

Technological advances in veterinary diagnostics: opportunities to deploy rapid decentralised tests to detect pathogens affecting livestock

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Summary

Sustainable food production capable of feeding a growing human population is a significant global challenge, and is a priority encompassed within the United Nations Millennium Development Goal to 'eradicate extreme poverty and hunger'. Infectious diseases reduce the productivity of farm animals, and the globalised trade of animals and their products increases the threat of disease incursion. Accurate and rapid diagnostic tests are an essential component of contingency plans to detect, control and eradicate such diseases. Diagnosis involves a 'pipeline' that normally starts with clinical suspicion, followed by collecting samples, transporting specimens to a centralised laboratory setting (e.g. national/international Reference Laboratories), analysing these samples using a range of diagnostic tests and reporting the results. However, the transport of specimens from the field to the laboratory can be a lengthy process that can delay critical decision-making and severely affect the quality of the samples. This important limitation of centralised diagnostic testing has motivated the development of tools for the rapid, simple detection of livestock pathogens. Recent advances in the development of technologies for personalised human medicine have motivated the development of prototype diagnostic tests for a wide selection of diseases of livestock. However, many of these tests are not yet routinely used or commercially available. This paper critically reviews the most promising examples of such assays, and highlights the challenges that remain to transition these tests from applied research and development into routine use.

Keywords

Decentralised testing – Detection – Diagnostics – Field diagnostics – Livestock – Molecular assays – Point-of-care testing – Rapid testing.

Introduction

Prompt and accurate detection of infectious agents is necessary, since many livestock diseases have severe economic consequences, yet local veterinary diagnosis can be confounded by diseases that share similar clinical signs. Therefore, the presence of notifiable diseases is usually confirmed in dedicated laboratories, using assays recommended by the World Organisation for Animal

Health (OIE). These tests are typically performed, analysed and interpreted by trained personnel, according to the recommendations of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)* (1). Although these laboratory-based tests can provide rapid results (2, 3), samples must be transported to the laboratory, which can negatively affect the quality of the specimens and delay or even hinder immediate crucial decision-making and the process of disease control.

Within the last decade, many technological advances have been made to improve diagnostics for infectious diseases of human health importance. Diagnostic assays based on the detection of antigens, antibodies or nucleic acid molecules have been developed and successfully introduced for the routine surveillance of human diseases, such as acquired immune deficiency syndrome (AIDS), tuberculosis (TB) and malaria (4, 5, 6). The deployment of new technologies has made a significant contribution to improving the capacity and expanding the extent of decentralised testing. For example, there are now World Health Organization (WHO) pre-qualified rapid diagnostic tests which are commercially available for human immunodeficiency virus (HIV), malaria and TB, with approximately 81 million HIV, 314 million malaria (2014) and 10 million TB (Xpert® TB cartridges) tests procured annually (7, 8, 9). Rapid tests for antigen and nucleic acid detection have also been adopted by WHO in emergency situations, such as the response to the most recent outbreak of Ebola in West Africa (10, 11), with seven tests currently available through the Emergency Use Assessment and Listing procedure (EUAL). Capitalising on these advances in the human diagnostics field, the veterinary industry is now exploiting the use of similar technologies to detect important livestock pathogens.

Opportunities and drivers for the development of simple, rapid diagnostics for livestock diseases

The development of simple, portable diagnostic devices is now considered a priority for animal diseases. This idea is echoed within various independent reports following major outbreaks of foreign animal diseases, such as foot and mouth disease (FMD) (12, 13, 14), and by research funded by the Department for Environment, Food and Rural Affairs (Defra) and the Economic and Social Research Council (www.genomicsnetwork.ac.uk/innogen). International organisations, such as the Food and Agriculture Organization of the United Nations (FAO) and the OIE, stress that governments could save billions of dollars by stepping up the control of high-impact animal diseases but, to achieve this, access to rapidly deployable, decentralised diagnostics that permit active case control of diseases is required.

A powerful illustration of the effectiveness of rapid point-of-care testing (POCT) diagnostics for veterinary diseases came during the latter stages of the rinderpest (RP) eradication programme. The use of a simple, disposable, antigen-detection test proved invaluable in countries such as Pakistan and Somalia and empowered field veterinarians

to stamp out the last remaining pockets of infection (15). The use of a similar strategy during the 2001 FMD outbreak in the United Kingdom (UK) could have prevented the 'slaughter-on-suspicion' policy being enforced and reduced the unnecessary culling of animals on 23% of suspect premises (16).

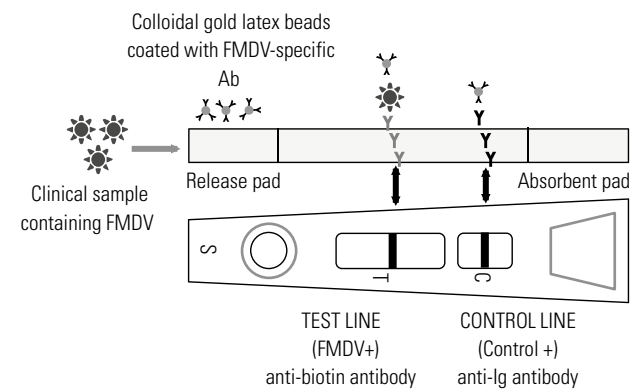
Epidemics of highly pathogenic avian influenza (HPAI) virus (potentially zoonotic) and equine influenza have repeatedly illustrated the importance of rapid diagnosis in animal health crisis management. For example, the 2007 equine influenza epidemic in Australia caused economic losses assessed at around AU\$ 10 billion (17). Other scenarios where POCT could be used include strangles in horses (*Streptococcus equi*), which is highly contagious and requires rapid testing; TB testing of badgers during single-capture events in the field; and diagnosing abortions due to *Brucella*, where culture is difficult, serology may be confounded by prior vaccination, and rapid decisions need to be made on isolating animals.

Recent technological advances in point-of-care testing diagnostics for veterinary diseases

The global veterinary diagnostics market is estimated to expand at a compound annual growth rate of 8.6% from 2016 to 2021, to reach US\$ 6.71 billion by 2021 (18). Its market drivers include innovation in new veterinary diagnostics. A search of the scientific literature in the National Center for Biotechnology Information (NCBI) PubMed index, using 'name of assay chemistry [Title] AND veterinary', identified a total of 251 isothermal, 383 real-time polymerase chain reaction (qPCR) and 122 immunoassay publications over the last five years. This paper will critically review a selection of these publications, focusing on antigen/antibody and nucleic acid detection technologies for economically and differentially important veterinary diseases.

