Report Cover Page

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Title
The use of new technologies for rapid, field-based (point-of-care) testing in the detection of emergency animal diseases
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Summary

Rapid advances are occurring in the field of point-of-care (POC) tests for infectious diseases, some of which are expected to be adopted as an aid in diagnosis of emergency animal diseases (EADs). It is not possible to predict which technologies will become available commercially but future POC tests are expected to be based on improved antigen detection systems with greater analytical sensitivity and/or replacement of existing antigen-based tests by tests based on nucleic acid detection, with or without amplification. These tests will cover multiple pathogens and/or multiple characteristics of pathogens. Tests will likely require minimal operator training to perform (sample-to-answer systems).

Prompt diagnosis of EADs minimises their economic and public health effects. POC tests can reduce the time required to make a diagnosis of these diseases. A decision by public sector agencies to use POC tests as an aid in managing EADs depends on the purpose of testing, including the stage of the outbreak and response, whether the benefits outweigh the costs of test and equipment deployment, and whether they offer advantages over existing protocols for disease management based on clinical suspicion and submission of samples to a laboratory. Suitable validated POC tests, once available, supported by appropriate quality management systems, could be deployed advantageously for a number of EADs in Australia if they meet one or more of these criteria.

As with all diagnostic tests there are risks that false positive or negative results will be generated and released. These risks will need to be anticipated and managed, and become more important if farmers have access to tests capable of detecting agents causing economically important EADs. Background information on new tests and technologies is provided as are examples of how new POC tests could be applied for several EADs including highly pathogenic avian influenza and foot-and-mouth disease.

The purpose of this report is to review the use of new technologies for rapid, field-based testing in the early detection of major emergency animal diseases.

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Executive Summary

Demand for point-of-care (POC) tests for animal and human diseases is increasing. Most of the POC and near-POC tests sold commercially have been for non-infectious conditions but rapid advances are occurring in the development of POC tests for infectious diseases. Some of these are expected to be adopted for POC tests for animal diseases.

Most POC tests available commercially for emergency animal diseases (EADs) use mature technologies based mainly on antibody capture of target antigens in samples; some of these tests have relatively low analytical sensitivity but are still suitable for 'rule-in' testing with appropriate sample selection. Any test based on nucleic acid amplification and detection has the potential to be adapted for use at POC or near-POC using portable equipment. Robust systems for conducting POC tests using nucleic acid amplification for EADs have been developed but have not been deployed widely in the field in Australia. Some of the equipment for these POC tests has been designed to allow assays to be monitored remotely providing scope for improved quality management for tests conducted in the field.

Prompt diagnosis of EADs minimises their economic and public health effects. POC and near-POC tests can reduce the time required to make a diagnosis of these diseases. At present, with the exception of anthrax, they play no role or only a minor supporting role in the diagnosis of these diseases in Australia. Highly specific (and, in some cases, highly sensitive) POC tests exist for many EADs that can be used to 'rule in' the presence of particular agents.

A decision by public sector agencies to use POC tests as an aid in managing EADs depends on the purpose of testing (including the stage of the outbreak), whether the benefits of POC testing (on site) outweigh the costs of deployment (including the cost of storage of equipment and reagents in multiple locations, therefore allowing rapid local deployment of the tests during an outbreak), whether they offer advantages over existing protocols for disease management based on clinical suspicion, and whether they offer significant advantages (especially time to diagnosis) over delivery to and testing of samples at a state or national laboratory and in reducing the work load in these laboratories during all phases of an outbreak and the subsequent response.

The areas where suitable POC tests could be deployed advantageously for EADs in Australia include:

i) pre-outbreak surveillance and preliminary diagnosis, with some testing conducted by producers using multiplexed test kits;

ii) testing of the index case for disease outbreaks in remote locations where delays in sample submission to a laboratory beyond 24 hours are expected;

iii) use as a triage tool for determining which samples to submit from an index farm;

iv) to support decisions on management of diseases of potential public health significance, especially if a positive result is obtained using a highly specific test;

v) to assist in taking early decisions on the fate of animals on dangerous contact premises or other high risk premises within the restricted area around the index case, including the use of POC tests to detect latently infected animals or animals with equivocal clinical signs;

vi) surveillance and investigation of farms in the restricted area and control area during the response to an outbreak;

vii) as a tool for assessing integrity of zones and compartments;

viii) to assist in product and animal movement during an outbreak; and

ix) in demonstrating freedom from infection.

POC tests should only be deployed if they have been validated for the purpose(s) for which they are being used and if appropriate quality management systems are in place to ensure delivery of valid results. If deployed, they will not replace laboratory testing but will reduce the load of samples submitted for urgent testing during an outbreak. A selection of samples that test negative and all samples that are positive on a POC test will still need to be submitted for further testing at a laboratory to obtain additional information to support disease control and epidemiological studies.

The technology for development of the next generation of POC tests is available or in the process of being refined through close collaborations between biologists and engineers. Developments in this area are expected to result in highly sensitive and specific assays capable of detecting a broad range of pathogens in one test run, using 'sample-to-answer' technology (i.e. little or no sample preparation required), that require minimal technical knowledge and training to perform. New technologies for POC tests are expected to be based on improved antigen detection systems with greater analytical sensitivity and/or replacement of existing antigen-based tests by tests based on nucleic acid detection, with or without amplification.

In human health, a range of new technologies is being used in clinical microbiology to replace culture. A gap still exists between ideal and actual performance for many tests, but is closing. The lessons learned from development and use of new generation POC or near-POC tests and technologies for human pathogens will prove invaluable in developing tests for EADs. One pertinent example from human health is the application of POC tests for rapid detection of drug resistant tuberculosis in resource-limited settings.

It is not possible to predict which of the specific technologies available now or undergoing development will be successful commercially as POC tests for EADs. A crucial factor in the development and commercialisation of POC tests is the presence of a suitable market for the end-product, which in the case of many EADs in Australia is limited despite the potential economic importance of some of these diseases (e.g. FMD). Demand for these tests is low until an outbreak (or suspected outbreak) occurs. In the future, some POC tests for production animals will probably cover infectious agents and diseases that are endemic in animals in Australia as well as those that cause EADs. It is expected that some producers will use these tests to assess the health status of their animals once they become widely available and affordable.

As more POC tests become available and especially if they are in a format suitable for use by producers, they will create a number of risks that will need to be managed. These include:

i) the many factors that can lead to misclassification of animals as infected or not infected;

ii) failure by private users to report results of POC tests for EADs (positive and negative) or to send samples for confirmation to veterinary authorities; and,

iii) premature announcement of incorrect results.

These risks, many of which also apply to existing tests, need to be balanced against the advantages offered by POC tests especially in the management of outbreaks once they occur. If POC or near-POC tests are deployed for EAD management the methods of use must be incorporated into emergency plans.

POC tests for five specific diseases (chosen as examples) have been considered in this review. For highly pathogenic avian influenza (HPAI), POC and near-POC tests could play an important role in disease surveillance prior to clinical outbreaks, for early detection of infection in wild birds, preliminary identification of infected flocks of poultry and in testing of

poultry for evidence of the disease during the response to an outbreak. Existing or improved immunochromatographic tests or tests based on nucleic acid amplification could be used, with the latter providing greater sensitivity but currently requiring technically-qualified personnel to conduct tests.

For FMD, POC tests could be used as a support tool during the course of an outbreak either through their use on suspected cases in remote locations, or for testing of suspect cases in the restricted area (RA) and control area (CA) during an outbreak. POC tests based on nucleic acid amplification have the potential to allow early detection of latently infected animals on targeted high risk premises but their use for this purpose would require sample collection and testing of a large part of suspect herds, which would tie up considerable manpower during an emergency response. POC tests for this purpose have not been adopted in other countries despite the availability of tests and suitable equipment to perform the tests. Currently available immunochromatographic tests for FMD antigen are valuable aids for detection of virus in vesicular epithelium but are of limited value in animals with old lesions. Serological tests could provide evidence of exposure to virus as early as 8 days after exposure. Sensitive and specific POC tests for antibodies to FMD virus covering all subtypes, if developed, could prove to be a useful adjunct for rapid testing of herds or flocks of animals with old or atypical lesions unsuitable for tests for detection of the pathogen.

For classical swine fever, existing rapid tests based on antigen detection require basic laboratory facilities and, once suitable tests are developed, could be replaced by multiplexed sample-to-answer test systems that incorporate assays for a range of pathogens capable of causing severe disease, including porcine reproductive and respiratory (PRRS) virus, porcine circovirus-2, classical swine fever virus and African swine fever virus. Such a test would appear to have a role to play in POC testing in pigs globally. Sensitive and specific POC tests for respiratory pathogens covering porcine reproductive and respiratory syndrome (PRRS), classical swine fever, swine influenza, mycoplasmas and other bacterial pathogens would also likely find a market. POC systems based on nucleic acid amplification for detection of classical swine fever virus have been developed and tests for differentiation of vaccine strains from field strains could be adopted for use in the field if required. POC tests could prove valuable for surveillance of feral pig populations in remote locations.

Existing POC tests for anthrax are already used and are highly sensitive and specific on recently dead animals. Future tests would need to be able to detect the agent or its toxins in animals dead for more than 48 hours if they are to replace existing POC tests. Concerns regarding bioterrorism have resulted in a number of devices capable of field deployment for detection of *Bacillus anthracis*, the causative agent.

Near-POC testing is used in other countries for bovine spongiform encephalopathy (BSE) but relies on detection of prion proteins in brain. Finding an alternative marker in blood or other readily collected samples from live animals (i.e. that do not require collection of samples of brain), if feasible, is an avenue being explored but a POC or near-POC test on ante mortem samples from live animals is unlikely to be available for some time.

1. Introduction

The past 50 years have seen quantum leaps in the technology available for detection of pathogens by methods other than culture of the causative agent, initially with the use of radioimmunoassays, followed by enzyme-linked immunosorbent assays (ELISAs) and then methods for amplification, detection and sequencing of nucleic acids, including development of microarrays[1] (see the Glossary for a brief description of various terms used in this review). Agents that could be detected only by culture (and some that could not even be cultured), and that previously took days or weeks to isolate, can now be identified in a few hours (and, in some cases, a few minutes), often in tests that allow detection of multiple agents (see, for example, Lim et al. 2005 for a review of developments).

Other tests available today not only detect infectious agents but also provide additional information on the agent such as its expected sensitivity to certain drugs. Some tests can also detect and measure biomarkers associated with specific disease processes (see, for example, Lipkin and Briese 2007).

Until relatively recently, diagnosis of emergency animal diseases (EADs) involving testing of samples was the province of central reference laboratories. Today, many of the techniques summarised above have been adapted (or are in the process of being adapted) for use in the field or in places outside laboratories, changing the way that diagnostic tests are viewed and performed, and also changing the role of laboratories.

Based on past and current trends, the next 10 to 20 years will almost certainly result in further rapid evolution of test methods and in changes in the role of laboratories, posing new challenges to policy-makers and regulators, who at present are developing or have policies and regulations in place to control how rapid tests for EADs are applied and who can use them. In the not too distant future, it is possible that rapid tests capable of testing animals for a range of pathogens simultaneously on one sample (multiplexed tests), including those associated with EADs, will become available commercially providing new opportunities to rapidly gather information in the field about disease status of individual animals or whole herds.

Methods and technologies viewed as cutting edge today will appear relatively ‘primitive’ in 30 years time, just as a look back to 1980 — the pre-polymerase chain reaction (PCR) era — demonstrates the rapid progress that has been made in diagnostic technologies since then, first with conventional, gel-based PCR and then real-time quantitative PCR. It is important to be prepared for the opportunities and the challenges that these new tests and techniques will afford. The availability of accurate rapid tests coupled with the free availability of information on diseases through the internet could even alter the way veterinary medicine is practised. Information regarding animal diseases and on specific cases is moving from the realm of the veterinary profession to the broader community, forming part of the process of ‘democratisation of information’.[2]

[1] See the following BioRad weblinks for an introduction to developments in the various techniques http://www.biorad.com/evportal/en/AU/evolutionPortal.portal?_nfpb=true&_pageLabel=SolutionsLandingPage&catID=LUSO4W8UU and http://www.biorad.com/evportal/en/AU/evolutionPortal.portal?_nfpb=true&_pageLabel=SolutionsLandingPage&catID=LUSM0E8UU

[2] Examples include websites of ProMED-mail, Flutrackers, US Center for Disease Control and Prevention, Food and Agriculture Organization (FAO), World Organisation for Animal Health (OIE), World Health Organisation (WHO), CIDRAP, The Pig Site and many others, all of which provide information to the public on animal and human diseases and their control prevention and treatment.

Many of the issues related to field-based tests referred to in this document as POC tests have already been considered in Australia by the Sub-committee on Animal Health Laboratory Standards (SCAHLS).[3] Legislation is already in place in Victoria that provides for control of the use of POC tests for notifiable diseases of animals to approved tests only conducted by approved and trained persons. It requires that adequate records are kept by the tester and that the Department of Primary Industries is informed of the results.[4]

However, this is not the end of the process. Once the next generation of POC tests (expected to cover multiple pathogens and requiring minimal training or skill to use) becomes available to farmers it may become more difficult to control access to the tests and to gain access to the results of tests. New ways of managing the risks associated with the use of POC tests will probably be needed. At the same time the availability of accurate and reliable tests will afford opportunities for producers to gain information on agents that will facilitate disease management.

This review focuses on the use of POC and near-POC testing for diagnosis of EADs, with an emphasis on new technologies and their application for EADs of production animals. It provides background information on POC tests (Section 2) and examines the situations under which POC tests could be used for EADs in Australia (Section 3). It explores the management of risks associated with POC tests (Section 4) and also examines some market factors that determine whether POC tests are developed (Section 5). The Appendices provide additional information on the technologies available or being developed (Appendix 1) and review POC tests for five selected important EADs, focusing on avian influenza including HPAI (Appendix 2) and FMD (Appendix 3), with brief reviews of classical swine fever, anthrax and bovine spongiform encephalopathy (BSE)(Appendix 4 to 6) — covering the role these tests play or might play in the future, especially tests based on new technologies, in the event of an outbreak or suspected outbreak of one of these diseases.

The review was prepared at an 'inflection' point along the pathway of POC test technologies. For agent detection, a shift is occurring away from established POC tests based on antibody/antigen reactions with relatively insensitive detection systems to a new generation of tests based around nucleic acid amplification and detection, or on better detection systems for antibody/antigen reactions. A shift is also occurring from tests for single agents to arrays and other tests that can detect multiple agents. Tests based on nucleic acid amplification are well established in central laboratories but are only now (in 2012) gaining some penetration as near-POC tests for selected human pathogens. They are also being developed for use for animal diseases. Tests involving antibody/antigen reactions are becoming more sophisticated and combinations of the two (antigen antibody reactions detected by nucleic acid amplification) are also being developed. This means that, in conducting the review, it has been necessary to look forward to the types of tests that might be developed and available commercially because it is expected that they will offer different advantages and disadvantages to existing tests, and also present new challenges to policy-makers and regulators.

[3] See SCAHLS newsletter December 2010 for a brief summary of deliberations on POC tests. Available at http://www.scahls.org.au/__data/assets/pdf_file/0011/1885133/SCAHLS_newsletter_issue_13_Dec_2010.pdf

[4] See Section 16 in the Livestock Disease Control Act 1994 available at http://www.austlii.edu.au/au/legis/vic/consol_act/ldca1994273/s16.html

2. Background information on point-of-care (POC) tests

2.1 What are point-of-care tests?

Point-of-care (POC) tests are used widely in human medicine as an aid to early diagnosis of diseases and other medical conditions. The market for POC tests for humans has been valued at more than $30 billion globally in 2005 and is dominated by tests for pregnancy, cardiac disease and glucose levels (Bissonette and Bergeron 2010). The market is growing rapidly.

In human medicine, various definitions have been used for POC tests including (Ehrmeyer and Laessig 2007):

> **'***patient specimens being assayed at or near the patient, with the assumption that test results will be available instantly or in a very short time frame, to assist care-givers with immediate diagnosis and/or clinical intervention.*'

POC tests have been used successfully in veterinary medicine for more than 40 years for simple biochemical assays on blood and urine. Near-POC tests for use in veterinary clinics that reduce the turnaround time for samples (especially for clinical chemistry and haematology) have proliferated.

In veterinary medicine, a range of terms has also been applied for tests used outside veterinary laboratories. Some of these terms were described in a review on diagnostic methods for FMD (Sammin et al. 2010):

> '*A confusing multiplicity of terminologies have been used to describe the concept of testing for FMD outside of a NRL (National Reference Laboratory); the terms "rapid testing", "pen-side testing", "on-farm testing", "on-site testing", "field testing", "devolved testing" and "decentralised testing" have all been used synonymously.*'

The term, 'decentralised testing', was preferred by these authors. Another term, 'agricultural screening tools', has also been used to describe tests or methods usually performed outside a laboratory. It has been defined (FAZD Center 2010) as 'A tool used to detect a potential disease or condition in an animal, group of animals, or animal product. The tool may be used in any phase of an outbreak response, and is not required to be confirmatory (diagnostic) in nature, but rather is intended for rapid initial detection.'

In this review, the term point-of-care (POC) test is used for tests used on farms or in the field, in the open or in a room or other structure, but any of the terms mentioned could have been applied. The term near-POC is used in this review for tests that can be used inside a room or other structure being used as a basic laboratory with minimal equipment needed to undertake tests rather than well equipped central or reference laboratory with specialist staff undertaking a range of other diagnostic tests.

2.2 Types of POC tests

POC or near-POC tests for infectious diseases mainly fall into three broad categories — tests for detecting antibody; tests for detecting antigens using immunological methods or aptamers; and, tests for detecting microbial nucleic acids after preliminary amplification. Other tests are also being developed. The range of tests available and developments occurring in this field are listed in Table 1 and discussed in more detail in Appendix 1.

All *in vitro* diagnostic tests should be supported by clinical or pathological diagnosis. Clinical and post mortem examination are sometimes underestimated as 'POC diagnostic tools', but they can be extremely accurate when performed by competent veterinary clinicians and pathologists. In many cases, the findings of clinicians and pathologists provide sufficient

justification for immediate action on a farm with a suspected EAD (e.g. ruminants with vesicles, or high mortality disease in poultry with subcutaneous oedema and widespread haemorrhages) without performing POC tests that identify specific pathogens in samples.

Development and use of POC tests for infectious diseases have lagged behind tests for biochemical and haematological parameters. Until the late 1980s POC tests (or near-POC tests) for EADs of animals were largely restricted to simple microscopy (e.g. smears stained with polychrome methylene blue undergoing the M'Fadyean reaction for detection of *Bacillus anthracis*), immunofluorescence (e.g. pancreatic impression smears for avian influenza (Selleck et al. 2003), and a few immunochromatographic tests such as those for detection of influenza viruses, developed for use in humans but applicable in animals (Waner et al. 1991). Since then, a wider range of immunochromatographic tests has become available,[5] including tests for companion animal diseases, some of which are capable of detecting multiple pathogens in one test.[6] In addition, many tests that amplify and detect nucleic acids are available. In theory, any test based on nucleic acid amplification and detection can be adapted for use at POC or near-POC given the availability of robust equipment and reagent packs for performing these tests, and a few have been developed as commercial applications[7]. However, the tests have to be assessed under field conditions before they are used as primary diagnostic tools or for surveillance, especially given the harsh conditions under which these tests may have to be used compared with the controlled environment in laboratories.

Most tests based on nucleic acid amplification still require relatively sophisticated equipment, precluding their use by most primary producers, other than large companies that can afford the necessary equipment and employ trained technicians required to conduct the tests. Portable equipment, although available, has not yet been deployed widely by veterinary services outside the existing laboratory networks, although the use of isothermal techniques for nucleic acid amplification that require only a heat block has made it possible to develop relatively simple field test packs.

2.3 Drivers for development of POC tests

One of the main advantages of POC tests is the time saved when accurate POC diagnostic tools are available (Bissonette and Bergeron 2010). In human medicine, developments in POC tests for infectious diseases are being driven by demands from clinicians for early and rapid information on pathogens in samples from patients. Such information can assist clinical management and also provide significant economic benefits through, *inter alia*, reduced mortality, reduced duration of hospitalisation of patients, reduced transmission of resistant bacteria within hospital settings, reduced use of pharmaceutical products and reduced post-surgical infection with multi-resistant organisms.

For example, time to initiation of appropriate antimicrobial therapy is a key determinant of survival for humans with septic shock (Kumar et al. 2006; Kumar et al. 2009). The potential benefits of rapid tests capable of detecting organisms in blood are clearly evident given existing methods using blood culture take more than 24 hours to produce a result. However,

[5] See for example the Binax Now range for human pathogens at http://www.binaxnow.com/

[6] See for example the range of in-clinic tests for dogs and cats sold by Idexx at http://www.idexx.com.au/html/en_au/smallanimal/inhouse/snap-in-house-tests.html

[7] See, for example, Smiths Detections at http://www.smithsdetection.com/media/Sample_Preparation_Unit_and_Reagent_Pack.pdf

this advantage can only be achieved if the rapid tests deliver accurate (sensitive and specific) information on the organisms involved and their likely antimicrobial sensitivity. Rapid tests that can be used at or near to POC to detect microbes in blood are now available (although some are still less than ideal (Josefson et al. 2011)). Similar tests to identify nasal carriage of methicillin-resistant *Staphylococcus aureus* are also available, allowing decisions to be made on suitability of patients and infection control procedures before medical and surgical interventions (see, for example, Creamer et al. 2010). A gap still exists between ideal POC tests and what is being achieved at present with most systems available commercially, but the gap is closing as each new round of tests is developed, as has been demonstrated with POC tests for *Mycobacterium tuberculosis* and rifampicin resistance (Boehme et al. 2010).

As expected, the economic benefits of POC testing in humans, based on the improved turnaround, are being promoted by those marketing new POC tests.[8] Early availability of test results can reduce costs or alter the course of treatment (see, for example, Boehme et al. 2010) but this is not always the case as was shown in a study using RT-PCR tests for respiratory pathogens in human paediatric patients in the Netherlands (Wishaupt et al. 2011). Improvements in turnaround time can also occur with accurate POC tests for EADs if tests are available for rapid field deployment. This issue is discussed further in Section 3. The costs and benefits of POC tests for EADs need to be assessed on a case-by-case basis. The advantages that accrue from earlier diagnosis should form part of this assessment.

Another major driver for the development of POC tests is the threat (perceived and actual) of bioterrorism and biological warfare (e.g. anthrax spores in the US Postal system (Dewan et al. 2002)). The process of development of these devices (some under development and others that are already in use) has been facilitated by funds from national defence and security budgets in a number of countries (see, for example, Wolcott et al. 2007).

