livet

livet – scientific white paper

Introduction

Highly contagious diseases such as Equine Influenza or Strangles are a central aspect in the hygiene and health management of horse farms. A fast and accurate diagnosis of the causative pathogens can prevent both further spreading in the herd and potentially high costs for the farm and the horse owner. However, rapid and, above all, reliable diagnostics are currently difficult in acute infections (Cummins et al.).

The standard procedure is to send a sample to be analysed to an external laboratory for the exact determination of the infectious pathogen. This analysis is time-consuming, which is why the faster option of unspecific medication is often preferred. Yet, this can exacerbate the problem of antimicrobial resistance (AMR) which was indicated as one of the major global risks at the World Economic Forum 2013 (WEF).

Infectious diseases in animals - Current diagnostic procedures

The current lack of reliable on-site veterinary diagnostic tests increases this problem for both the individual and the equine population as a whole. In addition, the likelihood of an unspecific therapy failing is increased by AMR (Weese et al.). Rapid diagnosis of infectious diseases is therefore of utmost importance. It ensures adequate treatment of the affected individual and prevents the spread of infection to animals in the same population (van de Sande-Bruinsma et al.).

Diagnosis in a reference lab

Reference laboratories usually use cumbersome analytical equipment that is complex to operate and therefore requires trained and experienced personnel to operate and interpret the data (de Paz et al.). Delays in diagnosis of up to several days and thus in the appropriate treatment of the patient may additionally be caused by factors such as transport or delivery of samples outside of opening hours (such as at weekends). The most widely used laboratory methods for the detection of microorganisms are bacterial cultures, enzyme-linked immunosorbent assay (ELISA; an antibody-based detection method) and polymerase chain reaction (PCR), see Table 1.

Table 1: Comparison of standard methods used in centralized laboratories for the detection of pathogens

Method	Time	Resources	Costs	Sensitivity
Bacterial culture	Several days to weeks	Trained and experienced technicians	Medium	+/++
ELISA (antigens/antibodies)	Hours	Trained and experienced technicians	Low/medium	+/++
PCR (real-time, end-point)	Hours to a full day	Trained and experienced technicians, special equipment	high	+++

livet ag
Freiburgstrasse 251, 3018 Bern, Switzerland
+41 (0) 31 552 07 17, info@li.vet, www.li.vet

Available point-of-care diagnostics

'Dipstick tests' (lateral flow immunoassays) are currently the most widely used tests for on-site diagnosis of infectious diseases in veterinary medicine (Busin et al.). These tests are often used in small animal clinics and include tests for Borreliosis, Ehrlichiosis, Anaplasmosis or Canine parvovirus. However, for the diagnosis of acute infectious diseases like respiratory infections, lateral flow assays suffer from poor sensitivity due to the initial absence of specific antibodies from the host (Figure 1).

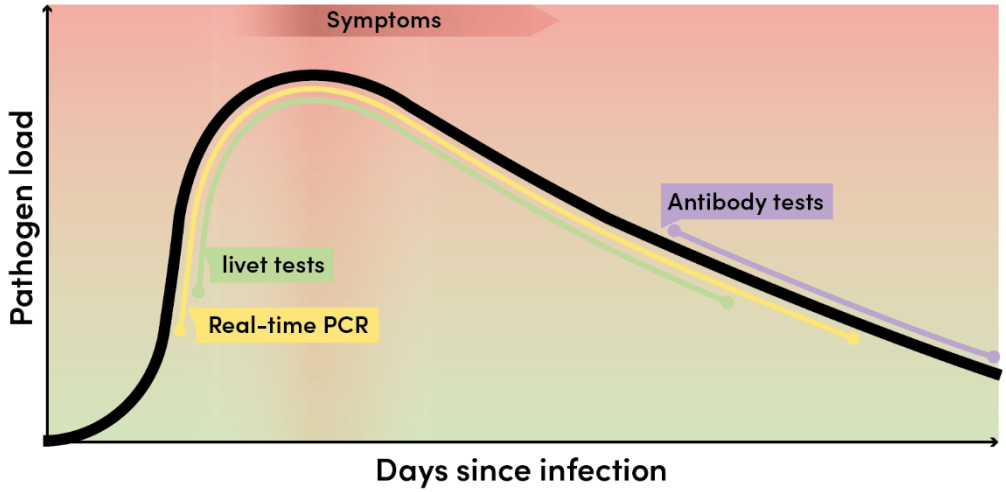

Figure 1: Progression of pathogen load in an infection and respective diagnostic methods over time

Our solution

The founding team of livet can look back on more than 16 years of experience in the development and implementation of microbiological diagnostics in the field of human medicine (Institute for Infectious Diseases, IFIK, University of Bern, Switzerland). With our knowledge, we developed a novel isothermal amplification method for real-time detection.