Antigen/antibody detection systems

Antigen detection has now been incorporated into portable immuno-chromatographic strip tests, also known as antigen-lateral flow devices (Ag-LFDs), which work by binding both viral antigen and antibody-coated detector particles to bands of capturing monoclonal antibody on a membrane (Fig. 1). Ag-LFDs are simple to use, highly portable, rapid (circa 10 minutes), disposable and inexpensive, making them an ideal assay format for



FMDV: foot and mouth disease virus
 Ab: antibody
 Anti-Ig: anti-immunoglobulin

Fig. 1

The mechanism of antigen-lateral flow devices

A positive result is signified by the presence of two red bands (test and lateral flow devices control line). Negative results are indicated by a single band (the lateral flow devices control line)

veterinary POCT diagnosis. For high-impact livestock viral diseases, there are a number of examples of commercially available Ag–LFDs. They include those developed for the detection of FMD virus (FMDV) (SVANODIP® FMDV–Ag) and RP virus (RPV) (SVANODIP® RPV–Ag) through Boehringer Ingelheim (Bracknell, UK), both of which have been successfully deployed for decentralised testing (19, 20), and for avian influenza virus (AIV) during outbreaks of H5N1 HPAI virus (HPAIV) in Egypt (21) and Viet Nam (22). Furthermore, FMDV-specific Ag–LFDs are moving towards routine use, appearing in the OIE *Terrestrial Manual* of 2017 as a recognised assay technology (1).

Prototype assays have also been developed for a number of other diseases, including swine vesicular disease virus (SVDV) (23), vesicular stomatitis virus (VSV) (24), peste des petits ruminants virus (25), and serotype-specific detection of FMDV (26, 27, 28, 29). However, these assays are not yet commercially available. LFDs can also be developed to detect specific antibody responses to identify animals that have been exposed to pathogens. For example, Boehringer Ingelheim currently offers over 20 livestock-specific, one equine-specific and three avian-specific assays in this format.

Validation studies show that these LFDs can offer a diagnostic sensitivity equivalent to that of a laboratory-based antigen enzyme-linked immunosorbent assay (Ag–ELISA) (23, 24, 26). Furthermore, combining these field-based assays with smartphones has enabled automated readouts and reporting of results for increased functionality (30). However, in common with other immunoassay formats, Ag–LFDs have lower analytical sensitivity when compared to molecular

tests, limiting their application to the acute clinical phase of disease and samples that contain large amounts of intact target organisms (31). Although sampling multiple affected animals can increase confidence in diagnosis at the herd level (32), confirming a negative disease status in animals can be difficult.

In addition to the value of LFDs for local testing, recent studies have shown that nucleic acid can be subsequently recovered from them, permitting further downstream molecular characterisation of the agent using polymerase chain reaction (PCR) (15) and sequencing approaches (15, 33). These findings are particularly relevant for disease surveillance in remote settings in sub-Saharan Africa and parts of Asia, where centralised diagnostic capacity is often limited. These exciting data suggest that LFDs could be a simple way to develop novel community-based surveillance systems, which have the capacity to provide immediate feedback to the farmer. Local testing by LFD could also be adopted to improve the triage and selection of good-quality positive samples at the farm (before shipping parallel specimens to the laboratory). Alternatively, positive LFDs could be ‘dry preserved’ for later molecular epidemiological characterisation at reference laboratories (simplifying logistics, lowering costs and reducing biohazard risk).

Real-time polymerase chain reaction

The majority of the peer-reviewed research papers that describe POCT diagnostic methods have used nucleic acid detection methods. Real-time PCR chemistry (qPCR) is now an established tool to detect and quantify nucleic acid in laboratory settings, offering superior analytical sensitivity for the detection of acute disease, in comparison to serologically based assays. As a result of this routine laboratory use, much progress has been made recently in transitioning qPCR onto POCT platforms (34, 35), with a number of technologies now commercially available (Table I) (36, 37).

Perhaps some of the most promising POC technologies are those that are fully automated: integrating nucleic acid extraction, thermal cycling and reporting results to a single instrument for minimal on-site expertise. For instance, the prototype Enigma® Field Laboratory (FL) (Enigma® Diagnostics, Porton Down, UK), a robust 19-kg platform which can be powered using a vehicle auxiliary, combines silica paramagnetic-bead-based nucleic acid extraction with lyophilised q(RT)–PCR reagents in a single cartridge (38), and has been successfully used to detect FMDV in field settings (39).

Its commercially available successor, the MiniLab™ (ML) (Enigma® Diagnostics), at 10–35 kg, is aimed at low-resource laboratory settings. Using similar technology to

Table I
Examples of molecular point-of-care test platforms

Sources: Holland & Kiechle (36) and Dineva *et al.* (37)

Platform	Manufacturer	Method	Capacity	Integrated sample preparation	Detection channels	Assay time	Power	Availability	Website
Enigma FL	Enigma Diagnostics, UK	PCR	1 sample	✓	6	1 h	Car auxiliary	Prototype	www.enigmadiagnostics.com
Enigma ML	Enigma Diagnostics, UK	PCR	1 sample/unit (up to 6 units)	✓	6	1 h	Mains	Commercial	www.enigmadiagnostics.com
SmartCycler®	Cepheid, USA	PCR	16–96 samples	X	4	<1 h	Mains	Commercial	www.cepheid.com
Genedrive®	Epistem Ltd, UK	PCR	1 sample (3-well capacity)	X (paper-based)	1	<1 h	Battery	Commercial	www.epistem.co.uk
R.A.P.I.D.®	Idaho Technologies, USA	PCR	32 samples	X	3	30 min.	Mains	Commercial	www.time.idahotech.com
POCKIT™	GeneReach, USA	iiPCR	8 samples	X	2	<1 h	Mains	Commercial	www.genereach.com
T-COR 8™	Tetracore, USA	PCR	8 samples	X	6	1–2 h	Battery	Commercial	www.tetracore.com
genesig q16	genesig, UK	PCR	16 samples	X	2	1–2 h	Mains	Commercial	www.genesig.com
Genie® II Genie® III	OptiGene Ltd, UK	LAMP	Genie® II: 16 samples Genie® III: 8 samples	X	Genie® II: 1 Genie® III: 2	<30 min.	Battery	Commercial	www.optigene.co.uk
T-8 T-16	TwistDx, UK	RPA	T-8: 8 samples T-16: 16 samples	X	T-8: 8 T-16: 3	<30 min.	T-8: Battery T-16: Mains	Commercial	www.twistdx.co.uk

