriomyza trifolii*)

### Background Information

*Diagnostician:* Mali Malipatil

*EPP Name:* Potato leaf miner (*Liriomyza cicerina*, *Liriomyza trifolii*)

*Date EPP protocol completed:* 2006

How many samples/yr do you screen for the EPP? 2 tests/yr

### Questions:

- Q1: *What diagnostic tests do you use for your EPP?*  
Morphology on adult flies  
PCR (RFLP) on Larvae
- Q2: *If PCR, how often do you check*  
– *primers?*  
Not yet, but should be checked every 3-6 months. (3 hrs)  
– *Positive and negative controls?*  
Check along with the primers, positive control is DNA.  
Also need to ensure Restriction enzymes are functional (3 hrs)  
– *Have new reagents been released which would improve the performance of your test?*  
No  
    ○ *If yes, what is required to incorporate these improvements into the protocol?*  
– *Other issues related to PCR.*  
Would like to be more validation work on larger number of populations
- Q3: N/A *If ELISA, how often do you*  
– *re-order antisera?*  
– *Check positive controls?*  
– *Have new reagents been released which would improve the performance of your test?*  
    ▪ *If yes, what is required to incorporate these improvements into the protocol?*  
– *other issues related to ELISA*
- Q4: N/A *If isolation and culturing is required, how often do you*  
– *prepare stock solutions for selective media?*  
– *Check controls (if present)?*  
– *Other issues related to fungal and bacterial isolations.*
- Q5 *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*  
There is a requirement to keep up-to-date with taxonomic activities globally both in the literature (20 hrs/yr) and specimens (10 hrs/yr).
- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
No

- *If yes, what is required to incorporate these changes into the existing manual?*
- *How do you validate these techniques against the existing protocol?*

- Q7: *Are you or your organization doing active surveillance for this EPP?*  
Vic DPI is not surveying for the pest, but I do know that AQIS is.  
*-If yes, how often?*  
At a passive level we receive around 1-5 specimens per year.  
*- If no do you think this would be worthwhile?*  
I do think it would be worthwhile to conduct surveys, particularly in potatoes and vegetable crops.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
Regularly, on a fortnightly basis.  
(6 hrs/yr)
- Q9: *Do you stay in touch with experts internationally?*  
Yes  
*- If yes, how often?*  
Fortnightly  
(6 hrs/yr)
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
No  
*- If yes, are they “up to speed” with the diagnostic protocol?*  
AQIS can do identify some of the morphological properties visually, but not all of them. AQIS are not trained to perform dissections of the leaf miners for complete ID.  
*What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
The best training is on-the-job training of staff (approx 10 days/yr)

## **2.4: *Fusarium oxysporum* form sp. *ciceris* (Chickpea wilt)**

### **Background Information**

*Diagnostician:* James Cunnington (Victorian DPI)

*EPP Name:* *Fusarium oxysporum* form sp. *ciceris* (Chickpea wilt)

*Date EPP protocol completed:* 2007 (is in draft form).

How many samples/yr do you screen for the EPP? Expect 2-3 specimens/yr

### **Questions:**

Q1: *What diagnostic tests do you use for your EPP?*

PCR only (We use standard basic media to generate cultures and harvest mycelium from selected fungal colonies).

- Q2: *If PCR, how often do you check*  
 – *primers?*  
 – *Positive and negative controls?*  
 For both primers and positive controls we only conduct checks when I do a test. The ideal situation would be to check both primers and DNA on an annual basis (1 day/yr)  
 – *Have new reagents been released which would improve the performance of your test?*  
 No  
 ○ *If yes, what is required to incorporate these improvements into the protocol?*  
 – *Other issues related to PCR.*  
 No
- Q3: N/A *If ELISA, how often do you*  
 – *re-order antisera?*  
 – *Check positive controls?*  
 – *Have new reagents been released which would improve the performance of your test?*  
 ■ *If yes, what is required to incorporate these improvements into the protocol?*  
 – *other issues related to ELISA*
- Q4: *If isolation and culturing is required, how often do you*  
 – *prepare stock solutions for selective media?*  
 Use standard non-selective media that used regularly in the General Pathology lab.  
*Check controls (if present)?*  
*Other issues related to fungal and bacterial isolations.*
- Q5 N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*
- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
 No  
 ○ *If yes, what is required to incorporate these changes into the existing manual?*  
 - *How do you validate these techniques against the existing protocol?*
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
 No.  
 -*If yes, how often?*  
 - *If no do you think this would be worthwhile?*  
 Yes, but I think there are higher priorities.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
 Once a month  
 (12 hr/yr)



Q9: *Do you stay in touch with experts internationally?*  
No  
- *If yes, how often?*

Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
No  
- *If yes, are they “up to speed” with the diagnostic protocol?*  
- *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
2 Day workshop

## **2.5: Colletotrichum truncatum – Lentil Anthracnose**

### **Background Information**

*Diagnostician:* Kurt Lindbeck

*EPP Name:* *Colletotrichum truncatum* – Lentil Anthracnose

*Date EPP protocol completed:* 2005

How many samples/yr do you screen for the EPP? We have not needed to use the protocol since it was completed in June 2005.

### **Questions:**

Q1: *What diagnostic tests do you use for your EPP?*  
Morphological properties of colonies  
Conventional and Quantitative PCR.

Q2: *If PCR, how often do you check*  
– *primers?*  
– *Positive and negative controls?*  
We have not checked the primers or positive controls since the protocol was developed. We would check the validity of these reagents on a needs basis.  
  
– *Have new reagents been released which would improve the performance of your test?*  
No.  
○ *If yes, what is required to incorporate these improvements into the protocol?*  
  
– *Other issues related to PCR.*  
The protocol is solid and has been adopted by Canada.

Q3: N/A *If ELISA, how often do you*  
– *re-order antisera?*  
– *Check positive controls?*  
– *Have new reagents been released which would improve the performance of your test?*  
▪ *If yes, what is required to incorporate these improvements into the protocol?*  
– *other issues related to ELISA*

- Q4: *If isolation and culturing is required, how often do you*  
 – *prepare stock solutions for selective media?*  
 – *Check controls (if present)?*  
 – *Other issues related to fungal and bacterial isolations.*  
 The media used for isolation and fungal culturing is non-selective media (oatmeal agar) and is easy to prepare and can be made up quickly when required.
- Q5: *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*  
 The taxonomy is standard as there very few *Colletotrichum* sp. that are pathogenic on lentils.
- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
 No, other countries have adopted the protocol that we have developed.  
 ○ *If yes, what is required to incorporate these changes into the existing manual?*  
 - *How do you validate these techniques against the existing protocol?*
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
 Our organization conducted surveys in 2000 and 2001.  
 -*If yes, how often?*  
 There may be a survey conducted in this coming season.  
 - *If no do you think this would be worthwhile?*
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
 Yes, I conduct routine searches and discover 5-10 research papers each year.  
 (10 hrs/yr)
- Q9: *Do you stay in touch with experts internationally?*  
 Yes.  
 - *If yes, how often?*  
 Via email on a regular basis.  
 (5 hrs/yr).
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
 Yes, Dr Rebecca Ford's lab at Melbourne Uni.  
 - *If yes, are they "up to speed" with the diagnostic protocol?*  
 Yes.  
 - *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
 A 2 day workshop would be required to train other diagnosticians.

