Opportunities and challenges for the application of microfluidic technologies in point-of-care veterinary diagnostics

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Abstract

There is a growing need for low-cost, rapid and reliable diagnostic results in veterinary medicine. Point-of-care (POC) tests have tremendous advantages over existing laboratory-based tests, due to their intrinsic low-cost and rapidity. A considerable number of POC tests are presently available, mostly in dipstick or lateral flow formats, allowing cost-effective and decentralised diagnosis of a wide range of infectious diseases and public health related threats. Although, extremely useful, these tests come with some limitations. Recent advances in the field of microfluidics have brought about new and exciting opportunities for human health diagnostics, and there is now great potential for these new technologies to be applied in the field of veterinary diagnostics. This review appraises currently available POC tests in veterinary medicine, taking into consideration their usefulness and limitations, whilst exploring possible applications for new and emerging technologies, in order to widen and improve the range of POC tests available.

Keywords

Point-of-care, veterinary diagnostics, microfluidics, lateral flow immunoassays, paper-based microfluidics, dipstick test.

1. Introduction

Point-of-care (POC) diagnostics is an area that has attracted considerable attention in the last decade. Testing at POC means that analytical procedures are carried out at the side of or near to the patient [1], for this reason, it is also sometimes referred to as "bed-side" testing [2]. The reasons for the considerable interest in the field of POC testing are numerous: the potential to decrease costs of diagnosis [3], increasing the accessibility of these types of test to disadvantaged populations [4], and reducing the time between sampling and a treatment decision [5].

Following the global trend towards more affordable and accessible diagnostic testing, the Sexually Transmitted Diseases Diagnostics Initiative (SDI) within the World Health Organization (WHO) recently established a set of benchmark criteria for the ideal rapid test, under the acronym "ASSURED" [6]: Affordable, Sensitive, Specific, User-friendly (simple to perform in a few steps, with minimal technical training), Robust and rapid (results available in less than 30 min), Equipment-free, Deliverable to those who need them. Ideally, POC tests should respect all or as many as possible of these characteristics [7].

2. Point of care testing and its scope in veterinary medicine

In the veterinary area, there is a similar need for low-cost, reliable and rapid diagnostic tests to be carried out at the POC [8]. So called on-site or animal-side tests will have considerable advantages over laboratory-based testing, which usually involves laborious and expensive laboratory techniques and dedicated technical personnel. All of the analytical processes involved in testing, from collection of the sample to communication of the results, could potentially be performed in a single step, considerably reducing the time between testing and treatment [9]. This can translate into more affordable veterinary care, reduced handling of animals, targeted treatments and rapid testing in more remote geographic areas.

The need for more affordable, rapid and accessible tests is a recurrent theme in the literature, in particular as an invaluable tool in dealing with diseases that either represent a threat to public health [10], have substantial impact on animal welfare [11] and/or are of economic importance [12], with particular

relevance to situations where laboratory facilities and funds are limited [13]. Furthermore, the general globalisation of trade of animals and animal products has greatly increased the risk of rapid and wide-ranging spread of emerging and exotic diseases, requiring timely and efficient ways of dealing with diseases that could have catastrophic repercussions for the individual farmer, as well as economic implications for the entire country and international trade [14]. In situations concerning disease outbreaks, where rapid propagation of infectious agents and/or high mortality are salient features, as in the case of the highly pathogenic H5N1 strain of avian influenza virus, a rapid "animal-side" test would represent a critical tool for both collecting surveillance data and for assisting in the control of outbreaks [11, 15]. Currently available veterinary POC tests offer a good opportunity for a truly "animal-side" diagnosis, but the analytical performances of "on-site" testing are still considered limited compared with laboratory-based testing [16], whilst the possibility offered by the support of a central laboratory in the interpretation of the results is still perceived as critical [17]. Recent advances in microfluidic technologies for POC testing in the human field could overcome these hurdles and might be applied in veterinary medicine. This review aims to appraise the current status of POC testing in veterinary medicine, describing their advantages and limitations, whilst also assessing the potential of microfluidic technologies to improve existing POC tests and solve some of their intrinsic limitations.