iiPCR: insulated isothermal polymerase chain reaction

LAMP: loop-mediated isothermal amplification

PCR: polymerase chain reaction

RPA: recombinase polymerase amplification

UK: United Kingdom

USA: United States of America

the FL, the ML has increased throughput, with the option of adding up to six processing modules (40). Lyophilised assays have been validated for a number of viruses using this platform, including FMDV, classical swine fever virus (CSFV), African swine fever virus (ASFV) and AIV (41) (Enigma® Diagnostics). The ML has increased its compatibility with decentralised testing by improving stability, storage and transportability, since reagents can remain at ambient temperatures. However, these tests are not commercially available at present.

Not all mobile PCR platforms incorporate nucleic acid extraction. For instance, some commercially available platforms – including the Cepheid SmartCycler® (Cepheid), T-COR™ 4 (Tetracore), T-COR™ 8 (Tetracore), genesig® (Primerdesign Ltd), R.A.P.I.D.® (Idaho Technologies) (42, 43) and Genedrive® (Epistem Ltd, Manchester, UK) –

combine amplification and fluorescence-based detection in a single tube, with sample preparation required separately (Table I).

Although some of these platforms have developed their own simple sample preparation methods – for example the Genedrive® uses comprehensive, paper-based extraction (34) – the majority of this testing has been performed on nucleic acid extracted with either manual kits or robotic platforms. These are often unsuitable for decentralised testing in the field or for use in low-resource laboratories. For instance, the SmartCycler has been successfully trialled using manual nucleic acid extraction kits in laboratory settings to detect FMDV (44, 45, 46), ASFV (47), CSFV (48) and VSV (49), and the T-COR™ 4 has been used to successfully differentiate between ASF and CSF, using robot-extracted nucleic acid (50). The T-COR™ 4 has also been

used successfully under field conditions to detect Newcastle disease virus (51).

Lyophilised qPCR assay kits are now commercially available for a number of these POCT platforms: Tetracore (Maryland, United States of America [USA]) offers assays for FMDV, bluetongue virus (BTV), ASFV, AIV and CSFV and genesig® (Primerdesign Ltd, Southampton, UK) offers lyophilised kits for 59 bovine pathogens (www.genesig.com) (52, 53). However, these kits are for research purposes only and are not yet licensed for diagnostic purposes. The adoption of PCR assays that are less sensitive to inhibition (54, 55) could increase demand by negating the requirement for complex sample preparation, offering an advantage over laboratory-based assays and opening up the real possibility of q(RT)-PCR use in decentralised settings.

Alternative polymerase chain reaction chemistries

Whilst the transferral of routine q(RT)-PCR assays onto POCT platforms maintains some level of consistency and confidence between laboratory and POCT assays, numerous drawbacks are still evident. For instance, in order to maintain the precise thermal regulation required for PCR, sophisticated instrumentation is still necessary, which can be expensive and difficult to decontaminate between premises. This requirement has resulted in an inevitable trade-off between POCT portability and sample throughput capabilities (Table I). As such, alternative PCR chemistries, such as insulated isothermal PCR (iiPCR) and linear-after-the-exponential PCR (LATE-PCR) (56, 57, 58), have been explored as potential veterinary diagnostic solutions.

In iiPCR, a horizontal temperature gradient is created across a PCR vessel by heating it from the bottom at a fixed temperature, with the top cooled by surrounding air. By removing the requirement for conventional thermal cycling, iiPCR reactions can be performed using relatively simple portable platforms, such as the commercially available POCKIT™ nucleic acid analyser (Table I) (GeneReach, Massachusetts, USA) (59). Recently, POCKIT™-compatible, lyophilised RT-iiPCR assays have been developed and analysed in laboratory settings for pan-specific detection of FMDV (shown to identify 63 FMDV strains across all seven serotypes) (60), BTV (detecting all 26 known BTV serotypes) (61), and CSFV (62), all with comparable sensitivity to their respective, laboratory-based q(RT)-PCR equivalents.

Furthermore, direct detection of CSFV from serum dilutions and FMDV from vesicular fluid samples has been shown to yield positive results, somewhat negating the requirement for sample preparation and increasing compatibility with field use (60, 62). The use of other clinical materials is yet to be determined. Although rapid, with detectable levels of amplicon generated within 30 min (63), platforms such

as the POCKIT™ are binary end-point systems. Although this is an advantage when used by less-skilled operators (since the results need no interpretation), assay flexibility is reduced (quantitative interpretation is not possible).

Isothermal technologies

An alternative approach to reducing the size, cost and complexity of molecular POCT platforms is to remove the requirement for thermal regulation and perform reactions at a single fixed temperature using isothermal chemistries. Numerous isothermal methodologies have been developed (64), with four having been most widely used to detect livestock and avian pathogens to date, namely: loop-mediated isothermal amplification (LAMP) (Fig. 2a), recombinase polymerase amplification (RPA) (Fig. 2b), nucleic acid sequence-based amplification (NASBA) (Fig. 2c) and helicase-dependent amplification (HDA) (Fig. 2d).

Loop-mediated isothermal amplification is a rapid nucleic acid amplification technique that uses a strand-displacing polymerase, multiple primers and auto-cycling under isothermal conditions (Fig. 2a) (65, 66). Its high sensitivity and rapid detection have led to the development of a multitude of (RT)-LAMP assays to identify livestock and avian viruses. They include assays for ASFV (67), FMDV (40, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78), SVDV (79), VSV (80), BTV (81, 82, 83), CSFV (84, 85, 86, 87) and AIV (88, 89, 90, 91, 92, 93, 94). However, these are not yet commercially available in kit formats.