## 2.6: *Phytophthora ramorum*

### Background Information

*Diagnostician:* James Cunningham

*EPP Name:* *Phytophthora ramorum*

*Date EPP protocol completed:* September 2006

*How many samples/yr do you screen for the EPP?* 1-2 tests/yr

### Questions:

- Q1: *What diagnostic tests do you use for your EPP?*  
Use QPCR for survey samples  
Use culturing and PCR/sequencing for initial diagnosis
- Q2: *If PCR, how often do you check*  
– *primers?*  
Only when I get a sample in.  
– *Positive and negative controls?*  
Only when I get a sample in  
Ideally the primers and positive controls would be checked on an annual basis (6-8 hrs/yr)  
– *Have new reagents been released which would improve the performance of your test?*  
A “new” Taqman-based QPCR has been published.  
○ *If yes, what is required to incorporate these improvements into the protocol?*  
This test would need to be validated on at least 50 plant samples selected randomly in Australia as part of a survey (4 weeks work)  
– *Other issues related to PCR.*
- Q3: N/A *If ELISA, how often do you*  
– *re-order antisera?*  
– *Check positive controls?*  
– *Have new reagents been released which would improve the performance of your test?*  
▪ *If yes, what is required to incorporate these improvements into the protocol?*  
– *other issues related to ELISA*
- Q4: *If isolation and culturing is required, how often do you*  
– *prepare stock solutions for selective media?*  
The two media used are Water Agar (non-selective) and a selective media for *Phytophthora sp.* This media is also used regularly by the General pathology lab.  
– *Check controls (if present)?*  
This is not an issue as we can use any *Pytophthora* species to check the selective media.  
– *Other issues related to fungal and bacterial isolations.*
- Q5 N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*

- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
There are possibly new techniques that have been published. – see earlier comment
- *If yes, what is required to incorporate these changes into the existing manual?*
  - *How do you validate these techniques against the existing protocol?*
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
No.  
-*If yes, how often?*  
- *If no do you think this would be worthwhile?*  
Yes as *P. ramorum* covers such a wide range of industries. It is a very significant EPP. Surveys are also useful to help maintain skills in the lab and conduct staff training.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
Once a month  
(4 hrs/yr)
- Q9: *Do you stay in touch with experts internationally?*  
Yes  
- *If yes, how often?*  
3-4 times per year  
(4 hours/yr)
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
Not sure.  
- *If yes, are they “up to speed” with the diagnostic protocol?*  
Not with the protocol that I have developed in this lab.  
- *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
2 day workshop would be required. But lab would have to have QPCR capabilities.

## 2.7: *Tilletia indica* (Karnal bunt)

### Background Information

*Diagnostician:* Gordon Murray

*EPP Name:* *Tilletia indica* (Karnal bunt)

*Date EPP protocol completed:* 2003

*How many samples/yr do you screen for the EPP?*

Apart from the 2004 incursion we have received very few samples for testing. The WA lab does receive a number of samples on a routine basis. We are actively working with the diagnostic protocol at the moment as part of a research project.

### Questions:

Q1: *What diagnostic tests do you use for your EPP?*

Visual examination, Spore wash and concentration followed by microscopic examination (morphology)  
Conduct a PCR test on germinated spores

Q2: *If PCR, how often do you check*

- *primers?*
- *Positive and negative controls?*

Both primers and positive controls are checked with each PCR run. This section of the diagnostic protocol is still under development.

- *Have new reagents been released which would improve the performance of your test?*

No

- *If yes, what is required to incorporate these improvements into the protocol?*

- *Other issues related to PCR.*

The PCR protocol is still under development.

Q3: N/A *If ELISA, how often do you*

- *re-order antisera?*
- *Check positive controls?*
- *Have new reagents been released which would improve the performance of your test?*
  - *If yes, what is required to incorporate these improvements into the protocol?*
- *other issues related to ELISA*

Q4: *If isolation and culturing is required, how often do you*

- *Prepare stock solutions for selective media?*

Culturing of spores for germination is required but the media used is a general non-selective media that is easily prepared and made up fresh on a needs basis.

- *Check controls (if present)?*

Because of the non-selective nature of the media, a karnal bunt positive control is not needed.

- *Other issues related to fungal and bacterial isolations.*

Technically, the germination of the spores is difficult as many spores are cracked etc via the handling process. It is critical to have experienced staff to maximize the chances of germinating viable spores if present.

- Q5: *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*  
As mentioned above, the washing and preparation of the spores is a critical step and it is important to have experienced staff on hand.
- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
The current diagnostic protocol is about to be superseded by a new method of DNA extraction directly from spores. This is a project that we are working on in collaboration with WA Ag.  
  - *If yes, what is required to incorporate these changes into the existing manual?*
  - *How do you validate these techniques against the existing protocol?*  
The research project is currently doing the validation.
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
No, my organization is not doing any surveillance; we rely on passive surveillance only. WA is doing some active surveillance.  
  
  - *If yes, how often?*
  - *If no do you think this would be worthwhile?*  
It would be worthwhile to do active surveillance. It is more a matter of “who pays” for the surveillance.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
I keep in touch with the literature on a weekly basis  
(30 minutes/wk, 26 hrs/year)
- Q9: *Do you stay in touch with experts internationally?*  
Yes  
  - *If yes, how often?*  
I keep in touch by regular email about once a week (approx 26 hrs/yr).
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
Yes, WA Ag  
  - *If yes, are they “up to speed” with the diagnostic protocol?*  
Yes, and we currently are collaborating closely together on a Karnal Bunt related research project.
  - *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
Training would take at least a 2 day workshop. Ideally provisions would be made to have follow-up meetings/conversations to assist with establishment of a protocol at a new workplace.

## 2.8: Pierces Disease (*Xyella fastidiosa*)

### Background Information

*Diagnostician:* Jo Luck

*EPP Name:* Pierces Disease (*Xyella fastidiosa*)

*Date EPP protocol completed:* 2004

How many samples/yr do you screen for the EPP? 4/yr

### Questions:

Q1: *What diagnostic tests do you use for your EPP?*  
PCR, Culturing

Q2: *If PCR, how often do you check*

- *primers?*
- *Positive and negative controls?*

Every time we do the test. We have controls and back-ups within the PCR system (HKG) which ensures that either or both the primers and positive controls are not functional.

- *Have new reagents been released which would improve the performance of your test?*

Yes

- *If yes, what is required to incorporate these improvements into the protocol?*

Immuno-capture PCR has been reported as well as Quantitative PCR. We do not think we need to introduce the immuno-capture PCR test into the protocol. The Real time PCR for *X. fastidiosa* has been implemented in to the protocol and was done as part of a related research project.

- *Other issues related to PCR.*

Q3: N/A *If ELISA, how often do you*

- *re-order antisera?*
- *Check positive controls?*
- *Have new reagents been released which would improve the performance of your test?*
  - *If yes, what is required to incorporate these improvements into the protocol?*
- *other issues related to ELISA*

Q4: *If isolation and culturing is required, how often do you*

- *prepare stock solutions for selective media?*

We use a semi-selective media for the culturing of *X. fastidiosa*. The media is usually made up when specimens arrive for testing. Ideally the semi-selective media would be made up every 3 months.

(4 days/yr).

- *Check controls (if present)?*

We do not have a live isolate of *X. fastidiosa* that we can use as a positive control.

- *Other issues related to fungal and bacterial isolations.*

No.