3. Point-of-care devices currently available in veterinary diagnostics

At present, the most widely used technologies for POC testing in veterinary medicine are: dipstick tests and lateral flow immunoassays.

3.1 Dipstick and strip test

These assays are based on the principle of immunoblotting and are made of paper strips with pads to analyse specific fluids. After the sample is introduced, the results are compared with a colour-coded chart to provide a semi-quantitative determination of the analyte(s). The most commonly used are test strips

104 developed for human urine analysis, allowing the simultaneous detection or monitoring of leukocytes,
105 nitrite, urobilinogen, protein, pH, haemoglobin, specific gravity, ketones, bilirubin and/or glucose (Fig. 1)
106 [18]. While it has been developed for human patients, there is a high correlation between the dipstick
107 results and other routinely used methods for urine analysis, which has resulted in this test being widely
108 used in small animal private practice for first-line diagnosis of chronic kidney disease, mainly through an
109 assessment of proteinuria [19, 20]. However, care should be taken when interpreting positive test results
110 with low levels of proteins (traces) due to the high rate of false positive results [21].

111 A smaller version of the urine dipstick, restricted to detection of glucose and ketone bodies, is also
112 largely applied for at-home management of pets with diabetes. This test is also widely used in farmed
113 ruminants for the diagnosis of ketosis in cattle [22] and pregnancy toxaemia in sheep. Due to some
114 variation in results, a further advance in the diagnosis of these diseases is the use of appropriate strips
115 combined with electronic hand-held meters to accurately measure both glucose and one of the main
116 ketone bodies, ß- bhydroxybutyrate (BHB) in blood, making diagnosis more reliable as well as sampling
117 potentially more successful and less stressful [23]. This POC test has shown great potential for the
118 quantitative detection of BHB, with improved sensitivity and specificity when compared with dipstick tests
119 for detection of ketones in urine or milk [24, 25]. Its use for glucose measurement, however, does not seem
120 to be reliable [23]. Dipstick tests have also been used for the detection of antibiotics in serum, milk and/or
121 meat samples [26]. These rapid tests allow the detection of antibiotics within the µg/ml range, permitting
122 on-site monitoring of non-authorised uses of antimicrobials, which could be especially useful in
123 slaughterhouses and food processing plants.

124 The main advantage of these POC tests is that they can be readily carried out by the owners, proving to
125 be particularly useful for the long term management of chronic diseases [27]. The major limitation,
126 however, can be the subjective interpretation of results, based on a personal evaluation of a colorimetric
127 reaction [19].

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129 *3.2 Lateral flow immunoassays (LFIAs)*

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These devices are based on the principle of capillary force: a liquid flowing on or through a strip of polymeric material, on or in which specific molecules (e.g., antigens, antibodies, DNA/RNA sequence) have been immobilized [28]. These strips usually consist of multiple pads: a sample application pad, a conjugate pad, a membrane for detection and an absorbent pad (Fig. 2), usually made of different materials (e.g., nitrocellulose, glass fibre paper and fused silica) encased in a plastic cage for protection of a fragile paper membrane [29]. The best known example of a lateral flow test is the pregnancy test [30], which is probably the most used POC test worldwide. The main advantages of lateral flow over traditional laboratory-based tests are their simplicity, rapidity and low cost. Compared with traditional laboratory-based tests, LFIAs are considerably less expensive, but, due to the different materials required, they are still relatively costly (~ $10 for a pregnancy test; [29]) for application in low-resource settings [31], and, due to the multi step processes involved, manufacturing time is extended, making them less suitable for high-throughput production.

They usually provide a qualitative or semi-quantitative result, and analytical performance is generally poorer than laboratory-based tests, mainly due to reduced sensitivity [32, 33] and the possibility of errors due to testing by untrained personnel [2]. When compared with reference laboratory tests, specificity tends to be comparable, while sensitivity can be as low as 16%, suggesting that a positive result might be trusted, whereas, in the case of negative results, further confirmatory laboratory testing may be advisable. Several studies have assessed quantitation, but such devices still require instrumentation [34, 35] and trained personnel, and are still limited to single analyte testing.