Much progress has been made in transitioning LAMP into POCT platforms. This is partly due to LAMP chemistry having increased resistance to inhibitors when compared to PCR. LAMP has shown higher sensitivity than q(RT)-PCR when identifying SVDV in porcine faecal RNA extractions (79) and has also been used to detect FMDV RNA directly from epithelial, serum and oesophageal-pharyngeal (OP) fluid dilutions in field settings, using lyophilised reagents (39, 77). Furthermore, numerous detection methods are possible. Low-cost, objective, end-point detection can be performed using molecular LFDs (Fig. 3) (67, 77, 78, 89, 95), or commercially available portable fluorimeters can be used for real-time detection (Genie® II/III: OptiGene Ltd) (Table I), both of which have been successfully trialled for detecting FMDV *in situ* (36).

In RPA, isothermal amplification of the template is achieved by combining recombinase-driven primer targeting with strand-displacement synthesis (Fig. 2b) (96). Tube scanners from Twist Dx™ (Cambridge, UK) and Qiagen (ESEQuant TS2), in combination with lyophilised reagents (Twist Dx™), have enabled RPA to enter the POCT market. For instance, during the 2012 FMDV outbreak in Egypt,

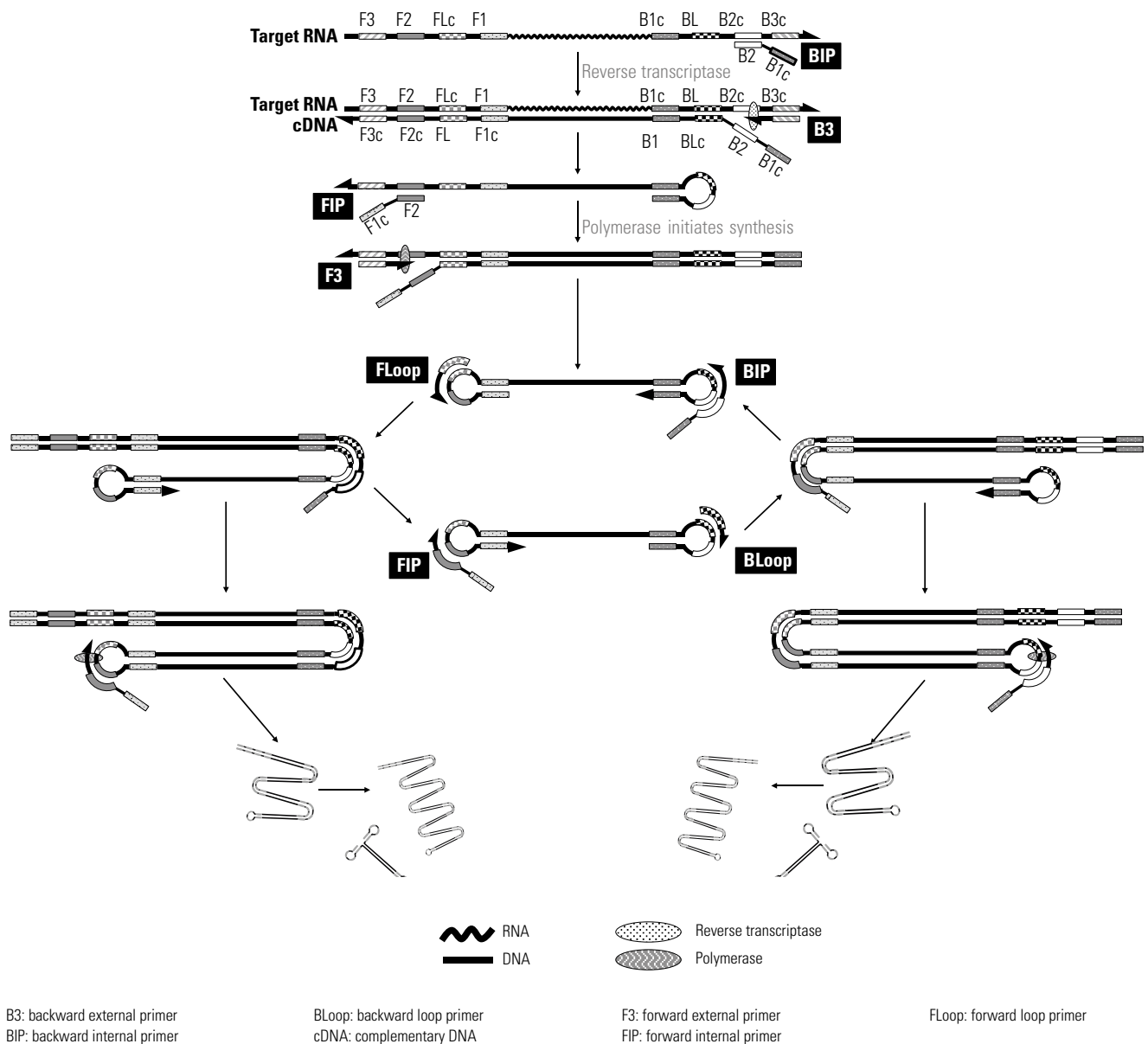
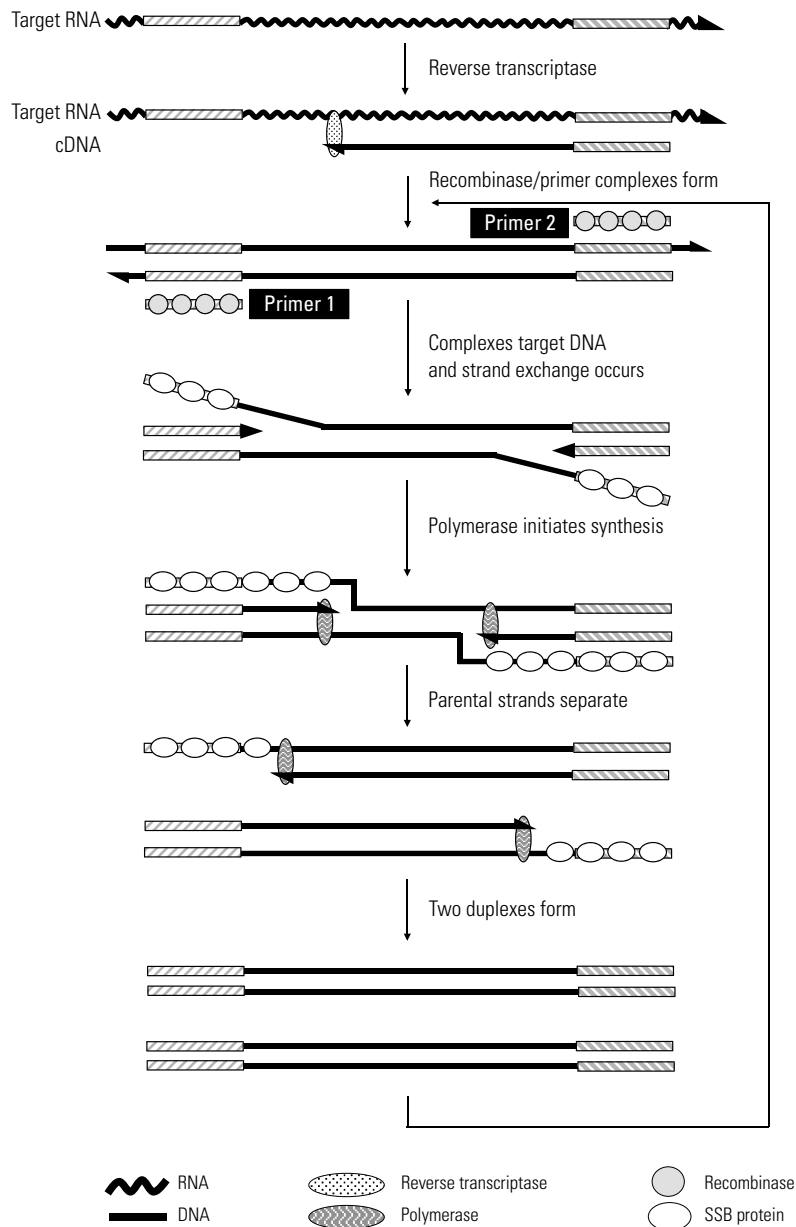