Work on POC and near-POC tests has also been funded by international donors for important infectious diseases of developing countries such as tuberculosis and malaria (see, for example, WHO 2009) and impressive results have been obtained using 'sample-to-answer' systems to detect *Mycobacterium tuberculosis* in patient samples and detection of resistance to rifampin (Boehme et al. 2010).

In the veterinary sphere, an increase in the number of outbreaks of EAD and emerging diseases, including diseases that have public health implications, has provided impetus for the development of POC tests[9] especially for a number of high profile diseases. Concerns about the pandemic potential of H5N1 avian influenza and the emergence of influenza viruses of probable porcine origin as a human pandemic strain in 2009 have driven major developments in tests for animal influenza viruses. POC tests have also been developed for important diseases of aquatic animals using similar technologies to those used for terrestrial animals (see, for example, Soliman and El-Matbouli 2010).

Development of POC tests is also benefitting from the rapid advances in human genomics given that similar techniques are used or can be adapted for use in POC tests (see, for

[8] See for example http://www.cepheid.com/media/files/brochures/Xpert%20MRSA_V5_6pgr%20US%20Brochure.pdf

[9] See, for example, the work on integrated portable systems for detection of Influenza A(H5N1) virus conducted through the Joint IAEA/FAO Program on Nuclear Techniques in Food and Agriculture at http://www-naweb.iaea.org/nafa/aph/stories/2010-transboundary-animal-diseases.html

example, He et al. 2010b). It is possible that near-POC gene sequencing systems will soon be available and could offer genetic information on both the pathogens and genetic characteristics of patients. This combination of results acquired either through sequencing or tests for specific markers in hosts and pathogens could assist in determining the most appropriate therapies, moving closer to the concept of personalised medicine for infectious diseases (see Hoggatt 2011 for a review of developments in this area). The main benefit of these technological advances for management of EADs is that the techniques developed can be applied in new tests for the detection of pathogens. They may also have some applications in management of EADs in the long term, especially if specific genetic markers for disease resistance or the response to specific EADs are identified.

Development of POC tests will be facilitated by the improved interface between engineering and biology that is regarded as very fertile ground for development of new technologies in the 21st Century (Economist 2011). Closer collaboration between these two disciplines has already produced a range of new diagnostic techniques (Melo et al. 2010). For POC tests some of the aims are to miniaturise and simplify tests (at least from an operator perspective), to improve detection limits and robustness (capacity to operate under less than ideal conditions outside laboratories) and to speed up existing assays, providing results in a matter of minutes or hours, rather than 24 hours or more if the sample has to be shipped over long distances, or if the agent has to be cultured. At the same time, the engineers and biologists developing the tests must aim to maintain the sensitivity and specificity of new tests to be equivalent to those of existing procedures on which they are based. They also have to ensure that the tests are cost-effective, providing opportunities for a market for the products once they are developed (see Section 5).

A number of reviews and papers provide information on the new technologies and trends towards miniaturisation of existing laboratory-based techniques (see, for example, Belak et al. 2009; Beyor et al. 2009; Bissonnette and Bergeron 2010; Lim et al. 2005; Miller and Tang 2009; WHO 2009). It is evident that the new technologies being developed and some already in use have the capacity to be used for POC or near-POC use in the near future. Bissonette and Bergeron (2010) expected the entrance of true POC molecular testing with integrated disposable devices within 5–10 years; some such tests have already become available within this timeframe. Further information on the various methods and techniques involved in POC tests (and potential POC tests) is provided in Appendix 1.

It appears inevitable that some of these tests will be adapted for use for animal diseases including EADs. Tests with the capacity to identify and subtype a number of microbial agents in a single test run will be available to field-based veterinarians and, potentially, producers providing information on the health status of animals in a matter of hours. Currently this testing is the province of central or reference laboratories — and only available if the person submitting the sample (or the laboratory or agency performing the tests) could afford to pay for testing for multiple agents. Some new tests can even detect and provide genetic information on previously ‘unknown’ or new pathogens (Rota et al. 2003), although the complexity of these tests means that they are still some way from being available as POC tests. POC tests have the potential to revolutionise delivery of animal health services, especially if they involve samples that are easy to collect by farm personnel such as oral fluids (Prickett and Zimmerman 2010).

Systems available or under development have the capacity to bring sophisticated POC tests to the field. At present, sample preparation is still required for many of these tests (e.g. concentration of organisms and extraction of nucleic acids). Until recently, this was more

difficult to perform under field conditions than in the laboratory. However, field extraction using products such as Zygem's prepGem allow this process to be conducted in the field in a single closed tube.[10] Other systems that will simplify nucleic acid extraction are available, including filter paper capture of nucleic acids (Bearinger et al. 2011), or are being developed. 'Sample-to-answer' technologies, in which all steps are undertaken in one reaction vessel, are required to overcome this problem, and some are already used in tests developed for human pathogens such as the Cepheid GeneXpert system[11] (Boehme et al. 2010) and the FilmArray system (Rand et al. 2011).

Apart from having the ability to meet the purpose for which it is designed, it has been proposed that an ideal POC test should require minimal instrumentation and be suitable for use by lay staff (or at least staff with minimal training). The so-called 'ASSURED' criteria (Urdea et al. 2006) — affordable, sensitive, specific, user-friendly, rapid, equipment-free and delivered to those in need — have been developed for POC tests for developing countries, and all but the last two of these criteria can also be applied to ideal POC tests for EADs in Australia. Some of the POC and near-POC tests now available, potentially available or under development for EADs still require equipment on which to perform tests but the cost of this equipment is likely to fall. Even with limited market penetration, equipment commercially available that is capable of running on site RT-PCR and licensed by USDA for testing for classical swine fever would cost approximately $AUD15,000.[12]

2.4 Quality management and POC tests

As with laboratory-based diagnostic tests, an appropriate quality management system is needed to ensure that POC tests are delivering the expected results. In veterinary medicine quality management systems for in-clinic devices are sometimes neglected13 — one of the major concerns for those regulating the use of POC tests for EADs (Sammin et al. 2010). There are many reasons why POC (and other) tests might not perform as expected including operator factors, equipment factors and reagent factors. In human medicine, systems are being developed and implemented for quality management of POC tests in clinics (Tirimacco et al. 2011). This process must include operator training. These issues, including the role of POC test managers have been considered in detail by SCAHLS14 and are discussed in papers on the use of POC testing (see, for example, Lewandrowski et al. 2011).

Nevertheless, a trend in current POC technologies is for the sample preparation phase to be included in the test system, thus minimising the input of the person preparing the sample and the risk of operator error (as well as reducing biosafety concerns to operators). This change does not eliminate the need for basic training (see, for example, Lawn and Nicol 2011) but it does mean that some POC tests can be reliably used by people without formal

[10] See http://www.zygem.com/Products/Products-PG.html

[11] See http://www.cepheid.com/systems-and-software/genexpert-system/

[12] The equipment from Tetrapak http://www.tetracore.com/real-time-pcr-detection/index.html Price estimate from www.warmwell.com

[13] Systems are available for ensuring quality but are not always applied. See, for example, the system from IDEXX that is used for in clinic biochemical analysers. However the systems in small practice laboratories rarely approach those in well managed laboratories. http://www.idexx.com/view/xhtml/en_us/smallanimal/inhouse/vetlab/quality-control-panel.jsf?SSOTOKEN=0

[14] Papers from SCAHLS meetings are not in the public domain

technical training or qualifications. Quality management can also be facilitated if the test process can be followed remotely via the internet by trained technical and professional staff in a distant laboratory. This is achievable with some of the equipment currently available for nucleic acid amplification and detection.

For animal health applications, POC tests and associated equipment need to be sufficiently robust to cope with the difficult field environment in which they are used. Already a number of systems have been developed that allow real-time PCR or similar techniques to be performed in the field. Some of the instruments do not even require an external power source (i.e. can use rechargeable batteries). A field diagnostic kit employing nucleic acid sequence based amplification (NASBA) is also available commercially.[15] These developments are small steps along the pathway that should result in POC tests being used more widely in the field for a range of purposes. (See Section 5 for more information on markets for POC tests and Section 4.2 on 'fitness for purpose' for these tests).

[15] See http://www.haikanglife.com/English/Bangladesh_training.html

Table 1 Developments in POC tests and in tests that could become POC tests

Test type	Main features of test	Examples	Developments/Comments
Detection of antibody			
ELISA	Mature technology. A number of test variations but mainly based on a single antigen adsorbed to a plate. Captured enzyme-labelled antibody produces coloured end product from substrate reacting with bound enzyme.	Many commercial kits available including tests for avian influenza .	Although a mature technology, equipment for reading reactions allows use as a near-POC test. Miniaturisation and multiplexing occurring.
Lateral flow immunochromat-ograhic devices	Sample is added to device and diffuses across membrane where it encounters labeled antigen that traps antibody.	Commercial tests for HIV antibody in humans	Mature technology but major developments in detection systems including biosensors, such as nanocarbon tubes, and techniques using amplification of oligonucleotide labels are increasing analytical sensitivity
Protein microarrays	A range of antigens arranged in an array that bind to different antibodies in sample.	Research tools for rinderpest and human influenza	Longer term potential for development as POC or near POC tests
Detection of antigens			
ELISA	See above, except in this case the test antigen is captured by an antibody bound to plate or in liquid phase.	Numerous commercial kits including avian influenza	Mature technology
Lateral flow devices	See above, except sample contains antigen and kit contains antibody. Often lower sensitivity than tests based on nucleic acid amplification and detection	Anthrax Influenza	New methods being developed for antigen detection that increase the analytical sensitivity of the tests
Aptamers	Usually oligonucelotides that are selected in vitro for their ability to bind specifically to targets based on shape recognition. Heat stable. Can detect chemicals that are poorly immunogenic and therefore not suitable for ELISA	Numerous examples in food testing	May prove to be of value in detecting agents of EADs

Tests based around thermal cycling for amplification of nucleic acids			
Polymerase chain reaction (PCR)	Extraction of nucleic acid is followed by amplification using heat tolerant polymerase and detection of amplified product usually on an agarose gel	Classical PCR not suitable for field use due to multiple steps in the process	
Real time (quantitative) polymerase chain reaction (RT-PCR)	As above but the use of fluorescent labels allows for quantitation of the amount of the target nucleic acid. Rapid and reduced risk of contamination compared with traditional PCR.	In theory any real time-PCR for infectious agents can be adapted for use in the field.	Major improvements in extraction techniques, detection systems and equipment that allow POC testing with minimal technical training
	Numerous variations such as LATE-PCR that has been used in robust machines for POC testing for EADs	Commercially available (or expected to be available) products for avian influenza, Newcastle disease and foot-and-mouth disease.	Equipment becoming more sophisticated. Assays that allow multiple targets to be detected in the one test are now possible (see below) Remote monitoring of assay performance is possible
Tests based around isothermal cycling for amplification of nucleic acids			
Various methods	Amplification occurs at a constant temperature using a heat block, avoiding the need for a thermal cycler.	Commercial NASBA kit available for near-POC testing for a number of agents	High potential for use of isothermal systems as POC tests given the minimal equipment requirements and potential to detect positive signal through visible colour change.
	Methods include nucleic acid sequence based amplification (NASBA) Loop-mediated isothermal amplification (LAMP), signal-mediated amplification of RNA technology (SMART), strand-displacement amplification, rolling circle amplification, isothermal multiple displacement amplification, helicase-dependent amplification, single primer isothermal amplification, and circular helicase-dependent amplification.	Experimental systems for Hendra virus.	

Tests based on nucleic acid amplification targeting multiple agents and/or detection of subtypes in a single sample, including microarrays			
Various methods **Multiplexed PCR and microarrays**	Extensions of the methods described above in that most rely on nucleic acid amplification followed by binding to an array of different oligonucleotide probes either on a solid surface or on beads in liquid.	Respiratory panels for human pathogens.	Some of these techniques have the potential to move to POC or near-POC tests in the future.
Miniaturisation of tests and 'sample to answer' systems			
'Lab on a chip'	Various methods built on those described above but the main distinguishing features are that the techniques can be performed by operators without formal technical qualifications (all steps in the assay are conducted in the one chip or cartridge). Tests rely on developments in engineering, microfluidics and nanotechnology to develop integrated systems.	Test for tuberculosis in humans detects presence of agent and drug resistance marker being applied at POC in resource- poor countries in Africa.	Many tests and techniques under development; some have been granted approval for diagnostic testing.

3. Possible applications of point- of-care (POC) tests for emergency animal diseases

Before, during and after an EAD outbreak, samples are collected from animals (and sometimes the environment) for a range of purposes including pre-outbreak active and passive surveillance; detection/confirmation of the index case and subsequent cases; demonstrating freedom from disease once the last clinical case in an outbreak is diagnosed and managed; and, ensuring business continuity. A key question addressed in this section of the review is whether and how POC tests, including those based on new technologies, can assist in the control and management of an outbreak of an EAD.

A report on the use of screening tests for FMD (FAZD Center 2010) suggests that they can have value at all stages of an outbreak — early detection, response, and recovery — and that the type of test, as well as how and when it will be used (and by whom), will vary depending on the stage of the outbreak.

Existing POC and near-POC tests have been used successfully as an aid in management of a number of EADs in a range of countries including highly pathogenic avian influenza (see, for example, Sims et al. 2003), although uptake in Australia and some other countries has been limited. The reasons for the limited uptake need to be understood. In part it has been a combination of lack of availability of suitable properly validated commercial tests and that the tests available (e.g. lateral flow devices for FMD) have not offered significant advantages over laboratory-based testing and clinical findings for diagnosis or management of cases. In the near future the first of these is not expected to be a limiting factor but before any new POC tests are applied as an aid in EAD management they must provide additional advantages over existing laboratory based (and POC) tests if they are to be adopted.

Four important benefits can arise from the use of POC tests for EADs:

i) The results of POC tests can potentially allow earlier action to be taken to control and/or eradicate the disease than would be the case if tests were performed at a laboratory.

ii) Results from POC tests can assist in identifying potential public health threats.

iii) The use of POC or near-POC tests can reduce the volume of testing that needs to be performed in laboratories, therefore reducing the potential for laboratory overload during the course of an outbreak.

iv) Positive results from properly validated and highly specific POC tests can provide greater confidence in decisions during an outbreak rather than relying only on clinical signs or using pre-emptive culling, potentially providing some protection against litigation by affected producers (assuming the tests have been fully validated for use in the field for that particular purpose).

This section provides information on the possible benefits arising from the use of current and potential POC tests for EADs in Australia. Information in the Appendices describes the range of new tests that could be developed (Appendix 1) and use (or potential uses) of existing and new POC or near-POC tests for five selected EADs — AI, FMD, classical swine fever, bovine spongiform encephalopathy (BSE) and anthrax (Appendix 2 to 6).

The potential benefits need to be weighed against the costs and risks associated with use of POC tests. In this review, areas that should be considered in assessing costs are discussed but specific cost-benefit analysis for existing tests is not done given that the cost of POC

tests will change over time and the many ways the tests can be applied. Tests that appear prohibitively expensive today are expected to become more cost effective as they evolve or if they become more efficient at detecting infectious agents, especially in animals displaying atypical symptoms. For example, at present conducting a field-based test involving nucleic acid amplification for FMD currently requires operators with specific technical expertise. Maintaining appropriately trained and qualified staff to perform these tests in locations outside laboratories, including the requisite quality management systems, is costly. The trend towards 'sample-to-answer' test systems and improvements in remote monitoring will permit tests to be conducted by staff with minimal training and no formal technical qualifications, reducing these costs. Such tests have been developed and used for near-POC testing for other diseases of humans such as tuberculosis (Boehme et al. 2010). The cost and probability of false positive and false negative results as a result of using POC or near-POC tests also have to be considered. These depend on the specific characteristics of each test and the purpose for which it is being used.

One of the main reasons proposed for using POC tests is to reduce the time taken to obtain a diagnosis of an EAD by providing test information earlier than if the samples had to be transported to a laboratory. As reported in a report of the lessons to be learned from the 2001 FMD outbreak in the UK, early diagnosis is crucial in disease outbreak management.[16]

Many factors influence the time to diagnosis of outbreaks of EADs. The owner of affected livestock has to recognise that his or her animals have a serious problem and then seek help for that problem. The person investigating the outbreak and providing diagnostic support (e.g. private or government veterinarians, stock inspector) must then suspect an EAD and submit appropriate samples for testing. Until recently, samples would be sent to Australia's national animal health laboratory for testing via relevant state or territory laboratories. However, in a number of recent outbreaks a preliminary testing and diagnosis was made at a state laboratory followed by confirmation at AAHL, a process that is being encouraged through the Laboratories for Emergency Animal Disease Diagnosis and Response (LEADDR) network.[17] In the case of anthrax, field testing is conducted. Testing could be streamlined further if appropriate POC or near-POC tests are available that offer accurate and rapid diagnosis of other EADs.

One alternative to use of POC tests is to improve transport of samples. However, closure of public sector regional veterinary laboratories in most Australian states and territories means that many farms, especially in the larger states, are now located long distances from a laboratory capable of conducting appropriate diagnostic tests or liaising with the national reference laboratory to ensure samples are dispatched.

Transport of samples over long distances can also be hampered by a range of factors including reluctance by carriers to transport samples containing potentially infectious material, (although this is not regarded as an important factor in Australia given that a single

[16] See National Archives of the United Kingdom at http://webarchive.nationalarchives.gov.uk/20100807034701/http://archive.cabinetoffice.gov.uk/fmd/fmd_report/report/index.htm

[17] See the following newsletter from SCAHLS for information on LEADDR http://www.google.com.au/url?sa=t&rct=j&q=leaddr%20network&source=web&cd=2&sqi=2&ved=0CC4QFjAB&url=http%3A%2F%2Fwww.scahls.org.au%2F__data%2Fassets%2Fpdf_file%2F0004%2F1486552%2FSCHAHLnewsNov09.pdf&ei=Y9Q6T4CDLMaUiQfw4fD4CQ&usg=AFQjCNEDBHxsjvt_hEfGrPih-rlVChjJjg

courier company has been contracted to deliver samples to the national reference laboratory), losses or delays in transit, and large scale natural disasters, such as flooding and cyclones. Availability of POC or near-POC tests would overcome some of the problems caused by these factors if the test kits are widely dispersed. However, the cost of stockpiling appropriate POC tests also has to be considered, especially for tests that are expected to be required infrequently. All test reagents have a finite shelf life and must be replaced once they expire. This is one reason for promoting inclusion of tests for EADs in multiplexed tests for endemic diseases of production animals (i.e. tests that would be used on a regular basis if available, reducing waste).

The cost of conducting POC tests for EADs (include maintaining stocks of tests, trained staff, quality management systems and portable equipment for diagnosis of diseases that may never occur or occur rarely) has to be outweighed by the benefits derived from the results of the tests. This will only occur if the results from POC tests facilitate the management of the outbreak, in particular, allowing earlier actions to be taken to prevent disease transmission as a direct result of the POC test results. In Australia this is more likely to be the case in remote locations (where there would be a delay between sample collection and sample testing in a laboratory) in line with findings in the UK on potential uses of POC tests for FMD (DEFRA 2011).

It also needs to be remembered that, when using POC tests on an index case, confirmatory tests will be conducted regardless of whether a positive or negative POC test result is obtained. If used for these cases the benefits that arise from test duplication need to be substantial.

In addition, when POC tests are used, all samples that test positive on a POC test should still be sent for confirmatory laboratory testing, not only to provide an additional level of certainty about the result but also to ensure specimens are not lost on infected premises, precluding their use in further molecular studies of isolates. Such studies can provide valuable information on the manner of transmission and epidemiological links between infected premises (Sammin et al. 2010). Confirmatory tests on samples can provide evidence in the event of legal action taken by farmers who believe their livestock were destroyed in error on the basis of POC tests. It is also important that results of all POC tests (positive and negative) are recorded so that they are captured in laboratory information management systems, outbreak management databases and epidemiological reports and investigations.

The potential risks associated with the use of POC and near-POC tests and their management are discussed in Section 4 but these risks need to be kept in perspective and balanced against the advantages of performing POC tests especially given that many of the risks also apply to any type of test.

The following sub-sections examine the potential uses of POC tests for EADs before, during and after an outbreak.

3.1 The use of point- of-care tests in the early detection of EADs, including scanning surveillance

As described in Section 2, one of the key trends in POC and near-POC test development is that the tests can detect multiple agents in a single run. If suitable, relatively inexpensive, multiplexed POC or near-POC tests become available that cover agents responsible for endemic diseases in Australia as well as EADs (e.g. a respiratory disease panel for chickens that includes tests for avian influenza viruses) it is expected that some farmers will use them to determine the health status of their animals. Positive results for an EAD should then trigger further investigations, allowing early diagnosis (but see management of risks in the next section).

In 2011, the cost of a post mortem examination and testing for a number of pathogens (e.g. enteric pathogens) for a grower pig undertaken at a state laboratory can exceed $1000 (including charges for carcass disposal). If suitable POC or near POC tests and test systems become available, it would be economically viable for some farms that want to monitor the health status of their herd to consider the option of multiplexed POC tests for pathogen detection, even if the cost of a single test run approaches the cost of a post mortem examination and ancillary tests. A full cost-benefit analysis cannot be conducted until these tests are available. In addition, other important information, including the correlation of gross and histopathological findings with agent detection, and opportunities to detect newly emerging diseases would be lost if only swabs are collected and tested. These aspects need to be factored into the cost-benefit equation.

Serological tests for antibody detection suitable for near POC use are also available for some EADs, including avian influenza, and could be used on a routine basis by producers or veterinary authorities to check for evidence of infection with avian influenza in otherwise healthy birds or in poultry displaying low grade respiratory disease.

In the case of avian influenza, a considerable quantity of testing for surveillance programs is performed on wild birds. At present most of these samples are tested in laboratories but field testing using portable equipment and reverse transcription real-time PCR with lyophilised reagents, has been used to minimise the effects of distance from a laboratory, maintenance of cold chains for samples and the time lag from sample collection to receipt and testing in a laboratory (which can also provide earlier warning of the presence of potential pathogens than laboratory-based test systems). The system used was equally specific as laboratory based tests but the analytical sensitivity was lower, which resulted in samples that contained low concentrations of viral RNA being misclassified as negative when compared with laboratory based systems (Takekawa et al. 2011). Use of these POC systems could be cost-effective for testing of migratory birds in remote locations in Australia.

Existing antigen detection lateral flow devices for avian influenza have relatively low analytical sensitivity and therefore only provide a positive result on samples that contain sufficient virus. The tests are generally more suitable for samples on sick and dead birds than on swabs from healthy poultry (Chua et al. 2007, Slomka et al. 2011) or wild birds. Testing of feathers was shown to be the most sensitive method in both chickens and ducks when using lateral flow devices to test infected poultry in Vietnam (Slomka et al. 2011). These tests could be of some use in remote locations provided the constraints of the tests are understood.