- Q5 N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*
- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
 No.
  - *If yes, what is required to incorporate these changes into the existing manual?*
 We have got the real time PCR going for *X. fastidiosa*, but have nothing published in this area. The protocol could be revised, updated as it is now 4 years old. Ideally the revision would be done on an annual basis.
- *How do you validate these techniques against the existing protocol?*
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
 No. Victorian DPI may be doing a survey in 07-08 but this will be a one-off survey if it goes ahead.  
*-If yes, how often?*  
*- If no do you think this would be worthwhile?*  
 Survey samples help to maintain a diagnostic capability. It is a good dry run for an incursion and it helps to keep staff trained, and importantly, to train new members of staff.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
 I do not keep up with the literature on *X. fastidiosa* and it is only by chance that I find out new developments. Ideally I would keep in touch with the literature on a weekly basis  
 (1 hr/wk, 52 hr/yr).
- Q9: *Do you stay in touch with experts internationally?*  
 No. I only contact global experts if there is a specific reason.  
*- If yes, how often?*
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
 There are two other labs in Australia that have the capacity to test for *X. fastidiosa*. One lab is a commercial lab and I have no idea what tests (PCR protocols ) they use for diagnosis. The second lab has the national protocol that we developed and follow on a routine basis.  
*- If yes, are they “up to speed” with the diagnostic protocol?*  
 We have had no “follow-up” training with the lab that uses the national protocol so we do not fully understand their capacity to conduct the protocol. It would be good to know that we had a second lab, but I do not think it is essential.  
  
*- What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
 The training would require a 2-3 day workshop.



## 2.9: *Erwinia amylovora* (fire blight)

### Background Information

*Diagnostician:* Brendan Rodoni

*EPP Name:* *Erwinia amylovora* (fire blight)

*Date EPP protocol completed:* 2002

How many samples/yr do you screen for the EPP? 6 samples/yr

### Questions:

Q1: *What diagnostic tests do you use for your EPP?*

PCR, Culturing

Q2: *If PCR, how often do you check*

- *primers?*
- *Positive and negative controls?*

*When I conduct a test (not routine)*

- *Have new reagents been released which would improve the performance of your test?*

*Yes*

- *If yes, what is required to incorporate these improvements into the protocol?*

New reagents (enzymes etc) are introduced into the system on a trial basis as samples are processed. Generic changes like enzymes are also screened using protocols for endemic pathogens.

- *Other issues related to PCR.*

The costs of lab consumables is an on-going issue for PCR.

Q3: N/A *If ELISA, how often do you*

- *re-order antisera?*
- *Check positive controls?*
- *Have new reagents been released which would improve the performance of your test?*
  - *If yes, what is required to incorporate these improvements into the protocol?*
- *other issues related to ELISA*

Q4: *If isolation and culturing is required, how often do you*

- *prepare stock solutions for selective media?*

Mostly when samples arrive we prepare the media. However the ideal scenario is to have the media prepared twice a year.

- *Check controls (if present)?*

Controls are checked as samples are submitted. This can prove a problem for the attenuated strain of *Erwinia amylovora* (Ea322) that we use as a positive control for the selective media as it can take some time to regenerate the culture from glycerols etc (viability issues).

- *Other issues related to fungal and bacterial isolations.*

It is not optimal to culture the positive controls on a needs-to basis. However, time constraints are the main reason why this happens.

- Q5 N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*
- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
 Yes.  
 ○ *If yes, what is required to incorporate these changes into the existing manual?*  
 We need to identify optimal PCR primers that will reliably detect all strains of *E. amylovora* and incorporate these primers in to the manual.  
 - *How do you validate these techniques against the existing protocol?*  
 There is no mechanism to do this at the moment. I do not know how I would incorporate a new test in to the manual. Generally speaking it relies on the outcomes of research projects to provide the techniques and the validation of the technique.
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
 No.  
 -*If yes, how often?*  
 The national survey for fire blight was completed 8 years ago and I was involved in a more recent but smaller survey for fire blight in 2005. Vic DPI may conduct a survey for this pathogen in the next 12 months, but this type of survey for fire blight is not routine.  
 - *If no do you think this would be worthwhile?*  
 Yes, survey samples are extremely valuable for the diagnostician. Active surveillance is a great way to ensure your diagnostic protocol works and that you have staff that can do the test competently.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
 On a monthly basis (6 hrs/yr)
- Q9: *Do you stay in touch with experts internationally?*  
 Yes  
 - *If yes, how often?*  
 Every three years I attend an ISHS International workshop on fire blight. (3 days/yr)  
 I contact experts on a needs basis.
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
 Yes  
 - *If yes, are they “up to speed” with the diagnostic protocol?*  
 Yes  
 - *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
 A DPI/PHA/Industry funded workshop was held in 2004 and pathologists were trained on the procedures outlined in the protocol. This was an extremely valuable exercise and should be a routine process, at least every 5 yrs.

## 2.10: Citrus Canker (*Xanthomonas axonopodis* pv. *Citri*)

### Background Information

*Diagnostician:* Deb Hailstones

*EPP Name:* Citrus Canker (*Xanthomonas axonopodis* pv. *Citri*)

*Date EPP protocol completed:* 2006

How many samples/yr do you screen for the EPP? 1-2

### Questions:

Q1: *What diagnostic tests do you use for your EPP?*  
Bacterial plating, PCR, Leaf inoculations

Q2: *If PCR, how often do you check*

– *primers?*

Check the primers as the need arises. A “Stock” tube of primers is also available if fresh workable solutions of primers are required.

– *Positive and negative controls?*

We have access to some quarantinable strains of citrus canker from a recent incursion. We have back up supplies of DNA as a back-up.

– *Have new reagents been released which would improve the performance of your test?*

Yes and No. The existing protocol is sufficient. However we have an Honours program at the moment that is evaluating new sets of primers for canker detection. This work may result in an improvement to the protocol. The honours project is part of an active research project on citrus canker at our institute.

○ *If yes, what is required to incorporate these improvements into the protocol?*

Based on the quality of the evaluation we would hope that these improvements would be incorporated directly in to the protocol.

– *Other issues related to PCR.*

Q3: N/A *If ELISA, how often do you*

– *re-order antisera?*

– *Check positive controls?*

– *Have new reagents been released which would improve the performance of your test?*

Q4: *If isolation and culturing is required, how often do you*

– *prepare stock solutions for selective media?*

The media used for plating *X. citri* is a non-selective media that is readily available from commercial suppliers.

– *Check controls (if present)?*

This is not required as there is media (Nutrient Agar) is not selective.

– *Other issues related to fungal and bacterial isolations.*

This work is to be conducted in a PC3 facility due to the quarantinable nature of the pathogen. Citrus canker work would represent about 1% of the diagnostic outputs of the PC3 lab. This cost needs to be included when considering the cost of maintaining an EPP.

- Q5: *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*  
 There are no ongoing activities that are required with respect to morphological properties of Citrus Canker.
- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
 No
  - *If yes, what is required to incorporate these changes into the existing manual?*
 - *How do you validate these techniques against the existing protocol?*
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
 No.  
 -*If yes, how often?*  
 - *If no do you think this would be worthwhile?*  
 The latest Citrus Canker incursion is a relatively recent event and additional surveys is not likely to add to the current knowledge about the distribution of Citrus Canker in Australia.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
 I keep up with the literature as part of our research project. I would specifically review the literature with respect to Citrus Canker diagnostics perhaps once every 2 months.  
 (3 hrs/yr).  
 I have no funding to support travel to relevant international conferences.
- Q9: *Do you stay in touch with experts internationally?*  
 Yes  
 - *If yes, how often?*  
 Once or twice per year, via email  
 (1 hr per year)
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
 Yes  
 - *If yes, are they “up to speed” with the diagnostic protocol?*  
 Yes  
 - *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
 I would like to run a workshop that included training for relevant staff in Australia on the protocols for *X. citri* detection. I have attended these in the past for other EPPs (PSTVd, Fire Blight) and found them to be very useful.