3.2.1 Companion animals

There is a significant market for the use of LFIAs for a range of acute and chronic diseases or conditions in companion animals (Table 1). These assays are usually easy to perform and interpret [32], with the possibility to improve sensitivity by using a dedicated LFIA reader, which allows an objective and quantitative interpretation of the results [28]. Although the cost of the test is an important consideration, reduced waiting time and the possibility of starting a therapy within the first visit are amongst the main

158 advantages of tests that can be carried out “in-house”. It is also important to consider that some diseases
159 have received considerably greater attention, notably viral infection diseases, such as FIV and FeLV in cats
160 and parvovirus in dogs, with an extended range of assays being available.
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162 *3.2.2 Livestock*
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164 For livestock, LFIAs have been focused mainly on illnesses that represent a substantial economic burden
165 and/or serious zoonotic or epidemic diseases (Table 2). OIE-listed diseases of the World Organization for
166 Animal Health, such as Foot-and-Mouth Disease (FMD) and Rinderpest have received considerable
167 attention, due to the crucial importance of a rapid diagnosis and, consequently, prompt intervention from
168 veterinary authorities. In the case of FMD, endemic areas are frequently in developing countries, and often
169 diagnosis is not reached due to the prolonged time between collection of samples, arrival at the reference
170 laboratory and subsequent testing [36]. In this case, both the economic constraints and accessibility to
171 remote areas are the dominant issues.
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173 *3.2.3 Food safety*
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175 Lateral flow tests have been successfully applied in two main areas: the detection of food-borne
176 pathogens and the detection of fraudulent substances in animal feed or in animal products (Table 3). In the
177 first case, much emphasis is placed on the prevention of zoonotic diseases, which represent a significant
178 and widespread public health threat. In the second case, recent feed-stuff scandals [37, 38] and increasing
179 reports of antimicrobial resistance dominate the scene, both in terms of research and public attention.
180 Here, and on-site test can be a powerful tool for rapid detection and subsequent surveillance, especially
181 when dealing with highly perishable products.
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183 **4. Microfluidic technologies available for POC diagnostics**
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One of the most promising technologies that has been applied recently in diagnostics is microfluidics, which involves the analysis of extremely small amounts (microlitres or nanolitres) of fluid using interconnected networks of channels measuring tens to hundreds of micrometres [39]. Since the introduction of microfluidics from the early 1990s [40], there has been a constant evolution of these methods, mainly following critical advances in microfabrication technologies [41]. Fluid transport in these devices is achieved by either passive (usually capillary forces) or active (generally pumping) mechanisms [42, 43], with the fluid flow being typically laminar [44].

Among the main advantages of microfluidics technologies for diagnostic applications are their portability and their low consumption of reagents; these attributes have made these devices inexpensive, rapid and generally easier to use compared with conventional (macroscale) testing [45]. The use of very small volumes, associated with shorter diffusional distances, results in significantly reduced time for analysis, making microfluidic assays significantly more rapid to perform than their macroscale equivalents [46]. Furthermore, being able to perform all necessary steps within one device and potentially in a single reaction represent a considerable advantage, allowing sample pre-treatment, analysis, signal detection and amplification in the same device [47]. Automated control of all steps can reduce inherent human error, which in turn increases the quality, reproducibility and reliability of assay results. The higher degree of control of fluid flow and the timing of binding reactions can also result in significantly improved analytical performance [48], while the opportunity for tests to be carried out simultaneously offers considerable potential for multiplexing [47]. Examples of the successful applications of these new technologies are in the clinical analysis of blood [49-51], pathogen identification [52, 53], genetic testing [54, 55], detection of environmental contaminants [56] and for drug screening [57].

Currently, two main types of microfluidic systems are used in the diagnostic field: micro total analysis systems (μTAS) and microfluidic paper-based analytical devices (μPADs). However, to date, there has been very limited application of this technology in the veterinary field [58].