3.2 Potential uses of point- of-care tests in the ‘Response Phase’

3.2.1 The index case

At present in Australia, the management of the index case of an EAD in production animals, other than anthrax, does not depend on the use of POC tests. If animals are showing signs typical of a major EAD, such as FMD, current Australian response plans[18] include immediate quarantine of the suspect premises and collection of samples for dispatch to a central

[18] Australian response plans/disease strategies are provided in Ausvetplan available at http://www.animalhealthaustralia.com.au/programs/emergency-animal-disease-preparedness/ausvetplan/disease-strategies/

laboratory. Similar actions are taken in other countries for EADs such as FMD (see, for example, DEFRA 2011).

Nevertheless, as time is crucial when managing the early stages of any EAD, a positive POC test result on-site might provide greater justification for taking additional preliminary measures both on and off the affected farm, to minimise the risk of disease transmission, until confirmatory results are obtained from a reference laboratory.

It can be argued that POC testing for an index case might be potentially more valuable if the affected animals are displaying clinical signs that are not entirely typical so that the suspicion of an EAD is relatively low (provided the POC test does not rely on samples from typical early lesions which is the case for some current FMD tests). In such cases, due to the low level clinical suspicion, movement controls might not be implemented until after test results from a laboratory are obtained. Use of a 'rule in' POC test (and a positive test result) would provide stronger grounds for farm quarantine until such time as confirmatory tests are performed in a laboratory. This assumes that suitable samples are available for testing.

The potential benefits of using POC tests can be seen in the following examples from EAD outbreaks in Australia and elsewhere. The initial clinical signs recorded in Australian HPAI outbreaks in 1976 and 1985 differed from those in the 1992 outbreak (in which there was very high mortality in one affected flock) and although HPAI was eventually considered in the differential diagnosis in the first two cases the clinical suspicion was initially lower than it was in 1992 (see description of the cases in Sims and Turner 2008). Had POC tests covering multiple agents been available and used at the time it is possible that these cases would have been diagnosed earlier if, in fact, an avian influenza virus was contributing to the disease in the early stages of these outbreaks, even if it had not yet developed into a highly pathogenic strain.

In contrast, the 1992 HPAI outbreak demonstrated that, if the clinical signs are highly suggestive of HPAI, results from POC tests are not required before taking actions, such as movement restrictions, on suspected infected premises. In this outbreak the clinical signs in affected poultry were sufficiently suggestive of HPAI for movement restrictions to be placed on the farm immediately after a farm visit (Sims unpublished). Results of laboratory tests were available within 12 hours of initial sampling (cf dead birds were sent to the Australian Animal Health Laboratory, AAHL) and although a rapid test on-site would have provided some comfort when placing movement restrictions on the farm, it did not affect the overall management of the outbreak. However, Bendigo, where this case occurred, is located only 200 km (3 hours drive) from AAHL, the national reference laboratory, so there was minimal delay getting samples to the national reference laboratory for testing. If the case had occurred in a more remote location, POC tests could have been more valuable.

The decision to close the main wholesale poultry market in Hong Kong in December 1997 due to the detection of chickens showing signs typical of highly pathogenic avian influenza (at a time when H5N1 HPAI virus was known to be circulating) would have been easier to make if POC test results demonstrating the dead birds in the market were virus positive rather than relying on clinical signs in affected birds (Sims unpublished).

In the case of FMD, precautionary quarantine measures would be implemented if animals have vesicular lesions. However if animals have been infected for more than a few days, typical vesicles (including ruptured vesicles with flaps of epithelium attached) suitable for testing, may not be available. This limits the value of current lateral flow antigen detection tests which use this tissue as the test material.

In a recent simulation exercise of an FMD outbreak in the United Kingdom (UK) it was concluded that lateral flow devices for antigen detection would not be of benefit in decision making given that the animals tested would need to have vesicular lesions, which would be

sufficient grounds to quarantine the premises anyway. The only places where these tests might be considered for use were those in remote locations (DEFRA 2011). The report on this exercise recommended the development of a policy for deployment of portable diagnostic equipment.

Once POC tests used in the field have equivalent analytical sensitivity and specificity to laboratory-based tests and are backed by appropriate quality management systems, it may be possible, on the basis of a POC test alone, to take action beyond movement restriction/quarantine on suspect premises. Although this prospect remains unlikely for most diseases in the near term, it should not be dismissed as a possibility in the future for some diseases.

Until such time as laboratory confirmation of a POC test result is no longer necessary before measures other than quarantine of the infected premises are taken, POC tests are expected to play a limited role in the diagnosis of the index case of an EAD outbreak, other than as a triage tool for determining which specimens to send on for further testing and as an additional warning to prepare for action on an EAD outbreak.

3.2.1.1 Index cases with potential public health significance

Some EADs, such as Hendra virus infection, HPAI and anthrax can have potentially serious immediate public health implications for those who have close contact with infected animals. An accurate POC test can potentially help in the management of such cases by using results to reduce the likelihood of human exposure. In the management of any diseased animal — especially one displaying signs suggestive of a serious zoonotic disease — management protocols for human safety, as defined in operating procedures and guidelines, should be adopted for suspected cases to protect human health, regardless of whether or not a POC test has been performed.[19] However, a positive POC test would heighten awareness of risks, especially if the case otherwise appeared to be atypical. Experiences from Hong Kong where rapid tests have been used for avian influenza suggest that the tests do not provide advantages for this purpose provided those visiting potentially infected premises follow standard procedures that include appropriate use of PPE (Sims unpublished).

Work is already in progress for a POC test for Hendra virus based on loop-mediated amplification of viral nucleic acid (Boyd et al. 2011). POC tests for anthrax are already being deployed and one of the advantages proposed for this test is that a positive result precludes the need for post mortem examination of carcasses (Hornitsky and Muller 2010) (although if there is a suspicion of anthrax this should not be done anyway).

If in the future vaccines are used for protection against an EAD with public health significance, such as Hendra virus infection, POC testing of vaccinated animals, if available, could also provide some indication of the susceptibility of the flock or herd (or individual animals) to the agent in question.

[19] See for example the guidance for dealing with suspected Hendra virus infected horses available at http://www.dpi.qld.gov.au/documents/Biosecurity_GeneralAnimalHealthPestsAndDiseases/Hendra-GuidelinesForVets.pdf

Testing for BSE by near-POC testing for suspect dead animals is used in other countries but in Australia any such cases would need to be confirmed at a reference laboratory given it would be an index case.

3.2.2 Subsequent cases in an outbreak

Once a definitive diagnosis is made on the index case of an EAD any additional suspect cases or premises detected through tracing or within the restricted and control areas are excellent candidates for use of appropriate POC tests or near-POC tests.

If an EAD is not confined to the index farm, especially if it spreads widely, testing of all subsequent cases will be required but will not necessarily be conducted at a laboratory. Sensitive and specific POC tests, if available, would assist in disease management in these situations and have been used elsewhere for this purpose. For example, near-POC tests for influenza virus used in Hong Kong in 2002 on dead poultry from farms in the control area allowed early detection of recently infected farms (Sims et al. 2003). Positive test results in the field using validated highly specific tests also provide additional certainty when applying control measures on farms, but there are costs associated with deployment.

In theory, POC tests have an advantage over laboratory based tests if they can be used to detect infected animals before they become infectious, especially if this window is short, as is the case with FMD. This advantage would apply if the time between collection of samples and receipt of results is at least 24 hours if samples are sent to a laboratory rather than being tested on site.

It has been recognized for some time that FMD virus can be detected in oesophago-pharyngeal samples from cattle infected with FMD virus prior to the development of clinical signs (potentially allowing animals incubating the disease to be detected through the use of rapid testing and culled before transmitting virus). For example, experimental studies on FMD in cattle (Callahan et al. 2002, Charleston et al. 2011) demonstrated that FMD viral nucleic acid can be detected in oesophago-pharyngeal fluids collected by probang 24 to 96 hours prior to the development of clinical signs. This potentially allows POC testing of cattle on dangerous contact premises before the detection of clinical signs. Yet, despite this information being available for 10 years, POC tests based on nucleic acid amplification have not been deployed or included in contingency plans for this disease in western countries, which has been a topic of criticism in the UK on some personal blogs[20] and discussed elsewhere in papers on the future for management of EADs (Breeze 2006).

Use of these tests for this particular purpose would only be possible if sufficient manpower was available to collect samples from enough animals on high risk premises to provide confidence that the virus is or is not present.[21] Experiences from EAD outbreaks suggest that availability of sufficient manpower for this purpose cannot be guaranteed. It also assumes that the test system used has the capacity for rapid throughput of multiple samples, that appropriate quality management systems for the POC test system are in place and the tests perform as well in the field as they do under laboratory conditions. All of these issues have been raised about the benefits of pre-clinical testing (ProMED-mail 2011). At present, equipment for use in the field for POC nucleic acid amplification is not designed for high throughput of samples (Sammin et al. 2010), suggesting it would not be suitable for this

[20] See for example www.warmwell.com

[21] For example, in a herd of 200 cattle it would be necessary to test a minimum of 53 animals (assuming the test has a sensitivity of 99.5%) to be 95% confident of detecting virus if ten animals (5% of the herd) were infected.

purpose. Other factors also need to be considered before adopting this measure including the effects of stress on the animals as a result of sample collection (e.g. would stressing the animals through sample collection speed up the onset of virus shedding therefore eliminating the advantage of early detection?) (ProMED- mail 2011).

3.2.3 Business continuity during an outbreak

A 2010 review (FAZD Center 2010) highlighted the issue of business continuity in outbreaks of FMD and the role that screening tests can play in this process. POC or near-POC tests should be developed and validated for pooled samples, such as bulk milk for this disease. These tests could also be used to monitor for cases when assessing freedom from disease after an outbreak. However, for the results to be credible, the tests would need to be highly sensitive to ensure that false negative results were rare. Not all POC tests will meet these criteria, again demonstrating that the test used must be validated for the purpose it will be applied.

POC tests could be used to demonstrate freedom from infection in compartments and zones but trading partners would need to be convinced of the validity of the test results and sampling system, discussed in detail below. In the case of HPAI, tests similar to those applied for detection of new cases could be used on sick or dead poultry to ensure that the compartment or zone remains free from infection even during an outbreak elsewhere in the country.

Movement of animals in the event of an EAD is an area where POC tests could play a role, including tests for antibody if immune status following vaccination is a condition of movement in EAD outbreaks where vaccination is used.

3.3 Recovery phase - demonstrating population freedom from infection

POC tests have the potential to be used to assist in demonstrating freedom from infection for specific pathogens. A key factor in selection and use of POC tests for this purpose is that the test methods and results must be recognised by trading partners. This, in turn, means that there will need to be an appropriate and extensive dossier of results demonstrating the value of the test for the purpose it is being used. This information may have to come from other countries for diseases that are exotic to Australia (e.g. FMD) and can also be developed during the response to an incursion (as occurred during the outbreak of equine influenza in Australia in 2007).

Appropriately validated POC tests can be used to demonstrate efficiency of an eradication policy provided the limitations of the test used are well reported and recognised when deploying it. Declaration of freedom will take into account all of the information available that supports the conclusion that a disease has been eradicated especially if approaches involving scenario tree modeling are used to demonstrate freedom.

Validated near-POC tests can play a role in determining the immune status of individual animals or herds (post-vaccination) if vaccination is used in an EAD response. Different tests and sample numbers are needed depending on the purpose of testing and expected prevalence of infection. For example, negative results for detection of an agent on a small number of samples do not guarantee freedom from infection for a low prevalence disease (see Section 4.11). (see Section 4.11).

4. Managing potential risks associated with use of point-of-care (POC) tests for EADs

POC tests offer a number of potential benefits as outlined in section 2 and 3 but a number of risks arise if POC tests are used for the diagnosis of EADs. Most of these risks also apply to other diagnostic tests but some are particularly important or specific for POC tests, especially if the testing is performed by producers. Only through an understanding of the risks is it possible to manage them so that the potential negative effects of improper use of POC tests are minimised and the positive aspects are accentuated. This section outlines a number of potential risks associated with the use of POC tests so as to ensure these are considered and addressed when POC tests are deployed. The risks that need to be considered and managed include:

i) the many factors that can lead to misclassification of animals as infected or not infected;

These factors, described in detail below, include issues such as use of the wrong type of test, collection and use of inappropriate samples, testing insufficient animals, or test reagents that have not been stored correctly,[22] all of which can apply to any type of diagnostic test. For example, the analytlcal sensitivity for some POC tests based on antigen detection is much lower than that of other laboratory based test systems based on nucleic acid amplification, and therefore false negative results can occur if the former tests are deployed in the field.

ii) failure to report results of POC tests (positive and negative) or to send samples for confirmation to veterinary authorities; and,

iii) premature announcement of incorrect results.

Release of information on a false positive POC test result suggesting Australia has an EAD is a particular concern. Such an announcement could have dramatic effects on Australian domestic and export markets. In the current world of rapid internet communications and social media such as Twitter, information (whether leaked or knowingly released by a test operator) about a positive POC test result to an EAD would propagate globally very quickly, especially given the number of agencies and groups dedicated to early capture of intelligence on outbreaks of emergency diseases. In contrast, a false negative POC test result on a farm might mean that samples from an outbreak of an EAD are not sent for diagnosis (e.g. a private company performs a multiplexed test for a range of avian diseases on a flock with avian influenza but the test provides a false negative result for this disease) or action to control a disease is delayed (e.g. false negative result on a POC sample in an outbreak of FMD in the Republic of Korea (ProMED-Mail 2010 – the reason for the false negative result in this case is not known). This will become particularly important when tests for multiple pathogens become available for POC and near-POC use. In such cases, a false negative result on a POC test would potentially delay diagnosis of the disease. Such a

[22] See for example the following discussion from Scientific American on the use of Twitter and other social media to assist in mapping outbreaks of disease http://www.scientificamerican.com/podcast/episode.cfm?id=social-media-tracks-disease-spread-12-01-09

situation could arise if operators are not properly trained, quality management systems are not in place, or the agent involved in the outbreak evolves so that it is not detected by the POC test. These and other factors that result in risk of misclassifying the infection status of animals as a result of POC tests are discussed in this Section.

4.1 Decisions to report disease and to test animals

When disease occurs, not all farmers choose to report it to authorities. Although existing laws in Australia make it compulsory to report notifiable diseases, the history of animal diseases globally demonstrates that some people choose to ignore such laws if they believe it is in their best interests to do so, especially if the likelihood of being caught is low. Reporting of a notifiable disease might also be delayed or because some diseases might not be recognised by farmers as being significant if they don't produce typical clinical signs or the signs are readily confused with those of endemic diseases that are not notifiable.

Using avian influenza in Australia as an example, it is presumed that most producers would report and want to test his or her poultry if experiencing an outbreak of HPAI, especially in highly susceptible species such as chickens and turkeys, given the high mortality rate associated with this disease. However, if poultry are infected with a low pathogenicity H5 or H7 virus, the farmer may not recognise that the poultry are infected because the infected birds may not show any clinical signs, may display only mild signs, or may develop signs of secondary bacterial disease (in which case the farmer may focus on the secondary infection and not realise that a notifiable disease is underlying the outbreak).

Some farmers may choose not to have animals tested or to report certain diseases (the so called 'shoot, shovel, shut up' strategy) if they believe that they will not be adequately compensated or fear the ramifications to trade of a case of a disease, such as bovine spongiform encephalopathy (BSE). Clearly, such actions are illegal where such disease are notifiable (as in Australia), but could occur and would be complicated further if simple POC tests for EADs become readily available, allowing farmers themselves to test the health status of their animals. Close cooperation between the livestock sector and veterinary authorities helps to reduce the risk of non-reporting and depends on the level of trust between farmers and authorities (Palmer et al. 2009).

Disease reporting can be viewed and assessed in a similar manner to screening tests. To ensure early detection of EADs, the 'sensitivity' of reports of diseases that meet the criteria of an EAD should approach 100%. If it doesn't, delays in diagnosis and implementation of control measures will occur.

4.2 Reason for testing and fitness for purpose of tests

As described in Section 3, during or after an EAD outbreak, samples are collected for a range of purposes (e.g. ongoing active and passive surveillance, demonstration of freedom from the disease once the last clinical case in an outbreak is diagnosed, ensuring business continuity etc.). A report on the use of screening tests for FMD (FAZD Center 2010) suggests that they can have value at all stages of an outbreak — early detection, response, and recovery — and that the type of test, as well as how and when it will be used (and by whom), will vary depending on the stage of the outbreak. In other words, POC tests are not necessarily fit for all purposes and must be validated with clear information on the analytical and diagnostic sensitivity and specificity of the test, and definition of the purposes for which the test can and should be used.

The World Organisation for Animal Health (OIE) has defined six main categories of use for diagnostic tests against which the 'fitness for purpose' of any test should be assessed during the process of test validation (OIE 2010c). These are:

i) To demonstrate population freedom from infection — with or without vaccination, historical freedom and re-establishment of freedom following outbreaks

A review conducted by SCAHLS[23] pointed out that a key factor in selection and use of POC tests for this purpose is that the test methods and results must be recognised by trading partners. This in turn means that there will need to be an appropriate and extensive dossier of results (recognised by OIE) demonstrating the value of the test for the purpose it is being used. This information may have to come from other countries for diseases that are exotic to Australia (e.g. FMD) and can be developed during the response to an incursion (as occurred during the outbreak of equine influenza in Australia in 2007).

ii) To demonstrate freedom from infection or agents in individual animals for trade purposes

The need for testing to demonstrate freedom from infection or agents in individual animals for export may be one of the main drivers for commercial development of POC tests. The cost of export certification and testing, in particular failures in export testing, is very high. Preliminary information on health status of a herd or flock provided via POC tests could alert a farm owner to a potential problem that would result in the herd or flock being ineligible for export, thus avoiding the need for costly tests.

iii) To demonstrate efficiency of eradication policy

Appropriately validated POC tests can be used to demonstrate efficiency of eradication policy provided the limitations of the test used are well reported and recognised when deploying it.

iv) To confirm or exclude a specific diagnosis in suspect clinical cases

As discussed earlier, POC tests can play a role in providing preliminary information ('triage tool') on the index case of an EAD. Their use would likely increase in the event of a widespread outbreak. The risk is that the sensitivity and specificity of the POC test may be lower than that of the laboratory-based testing system using the same samples, so that undue reliance on POC tests could result in farms being classified incorrectly as uninfected. Once POC tests used in the field have equivalent analytical sensitivity and specificity to laboratory-based tests and are backed by appropriate quality management systems, it may be possible to take action on an EAD on the basis of a POC test alone. Although this prospect remains unlikely for most diseases in the near term, it should not be dismissed as a possibility in the future.

v) To estimate prevalence of infection for risk analysis, classification of herd status, implementation of disease control

Validated POC tests conducted by approved operators could play a role in estimating prevalence of infection for risk analysis, classification of herd status, and implementation of disease control. Ongoing assessment of POC test results should be conducted through testing of selected samples in a reference laboratory and comparison of results with those from the POC test.

vi) To determine immune status of individual animals or herds (post-vaccination)

[23] Not in the public domain

Validated POC or near-POC tests can play a role in determining the immune status of individual animals or herds (post-vaccination) if vaccination is used in an EAD response.

Different tests and sampling regimes are needed for these different purposes and the expected prevalence of infection. For example, negative results for detection of an agent on a small number of samples do not guarantee freedom from infection for a low prevalence disease (see Section 4.11).

POC tests can be designed for 'rule-in' or 'rule-out' purposes (WHO 2009). For 'rule-in' tests the diagnostic specificity should approach 100%. 'Rule-out' tests require a sensitivity approaching 100% but can have a lower specificity. Both types of test can still be valuable, even if imperfect, provided their limitations are recognised and overcome, as use of rapid tests for influenza demonstrated in outbreaks of HPAI in 2002 (Chua et al. 2007).

4.3 Decision to use POC test rather than submit samples to a laboratory

The decision to use a POC tests obviously depends on whether a suitable test is available. The need to confirm POC test results for the index case of any EAD is regarded as essential (see, for example, Sammin et al. 2010). The decisions to use a POC test by a private operator and to not report the results (or not report them immediately) rather than submitting samples to a laboratory represents one of the biggest risks associated with use of POC tests. Legislative measures have been introduced to overcome this issue in parts of Australia.

4.4 POC test selection (type of POC test selected) and test attributes

The type of POC test chosen depends on the purpose of testing and test availability.[24]

The main risk in test selection is that the wrong test is chosen providing misleading information on the true infection status of the herd or flock. For example, serological tests for antibody detection (while very valuable in many other situations) early in the course of an outbreak or for a disease with a very high case fatality rate would have a high chance of failing to detect infected animals. In addition, the characteristics of the test need to be well defined so that the significance of positive and negative results can be determined. Examples for various test types are provided below.

4.4.1 ELISA for antibody detection

Data are available on the sensitivity and specificity of commercially available assays used as near-POC tests.[25] However, in many circumstances, serological tests for antibody detection are of limited value at the start of an outbreak except to demonstrate that an agent has not been circulating undetected in other livestock.

4.4.2 Lateral flow antigen ELISA/immunochromatographic tests

Numerous papers have been published on the analytical and diagnostic sensitivity and specificity under controlled conditions of lateral flow antigen ELISAs and related

[24] Includes testing at on-farm laboratories

[25] See, for example, the data sheet for *Mycoplasma hyopneumoniae* serology from Idexx available at http://www.idexx.com.au/pdf/en_au/livestock-poultry/mhyo-ab-test-brochure.pdf

immunochromatographic tests (see, for example, Chua et al. 2007, Ferris et al. 2009). In general terms, these tests usually have a lower analytical sensitivity (i.e. a higher threshold for detection) than PCR or culture, and have a low diagnostic sensitivity when the wrong sample type is used (e.g. scabs over old vesicular lesions rather than vesicular epithelium). The specificity of these tests is variable (see, for example, Marché and Van den Berg 2010).

4.4.3 Real-time PCR and other techniques based on nucleic acid amplification and detection

Key risks for tests based on nucleic acid amplification and detection are false positive and false negative results (as discussed in Appendix 1). These risks can be managed with appropriate quality management systems, including controls for each stage of the testing process. Miniaturisation of testing systems (see Appendix 1) may compromise quality management if it is no longer possible to run the same number of controls as used in reference laboratories for similar non-miniaturised tests. POC tests should ideally have the same or similar internal controls as their laboratory counterparts to ensure that all steps in the test have worked properly, including nucleic acid extraction.

Test sensitivity and specificity, both analytical and diagnostic (Saah and Hoover 1997), are major areas of focus for all new tests, including POC tests. Test sensitivity and specificity are influenced by many factors that may not be apparent under the controlled conditions in laboratories (i.e. the analytical sensitivity determined under controlled laboratory conditions is likely to represent an upper limit, especially for tests in the hands of novices or conducted under less than optimal field conditions). Diagnostic sensitivity and specificity of tests are affected if samples collected at inappropriate times are tested.