## 2.11: Citrus greening (*Liberibacter asiaticus*)

### Background Information

*Diagnostician:* Deb Hailstones

*EPP Name:* Citrus greening (*Liberibacter asiaticus*)

*Date EPP protocol completed:* 2006

*How many samples/yr do you screen for the EPP?* 2 tests/yr

### Questions:

Q1: *What diagnostic tests do you use for your EPP?*  
PCR

Q2: *If PCR, how often do you check*  
– *primers?*

Check the primers as the need arises. A “Stock” tube of primers is also available if fresh workable solutions of primers is required.

– *Positive and negative controls?*

I still have some dried tissue from some citrus trees that tested positive for Citrus greening several years ago.

– *Have new reagents been released which would improve the performance of your test?*

Yes

○ *If yes, what is required to incorporate these improvements into the protocol?*

New primer sets, and a quantitative PCR assay protocol have been published and both need validation. . This work is currently being funded as part of an ongoing research project.

– *Other issues related to PCR.*

Q3: N/A *If ELISA, how often do you N/A*

– *re-order antisera?*

– *Check positive controls?*

– *Have new reagents been released which would improve the performance of your test?*

▪ *If yes, what is required to incorporate these improvements into the protocol?*

▪ *other issues related to ELISA*

Q4: N/A *If isolation and culturing is required, how often do you*

– *prepare stock solutions for selective media?*

– *Check controls (if present)?*

– *Other issues related to fungal and bacterial isolations.*

Q5 N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*

- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
 No
  - *If yes, what is required to incorporate these changes into the existing manual?*
 - *How do you validate these techniques against the existing protocol?*
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
 No  
 -*If yes, how often?*  
 - *If no do you think this would be worthwhile?*  
 It would be difficult to survey the industry for citrus greening as we are still learning about how and where to conduct these surveys.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
 I check the literature on average about 6 times per year for this pathogen (approx. 1 hr/yr)
- Q9: *Do you stay in touch with experts internationally?*  
 Yes  
 - *If yes, how often?*  
 I stay in regular contact with the Citrus Greening Literature as I am actively involved in a research program on this pathogen,
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
 Yes  
 - *If yes, are they “up to speed” with the diagnostic protocol?*  
 This lab can do the PCR for Citrus Greening. We were involved in the training of the staff at the lab for the detection of this pathogen.  
 - *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
 It would be great to workshop this diagnostic protocol at the completion of the research project. We will need financial support to do this.

## 2.12: Maize Dwarf mosaic virus (MDMV)

### Background Information

*Diagnostician:* Andrew Geering

*EPP Name:* Maize Dwarf mosaic virus (MDMV)

*Date EPP protocol completed:* November 2004

*How many tests per year?:* 1-2 tests/year

### Questions:

*Q1: What diagnostic tests do you use for your EPP?*

ELISA

RT-PCR

*Q2: If PCR, how often do you check*

– *primers?*

Have not yet checked the primers. However ideally the primers would be checked every 6 months. (6 hrs to conduct PCR, 2 times per year)  
(12 hrs/yr)

– *Positive and negative controls?*

Have not yet checked the positive and negative controls. However, ideally this would be done every 6 months. (Same PCR as above).

– *Have new reagents been released which would improve the performance of your test?*

No

○ *If yes, what is required to incorporate these improvements into the protocol?*

– *Other issues related to PCR.*

None identified.

*Q3: If ELISA, how often do you*

– *re-order antisera?*

Have not done so. It is likely that the antisera is beyond its expiry date. (Suspect the used by date would be every 2 years)  
(To re-order antisera would cost \$500.00)

– *Check positive controls?*

Have not done so. Ideally this should be done every 6 months (6 hrs, twice yearly)

(12 hrs/annum)

– *Have new reagents been released which would improve the performance of your test?*

Not that I am aware of any new reagents.

▪ *If yes, what is required to incorporate these improvements into the protocol?*

– *other issues related to ELISA*

None identified.

*Q4: N/A If isolation and culturing is required, how often do you*

– *prepare stock solutions for selective media*

– *Check controls (if present)?*

– *Other issues related to fungal and bacterial isolations.*

Q5 N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*

Q6: *Do you believe that your diagnostic test is still relevant, or, do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*

I think that the protocol is still relevant. However, I cannot say with certainty that new strains of the virus, antisera or primers have been published on MDMV.

- *If yes, what is required to incorporate these changes into the existing manual?*

If there were changes that needed to be incorporated into the protocol, than you would need to conduct some research in silico for the PCR to match available sequence data with the primer sequences to ensure the new strain will be amplifiable by the primers recommended in the protocol. If changes were required than the new antisera or primers would need to be validated against the existing positive controls and optimal experimental conditions determined. If a new host was identified for MDMV than each test would need to be validated against that host tissue.

- *How do you validate these techniques against the existing protocol?*

If changes were required you would need to invest at least 1-2 weeks of work.

Q7: *Are you or your organization doing active surveillance for this EPP?*

No, there is no surveillance for MDMV by QDPI. I am not aware of anyone in Australia conducting any active surveillance at the moment. Past research projects on JGMV was a amore indirect of passive method of surveillance for MDMV. However there are no current research projects on this virus.

*-If yes, how often?*

*- If no do you think this would be worthwhile?*

It would be worthwhile in providing samples for the diagnostics lab to practise the protocols. It would also provide evidence for the absence of the pathogen. However it is likely that an active surveillance program would not increase the chance of finding the pathogen.

Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. Geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*

Have not done so to date. I keep up to date with plant virology literature, and therefore indirectly may come across new information on MDMV. But, I have not conducted a methodical literature search on MDMV. Ideally this should occur once yearly (10 hrs/year).

Q9: *Do you stay in touch with experts internationally?*

No, only on an ad-hoc basis

*- If yes, how often?*



Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*

No. This is a weakness of this protocol at the moment.

- *If yes, are they “up to speed” with the diagnostic protocol?*

Not applicable

- *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*

- *If no, what would be required for a second lab to be able to conduct the protocol?*

The second lab would need to purchase the reagents (primers, antisera etc – approx cost \$500.00). There would be a training component, likely to be straight forward as the second lab would be experienced in conducting these standard tests. I anticipate that this would take approximately 10 hours to do,. This would also need to conduct the 6 monthly test to ensure reagents and positive controls are working (10 hr/yr)

## **2.13: Pea early browning virus (PEBV)**

### **Background Information**

*Diagnostician:* Angela Freeman

*EPP Name:* Pea early browning virus (PEBV)

*Date EPP protocol completed:*2007

How many samples/yr do you screen for the EPP?

This protocol will be used routinely in our Post Entry Quarantine lab to screen faba bean lines (approx 50 tests/yr)

### **Questions:**

Q1: *What diagnostic tests do you use for your EPP?*

RT-PCR

QRT-PCR

We can also do Electron Microscopy and Herbaceous Indexing if required.

Q2: *If PCR, how often do you check*

– *primers?*

– *Positive and negative controls?*

We do the RT-PCR several times a year on a routine basis and so our primers and positive controls are constantly being tested and validated. We use the same set of primers for both RT-PCR and QRT-PCR. We also have a AQIS permit to import PEBV infected tissue and we are able to inoculate indicators in our PEQ glasshouse which helps us to have live PEBV tissue.

– *Have new reagents been released which would improve the performance of your test?*

We routinely do a serial dilution of our positive control to test the sensitivity of our test system. This is important as we routinely pool samples for screening.

○ *If yes, what is required to incorporate these improvements into the protocol?*

– *Other issues related to PCR.*

We have used this PCR system for the detection of PEBV for several years and we know that it works well.