4.1 Micro total analysis systems (μTAS)

These systems are also commonly known as “lab on a chip” (LOC) devices (Fig. 3), which use fluid as a working medium and can integrate a number of different functionalities on a micro scale [41]. One of the main advantages of µTAS devices is that they allow for all steps (from sample pre-treatment to signal detection) to be carried out at once, on the same device, allowing complicated molecular techniques (i.e. polymerase chain reaction (PCR)) to be transferred on the chip for POC testing. These devices are fabricated using techniques from the microelectronics industry [59], mostly using materials, such as silicon, glass and/or polymers [60]. At present, the most common materials used are thermoplastic polymers. These devices have reduced production costs, and have suitable mechanical, chemical and thermal properties [61].

Their diagnostic use is well established, with companies already commercialising POC devices on plastic platforms [62]. In the last decade, there has been a considerable focus on immunodiagnostic tests for the detection of disease markers, specifically for cardiac and cancer markers [63-66] and for the diagnosis of infectious diseases, including HIV/AIDS [67, 68], influenza [69] and hepatitis [70]. Some limitations of these devices are inherent in physical effects, such as the need for pressure-driven liquid flow, with the possible consequence of heat generation and, therefore, detrimental effects on biomolecules, or low grade mass transfer and/or reduced mixing capacity [41].

4.2 Microfluidic paper-based analytical devices (µPADs)

These devices are commonly referred to as paper-based microfluidics (Fig. 4), a concept that was first introduced by the Whiteside group at Harvard University, following on from initial research performed on paper strips for the determination of pH [71]. These devices allow inexpensive multiplexed analyses to be carried out [72], while maintaining the advantages of conventional microfluidic technology, such as size, speed and reduced sample volumes [7]. Paper has considerable advantages over other materials in that it is cheap, easy to source, biodegradable and naturally abundant, but also simple to modify chemically [73]. POC devices made from paper also have the advantage of not requiring external power sources, whilst fabrication techniques and machinery for production are usually less expensive than those for other

materials, with minimal technical expertise required [74]. Paper represents an excellent medium for diagnostic testing, due to its high surface to volume ratio, which allows reagents to be concentrated, enabling more rapid reaction times [75]. Although µTAS are renowned for being less expensive than conventional laboratory-based testing, materials such as glass and silicon can still be considered expensive, either in terms of their environmental footprint or in their production costs [47]. Therefore, one of the main advantages of choosing µPADs over µTAS as a diagnostic platform is their reduced cost. Also µPADs are considered to be "easier" to produce, with no requirement for valves or pumps, as they use capillary force to move fluids within the device [74]; however, there can be issues with sample retention and evaporation, making them less suited to the analysis of small volumes [76].

5. Possible applications of microfluidic technologies in veterinary medicine

POC is already widely applied in veterinary medicine, and new and emerging technologies could bring substantial improvements to both the range of tests available and their inherent performance. The reduction in cost and time coupled to the possibility of multiplexing and one-step applications make these devices attractive for cost-effective and on-site testing of animals. Although microfluidics are, at the moment, predominantly applied to human diagnostics (Table 4), there have been examples of applications to areas of veterinary interest [77-81]. Microfluidic platforms have been successfully used for detection of food-borne pathogens, such as *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium* [82] as well as for detection of progesterone in serum samples [83]. The use of microfluidics has also been applied to improve the range of existing POC tests available for the detection of subclinical ketosis in cattle [84], in particular, by allowing on farm testing of samples such as milk.

5.1 Nucleic acid amplification

Combining nucleic acid amplification techniques with microfluidic technologies to detect low concentrations of molecules in a rapid, reliable and economical way represents an opportunity for new POC

technologies. The most widely used amplification technique is PCR [85], which is often used for infectious disease diagnosis, especially for the detection of small amounts of pathogen DNA. A possible limitation of laboratory-based PCR techniques is their cost, and the time and expertise required for testing. A promising application of microfluidics is DNA amplification on silicon chips [86], although the need for a thermocycler limits its application in the field. For this reason, new amplification technologies, based on isothermal nucleic acid amplification have received considerable attention, and appear to allow for improvement of assay sensitivity, while providing a rapid and cost-effective approach [87]. Examples of the integration of this method into µTAS devices are growing [88-91], with existing applications to the detection of pathogens of veterinary importance, such as *Cryptosporidium parvum* [92, 93], *E. coli* [94], *Salmonella typhimurium* [77, 95] and *Suid herpesvirus 1* [96].