Detection of nucleic acid does not necessarily mean that an infectious pathogen is present in a sample or in the animal from which the sample was collected (Saah and Hoover 1997). This has implications for movement controls in disease outbreaks in which infected animals are quarantined (as occurs with equine influenza and swine influenza) rather than being destroyed. It has been shown that equine influenza virus nucleic acid can be detected for longer than viable virus in infected horses (Read et al. 2011).

4.5 Test / reagent storage

Some test reagents are heat labile, including capture antibodies and probes/primers. Heat lability is relevant to all types of tests but particularly relevant to POC testing when samples are tested under field conditions. This may be a risk if reagents are stored at remote locations under less than ideal conditions and quality management systems are not in place. Lyophilisation of reagents can assist in overcoming this problem (Madi et al. 2011). However, samples themselves are also heat labile and POC tests reduce the risk of sample degradation during transport by removing this step in the chain between sample collection and testing.

4.6 Species factors

Depending on the disease, the species tested can have a large effect on the quality of results from POC tests. In the case of HPAI caused by viruses of the H5N1 subtype, ducks are less likely to show clinical signs than chickens so are less likely to be tested if the decision to test is based on clinical signs. They also tend to shed lower quantities of virus, often below the detection limit of some POC tests (see Appendix 2). Differences occur in shedding patterns of virus and sites of viral multiplication (duration of shedding, main routes of shedding, tissue tropism when testing dead birds etc.). These factors are particularly relevant for those POC tests with a relatively low analytical sensitivity — they are only of value if the correct samples are collected.

4.7 Animal population factors and timing of sample collection

In the early stages of a disease the agent responsible may not have spread through the entire herd or flock. In such a situation, the sub-population of animals examined and tested is important — a random sample of the whole flock or herd may not detect disease and targeted sampling is usually preferable. These issues have been considered in detail by others (see, for example, Cameron 2011).

The value of a sample depends on the stage of the outbreak at the time the samples are collected. For example, serological testing for antibody detection is usually better than viral testing for historical information (but not very useful for highly pathogenic viruses that have a high case fatality rate). Tests on chickens at 24 hours post-infection for H5N1 HPAI can give negative results on immunochromatographic tests even though virus is being shed, but by 48 hours post-infection most infected chickens will return a positive test, reflecting the increased viral load in oral and cloacal samples at that time (Marché and Van den Berg 2010).

Detection of a PCR-positive sample may not be indicative of active infection if the sample is collected several weeks after an outbreak on a farm. For example, in equine influenza there can be prolonged detection of RNA beyond the infectious period (Read et al. 2011). A number of H5N1 PCR positive results were obtained from environmental samples collected in Cambodia but none was positive on culture; no culture positive samples were detected within 12 days of the last known clinical case (Vong et al. 2008).

4.8 Sample type (what samples to collect)

The type of sample collected influences the validity of results.

In the case of FMD, flaps of vesicular epithelium contain the highest concentration of virus. If these are not available and other samples such as scabs over ruptured vesicles are used, the virus will not be detected in most POC tests.

For BSE the location of sampling is extremely important. Tissue 1cm caudal or rostral to the obex is the preferred sample for near-POC tests (OIE 2010b).

Samples of faeces, semen and tissues from autolysed carcasses are all recognised as having significant potential to interfere with methods for nucleic acid amplification.

Dead or sick birds are more likely to yield a positive antigen detection test than clinically healthy bird for HPAI (the latter in an infected flock will only be positive if the birds tested is incubating disease, the sample is taken at a time when shedding has commenced and is at high enough levels to ensure detection).

For avian influenza there are differences in viral concentration in the cloaca, oropharynx, internal organs, faeces, feathers and general environmental swabs from areas where poultry are kept. POC tests with a low analytical sensitivity should not be used on samples with low concentrations of virus (e.g. faecal samples from healthy animals or environmental samples) (see, for example, Slomka et al. 2011).

Studies on human pandemic influenza (H1N1) also demonstrate that diagnostic sensitivity is influenced by sample selection, with some patients testing negative on nasopharyngeal swabs but positive on bronchoalveolar lavage (Singh et al. 2010) despite using laboratory-based real-time PCR with high analytical sensitivity to test the samples.

Given that the type of sample collected influences the validity of results and that POC tests may be used by lay or other operators who are not otherwise aware of this, guidance is required on sample selection when POC tests are used.

4.9 The method of sample collection and labeling of samples

The material used for collecting specimens can influence test results. Examples include the types of swab (e.g. RNase in wooden swabs, cotton versus Dacron swabs (CDC 2009), calcium in swabs (Menassa et al. 2010)) the type of transport medium (transport media containing glycerol will interfere with PCR), and excess blood or excess material around the swab (which can interfere with lateral flow devices, and with PCR if too much extraneous nucleic acid is present). In addition, contamination of samples can occur if care is not taken during collection when dealing with multiple animals.

Labelling of samples is also an issue but potentially less of a problem for samples tested on site than with those sent to a distant laboratory. Mislabelling should not be neglected as a potential cause of error in identification of infected animals if multiple samples are collected and for correlation of results once samples tested at POC are also tested in a central laboratory.

4.10 Disease prevalence

The positive predictive value of any test falls markedly as the prevalence of a disease falls (i.e. there is a higher proportion of false positives for low prevalence diseases due to the fact that no test is 100% specific). The number of animals that have to be sampled to ensure detection of disease in a given population also increases as prevalence falls, and the number required is also influenced by the test sensitivity and specificity. Many POC tests have lower analytical sensitivity and specificity than laboratory-based tests, so the number of samples required to be confident of results in surveys will usually be greater if POC tests are used. These test limitations need to be recognised and built in to survey design. This issue will become particularly important if POC tests for low prevalence disease are available to those without the background knowledge to consider and understand the effects of prevalence on positive predictive value.[26]

4.11 Sample preparation

Many POC tests require minimal sample preparation so that the test has to be sufficiently robust to deal with substances in the sample that could interfere with the test. For example, major problems have been encountered in developing suitable POC tests for saliva samples for detection of *Mycobacterium tuberculosis* because of the sample matrix. Some of these aspects are discussed in detail by Beyor et al. (2009) in the development of their prototype 'lab on a chip'. It is necessary to include systems in miniaturised POC tests to remove extraneous materials from the target before nucleic acid amplification commences. On the other hand, minimal sample handling also reduces the risk of cross contamination.

Inhibitors in samples or in reagents can be important unless appropriate measures are taken. As described earlier, excess mucus or tissue in lateral flow devices can clog the membrane and prevent the sample from interacting with the antibody in the device (Sims unpublished, Chua et al. 2007). Excess antigen can interfere with tests for antigen detection in lateral flow devices — the so-called 'hook effect' (O'Farrell 2009).

[26] The US CDC website contains information on the relationship between prevalence, specificity and positive predictive value for rapid diagnostic tests for influenza in the document entitled Rapid Diagnostic Tests for Influenza available at http://www.cdc.gov/flu/professionals/diagnosis/rapidlab.htm

Sample contamination during testing is a major potential concern (highlighting the importance of having appropriate controls) and can occur at any point in the process. Operator skill is also important in reducing the likelihood of contamination. For POC tests contamination can be less of a problem as there may be no nucleic acid contaminants from previous testing, (as can occur in a laboratory). However, problems can occur with small devices if appropriate steps are not taken to prevent carry over between samples.

Ultimately, all of these issues relate to quality management and highlight the need for good quality management systems. It may be harder to implement these systems when 'sample-to-answer' tests are used given that there is little operator control over these tests.

4.12 Environmental factors (including place where the test is conducted)

A range of factors including temperature, dust, wind, level of contamination in the environment, stability of electricity supply and quality of water can affect the validity of test results conducted in the field, especially if dealing with agents that persist in the environment or can be transported by air. Results obtained with rapid antigen testing of human samples for pandemic influenza A(H1N1) varied with location (Landry et al. 2011), suggesting an influence of different environments on POC test results.

4.13 Pathogen factors (antigenic or molecular variation in the organism)

As with all types of tests for pathogens, one of the major risks associated with all diagnostic tests, including POC) tests is evolution of infectious agents so that tests no longer recognise the pathogen for which it was originally designed (see, for example, Ferris et al. 2006). This can be a major issue for viruses that evolve rapidly such as influenza viruses and many other RNA viruses. The way to overcome this problem is to ensure that primers and probes (and antigens and antibodies) are based on current sequences and antigenic characteristics and, wherever possible, are based on highly conserved areas of the organism. The former is difficult to achieve if a new virus emerges such as the H1N1 human pandemic virus. To detect molecular changes it may be necessary to run two tests either in parallel or sequentially — one that is type-specific (e.g. influenza A) based on a conserved part of the organism and one that is subtype specific, which is more likely to be subject to variation as the agent evolves. This process applies to any type of test, but when laboratory tests are miniaturised there is a possibility that for some tests fewer sites will be targeted in the assay. If this is the case it may produce false negative results.

4.14 Machine/equipment factors (for new field-designed testing systems)

The quality of the engineering of the equipment, such as the machines used in 'lab on a chip' POC testing, and algorithms used to analyse the data (e.g. data from microarrays) influence the quality of results. Without a sound understanding of the assumptions on which these algorithms are based it may be difficult for test operators to assess the validity of test results. As POC tests are more likely to be operated by operators with limited knowledge of the tools they are using (in contrast to tests conducted in a laboratory performed by qualified technicians), there is a possibility that some incorrect results could be reported. This issue can be addressed through quality management systems.

4.15 Operator factors (experience and training of the test operator)

Operators without formal technical qualifications will have limited understanding of the factors that can influence test results. Human health authorities in Australia have some training programs in place for POC test operators. Turnover of staff is one of the important considerations in maintaining an appropriately trained group of POC operators (Shepherd et al. 2009).

The importance of an ongoing quality management program for all POC tests and test operators cannot be overstated as a means of reducing the risks of incorrect results because of improper test procedures.

Although not expected to affect test results, occupational health and safety aspects of some POC tests also need to be considered (e.g. when dealing with zoonotic agents such as some avian influenza viruses or Hendra virus).

Under laboratory conditions, samples potentially containing zoonotic infectious agents are handled using appropriate biosafety measures (such as biosafety cabinets). Staff involved in field-based POC testing would already be at risk of exposure to infected animals or carcasses and would be expected to wear appropriate personal protective equipment (PPE). The incremental risk of exposure from POC testing is likely to be low, unless the test procedure generates droplets or aerosols of potentially infectious particles or the test is conducted outside the restricted area on the premises and PPE is not worn.

Disposal of used tests kits should follow standard recommendations for medical waste containing (or possibly containing) infectious agents. Note that duplicate samples for all POC tests and all positive samples in which nucleic acid has been amplified should be kept for additional laboratory testing rather than being disposed of in the field. If used by farmers on farms limited access to medical waste collection or an autoclave creates a potential problem. If samples or used kits are disposed of incorrectly there is a low level risk of disease transmission to other animals and to humans.

A recent study on a rapid test for *M. tuberculosis* using the GeneXpert system (Banada et al. 2010) demonstrates the type of protective measures that should be implemented to ensure operators are not put at risk.

4.16 Quality management systems

Many of the factors described in this section relating to test performance can be overcome if suitable quality management systems are in place. All recommendations on the use of POC for EADs state the importance of having quality management systems in place (see for example, Sammin et al. 2010) but problems for quality management programs can still be encountered for any test that is used infrequently (i.e. lack of data to assess test performance over time). This has implications if equipment is stockpiled.

4.17 Frequency of non-reporting of positive findings from POC tests

Even though it is an offence in most jurisdictions in Australia not to report suspicion of a notifiable disease (and in some places, results of testing for notifiable diseases) it is still possible that some people will choose not to do so, potentially leading to delays in disease diagnosis and implementation of control measures. Bioethical implications of the increased availability of POC tests in human medicine have been considered (Kearns et al. 2010) and will provide fertile ground for similar studies if POC tests for EADs become widely available to farmers.

4.18 Transmission of disease through equipment used for POC tests

Any equipment for POC tests that is used at or near the site of an EAD outbreak must be properly decontaminated before being moved off site to avoid inadvertent spread of pathogens. The equipment must be designed so that it can be cleaned and disinfected, a feature of many of the rugged systems designed for field amplification and detection of nucleic acids.

4.19 Summary of risk factors affecting test results

Each of the factors listed in this Section can influence the quality of the information provided by POC tests and each needs to be considered when assessing the weight to be placed on a positive or negative POC test result for an EAD. Ultimately, the goal is to identify correctly the infection status of animals either individually or on a herd or flock basis. When assessing these factors the benefits that can accrue from the use of POC tests must also be considered. A risk management plan for each POC test (and specific uses of the test) could be developed to ensure that all of the risks associated with the use of the test are recognised and that appropriate mitigation measures are implemented.

5. Factors that influence the development and availability of point-of-care (POC) tests

A key determinant of whether a test is developed is the existence of a viable market for the product. This section considers some of the factors that may determine whether POC tests will be developed for Australian EADs.

A market exists for POC or near-POC tests for animal diseases, including tests for companion animals, as the products developed and sold by companies such as IDEXX, Symbiotics, Svanova and Prionics demonstrate. However, only a small number of POC antigen detection tests are available commercially for EADs, including tests for FMD and rinderpest.[27] Most commercially available POC tests for EADs are not yet based on nucleic acid amplification or microarrays, other than several POC or near-POC systems that have been developed but have not yet found a strong commercial market in animal disease diagnosis.

It is noteworthy that during an outbreak of equine influenza in Hong Kong in 1992 a commercially available POC test (Directigen) was used to assist in detecting infected horses. It proved to be extremely valuable as a tool in managing the outbreak (as a 'rule-in' test for quarantine purposes) even though the sensitivity was later found to be only 83% and specificity 78% (Chambers et al. 1994). The same test was used for rapid detection of infected chickens in outbreaks of HPAI in Hong Kong in the early 2000s (Sims et al. 2003) — see Section 3 and Appendix 2. The same test was also used in laboratories for detection of small quantities of virus in cultures for both equine and avian influenza, providing a rapid confirmation of the presence of influenza A virus in allantoic fluids from inoculated embryonated eggs containing a haemagglutinating agent.

However, the test used for these cases was not developed specifically for domestic animals. Rather, it was designed for use in medical clinics to assist in detection of influenza virus in human patients. Since then, there have been only a limited number of POC tests developed and released commercially for diseases of livestock and those that have been released for EADs (e.g. FMD lateral flow devices) have a relatively limited market in developed countries given that these diseases occur infrequently. These cases demonstrate that the market for POC tests for EADs in the livestock sector is not strong, and that many of the advances in POC testing will probably arise from adaptation of human health applications rather than through dedicated animal health diagnostics companies.

POC tests for EADs are normally developed either because veterinary or disease managers see a role for the test as part of their rapid response and control programs or if commercial companies identify an unmet demand, sufficient to justify the investment in test development, production and marketing. As discussed in Section 2, developments in POC tests for human health are being driven by concerns about bioterrorism, by demands for economically important diseases such as carriage of methicillin-resistant *Staphylococcus aureus*, or through aid agencies providing support for development of POC tests suitable for use in resource-poor settings for important diseases such as tuberculosis (WHO 2008, WHO 2009).

A WHO study (WHO 2009) on pathways to better diagnostic tests for tuberculosis provides valuable insight into the steps required during test development, starting with the needs

[27] See for example Svanova Biotech AB s product listing at http://www.svanova.com/showpage.asp?pageID=15

assessment to determine the desired features of diagnostic tests. This step is followed by feasibility studies that assess at an early stage whether it is possible to develop an appropriate test and to rule out techniques that are unlikely to be feasible. This step also includes assessment of intellectual property requirements that must be considered (patent issues etc.). Other legal issues (including legislation on how particular tests can be used and who can use them) also influence commercial decisions. A regulatory plan forms part of the feasibility assessment undertaken by companies deciding on whether to develop and commercialise specific tests. The third phase is the development and optimisation of the test and associated technology, including costing the test and redefining the business plan, development of prototypes, and early field-testing and user feedback. The next phase covers independent evaluation of the test and demonstration of its benefits. The final stage is an impact assessment under field conditions.

It has been suggested (FAZD Center 2010) that tests capable of detecting multiple endemic agents (e.g. using microarrays) of production animals are more likely to be attractive to commercial companies than those for emergency diseases because of the size of the potential market. If appropriate tests are developed and a suitable market for these tests exists it would be possible to add additional components to these tests for EADs, such as FMD or avian influenza. This process should be encouraged as the cost of incorporation of several additional targets in a multiplexed test is likely to be relatively low. Including elements that detect pathogens that cause EADs would allow early detection of these diseases if they occurred.

Time will tell whether a suitable market exists for multiplexed diagnostic tools for production animals or whether these will remain in the realm of research laboratories. The costs involved in development of tests can be prohibitive (e.g. microfluidics ‘sample-to-answer’ tests), especially for the development and production of niche products with a low volume of sales.[28]

Until then, the main source of POC tests for EADs will likely be state-funded organisations with a mandate to diagnose, control and prevent these diseases, or partnerships between national and international agencies and private partners.

[28] See interview with Leanna Levine at http://www.qmed.com/mpmn/article/29267/design-and-development-sample-answer-microfluidic-disposables-point-care-applicat

6. Conclusions

This review has examined the potential application associated with the use of existing and new POC and near-POC tests in the management of EADs in Australia.

In Australia at present, POC tests mainly play a minimal role in the management of EADs but they have the potential (if available) to assist in 'ruling in' cases, especially as an outbreak progresses, in selection of samples for testing at a reference laboratory (especially for the index case), and in providing additional immediate information for cases with potential public health implications. They can also play a role in surveillance testing prior to the detection, in detection of cases around an outbreak site, in movement management, assessing disease status of zones and compartments, and in determining freedom from infection.

If POC tests are used they must be readily available when an outbreak occurs. This means that protocols for use of these tests must be included in operational manuals and the required tests and necessary equipment have to be stockpiled in appropriate locations. The costs of stockpiling need to be considered against the costs if samples are sent to a laboratory for testing, the benefits of earlier diagnosis by POC testing, whether the information provided by the POC test exceeds that from clinical inspection and the extent to which use of POC tests will prevent laboratory overload. The tests also have to be validated as fit for purpose.

Use of POC tests offers advantages but also creates a set of risks that have to be managed, some of which are unique to POC tests. Given the small number of POC tests available for EADs at present, this process is relatively easy to regulate and manage. It is expected to become more difficult once simple, user-friendly, accurate, multiplexed POC tests for livestock diseases become available and are used by producers. Development of tests that fit this description is being promoted by animal health experts elsewhere; it is viewed as the most likely pathway for development of commercially viable tests for EADs given the small market for tests for these diseases. Tests that fit this description are available that can detect a range of respiratory pathogens in humans and have the potential for use as POC or near-POC tests. They could be adapted for use in animals.

It is not possible to predict which of the developing technologies will be viable commercially as POC tests in the animal health field. In theory, any test involving nucleic acid amplification can now be adapted for field use but to date the uptake of these technologies in POC tests has been limited, despite the development of field deployable equipment. In part this reflects the relative cost of deploying equipment and reagents to field locations versus the cost and time of transporting specimens to a laboratory. Methods for amplification and detection of pathogen nucleic acids that use isothermal amplification (such as LAMP) appear to offer some significant advantages over those that require thermal cycling and this technology is likely to become more commonly used in POC tests.

Although tests based on amplification and detection of nucleic acids appear to be favoured at present as the most likely new generation of POC tests, other technologies that improve the analytical sensitivity of existing antigen detection test systems or that are capable of detecting small quantities of an organism without amplification are also under development. Systems that require minimal operator handling of specimens and therefore minimal training offer considerable advantages and could result in wider adoption of POC tests for EADs. These have already been developed and deployed as POC tests for human pathogens.

Some POC tests (e.g. for influenza) used in animals have been developed for use in humans, and not specifically for use in animals. The experiences with POC tests for infectious diseases for the human health sector (especially POC tests for resource-limited settings in the developing world that approximate the conditions for POC tests conducted on farms in Australia) should be monitored to assess if similar tests are suitable for adoption or adaptation for EADs. A number of the assay systems currently under development appear to offer potential as POC tests for animal diseases.

Appendix 1. Point-of-care test methods and techniques

This section briefly reviews the types of POC tests available or likely to be available in the near future and the technologies that are under development that could be used to underpin POC tests. Further information on tests for five EADs, chosen as examples of different diseases and pathogen types, is provided in Appendices 2–6.

Three main test types are used or are expected to be used in the near future at POC for infectious diseases — tests for antibody, tests for antigen detection, and tests based on amplification and detection of microbial nucleic acids usually following amplification. Within these three broad types is a wide range of methods and techniques. These are summarised in Table 1 in Section 2.

A1.1 Tests for antibody detection

Simple serological tests based on ELISA technology have been used in the intensive animal industries for many years. Managers of large intensive farms undertake in-house tests on flocks or herds as near-POC tests to assess responses to vaccination or to detect infection with certain pathogens. Commercial companies (e.g. Idexx, Prionics, Svanova and Symbiotics) produce these kits and provide broad interpretative information for users. The tests are generally designed for use on a representative sample of a herd or flock rather than for use on a single animal. All require an ELISA reader and (with a few notable exceptions such as pullorum disease testing) use serum samples rather than whole blood and thus need a centrifuge to separate serum from red cells. These equipment requirements mean the tests have to be conducted in places with trained technicians. Field centrifugation is possible but is more easily conducted off-site. One advantage of serological testing over other methods is that it is less invasive to collect a blood sample than tissue samples.

Lateral flow immunochromatographic methods are available for antibody detection for a range of human diseases, although not all provide accurate results. For example, major concerns have been expressed about their effectiveness for the diagnosis of human tuberculosis (WHO 2008). In contrast, POC serological tests for human immunodeficiency virus have proved to be valuable in detecting infected patients (Anderson et al. 2011).

A recent development for rapid antibody detection is the use of multiple protein targets incorporated as a microarray into an assay system for antibodies to contagious bovine pleuropneumonia on a nitrocellose membrane (Gangtelius et al. 2010). Similar systems have been developed for antibody detection against different influenza virus subtypes in humans (Koopmans et al. 2012) and could potentially be adapted for other pathogens providing more information about immune responses than standard serological assays.

Most of the POC tests currently available only provide evidence of exposure to a particular type of pathogen or vaccine antigen (e.g. influenza serology in the commercially available tests is based on antigens conserved by all type A influenza viruses; it is not normally subtype-specific). Apart from some specific cases (discussed for individual diseases in Appendixes 2 to 6) serological tests do not distinguish antibodies induced by vaccines from those following natural exposure to virus. Nevertheless, in places where vaccines are not used and where infection with pathogens of the same type is rare, these tests can provide valuable information. For example, tests for avian influenza allow detection of a flock exposed to influenza A virus. Additional tests can then be conducted to determine if the virus involved is a notifiable avian influenza virus (i.e. H5 or H7 subtype) or some other low pathogenicity subtype (OIE 2010a).