- Q3: N/A *If ELISA, how often do you*
- *re-order antisera?*
  - *Check positive controls?*
  - *Have new reagents been released which would improve the performance of your test?*
- Q4: N/A *If isolation and culturing is required, how often do you*
- *prepare stock solutions for selective media?.*
- Q5 N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*
- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
 No, this protocol is current and does not need upgrading.
  - o *If yes, what is required to incorporate these changes into the existing manual?*
 - *How do you validate these techniques against the existing protocol?*  
 The quantitative RT-PCR protocol was developed and validated as part of an associated research project.
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
 No.  
*-If yes, how often?*  
 Vic DPI may be conducting a survey this season but we are unsure.  
*- If no do you think this would be worthwhile?*  
 Not really.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
 This is an ongoing process. We check the literature once a year specifically for papers and information on PEBV (8 hrs/yr). I keep in touch with literature on pulse viruses on a monthly basis (6hrs/yr).
- Q9: *Do you stay in touch with experts internationally?*  
 Not specifically for this virus. PEBV is more a problem in Europe and our major virus problems in pulses are more in line with those in the Middle East.  
*- If yes, how often?*
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
 Yes  
*- If yes, are they “up to speed” with the diagnostic protocol?*  
 No, not really. There has never been a need for this lab to run the test.  
*- What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
 A 3-4 hr work shop, and supply of appropriate positive controls would be required to get a lab “up –to-speed”.

## **2.14: Broad bean stain virus (BBSV)**

### **Broad bean true mosaic virus (BBTMV)**

### **Broad bean mottle virus (BBMV)**

#### **Background Information**

*Diagnostician:* Angela Freeman

*EPP Name:* Broad bean stain virus (BBSV)  
Broad bean true mosaic virus (BBTMV)  
Broad bean mottle virus (BBMV)

*Date EPP protocol completed:* 2007

How many samples/yr do you screen for the EPP?

Is part of the routine indexing of pulse lines screened for quarantinable viruses in our PEQ facility located at Horsham.

#### **Questions:**

Q1: *What diagnostic tests do you use for your EPP?*  
ELISA, TBIA

Q2: N/A *If PCR, how often do you check*  
– *primers?*  
– *Positive and negative controls?*  
– *Have new reagents been released which would improve the performance of your test?*  
    ○ *If yes, what is required to incorporate these improvements into the protocol?*  
– *Other issues related to PCR.*

It would be nice to get support to develop PCR tests for these three viruses.

Q3: *If ELISA, how often do you*  
– *re-order antisera?*  
We use ICARDA antisera. It is a polyclonal antiserum and both ICARDA and I have a good supply of the antisera. We do our own conjugation of Alkaline Phosphatase to the IgG and a subsequent serial dilution “Checkerboard” ELISA plate to optimise IgG/ conjugate concentrations. This process indirectly checks the sensitivity of the assay.  
(10 hour/yr)  
– *Check positive controls?*  
Because of our PEQ facility and a quarantine permit that I hold, I am able to import dried tissue from overseas and we are also able to inoculate fresh tissue with virus isolates in our PEQ isolate. Therefore limitations with positive controls are not issue for our protocols.  
(10 hours/yr)

– *Have new reagents been released which would improve the performance of your test?*

No, we use the traditional DAS ELISA technique.

▪ *If yes, what is required to incorporate these improvements into the protocol?*

– *other issues related to ELISA*

We use the tissue blot immunoassay (TBIA) in combination with our ELISA tests. We tend to use ELISA to test pooled samples and TBIA to screen individual plants.

It is very important to have well trained and experienced staff running the tests as this ensures a high standard of testing and maximizes your chances of detecting infected plants.

Q4: N/A *If isolation and culturing is required, how often do you*

– *prepare stock solutions for selective media?*

– *Check controls (if present)?*

– *Other issues related to fungal and bacterial isolations.*

Q5: N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*

Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*

Yes, in a way. It would be good to develop PCR protocols for these viruses.

○ *If yes, what is required to incorporate these changes into the existing manual?*

Improvements could be made as part of the on-going PEQ-AQIS project. However this project is not funded to support significant research exercises.

- *How do you validate these techniques against the existing protocol?*

I would validate any new protocol using the range of virus isolates that we hold under quarantine at Horsham DPI.

Q7: *Are you or your organization doing active surveillance for this EPP?*

Our organization may conduct a survey for these viruses this season.

-*If yes, how often?*

BBSV was surveyed for once as a one-off request from AQIS. I am not aware of any surveys for BBMV and BBTMV.

- *If no do you think this would be worthwhile?*

It would be worthwhile to survey for these viruses to confirm that these viruses are “known not to occur”. As it stands the status is “Not known to occur”.

Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*

I scan the literature on these viruses on a monthly basis and conduct a designated yearly review on each virus

(21 hrs/yr)

- Q9: *Do you stay in touch with experts internationally?*  
 Yes  
 - *If yes, how often?*  
 I have regular contacts with experts on these viruses, particularly from ICARDA (Syria).  
 (10 hours/yr)
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
 Yes  
 - *If yes, are they “up to speed” with the diagnostic protocol?*  
 Not really, as have not had to use the protocol.
- *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
 A 4 hour workshop is all that would be required.

## **2.15: Pea Enation mosaic Virus (PEMV)**

### **Background Information**

*Diagnostician:* Angela Freeman

*EPP Name:* Pea Enation mosaic Virus (PEMV)

*Date EPP protocol completed:* 2007

How many samples/yr do you screen for the EPP?

Is part of the routine indexing of pulse lines screened for quarantinable viruses in our PEQ facility located at Horsham.

### **Questions:**

Q1: *What diagnostic tests do you use for your EPP?*  
 ELISA, TBIA

Q2: **N/A** *If PCR, how often do you check*  
 – *primers?*  
 – *Positive and negative controls?*  
 – *Have new reagents been released which would improve the performance of your test?*  
     ○ *If yes, what is required to incorporate these improvements into the protocol?*  
 – *Other issues related to PCR.*

It would be good to develop PCR protocols for this virus. PEMV can exist in combination with an umbravirus and it be worthwhile to extend the diagnosis to detect this virus as well.

Q3: *If ELISA, how often do you*  
 – *re-order antisera?*  
 We use ICARDA antisera. It is a polyclonal antiserum and both ICARDA and I have a good supply of the antisera. We do our own conjugation of Alkaline Phosphatase to the IgG and a subsequent serial dilution “Checkerboard” ELISA plate to optimise IgG/ conjugate concentrations and indirectly check the sensitivity of the assay.  
 (10 hour/yr)

– *Check positive controls?*

Because of our PEQ facility and a quarantine permit that I hold, I am able to import dried tissue from overseas and we are also able to inoculate fresh tissue with virus isolates in our PEQ isolate. Therefore limitations with positive controls are not issue for our protocols.

(10 hours/yr)

– *Have new reagents been released which would improve the performance of your test?*

No, we use the traditional DAS ELISA technique.

▪ *If yes, what is required to incorporate these improvements into the protocol?*

– *other issues related to ELISA*

We use the tissue blot immunoassay (TBIA) in combination with our ELISA tests. We tend to use ELISA to test pooled samples and TBIA to screen individual plants.

It is very important to have well trained and experienced staff running the tests is this ensures a high standard of testing and maximizes your chances of detecting infected plants.

Q4: N/A *If isolation and culturing is required, how often do you*

– *prepare stock solutions for selective media?*

– *Check controls (if present)?*

– *Other issues related to fungal and bacterial isolations.*

Q5: N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*

Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*

Yes, in a way. It would be good to develop PCR protocols for these viruses.

○ *If yes, what is required to incorporate these changes into the existing manual?*

Improvements could be made as part of the on-going PEQ-AQIS project. However this project is not funded to support significant research exercises.

