

# Survey of the Large-Animal Diplomates of the American College of Veterinary Internal Medicine Regarding Knowledge and Clinical Use of Polymerase Chain Reaction: Implications for Veterinary Education

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## ABSTRACT

A questionnaire was developed to document the knowledge base of large-animal diplomates of the American College of Veterinary Internal Medicine (ACVIM) regarding polymerase chain reaction (PCR) technology and to identify the common use of this technology in equine practice. Ninety-three of the 278 mailed questionnaires were returned, for an overall response rate of 33.4%. Ninety respondents (99%) reported being familiar with the general principles of nucleic acid probe technology; however, only 52 (57%) knew the difference between conventional (traditional) and real-time (second-generation) PCR. The majority of the respondents (88%) emphasized the need for continuing education on molecular diagnostics. Eighty-four (92%) of the respondents regularly use PCR (conventional and/or real-time) for the detection of equine pathogens, and 80 (88%) commonly submit their samples to university/state veterinary laboratories. Blood, nasal swabs, and feces are the three equine specimens most commonly submitted for PCR analysis of *Streptococcus equi*, *Lawsonia intracellularis*, *Neorickettsia risticii*, equine herpesvirus 1/4, *Rhodococcus equi*, *Sarcocystis neurona*, and equine influenza virus. Diplomates reported costs associated with molecular diagnostics and unreliability of PCR as the most common limitations of PCR. Didactic training in veterinary curricula and during continuing-education opportunities continues to be necessary to produce veterinarians who have an understanding of the clinical applications of molecular diagnostics.

## INTRODUCTION

The development of polymerase chain reaction (PCR) technology in the late 1980s dramatically changed not only many aspects of research in molecular biology but also the diagnosis of infectious diseases in humans and animals.<sup>1</sup> PCR has become the most commonly used method of detecting nucleic acids (DNA and RNA) for diagnostic purposes. It is a nucleic acid–based amplification technique that has been described as “genetic photocopying,” a highly sensitive procedure for detecting infectious pathogens in host tissues even when only a small number of host cells is infected. PCR has distinct advantages, as a diagnostic tool, over conventional microbiology, especially in the detection of slow-growing, difficult-to-cultivate, or uncultivable microorganisms. PCR is best used in clinical situations for which conventional clinical microbiology diagnostic procedures are inadequate, time consuming, difficult, expensive, or hazardous to laboratory staff.<sup>2</sup> PCR can also be used successfully in situations in which inhibitory substances such as anti-microbials are present. Thanks to the stability of DNA, pathogen detection can be performed successfully on a variety of sample types and even on formalin-fixed tissue. PCR also has inherent disadvantages, which are often related to the high sensitivity of the assay. False positive signals can be generated as PCR product carry-over when PCR reaction tubes are opened to run gel electrophoresis or with the inappropriate use of positive controls, the handling

of plasmids (which may carry the target sequence of a PCR assay), or the contamination of samples during the nucleic acid purification process. Diagnostic PCR laboratories often address these issues by using separate rooms for reagent preparation, specimen processing, and amplification and detection, as well as by using automated and enclosed extraction systems and closed-tube PCR systems. New PCR platforms, such as the real-time PCR, were introduced in the mid-1990s and are slowly replacing the cumbersome conventional or traditional PCR assays.<sup>3</sup> Real-time PCR systems rely on the detection and quantitation of a fluorescent reporter released during PCR from an internal, fluorescently labeled probe, the signal of which increases in direct proportion to the amount of PCR product in a reaction.<sup>4</sup> These fluorescent signals are measured quantitatively through the tube, which therefore does not need to be opened. Advantages of closed-tube real-time PCR detection are significant for the diagnostic applications and include (1) quantitative quality control of the input target DNA; (2) elimination of post-amplification steps by means of closed-tube detection, resulting in the virtual absence of PCR product carry-over and false positive PCR product generation; (3) fast and efficient real-time PCR on 96- or 384-well plates, leading to short turnaround time; (4) standardized PCR protocols with uniform amplification specifications, leading to high reproducibility among diagnostic laboratories; and (5) the availability of stable and

quality-controlled PCR reagents, which contributes to high reproducibility and reliability.