Serological tests only provide evidence of previous exposure to the agent in question. Thus a positive flock or herd may no longer be infected with the agent at the time the samples are

collected. Serological tests are also of limited use in the early stages of an outbreak given the time required from infection to seroconversion. For the rare diseases that are virtually 100% fatal, such as HPAI caused by some strains of H5N1 subtype virus circulating in Asia since 1997, these tests may fail to detect infected animals unless a large number of samples is collected given that the vast majority of surviving animals are unlikely to have been exposed to the virus.

Serological tests will continue to play an important role in disease diagnosis, monitoring of responses to vaccination, and disease surveillance. Based on the directions of research for POC tests, which at present concentrates on methods for pathogen detection, serological tests are not likely to be the main focus for new POC tests for EADs. Serological tests are expected to remain as near-POC or laboratory based tests, although POC tests that allow differentiation of vaccinated from infected animals could be developed to support specific disease control programs. The potential for development and use of serological POC tests for FMD should also be explored for use in animals with equivocal clinical signs suggestive of healing vesicles given animals can seroconvert within 8 days of infection (see Charleston et al. 2011 for evidence of seroconversion).

A1.2 Tests for antigen detection

A wide range of POC tests and near-POC tests is available for detection of antigens in samples including ELISA tests described above.

A1.2.1 Antibody capture of antigen

Commercial tests based on detection of viral or bacterial antigen have been available for many years (e.g. tests for detecting influenza A viruses in clinical samples) and the range of tests, especially for use in human medicine, has increased steadily over time (see, for example, Charles 2008). Most of these tests rely on a reaction between a labelled antibody (in the test system) with antigen (the agent of interest) in the sample, if present, followed by a reaction with a secondary antibody or other trapping chemical (‘sandwich’ assay). Some of these tests work on a competitive basis with antigen in the sample competing with labelled antigen in the test for sites on detecting antibody.

Various media have been used including combinations of nitrocellulose membranes and antibody-coated beads or colloidal gold particles. Lateral flow devices involve the diffusion of antibody-coated beads or particles along the membrane to a line of trapping antibody. Tests can be qualitative or quantitative. The evolution of these devices has been reviewed in detail elsewhere (see, for example, O’Farrell 2009).

Antigen detection tests can be highly sensitive in outbreaks of EADs if used on appropriate samples at the right time (Chua et al. 2007). They usually have a lower analytical sensitivity (i.e. a higher threshold for detection of pathogens) than culture or tests that involve capture, extraction, amplification and detection of nucleic acids from pathogens (described in the next section). The reagents in these tests are also heat-labile, making them less suited for use in areas where they may be exposed to high temperatures (Bissonnette and Bergeron 2010).

As with serology, some antigen detection kits only detect a type-specific antigen (e.g. Influenza A) and any positive test has to be examined further to determine the subtype involved, through either nucleic acid amplification or culture.

Overall, lateral flow devices for detection of a number of human pathogens perform as well as laboratory-based alternatives (Sturenburg and Junker 2009) but some of the antigen detection tests in use in human medicine have very low sensitivity (van Dommelen et al. 2010). These tests are generally regarded as ‘rule-in’ tests only, with negative samples requiring additional testing.

Areas of focus for developers of POC tests based on antibody–antigen reactions include improvements in the sensitivity of detection (see, for example, Hong et al. 2011), improvements in reproducibility of the test through changes to the way reagents in the test are prepared and presented to the antigen[29] (Tang et al. 2010), and concentration of the antigen to improve sensitivity (Mashayekhi et al. 2010). Use of antibody fragments has also been explored as a way of improving binding kinetics with the target antigen (Lim et al. 2005).

A novel technique that combines antibody binding to proteins with nucleic acid amplification (proximity ligation) offers much greater sensitivity than standard immunological tests for detection of avian influenza antigen (Schlingemann et al. 2010) and does not require nucleic acid extraction as a preliminary step.[30] The technique has been used experimentally in assays for FMD virus (Nordengrahn et al. 2008). Experimentally, use of carbon nanotubes as a detection device can also increase the sensitivity, compared to that of other methods, of detection of influenza virus using antibody (Lee et al. 2011). Both of these techniques have the potential to be used in future POC tests. Testing for multiple targets is also being developed and in some cases deployed. It has been suggested that biosensor-based detection methods for immunoassays could be more cost-effective than methods based on nucleic acid amplification (Ince and McNally 2009) and a system has been developed for detection of avian influenza virus in swabs (Wang et al. 2011).

At present, the only immunochromatographic test used as a frontline POC diagnostic tool in Australia for EADs is a test for *Bacillus anthracis*. This test has high sensitivity and specificity, with the diagnostic sensitivity falling once the time from death to sample collection exceeds 48 hours. The test is based on detection of protective antigen of *Bacillus anthracis*. Those performing the test require appropriate training (Hornitzsky and Muller 2010) and according to the Australian Standard Procedure any positive test is still required to be confirmed by an appropriate laboratory test.

Lateral flow devices have also been developed and are available commercially for FMD and provide results of equivalent sensitivity and specificity to those of the laboratory-based ELISA (Ferris et al. 2009). Similar tests are also available for rinderpest (Bruning et al. 1999) but use lachrymal fluid as the sample. Some companies will build customised lateral flow device tests on demand which means that it is theoretically possible to develop POC tests based on this technology for virtually any agent.

Near-POC ELISA testing is possible in any location with basic laboratory facilities and an ELISA reader, and increases the range of agents that can be detected to cover many EADs.

POC tests based on antigen capture will continue to be used and there may be renewed interest in these methods if detection systems under development now can be converted to practical POC tests with marked improvements in analytical sensitivity.

A1.2.2 Tests using aptamers for binding of targets

Aptamers are oligonucleotides selected in vitro for their capacity to bind with a particular part of a target chemical, recognising the target by shape rather than sequence (Lim et al. 2005).

[29] See, for example, http://www.ivdtechnology.com/article/better-and-faster-improving-rapid-point-care-testing and http://www.chembio.com/newtechnologies.html

[30] See the following video for simple background information on the technology http://www.abnova.com/abvideo/abvideo_detail.asp?k=Proximity_Ligation_Assay_(PLA)

Aptamers provide an alternative to antibodies as a means of capture of microorganisms in test systems (Bruno et al. 2009). Experimental devices using aptamers are in development for rapid detection of micro-organisms in food, aiming to overcome the need for culture and the associated delays in obtaining results. Aptamers have the advantage of being easier to manufacture and are more heat stable than antibodies (Bissonnette and Bergeron 2010). Aptamers have also been used for proximity ligation detection of proteins (Fredriksson et al. 2002). The application of aptamers for detection of microbes has been reviewed elsewhere (Torres-Chavolla and Alocia 2009).

It is possible that tests based on aptamers will form part of POC tests for EADs in the future.

A1.3 Tests based around nucleic acid amplification and detection

The PCR in its various forms is currently the most frequently used method for nucleic acid amplification. Isothermal techniques have also been developed that offer some advantages, not the least of which is that they do not require an expensive thermocycler.

Tests based on nucleic acid amplification are now well-established as frontline diagnostic tools in many countries in subnational and national laboratories for a wide range of EADs (e.g. avian influenza, equine influenza, classical swine fever, FMD). However, such tests can give both false negative and false positive results through failure to extract the nucleic acid, mismatches of primers and probes to the agent (see, for example, Landry et al. 2008), or most importantly, contamination (false positive) of samples at any stage from sample collection to the assay process. All of these issues can be overcome if appropriate quality management systems are in place and primers and probes are regularly monitored for necessary modifications based on changes in the gene sequences of target organisms. Running tests targeting different conserved areas of the genome (e.g. M gene of influenza virus plus tests using specific primers for a particular influenza subtype, such as H5) can provide an early indication of genetic variation if the generic test is positive and the subtype test is negative.

The main advantage of these tests is that they can detect minute quantities of pathogens in samples from animals and from the environment. The main disadvantage is that, without further tests, it is not possible to determine whether the nucleic acid amplified is from a ‘viable’ (infectious) organism. The high sensitivity, achieved because of amplification, also makes them vulnerable to contamination.

Until recently, most tests based on nucleic acid detection targeted a single pathogen. However, in the past few years, multiple agents have been targeted using a broad multiplex PCR followed by detection of individual agents through hybridisation with oligonucleotides arranged as microarrays (see, for example, LeBlanc et al. 2009).

A1.3.1 Methods based on thermal cycling

PCR using heat-resistant polymerase enzymes and thermal cycling remains the most widely used means of nucleic acid amplification. The methods involved have developed at a remarkable rate since their first application and tests using PCR are now deployed worldwide for diagnosis of EADs, even in developing countries. The development of real-time PCR revolutionised the way that diagnostic laboratories operate. In some laboratories, real-time PCR has replaced viral isolation as the main method of diagnosis of certain diseases (such as avian influenza) and allows quantification of viral loads in samples at a lower cost than performing a similar assay using culture (I. Brown pers. comm.). For detection of RNA viruses, reverse transcription is included in the process.

A range of real-time PCR variations has been developed and applied for diagnosis of EADs (Rodriguez-Sanchez et al. 2008). TaqMan technology is widely used (see, for example,

Belak et al. 2009). Linear-after-the-exponential (LATE) PCR (Sanchez et al. 2004) has been adopted by Smiths Detections[31] as the method of choice for its field-based PCR equipment. Reverse-transcription LATE PCR has been used experimentally for detection of all serotypes of FMD virus. The dynamic range of the test was the same as the real-time reverse-transcriptase PCR used routinely in the reference laboratory conducting the trial (Reid et al. 2010, Pierce et al. 2010). Another variations used in the diagnosis of animal diseases is the technique referred to as light-upon-extension PCR (LUX PCR) (Belak et al. 2009).

Multiplex PCR (Weile and Knabbe 2009), miniaturisation of PCRs, and combinations of the two are paving the way towards POC tests able to detect and subtype multiple agents (Thaitrong et al. 2010). These methods are discussed below under miniaturization of tests.

A1.3.2 Isothermal methods

Isothermal amplification methods have been developed and include technologies such as transcription-mediated amplification, NASBA, signal-mediated amplification of RNA technology (SMART), strand-displacement amplification, rolling circle amplification, loop-mediated isothermal amplification (LAMP), isothermal multiple displacement amplification, helicase-dependent amplification, single primer isothermal amplification, and circular helicase-dependent amplification. The features of these tests, which more closely resemble *in vivo* methods of gene amplification than those using thermal cycling, and their potential application in POC tests has been reviewed elsewhere (Gill and Ghaemi 2008; Asiello and Baeumner 2011).

It is not yet clear which of these techniques (if any) will emerge as the backbone of commercially-viable POC tests for animal diseases in the future but their simplicity is appealing given they can be performed using only a heat block or water bath and basic laboratory equipment. However, LAMP currently appears to be the favoured method for those looking to develop simple POC tests based on isothermal nucleic acid amplification. The test result can be easily read using a number of simple methods including a lateral flow device (Belak et al. 2009). A field-deployable assay for Hendra virus using these methods demonstrated equivalent sensitivity to a TaqMan real-time PCR assay (Foord et al. 2011). LAMP has also been used for detection of viruses in fish, including cyprinid herpesvirus 3 (Soliman and El-Matbouli 2010). A multiplex microfluidic system using LAMP has been used experimentally to detect a range of human and porcine influenza viruses (Fang et al. 2010). A system using NASBA technology is already available in a field deployable kit and test kits are available for a range of EADs.

A1.3.3 Microarrays

The use of DNA (and other) microarrays in microbiology, has been thoroughly reviewed elsewhere providing details of many of the methods available and their strengths and weaknesses (Miller and Tang 2009). Such microarrays use specific DNA oligonucleotide probes, bound to a solid surface, usually as a printed array, or to microscopic beads in a liquid array, that are exposed to and hybridise with products (usually labelled) of multiplex PCR. Microarray technology has the potential to revolutionise clinical microbiology given it has the capacity to detect and characterise a range of organisms in a single test run.

Miller and Tang (2009) concluded that:

[31] See http://www.smithsdetection.com/veterinary_diagnostics.php

'The ideal microarray platform for the diagnostic laboratory is a low- to medium density array that offers limited, reliable, and straightforward results without the need for sophisticated equipment and data management. Indeed, platforms that have begun to meet these criteria have been developed, such as electronic microarrays and suspension bead arrays.'

They, and others (see Zhao et al. 2010), also suggested that one of the weaknesses of existing systems, including those using suspension bead arrays, is the potential for contamination of samples given that the assay systems require opening of post-amplification tubes and pipetting. The use of a closed system is one of the advantages of real-time PCR, avoiding the need for a separate post-amplification area in the laboratory when conducting tests (as is required with conventional gel-based PCR technology in which amplification and detection of amplified products are performed as separate steps). Other potential disadvantages of microarrays are concerns about interference between components, issues with optimisation of multiplex PCR used before hybridisation with probes, and changes in genetic characteristics of the target which results in non-reaction with probes in the array.

A number of products using microarrays for clinical microbiology are available and others are under development or available for research use.[32] Products developed for human microbiology for respiratory pathogens include the Infiniti Respiratory Viral Panel from AutoGenomics;[33] the MultiCode-PLx RVP from EraGen Biosciences (Arens et al. 2010), the ResPlex II assay produced by Qiagen (Balada-Llasat et al. 2011), the Ngen respiratory virus ASR assay produced by Nanogen (Li et al. 2007), and the xTAG RVP from Luminex Molecular Diagnostics.[34] Of these systems, three use suspension bead arrays (Miller and Tang 2009). Studies comparing these systems are now being conducted and some have been published (see, for example, Balada-Llasat et al. 2011).

Multiplexed tests that can detect viral agents in samples from birds have been developed using Luminex Xtag technology (Boyd et al. 2011), a system that has also been used for detection of human respiratory viruses. A test for detection of FMD viruses and other bovine viruses using multiplex PCR coupled with a microsphere array has also been described and evaluated (Hindson et al. 2008). A panviral microarray system referred to as Virochip has been used to detect porcine viruses in a range of respiratory samples (Nicholson et al. 2011).

Microarrays are not available as POC tests due to their complexity, but it is unlikely that this will remain the case for long. For example, a microarray has been developed experimentally capable of detecting H5N1 influenza virus (at about 10^3 TCID) that does not require preliminary PCR. Capture oligonucleotides in the microarray hybridise with viral RNA and an intermediate oligonucleotide hybridises with the captured viral RNA. The bound intermediate oligonucleotide is then detected via gold nanoparticle-labelled probes stained with silver. This technique has the potential for POC application but still requires RNA extraction and a simple system for identifying positive signals (Zhao et al. 2010).

[32] See, for example, http://www.akonni.com/

[33] See http://www.autogenomics.com/1/infectious_respiratory.php

[34] See http://www.luminexcorp.com/rvp/overview.html

A1.4 Miniaturisation of tests and 'sample-to-answer' systems

Although miniaturisation only represents a different way of applying the various techniques already described, the 'holy grail' of POC testing is to incorporate all elements of tests based on the techniques described above in one small reaction vessel that requires virtually no technical skill for sample loading and provides a clear unambiguous and accurate result. Such a test reduces the training needed by the operator, reduces the risk of cross-contamination during testing, and minimises reagent costs by using smaller volumes. These systems are also referred to as micro-total analysis systems (μ-TAS) or 'labs on a chip'.

It is only in the past few years that systems have been developed that allow miniaturisation of all steps from nucleic acid extraction or isolation and concentration through to reading of the results. These developments have been possible because of advances in the areas of microfluidics and nanotechnology. Numerous papers have been published on and entire journals are now devoted to this topic (e.g. the journal, *Lab on a Chip*, published by the Royal Society of Chemistry in the UK).

One paper (Beyor et al. 2009) serves to illustrate the remarkable progress that is being made (at least at the experimental level) in the development of miniaturised test systems that include all steps in the process from sample to result, for detection of specific microbes, using very small volumes of reagents and samples.

A review of the use of isothermal cycling in μ-TAS (Asiello and Baeumner 2011) commented that there was still room for progress in development of the sample preparation component of existing experimental or prototypic systems. This review also suggested that systems such as NASBA that allow amplification at lower temperatures offer some advantages in terms of energy use than those that are isothermal but operate at higher temperatures.

Others (Lee et al. 2010) commenting on advantages and disadvantages of miniaturisation of tests involving real-time PCR, concluded that **'***Real-time PCR chips are not perfectly accurate diagnostic tools for a laboratory but they have advantages over traditional techniques for point-of-care testing.'* This statement demonstrates that there are tradeoffs with miniaturisation and that the limitations of these systems compared with the parent test will need to be well defined and assessed before deployment. Unless the gap between what can be achieved at POC and in a laboratory is very narrow, POC tests will remain supportive tools only with verification of results always required in a laboratory.

Other systems in which a single-use cartridge contains all test reagents and materials to conduct the test have been developed, are being applied, and have the potential to revolutionise diagnosis of diseases such as tuberculosis (e.g. Cepheid GeneXpert system) (WHO 2009). One of these tests is being applied as a true POC tests in hospital wards for detection of methicillin-resistant *Staphylococcus aureus* (Brenwald et al. 2010). Another system developed by Idaho Technology referred to as the FilmArray allows detection of multiple (21) respiratory pathogens using a pouch system and samples that require no pretreatment or accurate pipetting (Poritz et al. 2011). All reagents are included in each pouch and the test is based on a nested multiplex PCR followed by specific PCR for the 21 agents in the panel and melting curve analysis. The system has been compared with the Luminex Xtag system for respiratory pathogens and provided results much faster and with comparable sensitivity (Rand et al. 2011).

These tests offer a glimpse of the types of techniques that will be available in the next 10 to 20 years and represent important steps in the evolution of POC tests. Some of these could be adapted for use for pathogens of farm animals. Systems such as the FilmArray system would appear to be ideal candidates for developing panels of tests for respiratory pathogens in livestock and poultry given the development of similar panels for human respiratory pathogens.

A1.5 Other potential POC technologies

A number of other technologies in the development pipeline (Lim et al. 2005) could play a role in future POC tests. These include techniques that detect and analyse volatile gases from samples from patients, including so-called artificial 'noses' (WHO 2009). Biosensors will form a key part of detection systems in new tests (Rodrigues Ribeiro Teles et al. 2010) and are already being applied experimentally. Other related detection technologies such as quantum dot-based nanosensors (Zhang and Hu 2010) could also play a role. It remains to be seen which of these technologies will emerge as the main components of new POC tests for animal pathogens.

Appendix 2. Point-of-care tests for avian influenza

POC or near-POC tests for avian influenza are available commercially and have been used widely (Chua et al. 2007). The features of these tests and likely developments in POC tests for avian influenza have also been reviewed elsewhere (see, for example, Belak et al. 2009).

The main POC tests for avian influenza antigen use immunochromatographic methods that are limited by their inability to detect low concentrations of virus (i.e. they have a low analytical sensitivity). Near-POC tests, based on nucleic acid amplification, have also been used successfully and some of these are now available (or will be available very soon) for application in the field.

This Appendix examines the various POC diagnostic methods available or under development, how they might be applied, and how their use might influence disease control programs in Australia.

A2.1 Preliminary diagnosis based on clinical signs

HPAI cannot be diagnosed definitively without a test for viral detection followed by subtyping and molecular characterisation of the virus (OIE 2010). Nevertheless, during outbreaks of disease in chickens caused by a virulent virus, the clinical signs of rapid and high mortality provide strong grounds for suspicion of HPAI and early introduction of measures to prevent onward transmission of the virus from the affected farm, such as movement controls. In such cases, a positive POC test would provide additional evidence for the presence of infection, especially if used on suitable samples at the appropriate time. However, in both cases confirmation of the diagnosis for the index case by a reference laboratory would still be required. As discussed in Section 3 when chickens display atypical signs the suspicion of avian influenza may be low and action on infected flocks can be delayed unless samples are taken for tests for avian influenza.

A2.2 Serological tests for antibody detection

Serological tests based on ELISA technology that can be conducted in small in-house laboratories are available commercially, such as products from Idexx and Symbiotics. These detect type-specific antibodies (i.e. detecting antibody to Type A influenza viruses only) and are designed for use on a flock basis rather than on individual birds. Subtype-specific ELISAs have been developed in research laboratories but heterosubtypic cross-reactions can occur, reducing their specificity (Postel et al. 2011).

As most chickens in Australia are free from antibodies to influenza A viruses, any positive result for antibody to Type A influenza virus warrants further investigation following internationally agreed protocols (OIE 2010a) and the Australian Veterinary Emergency Plan (AUSVETPLAN[35]).

Serological tests for detecting exposure to field virus in vaccinated poultry have also been developed and are available as near-POC competitive ELISA kits.[36] A competitive ELISA

[35] Available at http://www.animalhealthaustralia.com.au/programs/eadp/ausvetplan/ausvetplan_home.cfm

[36] See for example products from the Noak company http://www.noackgroup.com/Live/ProductCatalog_en.YoCms?GROUP=%24Z_POU_11_01

using monoclonal antibodies to influenza neuraminidase subtypes N1, N2 and N3 has been shown to be highly sensitive and specific (Moreno et al. 2009). However, these tests have not been validated in the field for H5N1 HPAI viruses currently circulating in Asia and Egypt. The time of sample collection will affect the diagnostic sensitivity of these tests given the time it will take for sufficient poultry in the flock to seroconvert to allow detection of a positive bird. Not all vaccinated poultry exposed subsequently to virus will develop a serological response because vaccination increases resistance to infection and systemic infection may not occur after exposure of vaccinated poultry to virus (van den Berg et al. 2008).

A2.3 Antigen detection

Immunofluorescence on respiratory swabs has been used in diagnosis of human influenza for many years (Dwyer et al. 2006). A rapid test based on mouse monoclonal antibody to influenza type A on pancreatic impression smears was developed after the 1985 HPAI outbreak in Australia (Selleck et al. 2003) and was used at AAHL in subsequent outbreaks. It was also used on dead birds submitted for active surveillance at a laboratory close to an outbreak of HPAI in Bendigo in 1992 for screening hundreds of routine dead poultry from farms in the restricted and control areas.

The need for a fluorescence microscope and areas for sample preparation and staining limited the application of this technique to a laboratory with appropriate facilities. The test required experienced trained operators to prepare and read large numbers of smears. However, this test demonstrated that antigen detection was an appropriate means of rapid provisional diagnosis for 'ruling in' cases — even if the diagnosis needed to be confirmed by isolation of virus or other means of viral detection, and the sensitivity was lower than that of isolation.

This method of surveillance and testing would no longer be used following an outbreak. Instead it is more likely that tests based on nucleic acid amplification and detection would be used on swabs collected from clinically affected or dead poultry in the control area rather than transporting dead birds to a laboratory near the infected premises for testing (a process that required strict infection control and quarantine of laboratory staff in the Regional Laboratory where samples from dead birds were collected) (Sims, unpublished).