- *How do you validate these techniques against the existing protocol?*

I would validate any new protocol using the range of virus isolates that we hold under quarantine at Horsham DPI.

Q7: *Are you or your organization doing active surveillance for this EPP?*

Our organization may conduct a survey for these viruses this season.

-*If yes, how often?*

- *If no do you think this would be worthwhile?*

It would be worthwhile to survey for this virus to confirm that these viruses are “known not to occur”. As it stands the status “Not known to occur”.

- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
 I scan the literature on these viruses on a monthly basis and conduct a designated yearly review on each virus  
 (21 hrs/yr)
- Q9: *Do you stay in touch with experts internationally?*  
 Yes  
 - *If yes, how often?*  
 I have regular contacts with experts on these viruses, particularly from ICARDA (Syria).  
 (10 hours/yr)
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
 Yes  
 - *If yes, are they “up to speed” with the diagnostic protocol?*  
 Not really, as have not had to use the protocol.  
 - *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
 A 4 hour workshop is all that would be required.

## **2.16: Plum pox virus**

### **Background Information**

*Diagnostician:* Brendan Rodoni

*EPP Name:* Plum pox virus

*Date EPP protocol completed:* 2004

How many samples/yr do you screen for the EPP? 2/yr

### **Questions:**

Q1: *What diagnostic tests do you use for your EPP?*  
 PCR, ELISA

Q2: *If PCR, how often do you check*  
 – *primers?*  
 When we do the test.  
 – *Positive and negative controls?*  
 When we do the test.  
 Ideally the primers and positive controls would be validated twice a year.  
 – *Have new reagents been released which would improve the performance of your test?*  
 Yes, new RT-PCR systems have been developed. We introduced this system into our lab using protocols for other endemic pathogens and it was relatively easy to transfer the changes into the PPV protocol. It has influenced the concentration of primers that we use in our standard reaction, which is different to that recommended in the original publication.

- *If yes, what is required to incorporate these improvements into the protocol?*

– *Other issues related to PCR.*

A Quantitative PCR protocol has been reported. We have adopted QPCR using Syber green, but we are also happy to persist with conventional PCR as well. These changes were developed and validated as part of a research project. We also introduced a high throughput capacity for nucleic acid from hosts of PPV. This process was also validated by an associated research project (Victorian Government funded).

Q3: *If ELISA, how often do you*

– *re-order antisera?*

Infrequently. This is a major problem with ELISA protocols for EPPs.

– *Check positive controls?*

We have run out of our positive control for PPV. We did have some “Expressed” PPV CP that we used. We are not allowed to import PPV infected tissue that we could use as a positive control for ELISA. This is also a problem with ELISA.

– *Have new reagents been released which would improve the performance of your test?*

No.

▪ *If yes, what is required to incorporate these improvements into the protocol?*

– *other issues related to ELISA*

Re-ordering antisera after the expiry date on the test kit is an important issue. ELISA kits cost money and no time is allocated to do “dummy” runs to ensure the kit works. This is also difficult to do in the absence of a positive control.

Q4: N/A

*If isolation and culturing is required, how often do you*

– *prepare stock solutions for selective media?*

– *Check controls (if present)?*

– *Other issues related to fungal and bacterial isolations.*

Q5: N/A

*If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*

Q6:

*Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*

New strains have been reported.

○ *If yes, what is required to incorporate these changes into the existing manual?*

I am confident that the PCR we use will detect the new strains but I am not sure if the ELISA test will detect these new strains.

- *How do you validate these techniques against the existing protocol?*

I discuss these issues with colleagues/Experts from overseas.



- Q7: *Are you or your organization doing active surveillance for this EPP?*  
 No.  
 -If yes, how often?  
 - If no do you think this would be worthwhile?  
 Active surveillance is good for diagnosticians as the receipt of samples on a regular basis ensures reagents etc are functional and it helps to train staff.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
 Every month  
 (6 hrs/yr)
- Q9: *Do you stay in touch with experts internationally?*  
 Yes  
 - If yes, how often?  
 I contact experts on issues about PPV about once a year. I also attend an ISHS international conference on viruses of small trees and berries that is held every three years.  
 (3 days/yr).
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
 Yes  
 - If yes, are they “up to speed” with the diagnostic protocol?  
 Yes  
 - What training is required to ensure that this and other labs are capable of conducting this EPP protocol?  
 A two day workshop would be required. We held a workshop in 2004 for PPV diagnostics. This was a valuable exercise and should be held routinely (? Every 5 years).

## **2.17: Red clover vein mosaic virus (RCVMV)**

### **Background Information**

*Diagnostician:* Angela Freeman

*EPP Name:* Red clover vein mosaic virus (RCVMV)

*Date EPP protocol completed:* 2007

How many samples/yr do you screen for the EPP?

Is part of the routine indexing of pulse lines screened for quarantinable viruses in our PEQ facility located at Horsham.

### **Questions:**

Q1: *What diagnostic tests do you use for your EPP?*

PCR – Commercial Kit

Q2: *If PCR, how often do you check*

– primers?

– Positive and negative controls?

The primers and positive control that we use for this virus are purchased from a commercial diagnostic company in the USA. A limitation with this test therefore is that we do not know what region of the RCVMV genome that we are

amplifying. We rely on the validation of the commercial company to ensure the quality of the reagents and that the select primers will detect most strains of this virus.

- *Have new reagents been released which would improve the performance of your test?*

Ideally it would be good to design our own primers.

- o *If yes, what is required to incorporate these improvements into the protocol?*

This would require a small research project (student honours) to complete this work.

- *Other issues related to PCR.*

Q3: N/A *If ELISA, how often do you*

- *re-order antisera?*
- *Check positive controls?*
- *Have new reagents been released which would improve the performance of your test?*
  - *If yes, what is required to incorporate these improvements into the protocol?*
- *other issues related to ELISA*

Q4: N/A *If isolation and culturing is required, how often do you*

- *prepare stock solutions for selective media?*
- *Check controls (if present)?*
- *Other issues related to fungal and bacterial isolations.*

Q5: N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*

Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*

Yes. It would be good to develop a PCR protocol for this virus so that we knew what region of the genome we were amplifying and validate these primers against our collection of positive controls for RCVMV. .

- o *If yes, what is required to incorporate these changes into the existing manual?*

Improvements could be made as part of the on-going PEQ-AQIS project. However this project is not funded to support significant research exercises.

- *How do you validate these techniques against the existing protocol?*

I would validate any new protocol using the range of virus isolates that we hold under quarantine at Horsham DPI.

Q7: *Are you or your organization doing active surveillance for this EPP?*

Our organization may conduct a survey for this virus this season.

-*If yes, how often?*

- *If no do you think this would be worthwhile?*

It would be worthwhile to survey for this virus to confirm that it is “known not to occur”. As it stands the status “Not known to occur”.

Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*

I scan the literature on these viruses on a monthly basis (3 hrs/yr) and conduct a designated yearly review on each virus (5 hrs/yr)

- Q9: *Do you stay in touch with experts internationally?*  
Yes  
- *If yes, how often?*  
I have regular contacts with experts on these viruses, particularly from ICARDA (Syria).  
(4 hours/yr)
- Q10: *Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*  
Yes  
- *If yes, are they “up to speed” with the diagnostic protocol?*  
Not really, as have not had to use the protocol.  
- *What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*  
A 4 hour workshop is all that would be required.