Similar to traditional PCR, this isothermal reaction amplifies a characteristic nucleic acid sequence with the help of enzymes and specific primers. Some examples of isothermal amplification technologies include LAMP (loop-mediated isothermal amplification; Notomi et al.), RPA (recombinase polymerase amplification; Piepenburg et al.) or SDA (strand displacement amplification; Walker et al.).

One of the many challenges of isothermal amplification is the low specificity, which can result in non-specific primer binding, and the resulting high complexity of primer design. Thanks to our experience in isothermal amplification methods, we were able to solve these problems. To ensure the absence of inhibitors or pipetting errors, an internal process control is built into our assays as standard. This is amplified in the absence of the target sequences. The enzyme used has helicase activity, hence the denaturation step at 95 °C is needed. Thus, the reaction takes place at 65°C, resulting in a simpler device the assay is run on.

The livet portfolio currently includes tests for Streptococcus equi equi, Equine Herpesvirus types 1 and 4 (EHV-1 and EHV-4) and Equine Influenza Virus (EIV). Tests for other pathogens will follow.

livet ag
Freiburgstrasse 251, 3018 Bern, Switzerland
+41 (0) 31 552 07 17, info@li.vet, www.li.vet

livet

Conclusion

Today, we are probably more than ever aware of the need for rapid and reliable diagnostics for infectious diseases. Rapid diagnoses lead to immediate and precisely tailored therapies and can thus help in protection against epidemics. With rapid and effective diagnostics, the release of antibiotics in large and preventive quantities can be mitigated and targeted therapeutics can be administered. This has the positive side effect of limiting resistance development.

Up until now, veterinary diagnostics of infectious diseases has been characterised by long waiting times due to time-consuming laboratory diagnostics or unreliable antibody-based tests. livet offers a fast and highly reliable method for on-site diagnosis of infectious diseases in animals.

References

Cummins BM, Ligler FS, Walker GM. Point-of-care diagnostics for niche applications. Biotechnol Adv. 2016 May-Jun;34(3):161-76. doi: 10.1016/j.biotechadv.2016.01.005

WEF Global Risks 2013 – Eight Edition, http://www3.weforum.org/docs/WEF_GlobalRisks_Report_2013.pdf

Weese J., Antimicrobial resistance in companion animals, Anim Health Res Rev. 2008 Dec;9(2):169- 76. doi: 10.1017/S1466252308001485.

van de Sande-**Bruinsma** N, Grundmann H, Verloo D, Tiemersma E, Monen J, Goossens H, Ferech M; European Antimicrobial Resistance Surveillance System Group; European Surveillance of Antimicrobial Consumption Project Group. Antimicrobial drug use and resistance in Europe, Emerg Infect Dis. 2008 Nov;14(11):1722-30. doi: 10.3201/eid1411.070467

Busin V, Wells B, Kersaudy-Kerhoas M, Shu W, Burgess ST, Opportunities and challenges for the application of microfluidic technologies in point-of-care veterinary diagnostics, Mol Cell Probes. 2016 Oct;30(5):331-341. doi: 10.1016/j.mcp.2016.07.004

de Paz HD, Brotons P, Muñoz-Almagro C., Molecular isothermal techniques for combating infectious diseases: towards low-cost point-of-care diagnostics, Expert Rev Mol Diagn. 2014 Sep;14(7):827- 43. doi: 10.1586/14737159.2014.940319.

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T, Loop-mediated isothermal amplification reaction using a nondenatured template, Nucleic Acids Res. 2000 Jun 15;28(12):E63.

Piepenburg O, Williams CH, Stemple DL, Armes NA (2006) DNA Detection Using Recombination Proteins. PLoS Biol 4(7): e204. https://doi.org/10.1371/journal.pbio.0040204

Walker GT, Fraiser MS, Schram JL, Little MC, Nadeau JG, Malinowski DP. Strand displacement amplification--an isothermal, in vitro DNA amplification technique. *Nucleic Acids Res*. 1992;20(7):1691-1696. doi:10.1093/nar/20.7.1691

livet ag
Freiburgstrasse 251, 3018 Bern, Switzerland
+41 (0) 31 552 07 17, info@li.vet, www.li.vet