5.2 Multiplexing

A significant benefit of microfluidic technologies is the possibility to perform different tests in or on the same device [97]. Multiplexing has particular relevance in situations where multiple agents are involved [98] or where clinical signs are similar between/among distinct diseases [99, 100]. In this case, a POC "package" could be offered, in order to screen a single sample for all key pathogens involved in a particular disease scenario or complex [101, 102]. Other advantages could be the parallel interpretation of different tests for the same condition, in order to significantly increase test sensitivity [103] and the analysis of multiple markers to specifically diagnose a disease or condition, especially in the case of a progressive disease; or for monitoring therapeutic effectiveness. Finally, performing multiple tests at once, can also result in a reduction in cost, time and sample use [104].

5.3 Telemedicine and surveillance

Perhaps the most important advance in diagnostics is the possibility of combining microfluidics technologies with mobile read outs and electronic data storage. Since mobile phones have become

household items across the world and smart-phone cameras are of a high quality [105], the combination of these technologies could truly represent the future for POC. Examples of mobile read out and telemedicine applied to microfluidics are increasing [106-109], and show great promise. They allow for remote and cost efficient diagnosis, and also for information to be stored and shared automatically, making the process time-efficient and reducing human error [110]. At the same time, animal tracking systems are becoming automated, with widespread use of electronic identification, in the format of microchips and electronic ear tags/boluses. From the veterinary perspective, exploiting the opportunities of "distance-diagnosis" with efficient animal tracking could have a tremendous impact on disease control and surveillance, with considerable advantages for the monitoring of notifiable and zoonotic diseases, as in the case of bovine spongiform encephalopathy (BSE) [111] and in screening for changes in disease patterns [112]. Surveillance schemes are mostly carried out by official laboratories at considerable cost [113]. Recent restructuring of some diagnostic services will inevitably have a significant impact on diagnostic capability [114], which means that the present scanning surveillance systems may not be sustainable in the long term, such that alternative options might be required.

5.4 Disposal and handling of biological material

One of the main advantages of microfluidic devices is the possibility for the safe disposal of biological material [115]. This is of particular relevance for paper, where disposal of bio-hazardous waste could be safely and quickly achieved by incineration [116]. The advantages are in the further reduction of waste management costs, but, more importantly in the reduced risks of handling biological samples that might represent a health and safety risk [117]. It has been shown that veterinary surgeons are often concerned about the health and safety of packaging samples entering the postal system, as they are responsible for proper packaging and the safety of the recipient [17]. Therefore, safe and low-cost disposal of potentially bio-hazardous material represents a substantial added benefit for these new technologies.

6. Challenges

One of the biggest challenges in the field of microfluidics is the translation from academic research to end-user products [118]. While the field of microfluidics has seen an exponential development in recent years, the launch of a commercialised platform that would revolutionise the concept of microfluidic technologies is still lacking [119]. Something similar to the breakthrough achieved by the pregnancy test may be required to enable microfluidics-based testing to be more widely accepted. Unfortunately, the fact that the diagnostic field is already quite mature, makes it harder to find companies willing to invest in new areas [116], and the difficulties in changing people's attitudes toward testing can represent an additional hurdle, especially when methods have been in place for many years. In this respect, the perception that analytical performances are still inferior to traditional laboratory-based tests remains a considerable constraint to the uptake of these technologies [2]. However, there is evidence that when a rapid result can achieve a better treatment rate, the sensitivity of a test can play a less important role [120]. This situation is extremely applicable in the veterinary field, where owners may struggle to find time for follow up visits after a test has been performed, or it may be problematic for farmers to re-gather animals days after testing [121]. Furthermore, as already in place for instrumental veterinary POC testing [122], specific guidelines should be put in place for the quality assurance of newly developed POCs, in order to provide a consistent and practical approach to evaluating their performance and increasing veterinarians' confidence in test results [123].

While some of the challenges faced in human healthcare have been addressed by the use of microfluidic technologies, this is not the case for animal health-related areas. For example, although the use of microfluidic technologies is suited for telemedicine, the handling or recording of data needs to be carefully organised. Data management systems are available for POC [124], which allow for valuable information to be stored and made available in real time. However, in the case of notifiable diseases, specific rules and strict controls will be required to ensure that legislation is followed. Another significant challenge will be the need for targeted solutions according to the specific situation, remembering that a beloved sick companion animal will require a different approach from livestock displaying signs of a potentially zoonotic disease.