## **2.18: Potato spindle tuber viroid (PSTVd)**

### **Background Information**

*Diagnostician:* Deb Hailstones

*EPP Name:* Potato spindle tuber viroid (PSTVd)

*Date EPP protocol completed:* 2004

How many samples/yr do you screen for the EPP? 20 – 30 tests/yr

### **Questions:**

- Q1: *What diagnostic tests do you use for your EPP?*  
RT-PCR, Bioassay
- Q2: *If PCR, how often do you check*  
– *primers?*  
Check the primers as the need arises. A “Stock” tube of primers is also available if fresh workable solutions of primers are required.  
– *Positive and negative controls?*  
We regenerate our positive controls from dried plant tissue that is infected with PSTVd. We inoculate our bioassay plants as a positive control if we need to store some more PSTVd-infected dry tissue.  
Maintenance of this material requires 1-2 hrs labor/year.  
– *Have new reagents been released which would improve the performance of your test?*  
No, we use the primers and enzyme systems that were available at the time the diagnostic protocol was compiled.  
○ *If yes, what is required to incorporate these improvements into the protocol?*

– *Other issues related to PCR.*

None. However, we also conduct a Bioassay for the detection of PSTVd. This test requires the maintenance of a quarantine glasshouse. At present this bioassay is the only test conducted in the glasshouse as part of a diagnostic protocol. Some research projects also use this facility and therefore it is difficult to estimate the annual cost of maintaining this facility. It is a cost that should be included when considering the cost of maintaining a diagnostic protocol.

- Q3: N/A *If ELISA, how often do you*
- *re-order antisera?*
  - *Check positive controls?*
  - *Have new reagents been released which would improve the performance of your test?*
    - *If yes, what is required to incorporate these improvements into the protocol?*
  - *other issues related to ELISA*
- Q4: N/A *If isolation and culturing is required, how often do you*
- *prepare stock solutions for selective media?*
  - *Check controls (if present)?*
  - *Other issues related to fungal and bacterial isolations.*
- Q5 N/A *If the protocol is based on taxonomy, what ongoing activities are required to ensure a diagnostic capacity for the EPP?*
- Q6: *Do you think the current protocol has been superseded by new platforms and/or the emergence of new strains/pathovars of the EPP?*  
No.
  - *If yes, what is required to incorporate these changes into the existing manual?*- *How do you validate these techniques against the existing protocol?*
- Q7: *Are you or your organization doing active surveillance for this EPP?*  
No.  
-*If yes, how often?*  
- *If no do you think this would be worthwhile?*  
I agree with the concept in principle. However I can see that this is an expensive exercise to both government and industry.
- Q8: *How often do you keep in touch with current literature relating to the EPP (i.e. geographic distribution, emergence of new diseases, changes in the host range of the EPP)?*  
I rely on Brendan Rodoni to provide input into new literature that has an impact on the PSTVd manual.
- Q9: *Do you stay in touch with experts internationally?*  
I rely on Brendan Rodoni to keep up to date.  
- *If yes, how often?*

Q10:

*Is there a second laboratory in Australia that has the capacity to verify the diagnosis of this EPP?*

Yes.

*- If yes, are they “up to speed” with the diagnostic protocol?*

Within my lab I have had to ensure training of technical staff. In the last 5 years I have had to train 2 staff to level that I am satisfied that they can do PSTVd testing on industry samples. Again a portion of this training cost needs to be included when considering the cost of maintaining a diagnostic protocol.

*- What training is required to ensure that this and other labs are capable of conducting this EPP protocol?*

See above.

### **Appendix 3: Responses to the General Questions (Q 11 – 13)**

#### **Diagnostician 1:**

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- *lab accreditation*

I can see why lab accreditation is important. The problem is that the maintenance of a “State of Accreditation” is time consuming and expensive and is generally under resourced. This in turn generates stress within the lab environment.

- *staffing*

This is a major issue. Training is expensive and experience is critical. It is becoming increasingly difficult to maintain staff due to soft money contracts.

- *other*

Resources for a well run lab are often lacking. Time is often required to optimize procedures, to make a diagnostic test better or to increase the efficiency of lab procedures. To do this takes time (i.e. 40 hours to optimize a new PCR protocol, or to create “multiplex” PCR to cut costs of diagnosis etc). Because this type of work is not seen as a priority money is not provided to support this work.

*Q12: Do you think “ring tests” between Australian labs would be useful?*

Yes, but again ring tests would require funding support. Ring tests would help maintain a diagnostic capability and is a great way to ensure that diagnostic labs can correctly use a protocol. I think it would be best if the Ring testing was managed from a national level. However I am not sure who would be the best group to run these ring tests.

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

No not really as we have had several EPP incursions in several years and we usually harvest infected material from our bioassays and store as dried tissue.

#### **Diagnostician 2:**

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- *lab accreditation*

We have AQIS accreditation for our labs and glasshouse as part of our AQIS contract. This is not a lab standard as such but more for security/containment reasons. We would look at some level of accreditation if it became a legal requirement to have accreditation to test for EPPs.

- *staffing*

Job security for staff is an issue. It is important that the diagnosticians and technicians are active and are using their tests/skills on a routine basis. This ensures that when screening AQIS material the tests are conducted to the highest standard. At least one person within the group should be employed on an on-going basis who has a broad experience in this field and who has experience in the interpretation of test results.

- other

For virus diagnostics PCR is going to continually increase, particularly as the current supply of antisera runs out. It will become important for labs to not only have a solid PCR capability but also an ability to screen large numbers of samples using PCR.

*Q12: Do you think “ring tests” between Australian labs would be useful?*

Ring tests would be good, it would certainly improve the confidence of a lab to conduct tests of EPPs.

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

No, we have a AQIS permit to import plant issue to support our AQIS program.

*General comments: (Please add any other comments that you see are relevant to the maintenance of an EPP protocol).*

Pest Risk Analysis is an important component of a diagnostic manual. I am aware of some protocols being generated at the moment that do not include this analysis and I believe it results in an inferior diagnostic capability. The standard of diagnostic protocols should be regulated at a federal level.

### **Diagnostician 3:**

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

Lab accreditation is an issue. Our lab is trying to establish a Laboratory Manual Quality System. This system is not as intense as NATA accreditation. We anticipate that to comply with this system will add 30% to the time that it would normally take to conduct a test. We anticipate that it would cost around \$10,000.00 per year to maintain this lab management system for an EPP.

Staffing is a major issue on an on-going basis. It is not so much the senior scientists but also for the junior scientists and technicians that actually do most of the lab work. It is very difficult to maintain skilled staff with short term contracts

*Q12: Do you think “ring tests” between Australian labs would be useful?*

Ring tests could form part of the 6 monthly screen of positive controls. The problem with the ring test is that an independent person or organization would have to organize the ring test and would therefore need to have the positive controls etc for each EPP. This would be a massive task, cost money and place extra time on the diagnostic laboratories. What may be a better approach is to have a ring test for PCR or ELISA and send samples of a common pathogen (i.e. TSWV) to all the labs and compare the results between the labs. This would test the competence of each lab to do a “type” of test. The responsibility of organizing the ring tests could be rotated through the key, major labs.

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

Yes. There appears to be great variation in the conditions of import permits for both plant material and antisera, I am aware of variation between import permit conditions between labs within Australia. This is frustrating. The permits need to be renewed every 2 years.  
(4 hours per annum)

**Diagnostician 4:**

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- *lab accreditation*

Is a requirement for diagnostic labs in the ideal world. However, the level of accreditation has to be at an appropriate level that is achievable and sustainable. NATA is the common industry standard. Several labs in Australia have this accreditation. All labs struggle to maintain this level of accreditation. Lab accreditation needs to be properly funded on an ongoing basis.

- *staffing*

Staff associated with this line of work should not be on short term funding. The level of expertise and knowledge of these diagnosticians needs to be appropriately respected and valued.