In 2001–02 when HPAI caused by viruses of the H5N1 subtype re-emerged in markets and farms in Hong Kong (after the outbreak in 1997), rapid tests (specifically the Type A influenza test Directigen) on cloacal and tracheal swabs, and sometimes on tissues, from dead poultry, were used successfully to detect infection. These tests were mainly performed in a veterinary laboratory given the proximity of poultry farms and markets to the laboratory, but in some cases were used in markets on samples from dead chickens (Sims unpublished). When results were compared with subsequent isolation of virus there were few false positives in the outbreak in the first half of 2002 and the system was capable of detecting all infected farms and markets (samples were also collected for culture and nucleic acid amplification) provided a minimum of three sick or dead birds was tested (Chua et al. 2007). A positive POC test on a dead chicken from a wholesale market was used to detect an infected consignment of poultry and subsequent traceback to the farm of origin for the index case in an outbreak in 2002. The farmer was selling sick birds. (Sims et al. 2003). The information provided by near-POC tests allowed early introduction of control measures on farms as soon as antigen-positive dead birds were detected. All test results were confirmed by NASBA or reverse transcription, real-time PCR and culture.

Five antigen detection tests for avian influenza were evaluated in one study including some that test for Type A influenza and others designed to detect H5 subtype viruses (Chua et al. 2007). The overall sensitivity of the five tests, including two POC tests that are commercially available, ranged from 36.3 to 51.4% (95% CI ranging from 31.0 to 57.0%) when using

samples that were positive on culture (although some of the samples had been stored for some time before testing and some had to be diluted due to limited quantities of sample). The samples used included material from faecal samples collected from healthy birds at markets as well as swabs from clinically affected poultry. The diagnostic sensitivity of the tests was greater for sick or dead chickens than for other types of samples.

Commercially available antigen detection tests have also been evaluated under field conditions in Nigeria (Meseko et al. 2010), Egypt (Soliman et al. 2010) and Vietnam (Slomka et al. 2011). One false positive result was detected in the study in Egypt from 62 swabs (negative M gene RT-PCR but positive on two different antigen detection kits) (Soliman et al. 2010), and some false positive results were also noted in another study (Marché and van den Berg 2010). As has been discussed previously, antigen detection using existing tests requires relatively high concentrations of virus, above a threshold, usually 10^4 to 10^6 TCID/mL (Chua et al. 2007, Marché and van den Berg 2010), and this threshold is not reached in cloacal or oropharyngeal swabs collected in many waterfowl infected with influenza viruses, in contrast to clinically affected chickens, chukar, quail and pheasants (Chua et al. 2007). The quantity of H5N1 HPAI virus excreted by clinically affected ducks is often too low to allow viral detection using these test on samples from oropharynx or cloaca but brain swabs from dead ducks were suitable, provided the brain tissue did not clog the membrane on which the test was performed (as occurred with one of the tests assessed) (Chua et al. 2007). A study conducted in Vietnam demonstrated that feather samples from clinically affected poultry were more likely to test positive than cloacal and oropharygeal swabs. None of the 21 swabs from ducks that tested positive using real time PCR were positive using two different lateral flow devices but 53% and 33% of feather samples were positive (Slomka et al. 2011).

Other avian influenza rapid antigen tests have also been tested and have similar sensitivity and specificity (Chua et al. 2007). Tests have been developed that are specific for H5 virus only and offer the advantage of providing increased evidence of involvement of that subtype (He et al. 2010a).

Rapid antigen tests are used for diagnosis of influenza in humans, often in clinics. It has been reported that these tests had a low sensitivity for H1N1 pandemic influenza viruses in humans (Ginocchio et al. 2009). One study in Turkey also demonstrated their relatively low sensitivity (Ciblak et al. 2010) and the authors called for more sensitive POC tests. Current antigen detection tests are not as sensitive or as specific as tests based on nucleic acid amplification that can generally detect much lower concentrations of virus. Likely developments in commercial POC tests for influenza to 2013 have been studied and include new lateral flow devices, tests based around nucleic acid amplification. A number of these tests are capable of determining the subtype of virus present (Tayo et al. 2011).

A2.4 Tests based on nucleic acid amplification

NASBA was used in Hong Kong in the early 2000s before the availability of real-time PCR assays for avian influenza virus and allowed rapid confirmation of the presence of influenza A (H5) virus in pooled samples. The main disadvantage of NASBA at the time was the high cost of reagents but given the lack of alternative systems the expense was deemed worthwhile (Sims, unpublished). A mobile unit developed by Haikang Life Corporations Limited allows amplification and detection of viral RNA using NASBA. The equipment is only suitable for indoor use and nucleic acid extraction has to be performed on samples before they can be tested.

A near-POC ‘sample-to-answer’ test for influenza viruses (Xpert Flu A system) has been developed. In one trial using clinical samples from humans and comparing results to an in house quantitative RT-PCR it was found to be highly specific. The only influenza viruses it

did not detect were an H9 subtype virus and some viruses that were either present in low concentrations or that were molecular variants that the RT-PCR test, run in tandem, also had difficulty detecting (Jenny et al. 2010).

POC diagnostics based on LATE-PCR technology under development by Smiths Detections (Landry 2011) can detect and differentiate avian influenza and Newcastle disease. The unit developed by Smith Detections (Bio-Seeq[37]) provides all the necessary tools for PCR in the field using a disposable system for nucleic acid extraction to work with a portable machine. A similar device has been developed by Idaho technology (RAZOR[38]) and uses TaqMan real-time PCR.

Another portable device also developed by Idaho Technology the Ruggedized Advanced Pathogen Identification Device (RAPID[39]) has been used experimentally to detect various influenza A viruses (Daum et al. 2007) and has been used in the field to test samples from wild birds (Takekawa et al. 2011).

An experimental rapid POC system based on RT-PCR that provides results in approximately 30 minutes has been developed (Abe et al. 2011). Other tests that are being developed include arrays using nanoparticles (Zhao et al. 2010). These do not require amplification of nucleic acid.

A technique using double-stranded molecular beacon probes (Kerby et al. 2008) coupled with isothermal amplification of influenza virus RNA can be used to assess a number of features regarding influenza viruses in the sample.

Although not yet applied to avian samples the respiratory panel using FilmArray from Idaho Technologies has been tested against a range of influenza subtypes in human samples (Rand et al. 2011).

A2.5 Microarrays, miniaturization and sample to answer systems

The FluChip (MChip) has been evaluated for detection of a range of influenza viruses in humans. This particular microarray uses subtle differences in M genes in influenza viruses to distinguish strains. Assessment of the assay depends on 'training' the system to recognise different viral strains (Heil et al. 2010) and it is not available as a POC test. In one assessment, this test was highly sensitive, but less so than RT-PCR, and although sample processing and testing took seven hours (much longer than the usual time required for a POC test) the test provided accurate subtype information (Mehlmann et al. 2007). A miniaturised PCR system has been developed to detect avian influenza based on a solid phase microarray and has a test duration of one hour (Sun et al. 2011).

Other techniques have been developed experimentally that allow very small quantities of avian influenza viral antigen to be detected in a microfluidic system using immunoagglutination, even in a faecal matrix, using a light detection system dependent on Mie scattering (Heinze et al. 2010). This method has the potential for POC use in the future.

[37] See http://www.smithsdetection.com/media/Bio-Seeq_Vet_Diag_Sys.pdf

[38] See http://www.idahotech.com/RAZOREX/index.html

[39] See http://www.idahotech.com/RAPID/

A2.6 Use of POC and near-POC tests in management of avian influenza in poultry

Tests available for POC or near-POC diagnosis of avian influenza have been used in other parts of the world as part of the diagnostic system for avian influenza outbreaks — e.g. Hong Kong SAR (Sims et al. 2003), Indonesia (Azhar et al. 2010), and Egypt (Soliman et al. 2010) — often as screening tests in which a positive result provides grounds for action or preliminary measures on the farm, property or household/village of origin.

The structure and size of the poultry industry globally provides market opportunities for the manufacture of diagnostic tests designed to detect pathogens of importance to the industry, as already occurs with simple antigen/antibody detection tests for avian influenza sold by companies such as Symbiotics and IDEXX. The production and availability of the current generation of POC tests suggests that once technological hurdles are overcome, POC tests based on new and evolving technologies will become available for a range of avian diseases. Some of these are expected to be capable of detecting and even typing multiple agents, including avian influenza viruses. Although not yet available as a true POC test, microarrays have been used to detect avian influenza viruses and porcine respiratory pathogens. Systems for detecting human respiratory pathogens, including influenza viruses, proved to be valuable in the recent (2009–11) human influenza A(H1N1) pandemic.

Commercially available near-POC serological tests offer the possibility of detecting (previous) infection with LPAI virus that would not otherwise be detected. Any such results would need to be confirmed by a reference laboratory and the subtype of virus determined through HI tests and, ideally, isolation of virus.

The situations in which POC tests for EADs (including avian influenza) could be used in Australia in the future once appropriate tests are available and shown to be fit for purpose have been discussed in Section 3. Table 2 provides information on the situations where tests could be of value prior to, during and after an outbreak in Australia.

Table 2 Potential uses of POC and near-POC tests for highly pathogenic avian influenza

Situation	Test type	Comments
Pre-outbreak [All of the test types could be used to detect infection with low pathogenicity (LP) AI viruses (or low grade HPAI) in flocks experiencing small increases in mortality, respiratory disease, egg drop, or feed or water consumption that would not raise suspicion of an outbreak of HPAI]	Serology for antibody detection	Available commercially as near-POC test. Any positive results require investigation
	Field-based RT-PCR for wild bird testing (active surveillance or investigation of mortalities)	Systems available for POC or near-POC use. Has been used elsewhere for wild birds in remote locations.
	Multiplexed test for avian diseases (e.g. respiratory panel containing AI system	Potential for development of panels for conduct of tests by commercial farms. Systems needed to capture the information generated (both positive and negative).
Index case in outbreak on a farm	Clinical signs	Not diagnostic of AI but in some cases might allow earlier diagnosis if it results in early use of POC tests or submission of samples to a laboratory.

	Antigen detection on dead or sick birds	Rule in test. May allow early detection of virus in atypical cases (e.g. including infection with notifiable low pathogenicity AI of the H5 or H7 subtype).
	Field based nucleic acid amplification and detection	Potentially useful in remote locations. Some poultry companies could perform tests in house and would have to report to veterinary authorities
	Multiplexed test for avian disease (e.g. respiratory panel)	Tests could be conducted by commercial producers if these become available[a].
		All tests on index farm need to be verified at a laboratory
Follow up testing in and outside restricted and control areas, and in areas in other states and territories[b]	Antigen detection or near-POC testing based on nucleic acid amplification on dead or sick birds	Reduces potential for laboratory overload and time from sample collection to test result
	Multiplex testing of any disease outbreaks if available	Testing by producers
Compartments and zones	Serology, antigen detection and nucleic acid Nucleic acid amplification and detection	Performed as required to document on-going freedom from infection
Demonstrating freedom after the outbreak	Routine dead bird testing and dead birds from disease outbreaks Serological testing for antibody detection (except for viruses causing very high mortality)	Information on negative results adds to the weight of evidence of freedom from infection provided the test results are recognised as reliable by trading partners

[a] Near-POC tests could be used by in-house by major poultry companies once reliable tests become available capable of detecting a broad range of pathogens (e.g. tests for a panel of respiratory pathogens from respiratory specimens). Such tests would be useful for early detection of LPAI viruses or low grade HPAI in flocks experiencing small increases in mortality, respiratory disease, drops in egg production and/or feed or water consumption that otherwise not be detected. A positive result would trigger an EAD investigation.

[b] POC or near-POC tests could be used for follow-up testing around the index farm during an outbreak of HPAI (e.g. routine dead bird pick up and testing) as part of the surveillance program in the control area reducing the magnitude of the surge in testing for veterinary laboratories. Depending on the location of the outbreak, local testing of samples using sensitive and specific POC and near-POC tests would be expected to provide earlier warning of additional infected premises than if samples have to be transported to a laboratory. Local testing also spreads the workload and is consistent with the concept of the LEADDR network. The objective would be to reduce workload on the local laboratory or reference laboratory, to which only a smaller number of samples would be sent for confirmation.

Benefits from using POC tests would only arise if the tests are sufficiently sensitive and probes and primers are designed to detect the influenza virus concerned.

There is probably little justification for taking a mobile PCR machine to an outbreak on an index farm with a high clinical suspicion of HPAI unless specimens could not be delivered to a laboratory within 24 hours (resulting in delays in testing) given results of POC tests on the

index farm will have to be confirmed by laboratory testing and preliminary measures can be taken on clinical suspicion.

A2.7 Management of risks associated with POC tests for avian influenza

The management of risks associated with POC tests have been described in Section 4. Briefly, the main risks are that a poultry company performing POC tests might cull or sell an infected batch of birds without informing authorities. This has occurred overseas (based on clinical observations rather than POC test results) but both situations are considered unlikely under Australian conditions, involving the large poultry companies, given the close working relationship between the public and private sector and the EAD Response Agreement that is in place.

Both false positive (rare) and false negative results (common in samples with low concentration of antigen) can occur with antigen detection devices. These tests are relatively easy to use but basic training is still required for test operators especially on choice of sample. Confirmatory testing would be expected to be completed within 24 hours of any POC test, allowing any potential damage from a false positive POC test result to be minimised.

The main risk is that a false negative result provides a sense of false security to a farmer and that additional testing is not conducted on the flock or flocks concerned, providing opportunities for transmission of virus.

Appendix 3. Point-of-care tests for foot-and-mouth disease (FMD)

Foot-and-mouth disease (FMD) is the EAD of greatest concern to Australia because of the magnitude of the consequences of an outbreak. POC and near-POC tests have been developed for this disease. They have not been deployed widely in recent outbreaks in other countries. In part this is because they have not been fully verified as being fit-for-purpose under field conditions but also because the advantages of using POC tests (in particular those available so far) have not outweighed the cost of deployment or, in the case of antigen detection methods, the tests are only valid on animals with typical lesions (Ferris et al. 2009, Longjam et al. 2011, Madi et al. 2011, DEFRA 2011).

A3.1 Preliminary diagnosis based on clinical signs

As with avian influenza, clinical assessment remains an important POC diagnostic tool for FMD. Accurate clinical diagnosis of a vesicular disease by veterinary clinicians is possible when cattle or pigs are infected and the disease is detected early. Even if the clinical diagnosis proves to be wrong, any animal with vesicular lesions should be treated as a suspect case of FMD. Lesions and clinical signs can be less readily apparent in small ruminants or in animals with lesions older than 7 days in which vesicles are no longer detectable, making clinical diagnosis harder.

In an outbreak of FMD reliance on clinical signs without laboratory confirmation will result in culling of some uninfected flocks (in the UK outbreak in 2001 where flocks with clinical signs were destroyed within 24 hours, some 23% of flocks and herds destroyed on the basis of clinical signs did not test positive in subsequent laboratory tests (Ferris et al. 2006)).

Infrared thermography has been tested as a possible aid in early detection of FMD and has the potential to be used as a POC diagnostic tool (FAZD Center 2010).

A3.2 Serological tests for antibody detection

Serotype-specific tests for FMD have been available for some time including liquid-phase and solid-phase competitive ELISA (Mackay et al. 2001) and rapid test kits have been developed to detect antibody to FMD non-structural protein.[40] This particular test uses sera or plasma and therefore requires equipment to centrifuge blood. The test is affected by temperature and humidity. As discussed in Section 3, the rapid development of antibodies in infected animals could be used as a means of assessing animals with old lesions on site if sensitive and specific POC tests for FMD antibody were available and used.

Recent research on serological tests has focused on tests for discriminating infected from vaccinated animals (Longjam et al. 2011).

[40] See, for example, the test from the company Anigen http://www.google.com.au/url?sa=t&rct=j&q=anigen%20foot%20and%20mouth%20disease&source=web&cd=1&sqi=2&ved=0CCQQFjAA&url=http%3A%2F%2Fwww.lifebioscience.com.au%2FLife_bio%2Fdocuments%2FInsertsheetofAnigenRapidFMDNSPAb.pdf&ei=h-k6T46JIYaViAfeoeiGCg&usg=AFQjCNG4CRFffqU_M54cZTfjAr9w0_NInQ

A3.3 Antigen detection

A lateral flow device capable of detecting all seven serotypes of FMD virus is available commercially and does not cross-react with swine vesicular disease or vesicular stomatitis viruses. Under laboratory conditions the sensitivity of the device for FMD virus was 84% compared to 85% for the reference method antigen ELISA. The diagnostic specificity of the device was approximately 99% compared to 99.9% for the ELISA demonstrating that the device was suitable as a 'rule-in' test in the field when used on appropriate samples. The test requires preparation of samples of epithelium from lesions by grinding (Ferris et al. 2009). It has not been validated against samples from lesions of different ages. It could play an important role as a field test for 'ruling in' cases provided sufficient test kits were available, which may require stockpiling of kits either by individual countries or internationally. However it was not recommended for use during a disease simulation exercise in the UK, except perhaps in remote locations (DEFRA 2011).

A3.4 Tests based on nucleic acid amplification and detection

Tests on animals using nucleic acid amplification have allowed detection of preclinically infected viraemic animals (Ryan et al. 2008) and from oesophago-pharyngeal fluids (Callahan et al. 2002; Charleston et al. 2011) (as discussed in Section 3) and use of tests for this purpose could be one advantage of a POC device using nucleic acid amplification and detection on suspect farms if the constraints associated with sample collection from a large number of animals can be overcome. Laboratory testing for FMD in the 2007 UK outbreak was performed using real-time reverse transcription PCR. The maximum number of samples tested per day was 269, which is adequate for a small outbreak (Reid et al. 2009). NASBA-based techniques have also been shown to have equivalent sensitivity as real-time PCR for detecting FMD virus (Lau et al. 2008). A portable LAMP-based system using filter paper to capture nucleic acid has also been described (Bearinger et al. 2011). These techniques are potentially deployable in the field. The performance of a field-deployable RT-PCR assay has been reported and was found to be equivalent to laboratory tests when using wet reagents. The report concluded that the device should be assessed using commercially viable lyophilised reagents and in countries with endemic FMD (Madi et al. 2011).

A3.5 Use of POC and near-POC tests in management of foot-and-mouth disease

The issues related to deployment of POC tests at various stages of an EAD outbreak has been discussed in Section 3. This section explores some additional issues related to use of POC tests for FMD.

Sammin et al. (2010) reviewed the utility of POC tests for FMD. They concluded that these tests could play a role in the diagnosis of FMD but also reaffirmed that the index case of any outbreak should always be confirmed in a reference laboratory. Following an FMD simulation exercise in the UK it was concluded that lateral flow devices would only be of value in remote locations.

Issues associated with testing field-based testing for FMD (Sammin et al. 2010) in the UK have been described. Four options for diagnostic testing for this disease were identified:

i) Maintain the current situation of centralised testing with improved sample transport;

ii) Perform PCR or other tests in a local, regional or mobile laboratory, in line with the Laboratories for Emergency Animal Disease Diagnosis and Response (LEADDR) network option currently being developed in Australia;

iii) Use lateral flow devices that can detect FMD virus through antigen/antibody reactions on farm; or

iv) Use portable PCR equipment on farm.

The validity of the last option was questioned even though the analytical sensitivity of the test would be greater than antigen capture lateral flow devices on farm, given that portable PCR equipment is likely to be stored in regional locations (i.e. not distributed widely) and it may be just as easy and cost effective to transport specimens from the field than to transport the portable equipment. Key trade-offs in shifting away from testing at a laboratory include throughput and quality management, both of which have the potential to be compromised when using POC tests given the conditions under which the tests would be performed such as variable temperatures and humidity (see, for example, Harding-Esch et al. 2011, for effects of these on a POC test for *Chlamydia trachomatis*) and the limited demands for tests for EADs during periods when these diseases are not present or present rarely (as discussed in Section 4).

Some of these questions were also addressed at a meeting in the US (FAZD Center 2010). Among the key conclusions of this meeting was that lateral flow devices for FMD would be suitable as a 'triage tool' for ranking the priority of samples for submission to a laboratory for confirmation — that is, for 'ruling in' (not ruling out) a herd or flock that has clinical signs consistent with those of FMD. Retaining business continuity in the face of an EAD was also considered and would require considerable quantities of testing on livestock and livestock products. Accurate screening tests, including POC and near-POC tests that are rapid, highly sensitive and easy to use would be of value in the screening process.

This meeting also concluded that screening tests for FMD should be developed as and form part of multiplexed tests including tests for other (endemic) pathogens that provide information of benefit to the livestock industry, thereby increasing the likelihood of commercial tests for EADs becoming available.

The meeting also stressed the need for POC tests that work effectively on non-standard samples, such as bulk milk, that could be used in assessing freedom from infection in the recovery phase following an outbreak. The meeting suggested that equipment for conduct of real-time PCR should be deployed to major livestock concentration points in the event of an outbreak.

As discussed in Section 3, POC tests have a potential role to play in all stages of an outbreak of FMD.

Commercially available POC tests based on nucleic acid amplification, validated under field conditions, will almost certainly be available in the very near future. Once these are available and deployed the public expectation may well be that early action will be taken on dangerous contact or suspect farms based on results from POC tests (based on the premise that virus can be detected before animals become infectious) and that the use of these tests will shorten the duration of an outbreak.[41] This expectation may not be met if the throughput of

[41] See for example www.warmwell.com

the devices is low or the manpower required for testing sufficient animals for early (pre-clinical) detection is not available. Before deployment it will be necessary to understand and document the potential strengths and pitfalls of their use, and how they will be used, perhaps through use of desktop simulations based on existing models of transmission.

Other options should also be explored including the deployment of laboratory based PCR systems in locations closer to the site of outbreaks (or in State and Territory laboratories) (Reid et al. 2008; Sammin et al. 2010), with inactivation of samples on farm prior to dispatch, so as to ensure the time from dispatch of specimens from a suspect infected farm to provision of test results is minimised so as to achieve same day turnaround of results.

Appendix 4. Point-of-care tests for anthrax

The diagnosis of anthrax has been well summarised in the Australian and New Zealand Standard Diagnostic Protocol for this disease (Hornitsky and Muller 2010). This protocol describes the use of lateral flow devices for field detection of *Bacillus anthracis.*

Others have also published information on the technologies for anthrax detection previously (Edwards et al. 2006). Much of the interest in POC tests for anthrax has arisen as a result of concerns regarding bioterrorism. In a study comparing a portable PCR machine, RAZOR, with another larger, less portable, PCR machine (AB 7300/7500) for detecting agents potentially used in bioterrorism, including *Bacillus anthracis,* RAZOR provided more rapid results (40 minutes), was easier to transport, and appeared to cope better with sub-optimal PCR conditions. Both systems could detect between 10 and 100 fg of target DNA and no cross-reactions occurred. Both systems required facilities for sample preparation and DNA extraction (Matero et al. 2011).

