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Review

Moving past serology: Diagnostic options without serum

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ABSTRACT

Detecting antibodies formed in serum in response to infection is the traditional function of serology. Diagnostic modalities have included complement fixation tests, agar gel immune-diffusion, radioimmunoassay, ELISA and immunofluorescence. More recent technology now allows for the direct detection of pathogens by PCR. This review details the options for diagnostic testing using specimen types other than serum, identifying the advantages and disadvantages of these options and providing evidence for more widespread use of these techniques and specimen types.

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Introduction

A quest for clean serum specimens to detect immunological responses to the presence of important pathogens has characterised the history of disease testing in veterinary medicine. Serum has traditionally been preferred over whole blood to decrease non-specific reactions, and give more accurate and reliable results. Assays such as the complement fixation test, dating from the very beginning of the last century (Bialynicki-Birula, 2008), agar gel immune-diffusion test (Ouchterlony, 1948), radioimmunoassay (Yalow and Berson, 1960) and immunofluorescence antibody testing (Voller, 1964) were some of the first tests used. In more recent years, ELISA (Engvall and Perlmann, 1971; Van Weemen and Schuurs, 1971) in its various permutations (direct, indirect, competitive, sandwich, capture) has been able to detect either antibody or antigen, and is popular because it is simple, inexpensive and rapid.

Newer technologies such as PCR and quantitative PCR (Mullis and Faloona, 1987) are pathogen detection methods and could readily be applied to a variety of a specimen types containing genetic material. Extraction and amplification clean-up steps now make PCR less prone to interference than earlier versions that relied on observation to detect lines or agglutination. Recent further developments in PCR technology, eliminating the need for expensive thermocyclers, have the potential to further revolutionise field diagnostics (Thekisoe et al., 2007).

Specimen types other than serum have commonly been collected and tested in recent years, but difficulties of obtaining a clean and reliable signal to confirm a diagnosis have had to be overcome. Improvements in laboratory science and accessibility to good laboratory services and practices have improved diagnostic efficiency and decreased turnaround times. Additionally, efficient courier services make it practical to transport suitable specimens to a well-equipped laboratory.

In some parts of the world it is still difficult to get good quality specimens reliably to a diagnostic laboratory, either because the necessary transport infrastructure is absent or distances within the country or between countries to the laboratory infrastructure are too great. Similarly, convenience and cost-effectiveness can affect specimen collection, as obtaining a blood specimen is often the domain of veterinary or para-veterinary personnel, adding to the expense of diagnostic testing. Other readily obtainable body fluids, excretions or tissues could be obtained by less skilled personnel or animal owners to save on collection costs. In the human diagnostic field there is currently significant interest in exploring alternative specimen analysis such as dried blood spot testing for the diagnosis of hepatitis C (Coats and Dillon, 2015).

Recently, there has been a move away from centralised laboratory services, to ‘in-house’ or practice-based laboratory services for a variety of clinical disciplines, including serology. The robustness and reliability of the sampling technology has improved and has also started to venture out towards more novel applications, including point-of-care single specimen assays.

This review discusses a number of testing modalities with some examples from the authors’ experience. Since this field is constantly evolving, the examples are not comprehensive, but will hopefully encourage others to explore more novel testing options.

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Milk

Milk can be a suitable medium for animal disease testing as it is generally easy to obtain (often without any specialised equipment), and in dairy cattle it is often available throughout the year. Using milk as a specimen, a wide range of animal diseases can be tested for in individual animals and in pooled specimens from herds. Tests for antibodies against the following pathogens are available: *Brucella abortus* (Nielsen and Gall, 2001), bovine viral diarrhoea virus (BVDV; Lanyon et al., 2014b), enzootic bovine leukosis (EBL) and bovine herpes virus 1 (BoHV1; Reber et al., 2012), *Neospora caninum* (Schares et al., 2004; Hall et al., 2006), liver fluke (*Fasciola hepatica*; Reichel et al., 2005), Johne's disease (*Mycobacterium avium* subspecies *paratuberculosis*; MAP; Collins et al., 2005) and *Ostertagia ostertagi* (Charlier et al., 2005; Forbes et al., 2008) in cattle. Single animal testing can be performed and tank milk from herds of dairy cows is a ready-made pool for testing groups of animals. Tank milk presents a natural pool of animal biological specimens that, with adequate test analytical sensitivity, enables the tester to screen large numbers of animals for the presence or absence of disease. While testing for EBL by antibody ELISA, milk pools rarely exceeded 100–200 cows (Ridge and Galvin, 2005), but PCR testing for BVDV is now routinely performed on pools in excess of 400 because high test analytical sensitivity provides a very cost-effective way of screening large herds and, more broadly, whole dairy industry. Herds of >400 cows are typical for the New Zealand dairy industry (Hill et al., 2010).

Historically, milk testing for pathogens was used to test for *B. abortus* infection using the 'milk ring test' (Fleischhauer, 1955), where a drop of stained *B. abortus* antigen was added to a pooled milk specimen. If antibody to *B. abortus* was present, an antibody–antigen complex was formed, adhering to milk fat globules and rising to the surface of the milk as a coloured ring (Fleischhauer, 1955). False positive results for *B. abortus* antibody can occur in cattle vaccinated against *B. abortus* <4 months prior to testing, or in milk containing colostrum or from cows with mastitis. Infection with other pathogens, such as *Yersinia enterocolitica*, can also cause non-specific positive reactions (Kittelberger et al., 1997). Comparable results are obtainable with the fluorescence polarisation assay (Nielsen and Gall, 2001), and this newer test can be used in the field.