Fig. 2a
Schematic representation of the amplification mechanism of reverse transcription-loop mediated isothermal amplification assay

a pan-serotypic FMDV assay and portable RPA laboratory was trialled with manual RNA extraction (Dynabeads® Silane viral nucleic acid kit: Invitrogen), achieving 98% sensitivity over 45 samples, with an analytical sensitivity of 1,436 FMDV RNA molecules (97). A similar assay has also been developed for AIV H7N9 (98) but has not yet been trialled in field settings.

Nucleic acid sequence-based amplification is a continuous isothermal technique used primarily to detect single-stranded RNA. The reaction depends on the activities of three enzymes: a reverse transcriptase, T7 RNA polymerase,

and ribonuclease H, in addition to a single pair of specially designed oligonucleotide primers (Fig. 2c), with optimal conditions allowing 10^9 – 10^{12} -fold amplification after 2 h (99, 100). Using NASBA-electro-chemiluminescence for real-time detection, assays have been developed for numerous animal viruses, including FMDV and AIV (101, 102, 103, 104), with sensitivity reported to be equivalent to that of q(RT)-PCR. In addition, NASBA-enzyme-linked oligonucleotide capture has now been developed and trialled for FMDV detection, offering an increasingly portable solution, since only a temperature-controlled water bath and a 96-well microplate spectrophotometer are required for the amplification and



cDNA: complementary DNA

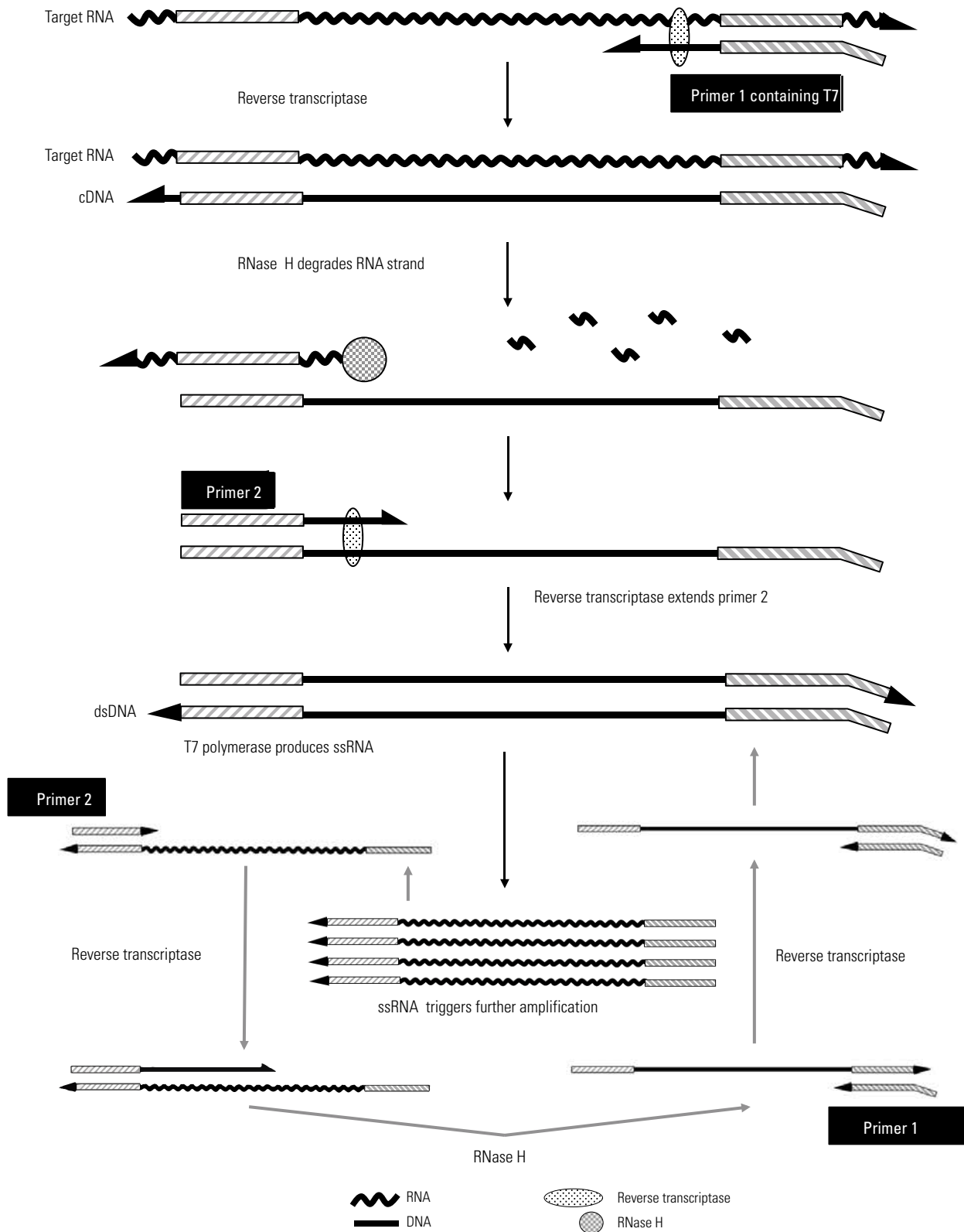
SSB protein: single-stranded DNA-binding protein

Fig. 2b
Schematic representation of the amplification mechanisms of reverse-transcription recombinase polymerase amplification

detection stages (105). However, to date, these tests remain in research and development, using extracted nucleic acid; further work is required to transition this chemistry into a POCT format.