Despite the fact that molecular diagnostics are routinely offered by university, state, and private veterinary laboratories, the veterinary literature is short on articles reviewing the use of PCR technology and its clinical applications as a molecular diagnostic tool.<sup>3,5,6</sup> Veterinarians often rely on continuing education offered at local or national meetings to improve their knowledge of molecular diagnostics. As more and more equine practitioners are using PCR to diagnose infectious diseases, it is important for them to have some background in this technology in order to properly interpret results. The objective of the study described here was to document the knowledge base of large-animal diplomates of the American College of Veterinary Internal Medicine (ACVIM) regarding PCR technology and to identify the common uses of this technology in equine practice. Based on this information, we make some suggestions for the improvement of education in this area.

## MATERIALS AND METHODS

A questionnaire was designed to appraise individual knowledge about PCR (familiarity, difference between conventional and real-time PCR, need for education), individual experience, sample type and assay commonly requested, and limitations associated with PCR (see Appendix). Further, veterinarians were asked about their practice situation (i.e., working in field practice, at a referral hospital, or in academia). The questionnaire was mailed to 278 large-animal internal medicine diplomates listed in the directory of the ACVIM. We restricted the survey to veterinarians practicing veterinary medicine in the United States. It should be noted that the directory is not divided into equine and food-animal specialties. The questionnaires were mailed on June 20, 2005, and the surveys were returned in a pre-paid, self-addressed envelope between June 28, 2005, and September 27, 2005. The survey responses were tabulated and analyzed using Microsoft Excel.<sup>a</sup> Categorical data were evaluated by means of the chi-square ( $\chi^2$ ) test.

## RESULTS

Ninety-three of the 278 questionnaires mailed were returned, for an overall response rate of 33.4%. Fifty (53.8%) respondents practiced in academia, 25 (26.9%) at a referral equine hospital, and 16 (17.2%) in a field practice setting, while 2 (2.1%) were not working as practitioners at the time of the survey. The two non-practicing veterinarians were excluded from the study, leaving 91 questionnaires to be evaluated.

Ninety respondents (99%) reported being familiar with the PCR technology; the only person not familiar with PCR was working in academia. Fifty-two of the diplomates (57%) knew the difference between conventional PCR and real-time PCR, while 39 (43%) did not. Most of the diplomates (78%) working in academia were aware of this technological difference, in comparison to veterinarians practicing in a referral hospital (40%;  $\chi^2=10.6$ ,  $p<0.01$ ) or in a field practice (19%;  $\chi^2=18.4$ ,  $p<0.001$ ; see Table 1). No statistical difference was noted between diplomates working in a

referral hospital and those working in a field practice ( $\chi^2=2.0$ ,  $p<0.2$ ). When asked if enough information regarding PCR was available to them via journals or textbooks, 16 (18%) answered "yes" and 75 (82%) answered "no"; there was no statistical difference between the groups regarding availability of information on PCR. Eighty diplomates (88%) felt that education on molecular diagnostics was necessary, while 11 (12%) did not. The need for continuing education on PCR was expressed in all occupational groups. Two diplomates (2%) did not use PCR as a molecular diagnostic modality, while 89 (98%) were either occasional (5, 6%) or regular (84, 92%) users. The percentage of occasional and regular PCR users was not statistically different between the three occupational groups ( $p>0.05$ ). According to the 89 occasional and regular PCR users, molecular analyses were commonly performed at university/veterinary state laboratories (88%) and commercial veterinary laboratories (47%). Only two diplomates reported using commercial human laboratories for PCR analysis.