Viral pathogens such as bovine leukaemia virus (causative agent of EBL) and BoHV1 (causative agent of infectious bovine rhinotracheitis; Witte et al., 1989) can also be tested for in milk.

BVDV is widely tested for in milk specimens and testing can be simultaneously performed to detect virus (by PCR) and antibody (by ELISA), thereby providing a means of establishing both the presence and absence of the virus and measuring herd immunity. Antibodies formed against BVDV are excreted into milk and correlate well with serum antibody titres. Pooled testing provides a quantitative ELISA assessment strongly correlated with within-herd prevalence (Lanyon et al., 2014b). High levels of antibody in the tank milk suggest sufficient exposure to virus to reduce the need for vaccination, while low tank milk antibody levels suggest the need for biosecurity measures or vaccination to prevent infection. The ability to monitor the relative changes in pooled antibody levels, and therefore, within-herd prevalence is particularly valuable. A sudden increase may be indicative of a recent incursion of infection, a change that can be difficult and expensive to detect when relying on individual animal testing, and would suggest the need for further investigation, beginning with bulk milk PCR testing.

As a logical follow-on after eradication of BVDV from the national dairy herd in Switzerland, on-going monitoring for continued freedom from BVDV infection is now based on regular bulk milk surveillance in that country (Presi et al., 2011). Surveillance testing for recently emerging animal diseases, such as bluetongue and Schmallenberg viruses, has also been based on bulk milk testing by ELISA (Balmer et al., 2014).

Pooled milk testing has also been successfully applied for EBL detection. Screening of all dairy herds in New Zealand by testing pools of milk from groups of 20 dairy cows using EBL ELISA found no evidence of infection by 2011 (Voges, 2011). While primary testing was on pooled milk specimens, further testing was required for any suspicious or positive results using individual serum antibody ELISA and PCR tests. By contrast, the EBL status of the beef industry in New Zealand remains unknown.

In Switzerland, a bulk tank milk specimen to test for BoHV1 reported significant cost savings by using milk instead of serum to detect antibodies. If the expenditure was identical, testing bulk milk yielded significant increases in test sensitivity (Reber et al., 2012), thus improving diagnostic outcomes.

Diagnostic outcomes have also been improved through the use of *N. caninum* bulk milk ELISA testing to predict the prevalence of infection in dairy herds in Australia (Hall et al., 2006). The stage of lactation affected the accuracy of the comparison between serum and milk (Schares et al., 2004), and milk testing was more sensitive.

Excellent accuracy has also been demonstrated when testing individual milk specimens for antibodies against *F. hepatica*, with very high sensitivity and specificity close to 100%. However, when bulk tank milk specimens were tested there was a decrease in sensitivity, so only dairy herds where the prevalence of *F. hepatica* was >60% could be identified (Reichel et al., 2005).

Serological tests for Johne's disease have low sensitivity but reasonable specificity. Testing of individual milk specimens yielded a sensitivity of 28% (Collins et al., 2005), slightly higher than serum, and sensitivity increased with age of animal tested (Nielsen et al., 2013). PCR can also be used to test for the presence of MAP DNA in milk (Buergelt and Williams, 2004), as can the peptide-mediated magnetic separation-phage (PMS-phage) assay (Foddai et al., 2011). However, advances in PCR testing for MAP in faeces could negate the need to use antibody based tests.

Antibody based tests (ELISA) are available to measure antibodies in bulk tank milk to the abomasal parasite *O. ostertagi* (Forbes et al., 2008). Only an association between ELISA values and milk yield can be made using these test results, rather than confirming true positive nematode infections in the herds, so additional diagnostic testing is required to establish the parasite status of the herd.

In sheep, Q-fever (*Coxiella burnetii*; Klaasen et al., 2014), *Brucella melintensis* (Hamidi et al., 2015) and *Mycoplasma agalactiae* (Poumarat et al., 2012) can be tested for using milk; in goats, milk specimens can be used to test for caprine arthritis and encephalitis (Nagel-Alne et al., 2015). Q-fever outbreaks in humans are associated with *C. burnetii* infection in small milking ruminants in Africa (Klaasen et al., 2014). Shedding of the organism is intermittent, thus infection was not always detected by PCR and serological tests might also be required. In contrast, PCR testing of milk for *B. melintensis* detected was more sensitive than serology in one study (Hamidi et al., 2015). Accurate serological classification of the *M. agalactiae* status of sheep is difficult and PCR testing of milk specimens with two PCRs should be used to confirm the presence of the organism. The resultant PCR results also require cross checking with a dot-immunobinding technique (Poumarat et al., 1991).

Milk testing can be utilised for detection of non-infectious conditions. For example, lateral flow devices to test for progesterone concentrations in milk present opportunities to define the oestrus cycle and pregnancy status of cows (Waldmann and Raud, 2016) and technological modifications may allow for testing to occur during milking (Dobson, 2016).

Colostrum

Colostrum is another medium that can be used for animal disease testing instead of milk. Its availability is restricted to a shorter time period, but provides other testing and diagnostic advantages. Testing

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