Helicase-dependent amplification (HDA) is an isothermal amplification method similar to conventional PCR, but which uses the action of a helicase enzyme, rather than heat, to denature nucleic acids (106). The addition of

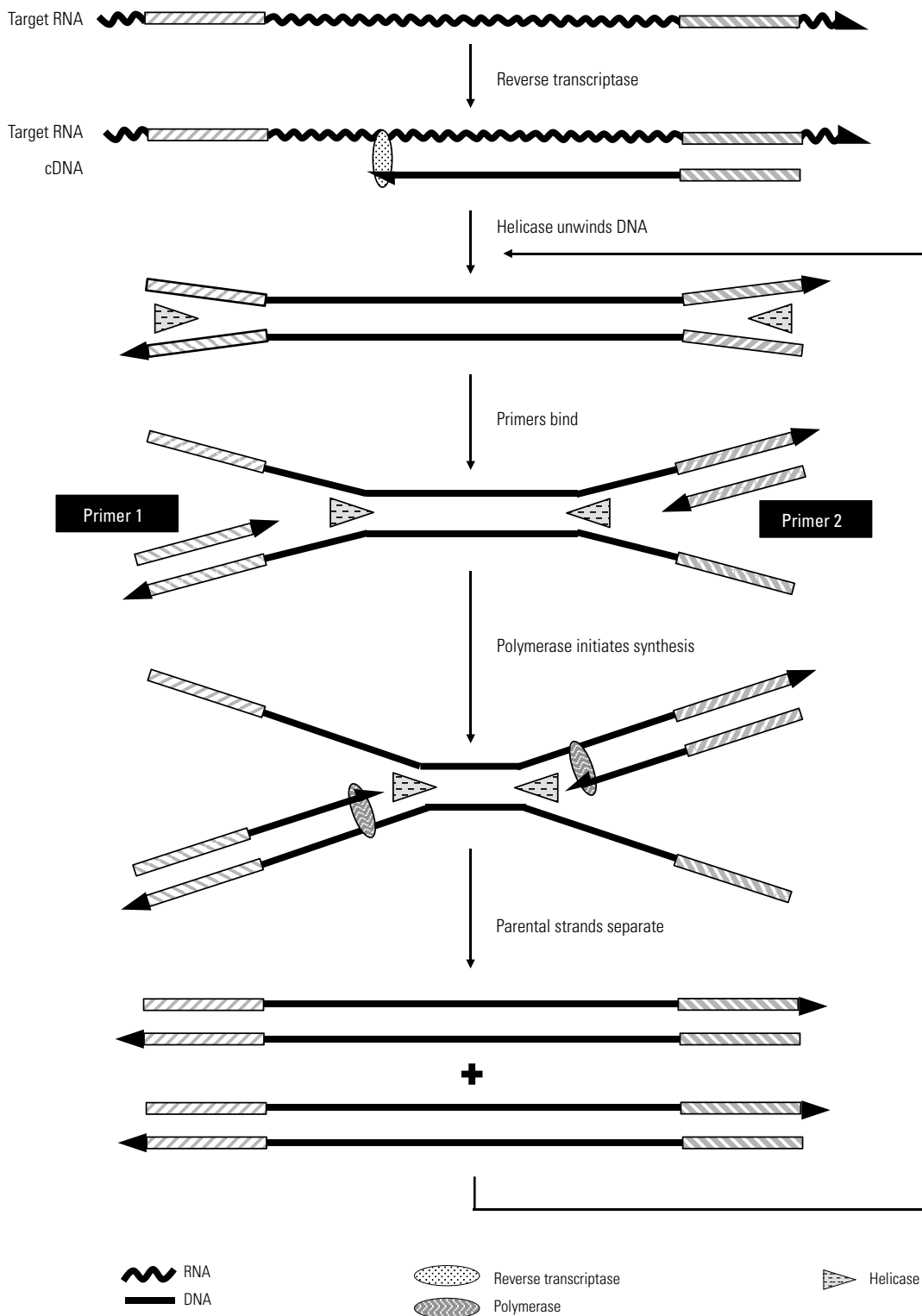
intercalating dyes has enabled HDA to be performed in real time (107), generally taking between 30–90 min for relatively short targets (70–120 base pairs) (108). Assays to detect FMDV have been developed and tested in laboratory settings (109), with detection limits reported to be ten-fold higher than with RT-PCR. However, as yet, these assays have not been transferred onto portable platforms. Thus, more work is required to transition and validate this chemistry as a POCT.



cDNA: complementary DNA
dsDNA: double-stranded DNA

RNase H: ribonuclease H
ssRNA: single-stranded RNA

Fig. 2c
Schematic representation of the amplification mechanisms of reverse-transcription nucleic acid sequence-based amplification



cDNA: complementary DNA

Fig. 2d
Schematic representation of the amplification mechanisms of reverse-transcription helicase-dependent amplification