The good performance of the lateral flow device in infected animals including its high specificity (Muller et al. 2004) means that even though field-deployable nucleic acid amplification systems could be used for this disease they probably offer few advantages over the lateral flow device for diagnosis of this disease in animals in Australia.

As with all EADs a positive POC sample should be backed by confirmatory testing. The lateral flow device is less reliable (reduced diagnostic sensitivity) for animals dead for more than 48 hours.

Appendix 5. Point-of-care tests for classical swine fever

Clinical signs of classical swine fever are quite variable and depend on the virulence of the virus, precluding reliance on clinical signs alone for diagnosis of this disease. The emergence of other porcine pathogens such as PRRS virus and porcine circovirus-2 has also produced diseases that can be difficult to distinguish clinically from classical swine fever and co-infections do occur complicating the diagnosis of these diseases. Laboratories conducting disease investigations in places where these viruses are endemic are required to perform tests for these agents (and other agents) in any case with high mortality, chronic wasting or respiratory disease. This provides a strong incentive for development of multiplexed tests for use within the pig industry.

Antigen detection test kits using either fluorescent antibody or ELISA have been available for many years. Various real-time PCR assays and isothermal methods have been developed for viral detection and could in theory be conducted at POC or near-POC provided quality management issues can be overcome, especially those related to nucleic acid extraction. The methods available have been described recently (Penrith et al. 2011; Rodriguez-Sanchez et al. 2008). One system using RT-PCR designed for portable equipment has been licensed for use in the US.[42]

Multiplex PCR testing for pestiviruses combined with a microarray has been developed and includes the capacity to detect and differentiate classical swine fever virus from other pestiviruses (LeBlanc et al. 2009, Le Blanc et al. 2010), an important issue in diagnosis of this disease given the susceptibility of pigs to pestiviruses from other animal species. This is not yet available as a POC test.

Other laboratory tests based on nucleic acid amplification can distinguish field strains from attenuated vaccine strains (Wen et al. 2011), which would be of value in places where vaccination is used.

The availability of portable and reliable test systems could be of benefit for testing of pigs in remote locations and would appear to be a very useful tool for testing of feral pigs, which are widely dispersed across many remote areas of Australia in the event that this disease becomes established in this population.

Once suitable tests are developed, multiplexed sample-to-answer test systems that incorporate assays for a range of pathogens capable of causing severe disease in pigs, including porcine reproductive and respiratory (PRRS) virus, porcine circovirus-2, classical swine fever virus and African swine fever virus would appear to have a role to play in POC testing in pigs globally. Sensitive and specific POC tests for respiratory pathogens covering porcine reproductive and respiratory syndrome (PRRS), classical swine fever, swine influenza, mycoplasmas and other bacterial pathogens would also likely find a market. POC systems based on nucleic acid amplification for detection of classical swine fever virus have been developed and tests for differentiation of vaccine strains from field strains could be adopted for use in the field.

[42] See Tetracore at http://www.tetracore.com/t-cor/index.html

Appendix 6. Point-of-care tests for bovine spongiform encephalopathy (BSE)

Clinical suspicion of BSE occurs when cattle display unusual neurological signs and testing for BSE is conducted routinely on fallen stock. However, testing is required to confirm the diagnosis.

Near-POC tests based on Western Blot and ELISA for BSE are used in central and slaughterhouse laboratories, with sample turnaround times as short as 100 minutes. The key to testing is the collection of appropriate samples (adjacent to the obex) (OIE 2010b).

Carra et al. (2009) assessed the performance of three tests used in laboratories conducting screening tests for BSE in Italy. The ELISA tests gave a high number of false positive results on samples from fallen cattle. The training time for technicians for the simplest of these tests was 10 days.

Work is being performed to detect markers of prion diseases in humans and animals (Edgeworth et al. 2011; Plews et al. 2011) but it is unlikely that POC or near POC tests applicable for ante mortem use in live animals will become available for this disease in the foreseeable future.[43] Current screening systems using existing tests on brain samples have proved to be highly valuable and will continue to be used.

[43] See for example the economic analysis commissioned by the Alberta Prion Research Institute in Canada at http://www.prioninstitute.ca/forms/BSE%20Testing%20Final-revised%20%20Plus%20App%20C%20AM%20Mar%2029.pdf

Acronyms and Abbreviations

AAHL	Australian Animal Health Laboratory
BSE	Bovine spongiform encephalopathy
CA	Control area
DCP	Dangerous contact premises
EAD	Emergency animal disease
ELISA	Enzyme-linked immunosorbent assay
FMD	Foot-and-mouth disease
HPAI	Highly pathogenic avian influenza
LAMP	Loop-mediated isothermal amplification
LEADDR	Laboratories for Emergency Animal Disease Diagnosis and Response
LPAI	Low pathogenicity avian influenza
NASBA	Nucleic acid sequence-based amplification
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PPE	Personal protective equipment
POC	Point-of-care
RA	Restricted area
SCAHLS	Sub-Committee of Animal Health Laboratory Standards
SMART	Signal-mediated amplification of RNA technology

Glossary

Term	Definition
Analytical sensitivity	The smallest amount of a substance that can be detected by the assay in a sample
Analytical specificity	Refers to the a of an assay to detect a specific organism or substance, rather than others, in a sample
Aptamer	An oligonucleotide that binds to a specific chemical target b shape recognition
Diagnostic sensitivity	The percentage of animals with a certain disease that are identified as detected as having that disease through the use of the assay
Diagnostic specificity	The percentage of animals that do not have a certain disease that are identified correctly as not having the disease through the use of the assay
Enzyme-linked immunosorbent assay	A test in which an antibody labelled with an enzyme binds with the target antigen and after unbound antibody is removed a chemical is added that is converted to a coloured product by the enzyme.
Highly pathogenic avian influenza	A form of avian influenza caused by viruses of either the H5 or H7 subtype that cause severe illness in poultry that meet a specific definition based on a virulence test in chickens and/or on the presence of multiple basic amino acids at the cleavage site of the haemagglutinin protein
Lab on a chip	Miniaturisation and integration of all steps in the test pathway
Lateral flow device **(lateral flow immunochromatographic device)**	A simple hand held device that combines binding of the target antibody or antigen in the sample in a system using capillary flow of labeled antibody/antigen microparticles along a membrane to a line or area of secondary trapping antibody.
Loop mediated isothermal amplification	A method for amplification of nucleic acid at a constant temperature. The reaction product can be detected visually.
Microarrays	Tests in which multiple proteins or oligonucleotides are used to bind to antibodies or amplified nucleic acid in the sample. The pattern binding is used to assess what was present in the sample.
Mulitplex(ed)	Used for assays in which multiple tests are performed on the one sample simultaneously.
Nucleic acid sequence based amplification	An method for amplification and detection of nucleic acids conducted at a constant temperature.
Polymerase chain reaction	System for amplification and detection of specific nucleic acid using a two-step process (amplification and detection) with the first step relying on heat stable polymerase enzymes and rapid thermal cycling. The second step is the detection of end products on a gel.

Point-of-care test	A test performed at or near an animal from which a sample is collected (also referred to as pen side tests).
Radioimmunoassay	A test in which an antibody labeled with a radioactive chemical binds with the target antigen. After unbound antibody is washed away the amount of radioactive material is measured.
Real-time polymerase chain reaction (or quantitative PCR)	As per PCR but the reaction can be followed in real time through use of fluorescent markers
Reverse transcription polymerase chain reaction	Use of an enzyme reverse transcriptase to convert RNA to complementary DNA for subsequent use in the polymerase chain reaction. Used for detection of RNA viruses including influenza and foot-and-mouth disease.
Sample to answer	Assays in which an unprocessed sample is introduced into the assay system and all subsequent steps in the assay are independent of the operator up to the issue of results.

References

Abe T, Segawa Y, Watanabe H, Yotoriyama T, Kai S, Yasuda A, Shimizu N, Tojo N (2011) Point-of-care testing system enabling 30 min detection of influenza genes. *Lab Chip.***11,** 1166–1167.

Anderson DA, Crowe SM, Garcia M (2011) Point-of-Care Testing. *Curr.HIV./AIDS Rep.***8,** 31—37**.**

Arens MQ, Buller RS, Rankin A, Mason S, Whetsell A, Agapov E, Lee WM, Storch GA (2010) Comparison of the Eragen Multi-Code Respiratory Virus Panel with conventional viral testing and real-time multiplex PCR assays for detection of respiratory viruses. *J.Clin.Microbiol.* **48,** 2387–2395.

Asiello PJ, Baeumner AJ (2011) Miniaturized isothermal nucleic acid amplification, a review. *Lab Chip.*March 9, 2011. Epub. ahead of print.

Azhar M, Lubis AS, Siregar ES, Alders RG, Brum E, McGrane J, Morgan I, Roeder P (2010) Participatory disease surveillance and response in Indonesia: strengthening veterinary services and empowering communities to prevent and control highly pathogenic avian influenza. *Avian Dis.* **54,** 749-753.

Balada-Llasat JM, LaRue H, Kelly C, Rigali L, Pancholi P (2011) Evaluation of commercial ResPlex II v2.0, MultiCode-PLx, and xTAG respiratory viral panels for the diagnosis of respiratory viral infections in adults. *J.Clin.Virol.* **50,** 42-45.

Banada PP, Sivasubramani SK, Blakemore R, Boehme C, Perkins MD, Fennelly K, Alland D (2010) Containment of bioaerosol infection risk by the Xpert MTB/RIF assay and its applicability to point-of-care settings. *J.Clin.Microbiol.* **48,** 3551-3557.

Bearinger JP, Dugan LC, Baker BR, Hall SB, Ebert K, Mioulet V, Madi M, King DP (2011) Development and Initial Results of a Low Cost, Disposable, Point-of-Care Testing Device for Pathogen Detection. *IEEE Trans.Biomed.Eng* **58,** 805-808.

Belak S, Kiss I, Viljoen GJ (2009) New developments in the diagnosis of avian influenza. *Rev.Sci.Tech.* **28,** 233-243.

Beyor N, Yi L, Seo TS, Mathies RA (2009) Integrated capture, concentration, polymerase chain reaction, and capillary electrophoretic analysis of pathogens on a chip. *Anal.Chem.* **81,** 3523-3528.

Bissonnette L, Bergeron MG (2010) Diagnosing infections — current and anticipated technologies for point-of-care diagnostics and home-based testing. *Clin.Microbiol.Infect.* **16,** 1044-1053.

Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD (2010) Rapid molecular detection of tuberculosis and rifampin resistance. *N.Engl.J.Med.* **363,** 1005-1015.

Boyd V, Foord A, Heine H (2011) Detection and differentiation of avian and zoonotic pathogens by a multiplex bead array assay. *Poster, One Health Conference, Melbourne, March 2011*.

Breeze RG (2006) Technology, public policy and control of transboundary livestock diseases in our lifetimes. *Rev.Sci.Tech.* **25,** 271-292.

Brenwald NP, Baker N, Oppenheim B (2010) Feasibility study of a real-time PCR test for meticillin-resistant Staphylococcus aureus in a point of care setting. *J.Hosp.Infect.* **74,** 245-249.

Bruning A, Bellamy K, Talbot D, Anderson J (1999) A rapid chromatographic strip test for the pen-side diagnosis of rinderpest virus. *J.Virol.Methods* **81,** 143-154.

Bruno JG, Phillips T, Carrillo MP, Crowell R (2009) Plastic-adherent DNA aptamer-magnetic bead and quantum dot sandwich assay for Campylobacter detection. *J.Fluoresc.* **19,** 427-435.

Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J, Ellis SJ, Shoulars KS, Gaffney KL, Rock DL, Nelson WM (2002) Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. *J.Am.Vet.Med.Assoc.* **220,** 1636-1642.

Cameron A (2011) Surveillance needs tools and options: Experiences between developed and developing world. In FAO. 2011. *Challenges of animal health information systems and surveillance for animal diseases and zoonoses.* Proceedings of the international workshop organized by FAO, 23-26 November 2010, Rome, Italy. FAO Animal Production and Health Proceedings, No. 14. Rome, Italy. 91-94 Available at http://www.fao.org/docrep/014/i2415e/i2415e00.pdf

Carpenter TE, Thurmond MC, Bates TW (2004) A simulation model of intraherd transmission of foot and mouth disease with reference to disease spread before and after clinical diagnosis. *J.Vet.Diagn.Invest* **16,** 11-16.

Carra E, Taddei R, Barbieri I, Botti G, Tranquillo V, Iori A, Gibelli L, Cerioli M, Cavadini P, Gelmetti D, Pongolini S, Capucci L (2009) Evaluation of three rapid diagnostic tests used in bovine spongiform encephalopathy monitoring in Italy. *J.Vet.Diagn.Invest* **21,** 830-836.

Centers for Disease Control and Prevention (CDC) (2009) Interim guidance of specimen collection, processing and testing for patients with suspected novel influenza A (H1N1) virus infection. Available at: http://www.cdc.gov/h1n1flu/specimencollection.htm

Chambers TM, Shortridge KF, Li PH, Powell DG, Watkins KL (1994) Rapid diagnosis of equine influenza by the Directigen FLU-A enzyme immunoassay. *Vet.Rec.* **135,** 275-279.

Charles PG (2008) Early diagnosis of lower respiratory tract infections (point-of-care tests). *Curr.Opin.Pulm.Med.* **14,** 176-182.

Charleston B, Bankowski BM, Gubbins S, Chase-Topping ME, Schley D, Howey R, Barnett PV, Gibson D, Juleff ND, Woolhouse ME (2011) Relationship between clinical signs and transmission of an infectious disease and the implications for control. *Science* **332,** 726-729.

Chua TH, Ellis TM, Wong CW, Guan Y, Ge SX, Peng G, Lamichhane C, Maliadis C, Tan SW, Selleck P, Parkinson J (2007) Performance evaluation of five detection tests for avian influenza antigen with various avian samples. *Avian Dis.* **51,** 96-105.

Ciblak MA, Kanturvardar M, Asar S, Bozkaya E, Yenen OS, Badur S (2010) Sensitivity of rapid influenza antigen tests in the diagnosis of pandemic (H1N1)2009 compared with the standard rRT-PCR technique during the 2009 pandemic in Turkey. *Scand.J.Infect.Dis.* **42,** 902-905.

Creamer E, Dolan A, Sherlock O, Thomas T, Walsh J, Moore J, Smyth E, O'Neill E, Shore A, Sullivan D, Rossney AS, Cunney R, Coleman D, Humphreys H (2010) The effect of rapid screening for methicillin-resistant Staphylococcus aureus (MRSA) on the identification and earlier isolation of MRSA-positive patients. *Infect.Control Hosp.Epidemiol.* **31,** 374-381.

Daum LT, Canas LC, Arulanandam BP, Niemeyer D, Valdes JJ, Chambers JP (2007) Real-time RT-PCR assays for type and subtype detection of influenza A and B viruses. *Influenza.Other Respi.Viruses.* **1,** 167-175.

DEFRA (2011) Exercise Silver Birch 2010 National foot and mouth disease exercise evaluation and lessons identified report Available at http://www.dpi.vic.gov.au/agriculture/pests-diseases-and-weeds/animal-diseases/vetsource/hendra-virus-investigation-procedures

Dewan PK, Fry AM, Laserson K, Tierney BC, Quinn CP, Hayslett JA, Broyles LN, Shane A, Winthrop KL, Walks I, Siegel L, Hales T, Semenova VA, Romero-Steiner S, Elie C, Khabbaz R, Khan AS, Hajjeh RA, Schuchat A (2002) Inhalational anthrax outbreak among postal workers, Washington, D.C., 2001. *Emerg.Infect.Dis.* **8,** 1066–72.

Dwyer DE, Smith DW, Catton MG, Barr IG (2006) Laboratory diagnosis of human seasonal and pandemic influenza virus infection. *Med.J.Aust.* **185,** S48–S53.

Economist (2011) The Global Campus. Jan 20, 2011 Available at: http://www.economist.com/node/17928981?story_id=17928981&CFID=160938534&CFTOKEN=74539441

Edgeworth JA, Farmer M, Sicilia A, Tavares P, Beck J, Campbell T, Lowe J, Mead S, Rudge P, Collinge J, Jackson GS (2011) Detection of prion infection in variant Creutzfeldt-Jakob disease: a blood-based assay. *Lancet* **377,** 487-493.

Edwards KA, Clancy HA, Baeumner AJ (2006) *Bacillus anthracis*: toxicology, epidemiology and current rapid-detection methods. *Anal.Bioanal.Chem.* **384,** 73-84.

Ehrmeyer SS, Laessig RH (2007) Point-of-care testing, medical error, and patient safety: a 2007 assessment. *Clin.Chem.Lab Med.* **45,** 766-773.

FAZD Center (2010) Protecting Agricultural Infrastructure: Defining the needs and requirements for agricultural screening tools. Available at: http://fazd.tamu.edu/files/2011/01/AST-Workshop-Report-FAZD-Center-FINAL-1.2011.pdf

Fang X, Chen H, Yu S, Jiang X, Kong J (2010) Predicting viruses accurately by a multiplex microfluidic loop-mediated isothermal amplification chip. *Anal.Chem.**83,***690-695*.*

Ferris NP, King DP, Reid SM, Shaw AE, Hutchings GH (2006) Comparisons of original laboratory results and retrospective analysis by real-time reverse transcriptase-PCR of virological samples collected from confirmed cases of foot-and-mouth disease in the UK in 2001. *Vet.Rec.* **159,** 373-378.

Ferris NP, Nordengrahn A, Hutchings GH, Reid SM, King DP, Ebert K, Paton DJ, Kristersson T, Brocchi E, Grazioli S, Merza M (2009) Development and laboratory validation of a lateral flow device for the detection of foot-and-mouth disease virus in clinical samples. *J.Virol.Methods* **155,** 10-17.

Foord A, Boyd V, Heine H (2011) A loop mediated isothermal amplification assay for detection of Hendra virus in the field. *Poster, One Health Conference, Melbourne, March 2011.*

Fredriksson S, Gullberg M, Jarvius J, Olsson C, Pietras K, Gustafsdottir SM, Ostman A, Landegren U (2002) Protein detection using proximity-dependent DNA ligation assays. *Nat.Biotechnol.* **20,** 473-477.

Gantelius J, Hamsten C, Neiman M, Schwenk JM, Persson A, Andersson-Svahn H (2010) A lateral flow protein microarray for rapid determination of contagious bovine pleuropneumonia status in bovine serum. *J.Microbiol.Methods* **82,** 11-18.

Gill P, Ghaemi A (2008) Nucleic acid isothermal amplification technologies: a review. *Nucleosides Nucleotides Nucleic Acids* **27,** 224-243.

Ginocchio CC, Zhang F, Manji R, Arora S, Bornfreund M, Falk L, Lotlikar M, Kowerska M, Becker G, Korologos D, de GM, Crawford JM (2009) Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. *J.Clin.Virol.* **45,** 191-195.

Harding-Esch EM, Holland MJ, Schémann JF, Molina S, Sarr I, Andreasen AA, Roberts C, Sillah A, Sarr B, Harding EF, Edwards T, Bailey RL, Mabey DC (2011) Diagnostic accuracy of a prototype point-of-care test for ocular Chlamydia trachomatis under field conditions in The Gambia and Senegal *Plos. Negl. Trop. Dis*. **5**, e1234.

He F, Soejoedono RD, Murtini S, Goutama M, Kwang J (2010a) Complementary monoclonal antibody-based dot ELISA for universal detection of H5 avian influenza virus. *BMC.Microbiol.* **10,** 330.

He Y, Zeng K, Gurung AS, Baloda M, Xu H, Zhang X, Liu G (2010b) Visual detection of single-nucleotide polymorphism with hairpin oligonucleotide-functionalized gold nanoparticles. *Anal.Chem.* **82,** 7169–7177.

Heil GL, McCarthy T, Yoon KJ, Liu S, Saad MD, Smith CB, Houck JA, Dawson ED, Rowlen KL, Gray GC (2010) MChip, a low density microarray, differentiates among seasonal human H1N1, North American swine H1N1, and the 2009 pandemic H1N1. *Influenza.Other Resp.Viruses.* **4,** 411-416.

Heinze BC, Gamboa JR, Kim K, Song JY, Yoon JY (2010) Microfluidic immunosensor with integrated liquid core waveguides for sensitive Mie scattering detection of avian influenza antigens in a real biological matrix. *Anal.Bioanal.Chem.* **398,** 2693-2700.

Hindson BJ, Reid SM, Baker BR, Ebert K, Ferris NP, Tammero LF, Lenhoff RJ, Naraghi-Arani P, Vitalis EA, Slezak TR, Hullinger PJ, King DP (2008) Diagnostic evaluation of multiplexed reverse transcription-PCR microsphere array assay for detection of foot-and-mouth and look-alike disease viruses. *J.Clin.Microbiol.* **46,** 1081-1089.

Hoggatt J (2011) Personalized medicine--trends in molecular diagnostics: exponential growth expected in the next ten years. *Mol.Diagn.Ther.* **15,** 53-55.

Hong HB, Krause HJ, Song KB, Choi CJ, Chung MA, Son SW, Offenhausser A (2011) Detection of two different influenza A viruses using a nitrocellulose membrane and a magnetic biosensor. *J.Immunol.Methods* **365,** 95-100.

Hornitsky MA, Muller JD (2010) Australia and New Zealand Standard Diagnostic Procedures: Anthrax Available at: http://www.scahls.org.au/__data/assets/pdf_file/0003/1280829/Anthrax_ANZSDP_2010_FINAL.pdf

Ince J, McNally A (2009) Development of rapid, automated diagnostics for infectious disease: advances and challenges. *Expert.Rev.Med.Devices* **6,** 641-651.

Institute of Medicine (US) Forum on Microbial Threats. Washington (DC): National Academies Press (US); 2007. Available at http://www.ncbi.nlm.nih.gov/books/NBK52875/#ch3.s12

Jenny SL, Hu Y, Overduin P, Meijer A (2010) Evaluation of the Xpert Flu A Panel nucleic acid amplification-based point-of-care test for influenza A virus detection and pandemic H1 subtyping. *J.Clin.Virol.* **49,** 85-89.

Josefson P, Stralin K, Ohlin A, Ennefors T, Dragsten B, Andersson L, Fredlund H, Molling P, Olcen P (2011) Evaluation of a commercial multiplex PCR test (SeptiFast) in the etiological diagnosis of community-onset bloodstream infections. *Eur.J.Clin.Microbiol.Infect.Dis.* March 4, 2011. Epub ahead of print.

Kearns AJ, O'Mathuna DP, Scott PA (2010) Diagnostic self-testing: autonomous choices and relational responsibilities. *Bioethics.* **24,** 199-207.

Kerby MB, Freeman S, Prachanronarong K, Artenstein AW, Opal SM, Tripathi A (2008) Direct sequence detection of structured h5 influenza viral RNA. *J.Mol.Diagn.* **10,** 225-235.