Finally, in order to fully exploit the potential of the new technologies at the POC, a higher degree of collaboration between engineers and biologists is required. Whilst, at the moment, the majority of the publications regarding microfluidics are in engineering journals [125], increasing publication of these topics in biological journals would help overcome some of the existing barriers. From an engineering point of view, research may focus predominantly on resolving the physical and chemical barriers posed by microfluidic technologies, while, from a veterinary diagnostics perspective, practical solutions are the main focus. By facilitating better communication between technology designers and end-users, a truly interdisciplinary approach could be achieved, which will help to solve the issue of translation of these technologies to the veterinary field.

7. Conclusions

Considering the wide array of veterinary conditions and the nature of veterinary diagnostics, POC testing offers distinct advantages over traditional laboratory-based testing. The advent of microfluidic technologies has further increased the opportunities for wider and more valuable use of POC testing. Although these technologies have not yet been applied as widely to veterinary medicine as they have in human medicine, they still offer great potential. Many of the hurdles encountered in diagnostics are commonly shared in human and animal medicine; advances in one field will therefore provide benefits to both sides, as long as specific needs faced from an animal health point of view are kept in mind. Importantly, a close collaboration between engineers, developing new and existing technologies and those at the end point in need of improved solutions will be of paramount importance.

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Figure Legends

Fig. 1. Urine analysis performed using a dipstick test. The test strip is immersed in the urine sample for a few seconds, and, after a few minutes, the colour resulting from the reaction can be visually compared against the chromatic scale provided.

Fig. 2. Schematic representation of a lateral flow strip. A liquid sample is deposited on to the sample pad, migrating through a conjugate pad and a porous membrane for detection in a final absorbent pad. In most strip tests, the appearance of the control line indicates a valid test, while the appearance of a second test line indicates a positive test result.

Fig. 3. Stand-alone, self-powered integrated microfluidic blood analysis system (SIMBAS) [51]. The microfluidic platform integrates plasma separation from whole-blood with multiple immunoassays (A). Cross section of the device (B): fabrication materials (1); storage of the device in a vacuum package (2); addition of 5 ml of whole-blood sample on the inlet, degas-driven flow propels the sample into the device (3); blood cells sediment gravitationally and are filtered, while plasma flows into the channel (4); detection of multiple biomarkers (5); the flow is stopped by the suction chamber (6).

Fig. 4. 3D Origami-based microfluidic paper based analytical device [126]. Schematic representation, size and shape of the 3D origami-based device (A); the front and back surface of the device (B); binding of a

baked thin wax-patterned blotting paper on each waste tab, front (C) and back (D); binding of an unbaked thick wax-patterned blotting paper on each waste tab (E, F). The assay procedure is carried out by folding the different tabs above the test pad and adding the reagents sequentially, with the aid of a customised device folder.

Table 1. Examples of lateral flow immunoassays for POC diagnosis currently available for companion animals.