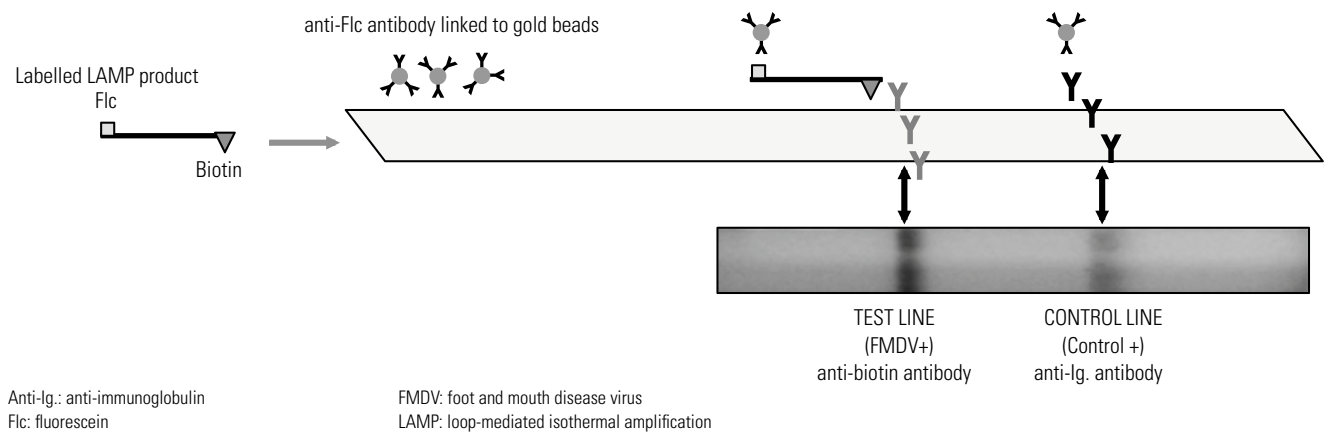
**Fig. 3**

Illustration of reverse transcription-loop mediated isothermal amplification assay – reaction detected using molecular lateral flow devices

A positive result is signified by the presence of two blue bands (test and lateral flow devices control line); negative results are indicated by a single band (the lateral flow devices control line)

The future of veterinary diagnostic strategies and opportunities to integrate other technologies into rapid-testing pipelines

It is believed that micro- and nano-technologies will play a leading role in global veterinary diagnostics in the near future, based on methods such as microfluidics, microarrays and lab-on-a-chip devices (110, 111). For instance, by 2013, a total of ten different POCT-appropriate biosensors had been developed to detect *Mycobacterium tuberculosis* for human diagnosis of TB (112). Progress with these micro- and nano-technologies has also been made in the veterinary field. A portable impedance biosensor for AIV detection (113) has been developed, intended for use in the field, and a microarray-based assay to differentiate between FMDV, VSV and SVDV (114) has been developed for laboratory use.

The availability of novel sequencing methods may change the way that diagnosis is performed in the future, providing an unbiased platform for sample analysis. The MinION (Oxford Nanopore, Oxford, UK), for example, is a commercially available portable sequencer that can be used for real-time biological analysis. This platform was recently applied in human diagnostics for real-time genomic surveillance of the Ebola virus in local laboratory settings, with sequencing results generated in less than 24 h after receiving Ebola-positive samples (115).

Sequencing technologies currently still in the development stage (e.g. developments by Genapsys, California, USA, and QuantuMDX, Newcastle upon Tyne, UK) may in the future enable the decentralised sequencing of targeted pathogen genome regions, complete genomes or metagenomes, although it is currently unclear if and when they will become commercially available. (For further information, see allseq.com/knowledge-bank/kb-category/emerging-technologies and www.nextgenerationsequencing.info/ngs-products/ngs-technologies/other-ngs-technologies.)

The future availability of field-based sequencing technologies, combined with our current knowledge of simple sample preparation strategies, such as the elution of nucleic acid from LFDs, could make *in situ* sequencing a real possibility in the next decade.

Challenges associated with the implementation of veterinary point-of-care testing

The benefits of transitioning diagnostic technologies into POCT formats are clear in terms of accelerating diagnostic confirmation, strengthening veterinary surveillance systems and providing increased flexibility in disease control. However, despite these benefits and numerous technological advances, the uptake of molecular POCT remains relatively low for veterinary diagnostic use, with all above assays remaining in the research and development phase. The future success and implementation

of POCT methods into the veterinary market depend largely upon several factors.

Quality assurance and approval

Although many POCT technologies and assays to detect agents causing notifiable diseases have been published in peer-reviewed journals, none yet appears in the OIE *Terrestrial Manual* as an approved diagnostic test (1), with the exception of the Ag-LFD for FMDV. In addition, performing assays outside recognised quality assurance systems (such as ISO 17025) leads to concerns about the acceptance of test data by national authorities and the farming community. Consequently, it is expected that the confirmation of primary cases of high-impact livestock viral diseases in previously disease-free countries (or zones) will always depend upon the results of reference laboratory tests. However, once an incursion of disease has been confirmed, POCT could be used to diagnose secondary cases, providing tools to quickly support the results of veterinary examination. By enabling rapid decisions to be made *in situ*, these technologies have the potential to decrease the costs associated with outbreak control, through measures such as reducing the unnecessary slaughter of uninfected animals.

What test and who will run it?

The transition of these assays into current diagnostic strategies must be considered in conjunction with how these assays would work in parallel with (or replace) existing methodologies. Without proper management, decentralised testing could put further stress on veterinary systems by increasing requirements for training and quality assurance, as well as complicating decisions about the most appropriate tests to use. Despite these concerns, there is an expectation from many stakeholders (including farmers and veterinarians) that these tests should be made freely available. This would not, however, remove the requirement for trained veterinary officials who are able to report and recognise the clinical signs of disease.

Furthermore, consideration must be given to reporting and storing test results, as well as the decisions that can be taken using POCT. Some of the newer POCT platforms, such as the T-COR™ 8 (Tetracore), are equipped with data transmission capacity, enabling digital linkage between POCT and laboratories for timely reporting and monitoring of test quality. However, without the incorporation of such POCT protocols into current veterinary contingency plans, such as the WHO recommendations on the use of rapid testing for human influenza diagnosis (116), these technologies are likely to remain underused.

Point-of-care tests may prove to be very useful in developing countries, where transporting samples to laboratories and

getting the results rapidly can be problematic. In these settings, they may be used by veterinary or para-veterinary staff. The field use of tests for FMD or *Brucella* in endemic countries does not cause the same amount of official concern as in countries that have eradicated these diseases. It may be that the use of POCTs for endemic diseases will lead to further development of these platforms and greater confidence in their use.