Koopmans M, de Bruin E, Godeke GJ, Friesema I, van Gageldonk R, Schipper M, Meijer A, van Binnendijk R, Rimmelzwaan GF, de Jong MD, Buisman A, van Beek J, van de Vijver D, Reimerink J (2011) Profiling of humoral immune responses to influenza viruses by using protein microarray. *Clin. Microbial. Infect.* October 19 2011 Epub ahead of print.

Kumar A, Ellis P, Arabi Y, Roberts D, Light B, Parrillo JE, Dodek P, Wood G, Kumar A, Simon D, Peters C, Ahsan M, Chateau D (2009) Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. *Chest* **136,** 1237-1248.

Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, Kumar A, Cheang M (2006) Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med.* **34,** 1589-1596.

Landry ML (2011) Diagnostic tests for influenza infection. *Curr.Opin.Pediatr.* **23,** 91-97.

Landry ML, Eid T, Bannykh S, Major E (2008) False negative PCR despite high levels of JC virus DNA in spinal fluid: Implications for diagnostic testing. *J.Clin.Virol.* **43,** 247-249.

Lau LT, Reid SM, King DP, Lau AM, Shaw AE, Ferris NP, Yu AC (2008) Detection of foot-and-mouth disease virus by nucleic acid sequence-based amplification (NASBA). *Vet.Microbiol.* **126,** 101-110.

Lawn SD, Nicol MP (2011) Xpert(R) MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future.Microbiol.* **6,** 1067-1082.

LeBlanc N, Gantelius J, Schwenk JM, Stahl K, Blomberg J, Andersson-Svahn H, Belak S (2009) Development of a magnetic bead microarray for simultaneous and simple detection of four pestiviruses. *J.Virol.Methods* **155,** 1-9.

LeBlanc N, Leijon M, Jobs M, Blomberg J, Belak S (2010) A novel combination of TaqMan RT-PCR and a suspension microarray assay for the detection and species identification of pestiviruses. *Vet.Microbiol.* **142,** 81-86.

Lee D, Chander Y, Goyal SM, Cui T (2011) Carbon nanotube electric immunoassay for the detection of swine influenza virus H1N1. *Biosens.Bioelectron.***26,** 3482-3487.

Lee D, Chen PJ, Lee GB (2010) The evolution of real-time PCR machines to real-time PCR chips. *Biosens.Bioelectron.* **25,** 1820-1824.

Lewandrowski K, Gregory K, Macmillan D (2011) Assuring quality in point-of-care testing: evolution of technologies, informatics, and program management. *Arch.Pathol.Lab Med.* **135,** 1405-1414.

Li H, McCormac MA, Estes RW, Sefers SE, Dare RK, Chappell JD, Erdman DD, Wright PF, Tang YW (2007) Simultaneous detection and high-throughput identification of a panel of RNA viruses causing respiratory tract infections. *J.Clin.Microbiol.* **45,** 2105-2109.

Lim DV, Simpson JM, Kearns EA, Kramer MF (2005) Current and developing technologies for monitoring agents of bioterrorism and biowarfare. *Clin.Microbiol.Rev.* **18,** 583-607.

Lipkin WI, Briese T (2007) Emerging Tools for Microbial Diagnosis, Surveillance and Discovery: Assessing the Challenges-Finding Solutions, Workshop Summary, Institute of Medicine (US) Forum on Microbial Threats. Washington (DC): National Academies Press (US); 2007. Available at http://www.ncbi.nlm.nih.gov/books/NBK52875/#ch3.s12

Loeffelholz MJ, Pong DL, Pyles RB, Xiong Y, Miller AL, Bufton KK, Chonmaitree T (2011) Comparison of the FilmArray Respiratory Panel and Prodesse Real-Time PCR Assays for Detection of Respiratory Pathogens. *J.Clin.Microbiol.* **49,** 4083-4088.

Longjam N, Deb R, Sarmah AK, Tayo T, Awachat VB, Saxena VK (2011) A Brief Review on Diagnosis of Foot-and-Mouth Disease of Livestock: Conventional to Molecular Tools. *Vet.Med.Int.* **2011,** 905768.

Mackay DK, Bulut AN. REndle T, Davidson F, Ferris NP (2001) A solid phase competition ELISA for measuring antibody to foot-and-mouth disease virus. *J Virol Methods*. **97**, 33-48.

Madi M, Hamilton A, Squirrell D, Mioulet V, Evans P, Lee M, King DP (2011) Rapid detection of foot-and-mouth disease virus using a field-portable nucleic acid extraction and real-time PCR amplification platform. *Vet.J.* Nov. 22, 2011. Epub ahead of print.

Marché S, van den Berg T (2010) Evaluation of rapid antigen detection kits for the diagnosis of highly pathogenic avian influenza H5N1 infection. *Avian Dis.* **54,** 650-654.

Mashayekhi F, Chiu RY, Le AM, Chao FC, Wu BM, Kamei DT (2010) Enhancing the lateral-flow immunoassay for viral detection using an aqueous two-phase micellar system. *Anal.Bioanal.Chem.* **398,** 2955-2961.

Matero P, Hemmila H, Tomaso H, Piiparinen H, Rantakokko-Jalava K, Nuotio L, Nikkari S (2011) Rapid field detection assays for *Bacillus anthracis*, *Brucella* spp., *Francisella tularensis* and *Yersinia pestis*. *Clin.Microbiol.Infect.* **17,** 34-43.

Mehlmann M, Bonner AB, Williams JV, Dankbar DM, Moore CL, Kuchta RD, Podsiad AB, Tamerius JD, Dawson ED, Rowlen KL (2007) Comparison of the MChip to viral culture, reverse transcription-PCR, and the QuickVue influenza A+B test for rapid diagnosis of influenza. *J.Clin.Microbiol.* **45,** 1234-1237.

Melo MR, Clark S, Barrio D (2010) Review: Miniaturization and globalization of clinical laboratory activities. *Clin.Chem.Lab Med.*Dec. 23, 2010. Epub ahead of print.

Menassa N, Bosshard PP, Kaufmann C, Grimm C, Auffarth GU, Thiel MA (2010) Rapid detection of fungal keratitis with DNA-stabilizing FTA filter paper. *Invest Ophthalmol.Vis.Sci.* **51,** 1905-1910.

Meseko CA, Oladokun AT, Ekong PS, Fasina FO, Shittu IA, Sulaiman LK, Egbuji AN, Solomon P, Ularamu HG, Joannis TM (2010) Rapid antigen detection in the diagnosis of highly pathogenic avian influenza (H5N1) virus in Nigeria. *Diagn.Microbiol.Infect.Dis.* **68,** 163-165.

Miller MB, Tang YW (2009) Basic concepts of microarrays and potential applications in clinical microbiology. *Clin.Microbiol.Rev.* **22,** 611-633.

Moreno A, Brocchi E, Lelli D, Gamba D, Tranquillo M, Cordioli P (2009) Monoclonal antibody based ELISA tests to detect antibodies against neuraminidase subtypes 1, 2 and 3 of avian influenza viruses in avian sera. *Vaccine* **27,** 4967-4974.

Muller JD, Wilks CR, O'Riley KJ, Condron RJ, Bull R, Mateczun A (2004) Specificity of an immunochromatographic test for anthrax. *Aust.Vet.J.* **82,** 220-222.

Nicholson TL, Kukielka D, Vincent AL, Brockmeier SL, Miller LC, Faaberg KS (2011) Utility of a Panviral Microarray for Detection of Swine Respiratory Viruses in Clinical Samples. *J.Clin.Microbiol.* Jan 26, 2011. Epub ahead of print.

Nordengrahn A, Gustafsdottir SM, Ebert K, Reid SM, King DP, Ferris NP, Brocchi E, Grazioli S, Landegren U, Merza M (2008) Evaluation of a novel proximity ligation assay for the sensitive and rapid detection of foot-and-mouth disease virus. *Vet.Microbiol.* **127,** 227-236.

O'Farrell B (2009) Evolution of lateral flow-based immunoassay systems. In, RC Wong, Hy TSE (eds) *Lateral Flow Immunoassay*. Humana Press, New York. 1-34.

OIE (2010a) Avian influenza, in *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Available at:
http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.04_AI.pdf

OIE (2010b) Bovine spongiform encephalopathy, in *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.06_BSE.pdf

OIE (2010c) Principles of validation of diagnostic assays for infectious diseases, *in Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.* Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/1.1.04_VALID.pdf

Palmer S, Sully M, Fozdar F (2009) Farmers, animal disease reporting and the effect of trust: A study of West Australian sheep and cattle farmers Rural Society 19, 32-48.

Pierce KE, Mistry R, Reid SM, Bharya S, Dukes JP, Hartshorn C, King DP, Wangh LJ (2010) Design and optimization of a novel reverse transcription linear-after-the-exponential PCR for the detection of foot-and-mouth disease virus. *J.Appl.Microbiol.* 109, 180-189.

Pierce VM, Elkan M, Leet M, McGowan KL, Hodinka RL (2011) Comparison of the Idaho Technology FilmArray System to Real-Time PCR for Detection of Respiratory Pathogens in Children. *J.Clin.Microbiol.* Nov. 23, 2011. Epub ahead of print.

Penrith ML, Vosloo W, Mather C (2011) Classical Swine Fever (Hog Cholera): Review of Aspects Relevant to Control. *Transbound.Emerg.Dis.* Feb. 9, 2011. Epub ahead of print.

Perkins MD, Small PM (2007) Partnering for better microbial diagnostics in Global Infectious Disease Surveillance and Detection: Assessing the Challenges—Finding Solutions, Workshop Summary, Institute of Medicine (US) Forum on Microbial Threats. Washington (DC): National Academies Press (US); 2007. Available at http://www.ncbi.nlm.nih.gov/books/NBK52875/#ch3.s12

Plews M, Lamoureux L, Simon SL, Graham C, Ruddat V, Czub S, Knox JD (2011) Factors affecting the accuracy of urine-based biomarkers of BSE. *Proteome.Sci.* **9,** 6.

Poritz MA, Blaschke AJ, Byington CL, Meyers L, Nilsson K, Jones DE, Thatcher SA, Robbins T, Lingenfelter B, Amiott E, Herbener A, Daly J, Dobrowolski SF, Teng DH, Ririe KM (2011) FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. *PLoS.One.* **6,** e26047.

Postel A, Ziller M, Rudolf M, Letzel T, Ehricht R, Pourquier P, Dauber M, Grund C, Beer M, Harder TC (2011) Broad spectrum reactivity versus subtype specificity — trade-offs in serodiagnosis of influenza A virus infections by competitive ELISA. *J.Virol.Methods*.ProMED-mail (2010) Foot and mouth disease: South Korea (09) North Gyeonsang 20101130:4301 30 November, 2010. http://www.promedmail.org

Prickett JR, Zimmerman JJ (2010) The development of oral fluid-based diagnostics and applications in veterinary medicine. *Anim Health Res.Rev.* **11,** 207-216.

ProMED-mail (2010) Foot & mouth disease – South Korea (09) (North Gyeongsang) OIE 30 November 2010 20101130.4301 http://www.promedmail.org

ProMED-mail (2011) Foot & mouth disease – UK: research. 9 May 2011. 20110509.1431 http://www.promedmail.org

Rand KH, Rampersaud H, Houck HJ (2011) Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. *J.Clin.Microbiol.* **49,** 2449-2453.

Read AJ, Arzey KE, Finlaison DS, Gu X, Davis RJ, Ritchie L, Kirkland PD. (2011) A prospective longitudinal study of naturally infected horses to evaluate the performance characteristics of rapid diagnostic tests for equine influenza virus *Vet Microbiol*. 2011 Nov 6. E-published ahead of print.

Reid SM, Ebert K, Bachanek-Bankowska K, Batten C, Sanders A, Wright C, Shaw AE, Ryan ED, Hutchings GH, Ferris NP, Paton DJ, King DP (2009) Performance of real-time reverse transcription polymerase chain reaction for the detection of foot-and-mouth disease virus during field outbreaks in the United Kingdom in 2007. *J.Vet.Diagn.Invest* **21,** 321-330.

Reid SM, Pierce KE, Mistry R, Bharya S, Dukes JP, Volpe C, Wangh LJ, King DP (2010) Pan-serotypic detection of foot-and-mouth disease virus by RT linear-after-the-exponential PCR. *Mol.Cell Probes* **24,** 250-255.

Rodrigues Ribeiro Teles FS, Pires de Tavora Tavira LA, Pina da Fonseca LJ (2010) Biosensors as rapid diagnostic tests for tropical diseases. *Crit Rev.Clin.Lab Sci.* **47,** 139-169.

Rodriguez-Sanchez B, Sanchez-Vizcaino JM, Uttenthal A, Rasmussen TB, Hakhverdyan M, King DP, Ferris NP, Ebert K, Reid SM, Kiss I, Brocchi E, Cordioli P, Hjerner B, McMenamy M, McKillen J, Ahmed JS, Belak S (2008) Improved diagnosis for nine viral diseases considered as notifiable by the world organization for animal health. *Transbound.Emerg.Dis.* **55,** 215-225.

Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, Peneranda S, Bankamp B, Maher K, Chen MH, Tong S, Tamin A, Lowe L, Frace M, DeRisi J, Chen Q, Wang D, Erdman DD, Peret TC, Burns C, Ksiazek TG, Rollin PE, Sanchez A, Liffick S, Holloway B, Limor J, McCaustland K, Olsen-Rasmussen M, Fouchier R, Gunther S, Osterhaus AD, Drosten C, Pallansch MA, Anderson LJ, Bellini WJ (2003) Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* **300,** 1394-1399.

Ryan E, Gloster J, Reid SM, Li Y, Ferris NP, Waters R, Juleff N, Charleston B, Bankowski B, Gubbins S, Wilesmith JW, King DP, Paton DJ (2008) Clinical and laboratory investigations of the outbreaks of foot-and-mouth disease in southern England in 2007. *Vet.Rec.* **163,** 139-147.

Saah AJ, Hoover DR (1997) "Sensitivity" and "specificity" reconsidered: the meaning of these terms in analytical and diagnostic settings. *Ann.Intern.Med.* **126,** 91-94.

Sammin D, Ryan E, Ferris NP, King DP, Zientara S, Haas B, Yadin H, Alexandersen S, Sumption K, Paton DJ (2010) Options for decentralized testing of suspected secondary outbreaks of foot-and-mouth disease. *Transbound.Emerg.Dis.* **57,** 237-243.

Sanchez JA, Pierce KE, Rice JE, Wangh LJ (2004) Linear-after-the-exponential (LATE)-PCR: an advanced method of asymmetric PCR and its uses in quantitative real-time analysis. *Proc.Natl.Acad.Sci.U.S.A* **101,** 1933-1938.

Schlingemann J, Leijon M, Yacoub A, Schlingemann H, Zohari S, Matyi-Toth A, Kiss I, Holmquist G, Nordengrahn A, Landegren U, Ekstrom B, Belak S (2010) Novel means of viral

antigen identification: improved detection of avian influenza viruses by proximity ligation. *J.Virol.Methods* **163,** 116-122.

Selleck PW, Lowther SL, Russell GM, Hooper PT (2003a) Rapid diagnosis of highly pathogenic avian influenza using pancreatic impression smears. *Avian Dis.* **47,** 1190-1195.

Shephard MD, Mazzachi BC, Watkinson L, Shephard AK, Laurence C, Gialamas A, Bubner T. (2009) Evaluation of a training program for device operators in the Australian Government's Point of Care Testing in General Practice Trial: issues and implications for rural and remote practices Rural and Remote Health Journal. Available at: http://www.rrh.org.au/articles/showarticleaust.asp?ArticleID=1189

Sims LD, Ellis TM, Liu KK, Dyrting K, Wong H, Peiris M, Guan Y, Shortridge KF (2003) Avian influenza in Hong Kong 1997-2002. *Avian Dis.* **47,** 832-838.

Sims LD, Turner AJ (2008) Avian influenza in Australia, in D. Swayne (ed) *Avian Influenza*, p 251-286, Blackwell, Ames, USA

Singh K, Vasoo S, Stevens J, Schreckenberger P, Trenholme G (2010) Pitfalls in diagnosis of pandemic (novel) A/H1N1 2009 influenza. *J.Clin.Microbiol.* **48,** 1501-1503.

Slomka MJ, To TL, Tong HH, Coward VJ, Mawhinney IC, Banks J, Brown IH (2011) Evaluation of lateral flow devices for identification of infected poultry by testing swab and feather specimens during H5N1 highly pathogenic avian influenza outbreaks in Vietnam. *Influenza.Other Respi.Viruses.* Dec. 12, 2011. Epub ahead of print.

Soliman H, El-Matbouli M (2010) Loop mediated isothermal amplification combined with nucleic acid lateral flow strip for diagnosis of cyprinid herpes virus-3. *Mol.Cell Probes* **24,** 38-43.

Soliman M, Selim A, Coward VJ, Hassan MK, Aly MM, Banks J, Slomka MJ (2010) Evaluation of two commercial lateral flow devices (LFDs) used for flockside testing of H5N1 highly-pathogenic avian influenza infections in backyard gallinaceous poultry in Egypt. *J.Mol.Genet.Med.* **4,** 247-251.

Sturenburg E, Junker R (2009) Point-of-care testing in microbiology: the advantages and disadvantages of immunochromatographic test strips. *Dtsch.Arztebl.Int.* **106,** 48-54.

Sun Y, Dhumpa R, Bang DD, Hogberg J, Handberg K, Wolff A (2011) A lab-on-a-chip device for rapid identification of avian influenza viral RNA by solid-phase PCR. *Lab Chip.* Mar. 2, 2011. Epub ahead of print.

Takekawa JY, Hill NJ, Schultz AK, Iverson SA, Cardona CJ, Boyce WM, Dudley JP (2011) Rapid diagnosis of avian influenza virus in wild birds: use of a portable rRT-PCR and freeze-dried reagents in the field. *J.Vis.Exp.*

Tang D, Tang J, Su B, Ren J, Chen G (2010) Simultaneous determination of five-type hepatitis virus antigens in 5 min using an integrated automatic electrochemical immunosensor array. *Biosens.Bioelectron.* **25,** 1658-1662.

Tayo A, Ellis J, Phillips LL, Simpson S, Ward DJ (2011) Emerging point of care tests for influenza: innovation or status quo *Influenza Other Resp. Viruses* 22 Nov 2011 Epub ahead of print.

Thaitrong N, Liu P, Briese T, Lipkin WI, Chiesl TN, Higa Y, Mathies RA (2010) Integrated capillary electrophoresis microsystem for multiplex analysis of human respiratory viruses. *Anal.Chem.* **82,** 10102-10109.

Tirimacco R, Glastonbury B, Laurence CO, Bubner TK, Shephard MD, Beilby JJ (2011) Development of an accreditation program for Point of Care Testing (PoCT) in general practice. *Aust.Health Rev.* **35,** 230-234.

Torres-Chavolla E, Alocilja EC (2009) Aptasensors for detection of microbial and viral pathogens. *Biosens.Bioelectron.* **24,** 3175-3182.

Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, Shepherd A, Wilson P, Dahl CA, Buchsbaum S, Moeller G, Hay Burgess DC (2006) Requirements for high impact diagnostics in the developing world. *Nature* **444 Suppl 1,** 73-79.

van den Berg T, Lambrecht B, Marche S, Steensels M, Van BS, Bublot M (2008) Influenza vaccines and vaccination strategies in birds. *Comp Immunol.Microbiol.Infect.Dis* **31,** 121-165.

van Dommelen L, van Tiel FH, Ouburg S, Brouwers EE, Terporten PH, Savelkoul PH, Morre SA, Bruggeman CA, Hoebe CJ (2010) Alarmingly poor performance in Chlamydia trachomatis point-of-care testing. *Sex. Transm.Infect.* **86,** 355-359.

Vong S, Ly S, Mardy S, Holl D, Buchy P (2008) Environmental contamination during influenza A virus (H5N1) outbreaks, Cambodia, 2006. *Emerg.Infect.Dis.* **14,** 1303-1305.

Waner JL, Todd SJ, Shalaby H, Murphy P, Wall LV (1991) Comparison of Directigen FLU-A with viral isolation and direct immunofluorescence for the rapid detection and identification of influenza A virus. *J.Clin.Microbiol.* **29,** 479-482.

Wang R, Lin J, Lassiter K, Srinivasan B, Lin L, Lu H, Tung S, Hargis B, Bottje W, Berghman L, Li Y.(2011) Evaluation study of a portable impedance biosensor for detection of avian influenza virus.*J Virol Methods* **178,**852-8.

Weile J, Knabbe C (2009) Current applications and future trends of molecular diagnostics in clinical bacteriology. *Anal.Bioanal.Chem.* **394,** 731-742.

Wen G, Zhang T, Yang J, Luo Q, Liao Y, Hu Z, Zhang R, Wang H, Ai D, Luo L, Song N, Shao H (2011) Evaluation of a real-time RT-PCR assay using minor groove binding probe for specific detection of Chinese wild-type classical swine fever virus. *J.Virol.Methods* **176,** 96-102.

WHO (2008) Diagnostics evaluation series 2: Laboratory-based evaluation of 19 commercially available rapid diagnostic tests for tuberculosis. WHO, Geneva.

WHO (2009) Pathways to better diagnostics for tuberculosis. A blueprint for the development of TB diagnostics. Available at: http://apps.who.int/tdr/publications/tdr-research-publications/tb-blueprint/pdf/pathways-tuberculosis.pdf

Wishaupt JO, Russcher A, Smeets LC, Versteegh FG, Hartwig NG (2011) Clinical impact of RT=PCR for pediatric acute respiratory infections: a controlled clinical trial *Pediatrics* **128**,1113-20.

Wolcott MJ, Schoepp RJ, Norwood DA, Shoemaker DR (2007) Rapid infectious disease diagnostic assays in Global Infectious Disease Surveillance and Detection: Assessing the Challenges—Finding Solutions, Workshop Summary. Institute of Medicine (US) Forum on Microbial Threats. Washington (DC): National Academies Press (US); 2007. Available at http://www.ncbi.nlm.nih.gov/books/NBK52875/#ch3.s12

Zhang CY, Hu J (2010) Single quantum dot-based nanosensor for multiple DNA detection. *Anal.Chem.* **82,** 1921-1927.

Zhang XJ, Sun Y, Liu L, Belak S, Qiu HJ (2010) Validation of a loop-mediated isothermal amplification assay for visualised detection of wild-type classical swine fever virus. *J.Virol.Methods* **167,** 74-78.

Zhao J, Tang S, Storhoff J, Marla S, Bao YP, Wang X, Wong EY, Ragupathy V, Ye Z, Hewlett IK (2010) Multiplexed, rapid detection of H5N1 using a PCR-free nanoparticle-based genomic microarray assay. *BMC.Biotechnol.* **10,** 74.