Disease/condition	Sample	Target analyte/pathogen	Target species	Commercial name	References
Addison's and Cushing disease	Serum	Cortisol	Dogs	SNAP® Cortisol Test	http://www.idexx.co.uk
Arthropod-borne diseases	Blood, serum or plasma	*Anaplasma phagocytophilum* and *platys*, *Ehrlichia canis* and *ewingii*, *Borrelia burgdorferi*, *Leishmania infantum*, *Dirofilaria immitis*	Cats and dogs	SNAP® 4Dx® Plus Test SNAP® 3Dx® Test SNAP Leishmania	[127, 128]
Cardiomyopathies	Serum or plasma	N-terminal pro-brain natriuretic peptide (NTproBNP)	Cats	SNAP® Feline proBNP Test	[129]
FIV and FeLV	Blood, serum or plasma	FIV and FeLV	Cats	BioSign® FeLV; BioSign® FIV; BioSign® FeLV/FIV; EVL One-step test; FASTest FeLV-FIV; SNAP® FIV/FeLV Combo Test; SNAP® Feline Triple® Test Speed Duo FELV/FIV; Witness FeLV-FIV	[130]
Giardiasis	Faeces	*Giardia*	Cats and dogs	SNAP® Giardia Test VetScan® Giardia Rapid Test	http://www.idexx.co.uk
Heartworm	Blood, serum or plasma	*Dirofilaria immitis*	Cats and dogs	BioSign® CHW; SNAP Feline Heartworm SNAP® Feline Triple® Test; WITNESS® DIROFILARIA	[131]
Liver disease	Serum	Bile acid	Cats and dogs	SNAP® Bile Acids Test	[132]
Lungworm	Serum or plasma	*Angiostrongylus vasorum*	Dogs	Angio Detect™ Test	http://www.idexx.co.uk
Pancreatitis	Serum	Pancreas-specific lipase	Cats and dogs	SNAP® fPL™ Test; SNAP® cPL™ Test	[133]
Parvovirus	Faeces	Parvovirus	Dogs	FASTest parvo strip; Witness parvo card; SAS™ Parvo; SNAP® Parvo Test; VetScan Canine Parvovirus Rapid Test; Speed® Parvo	[32]
Passive transfer of immunity	Blood, serum or plasma	IgG	Foals	SNAP® Foal IgG Test	http://www.idexx.co.uk
Thyroid disease	Serum or plasma	T4	Cats, dogs and horses	SNAP® Total T4 Test SNAP® T4 Test	http://www.idexx.co.uk
Thromboembolic disease	Citrated plasma	D-dimer	Dogs	NycoCard D-dimer test	[134]
Toxoplasmosis	Serum	*Toxoplasma gondii*	Cats	N/A	[135]

Table 2. Examples of lateral flow immunoassays for POC diagnosis currently available for livestock.

Disease/condition	Sample	Target analyte/pathogen	Target species	Commercial name	References
Anaplasmosis	Serum	*Anaplasma marginale*	Cattle	N/A	[136]
Aujeszky's Disease (Pseudorabies)	Serum	*Suid herpesvirus* type 1	Pigs	N/A	[137]
Bovine viral diarrhoea (BVD)	Serum and ear notch	Bovine viral diarrhoea virus (BVDV)	Cattle	SNAP® BVDV Test	http://www.idexx.co.uk
Classical Swine Fever	Blood	Classical swine fever virus	Pigs	CSFV Ab Test	http://www.idexx.co.uk
Foot and mouth disease (FMD)	Vesicular epithelium and fluid, blood	Foot and mouth disease virus	Ruminants and pigs	BioSign™ FMDV BioSign™ FMDV-Ag	[36, 138-140]
Infectious bursal disease	Bursa	Infectious bursal disease virus	Chickens	N/A	[141]
Neonatal diarrhoea	Faeces	Bovine rotavirus	Cattle	Rainbow Calf Scour Diagnostic Test	[33]
Passive transfer of immunity	Serum or plasma	IgG	Cattle	Midland quick test kit-calf IgG	[142]
Peste des petit ruminant	Lachrymal fluids	Peste des petit ruminant virus	Sheep and goats	N/A	[143]
Porcine reproductive and respiratory syndrome	Serum	Porcine reproductive and respiratory syndrome virus (PRRSV)	Pigs	BioSign® PRRSV	[12] [144]
Reproductive status	Milk	Progesterone	Cattle	N/A	[145]
Rinderpest	Lachrymal fluids	Rinderpest virus	Cattle	N/A	[146]
Tuberculosis	Blood, serum or plasma	*Mycobacterium bovis*	Red deer, wild boar, elephants, cattle and non-human primates	CervidTB STAT-PAK; VetTB STAT-PAK test; DPP® CervidTB; DPP® VetTB Assay for Elephants; PrimaTB STAT-PAK	[147-152]

Table 3. Examples of lateral flow immunoassays for POC diagnosis currently available for food safety.