*Q12: Do you think “ring tests” between Australian labs would be useful?*

Yes. I am not sure who is the correct body in Australia to run the ring tests. It should be organized by a national body.

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

Not at the moment.

**Diagnostician 5:**

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- *lab accreditation*

Lab accreditation is important and if established correctly can take enormous pressure off the lab diagnostician. There are more and more court cases in which diagnostic test results are submitted as evidence and industry want surety in diagnosis. The down-side of accreditation such as NATA is the costs to implement the systems to support the accreditation and the increased on-going costs to maintain accreditation.

- *staffing*

It is important that more than one person knows how to use the diagnostic protocol as they are a back-up if you are not present.

- *other*

Maintenance of positive controls is an issue. Non-designated labs/poor facilities can also cause problems. It would be nice to conduct pathogenicity assays for the exotics, but it is not essential for this protocol as sequencing data of PCR products is the ultimate information required to confirm a positive test result. For other pathogens however this may not be the case and it would be good if we had facilities that allowed us to do a pathogenicity assay. Pathogenicity assays are essential if we are trying to understand how an EPP may behave under Australian conditions on Australian host plants.



*Q12: Do you think “ring tests” between Australian labs would be useful?*

It would be great if this was to happen. The organization to run or oversee this process should be federal, possibly OCCPO.

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

I had to travel overseas to get the positive DNA for the EPP test and on the same trip I also validated test protocols in the manual. It is becoming more and more difficult to get positive controls in to the country.

*General comments: (Please add any other comments that you see are relevant to the maintenance of an EPP protocol).*

A steady flow of samples is ideal for maintaining an EPP protocol. Ring testing would be a good activity to be involved in.

### **Diagnostician 6:**

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- *lab accreditation*

It takes a lot of time and energy to obtain lab accreditation. We currently have ISO accreditation and I do not believe it is worthwhile for my lab to go to the level of NATA accreditation. It is frustrating when our managers insist that our labs reach an accreditation level and do not provide adequate resources to maintain the desired level of operation.

- *staffing*

Retaining experienced staff is the most critical area in maintaining a diagnostic capability for EPPs. This is not only staff that are experienced in the lab, but also field based staff who are familiar with the industry, the agronomy of crops and who have a strong handle on the endemic pests and pathogens that affect the crop in the field. Fortunately we are currently receiving this support from our organization.

- *other*

There is a constant trade-off within the organization between staff working on problems that we already have in Australia and working on problems (EPPs) that we do not have yet. This is a fine balance. It is important to have people working in the field as these are the people that are most likely to detect the problem.

*Q12: Do you think “ring tests” between Australian labs would be useful?*

Yes, ring tests are a good idea, and should be conducted between labs working on the EPP within Australia as well as labs from overseas. The overseas labs are important as we need to ensure that the diagnosis that we generate is accepted off-shore. Participation in global ring tests assures this acceptance. Ring tests also foster contact between fellow “like-minded” scientists and fuels collaboration.

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

No problems with importing DNA.

*General comments: (Please add any other comments that you see are relevant to the maintenance of an EPP protocol).*

I have experienced some problems with a lack of corporate memory within some federal bodies due to the high turnover of staff. Unfortunately the new staff are not sure what work has been commissioned in past years and I worry that they will commission work that has already been done.

**Diagnostician 7:**

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- *lab accreditation*

I don't think it essential but it is desirable. My organization is considering accreditation because it will be mandatory, nationally.

- *staffing*

On-going funding for staff is a major issue, as is equipment maintenance, Recognition by your employer that EPP diagnostics is what you should be doing as important, as this recognition helps secure funds.

*Q12: Do you think "ring tests" between Australian labs would be useful?*

Yes, ring tests are a good idea. The National Diagnostic Network should be involved in this process

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

Yes, I did have difficulties in getting DNA for the EPP. I tried four different labs and finally a colleague in Scotland who I know well got some DNA for me.

DNA for other EPPs has been easier. AQIS has recently changed the rules and I now need 1 permit for each species of dried specimens.

*General comments: (Please add any other comments that you see are relevant to the maintenance of an EPP protocol).*

It is not clear how to get your EPP diagnostic protocol accepted nationally. SPHDS role to drive this process and advise/inform the PHC.

**Diagnostician 8:**

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- *lab accreditation*

No comment

- *staffing*

Costs associated with relevant staff is a major issue. Currently there are no funds to support relevant staff to work on or maintain the EPP protocol. Current projects do not support staff to ensure that we are "lab ready" to conduct the EPP protocol.

*Q12: Do you think "ring tests" between Australian labs would be useful?*

Could be useful.

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

*General comments: (Please add any other comments that you see are relevant to the maintenance of an EPP protocol).*

We have had no feedback from the funding body that supported the development of the protocol. We do not know how the protocol has been received, or if in fact it is the national protocol that would/should be used by other labs. We do not know if the funding body has circulated the protocol to third parties. I have found it disappointing that there is no clear direction at the national level on what processes are involved to establish a protocol as the national diagnostic standard.

**Diagnostician 9:**

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- lab accreditation
- staffing

Retaining skills and expertise is the biggest challenge.

- other

Operating expenses, particularly for PCR is a problem. Maintenance of reference collections and associated databases is also critical and funds are often limited to support this work.

*Q12: Do you think "ring tests" between Australian labs would be useful?*

Ring tests for PCR testing is very important and is a great way to standardize the test at a national level.

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

We have had no difficulties in obtaining reference specimens for this pest as we can import preserved specimens.

*General comments: (Please add any other comments that you see are relevant to the maintenance of an EPP protocol).*

I am very supportive of this survey. For this pest it would be worthwhile to have a second lab that could complete the test as this help preserve the expertise nationally.

**Diagnostician 10:**

*Q11: What issues do you see as important at your work place to ensure a diagnostic capacity for an EPP?*

- lab accreditation

Gaining accreditation is a huge effort and requires significant support from your organisation. Once established there are some significant benefits with NATA accreditation and includes an assurance in the quality of the test and a commitment to ongoing training of staff. All staff must be a part of the accreditation system. I anticipate that between 5-10% of staff time is dedicated to maintaining a NATA standard. A downside with NATA accreditation is that there may be a reluctance to change or upgrade a protocol due to the amount of time and energy it takes to incorporate the changes in to a NATA protocol.

- *staffing*

Maintaining skilled staff is an on going and most pressing issue. Most of our trained staff are casuals and have been so for several years. One worker wanted to be an employee of the organization after working for nearly 10 years as a casual employee. Unfortunately the organization did not offer this person a more permanent job and this person has now left our department and has been hard to replace.

- *other*

Annual training of staff at our institute and nationally is critical.

*Q12: Do you think “ring tests” between Australian labs would be useful?*

Yes. Assessment ensures diagnosticians are familiar with the protocol and is an effective way to ensure that the protocol is working and that staff are trained. Ring tests or “inter-lab” tests are a mandatory requirement of our NATA accreditation.

*Q13: Do you have any difficulties in obtaining appropriate positive controls for your diagnostic tests?*

We have no problems in getting material safely into Australia. The big problem is getting people from overseas to actually send the material. It is difficult to get people to respond to your requests and usually comes down to your own established networks. It may be easier if we could pay international scientists for the specimens they send us, or involve them in our ring tests.

*General comments: (Please add any other comments that you see are relevant to the maintenance of an EPP protocol).*

Accreditation helps with using a protocol for EPPs. It is just a significant investment getting the protocol to the NATA level. Rationalisation of labs and the expertise and tests they offer across Australia is required to improve the efficiency of our diagnostic labs across Australia.