The cost model

The uptake of POCT will rely upon investment in the technology, leading to performance and cost advantages over the existing approaches used to control disease outbreaks. Although this review has outlined the performance advantages of POCT, the development of molecular assays into portable formats has resulted in inevitable compromises between cost and simplicity, with many of the more sophisticated and integrated platforms being less affordable. Almost all POCTs are platform tests, relying on some level of instrumentation. This makes them harder to implement than simply another laboratory-based ELISA or PCR. This leads to concern over whether a viable market for these assays currently exists in veterinary diagnostics as the cost per test still remains relatively high; the uptake and establishment of POCT therefore hinges upon future commercial market forces. In addition to the purchase of POCT platforms and consumables, the decentralisation of tests also incurs additional associated costs, including labour, training and quality assurance. The advantages of POCT programmes (e.g. rapid response) therefore need to outweigh such costs if POCTs are to be integrated into control programmes.

Conclusion

In conclusion, the early identification of infectious diseases in domestic and wild animals is crucial to improve our control capabilities. Point-of-care assays are usually developed with a view to their use in developed countries where these livestock diseases are often absent. However, these technologies also have huge potential to improve surveillance in countries in the developing world, where centralised diagnostic capacity is not always well established.

There have been great advances in POCT. However, the commercialisation of assays and their uptake remain low, with most diagnostic work for diseases notifiable to the OIE remaining in diagnostic laboratories. In some sectors, such as plant disease diagnostics, POCTs are more widely exploited. It is anticipated that the future success and implementation of POCT methodologies for livestock will be driven by the commercial sector and largely depend upon several factors,

including assay accreditation and the transitioning of POCT methodologies into current diagnostic strategies.

At a time when these technologies are rapidly evolving, probably the biggest single challenge is to identify those chemistries and platforms that offer the greatest potential and then suitably validate them for routine use. This crucial step has been recognised by major funding bodies such as the European Commission which, over the next few years, will fund collaborative research consortiums dedicated to validating diagnostic tools for animal and plant health through Horizon 2020. These projects will undertake ring tests, develop reference materials and harmonise or adapt tests for implementation in field conditions, with the expected impact of delivering the next generation of market-ready veterinary diagnostic tests.

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Les progrès technologiques dans le domaine du diagnostic vétérinaire : perspectives de déploiement de tests décentralisés rapides pour la détection des agents pathogènes affectant le bétail

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Résumé

La production durable de denrées alimentaires pour nourrir une population humaine en constante augmentation constitue un vaste enjeu planétaire ainsi que l'une des priorités définies par les Nations Unies dans le cadre des Objectifs du Millénaire pour le développement visant à « éradiquer l'extrême pauvreté et la faim dans le monde ». D'une part, les maladies animales réduisent la productivité des animaux d'élevage ; d'autre part, la mondialisation des échanges d'animaux et de produits d'origine animale intensifie les risques d'incursion de maladies. L'utilisation de tests de diagnostic rapides et fiables est une composante essentielle des plans d'urgence visant à détecter, contrôler et éradiquer ces maladies. Une procédure de diagnostic est généralement constituée de plusieurs opérations, depuis la suspicion clinique, la collecte d'échantillons, leur transport vers un laboratoire central (par exemple un laboratoire de référence national/international), jusqu'à l'analyse de ces échantillons au moyen d'une série de tests diagnostiques et la notification des résultats. Néanmoins, le transport des échantillons depuis le terrain jusqu'au laboratoire est parfois un processus très long qui peut retarder la prise de décisions cruciales, voire compromettre gravement la qualité des échantillons. Cette limitation importante des procédures diagnostiques centralisées a incité à mettre au point des outils permettant une

detección rápida y aísée des agents pathogènes affectant le bétail. Les progrès récents accomplis dans les technologies relevant de la médecine humaine personnalisée ont encouragé le développement de prototypes d'épreuves de diagnostic pour nombre de maladies du bétail. Toutefois, plusieurs de ces tests ne sont pas encore utilisés en routine ni disponibles commercialement. Les auteurs font le point sur les exemples les plus prometteurs de ces tests et soulignent les difficultés restant à résoudre pour que ces tests puissent évoluer d'une application en recherche et développement à une utilisation en routine.

Mots-clés

Bétail – Détection – Diagnostic – Diagnostic de terrain – Diagnostic décentralisé – Examen diagnostique sur le lieu d'intervention – Test moléculaire – Test rapide.



Avances tecnológicos en el diagnóstico veterinario: posibilidades de implantar pruebas rápidas descentralizadas para detectar patógenos del ganado

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Resumen

El logro de una producción sostenible de alimentos en cantidad suficiente para abastecer a una población humana cada vez más numerosa es una difícil empresa que el mundo tiene ante sí, que además entronca con una de las prioridades plasmadas en los Objetivos de Desarrollo del Milenio de las Naciones Unidas: «erradicar la pobreza extrema y el hambre». Las enfermedades infecciosas merman la productividad de los animales de granja, al tiempo que el comercio mundializado de animales y sus derivados amplifica la amenaza de incursiones infecciosas. La existencia de pruebas de diagnóstico rápidas y exactas es un elemento básico de todo plan de emergencia encaminado a detectar, controlar y erradicar esas enfermedades. Las labores de diagnóstico entrañan un «circuito» que normalmente empieza con la sospecha clínica, sigue con la obtención de muestras, su transporte a un laboratorio central (como un laboratorio de referencia nacional o internacional) y su análisis mediante diversas pruebas de diagnóstico y culmina con la notificación de los resultados. Sin embargo, el transporte hasta un laboratorio de las muestras obtenidas sobre el terreno es a veces un proceso lento, que puede retrasar la adopción de decisiones cruciales y merman sensiblemente la calidad de las muestras. Este importante inconveniente derivado de la realización centralizada de pruebas ha llevado a concebir herramientas que permitan detectar de forma rápida y sencilla patógenos presentes en el ganado. Los avances registrados últimamente en la obtención de tecnologías destinadas a la medicina humana personalizada han propiciado también la elaboración de prototipos de pruebas para diagnosticar

numerosas enfermedades del ganado, aunque muchas de ellas todavía no se utilizan sistemáticamente ni están comercializadas. Los autores, tras examinar en clave crítica los más prometedores ejemplos de estos nuevos ensayos, señalan las dificultades que aún subsisten para que estas pruebas puedan pasar del ámbito de la investigación aplicada y el desarrollo al de su utilización sistemática.

Palabras clave

Detección – Diagnóstico – Diagnóstico sobre el terreno – Ensayos moleculares – Ganado bovino – Pruebas rápidas – Realización de pruebas en el lugar de tratamiento – Realización descentralizada de pruebas.



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