Disease/condition	Sample	Target analyte/pathogen	Target species	Commercial name	References
Acute bloody diarrhoea	Milk	*Escherichia coli* O157:H7	Cattle	N/A	[153]
Anabolic steroid residues	Urine and liver	Medroxyprogesterone acetate (MPA)	Pigs	N/A	[154]
Antibiotic contamination	Milk, eggs, meat and urine	Beta-lactam, oxytetracycline, (dihydro)streptomycin gentamicin and sulfamethazine	Cattle, pigs, poultry, sheep and goats	Betastar® Combo Rapid Test; BioSign™ Sulfamethazine; Charm SL6™ Beta-lactam Test SNAP® Beta-Lactam ST Test; SNAPduo™ Beta-Tetra Test; SNAPduo™ Beta-Tetra Test ST; SNAP® Gentamicin Test; SNAP® MRL Test; SNAP® NBL Test; SNAP® Tetracycline Test; SNAP® Sulphamethazine Test	[155-160]
BSE	Brain	PrPBSE	Cattle	Prionics-Check PrioSTRIP	[161]
Clenbuterol contamination	Urine	Clenbuterol	Pigs	N/A	[162]
Mammalian proteins contamination of feed stuff	Plasma	Bovine IgG	Cattle	N/A	[163]
Melamime	Milk	Melamime	Cattle	SNAP® Melamine Test	[164]
Mycotoxins contamination	Feed matrix and milk	Aflatoxin B1 and M1, deoxynivalenol and zearalenone	Cattle and pigs	SNAP® AFM1 Test ROSA Mycotoxin Strips	[165, 166]
Nicarbazin residues	Feed matrix	Nicarbazin	Poultry	N/A	[167]
Salmonellosis	Meat	S*almonella typhimurium* and *enteritidis*	Poultry	N/A	[168]
Trichinellosis	Blood, serum and meat	*Trichinella spiralis*	Pigs	N/A	[169], [170]

Table 4. Examples of commercially available microfluidic-based POC technologies for human diagnostic applications.

Diagnostic application	Time to results	Sample type	Technology	Commercial name	Company homepage
Blood analysis (blood gas, electrolyte, haematology and metabolite panel)	30 seconds 2 minutes	Whole blood (100µl) Whole blood (2-3 drops)	Test-card with card reader Test cartridge with handheld analyser	epoc BGEM™ i-STAT	http://epocal.com https://www.abbottpointofcare.com
Blood typing	Few minutes	Fingerprick blood sample	Disposable, credit card sized microfluidic device	ABORhCard®	http://www.micronics.net[155-160][155-160][157-162]
Cardiac Troponin-I assay	10 minutes	Fingerprick blood sample	Integrated, self-contained assay cartridge with handheld reader	Nanōmix eLab	http://nano.com
Cancer diagnosis	100 minutes	Plasma (1ml)	Test cartdridge with platform	Idylla™ ctBRAF Mutation assay	https://www.biocartis.com
Coagulation monitoring (Prothrombin Time test)	3 minutes	Whole blood (5µl)	Memory microchip with a meter	CoagMax®	http://www.microvisk.com
Fertility (FSH, LH, PL)	30 minutes	Serum (20µl)	Test cartdridge with reader	Acix 100	http://www.achiralabs.com
Gastrointestinal infection (*Clostridium*	Less than 2 hours	Stool	Test cartdridge with reader	Verigene® *C.*	http://www.nanosphere.us

difficile)				*difficile* Test (CDF)	
HIV	50 minutes	Whole blood or plasma (100µl)	Test cartdridge with analyzer unit	EOSCAPE-HIV	http://www.wave80.com
Inflammation (C-reactive protein)	Few minutes	Whole blood, serum or plasma (5µl)	DVD-like disc with reading instrument	spinit® CRP	http://biosurfit.com
Lithium levels (bipolar disorder)	Few minutes	Fingerprick blood sample	Disposable lab-chip with a meter	Medimate MiniLab	https://www.medimate.com
Prostate Specific Antigen (PSA)	3 minutes	Serum or plasma (30µl)	Disposable cartridge with analyzing instrument	FREND™ PSA plus	http://nanoentek.com
Septicaemia	2-3 hours	Whole blood (10ml)	USB size chip with purpose-designed platform	Genalysis®	http://www.dnae.com/index.html