The types of sample specimen commonly submitted by diplomates for PCR analysis were blood (77%) and feces (77%), followed by nasal swabs (62%) and others (42%; see Table 2). Other type of specimens submitted included cerebrospinal fluid (12), transtracheal wash/bronchoalveolar lavage fluid (11), organ tissue (7), guttural pouch lavage fluid (5), urine (1), purulent aspirate (1), and culture isolate (1). No statistical differences ( $p>0.05$ ) were found on the type of specimen submitted between the three occupational groups. Commonly requested diagnostics were, in descending order, *Streptococcus equi* (63%), *Lawsonia intracellularis* (44%), *Neorickettsia risticii* (40%), equine herpesvirus 1/4 (37%), *Rhodococcus equi* (29%), *Sarcocystis neurona* (29%), and equine influenza virus (14%). When mentioned on the survey, other pathogens detected by PCR included equine viral arteritis, *Anaplasma (Ehrlichia) phagocytophilum*, *Corynebacterium pseudotuberculosis*, and *Salmonella* sp. No statistical differences ( $p>0.05$ ) were found on the common pathogen tested between the three occupational groups.

Respondents reported costs associated with molecular diagnostics as the most common limitation (33%) of PCR. Unreliability of PCR as a result of either false negative or false positive results was reported by 25% and 31% of respondents, respectively. Twenty-four respondents (11%) did not indicate any limitation of PCR, while seven mentioned the lack of reported validation data (sensitivity, specificity, predictive values) as a limitation. No statistical differences ( $p>0.05$ ) were found on PCR limitations between the three occupational groups.

## DISCUSSION

Molecular biological methods such as PCR have become increasingly applicable to the diagnosis of infectious diseases. To become widely used, these methods need to be standardized, safe, sensitive, and reproducible. Further, knowing the principles of diagnostic procedures is important for choosing the most suitable molecular diagnostic assay for a particular case. In an attempt to investigate the need for education in the field of applied molecular diagnostics for infectious equine diseases, we surveyed large-animal diplomates of the ACVIM. ACVIM diplomates play a key role in the education of equine general

**Table 1: Responses to a survey on PCR by large-animal diplomates of the ACVIM**

	<b>Knowledge of PCR*</b>	<b>Conventional vs. Real-Time PCR</b>	<b>Sufficient Information Available on PCR</b>	<b>Need for Education in PCR</b>	<b>Use of PCR</b>	<b>Diagnostic Laboratory***</b>
Academia** (N= 50, 55%)	49 yes (98%) 1 no (2%)	39 yes (78%) 11 no (22)	9 yes (18%) 41 no (82%)	45 yes (90%) 5 no (10%)	1 occasional (2%) 49 regular (98%)	25 commercial veterinary (50%) 1 commercial human (2%) 48 university/state (96%)
Referral hospital** (N= 25, 27%)	25 yes (100%)	10 yes (40%) 15 no (60%)	4 yes (16%) 21 no (84%)	21 yes (84%) 4 no (16%)	2 occasional (8%) 23 regular (92%)	15 commercial veterinary (60%) 18 university/state (72%)
Field Practice** (N= 16, 18%)	16 yes (100%)	3 yes (19%) 13 no (81%)	3 yes (19%) 13 no (81%)	14 yes (88%) 2 no (12%)	2 no (12.5%) 2 occasional (12.5%) 12 regular (75%)	3 commercial veterinary (19%) 1 commercial human (6%) 14 university/state (88%)
Total (N=91)	90 yes (99%) 1 no (1%)	52 yes (57%) 39 no (43%)	16 yes (18%) 75 no (82%)	80 yes (88%) 11 no (12%)	2 no (2%) 5 occasional (6%) 84 regular (92%)	43 commercial veterinary (47%) 2 commercial human (2%) 80 university/state (88%)

\*Refers to the specific questions asked in the questionnaire (see Appendix).

\*\*Refers to the working situation of the diplomates.

\*\*\*Refers to the laboratory where samples are submitted: commercial veterinary, commercial human, university/state veterinary laboratory.

practitioners and are often consulted by them on particular cases or for interpretation of specific diagnostics. Further, active diplomates have many opportunities to gain the necessary education, either through their specialty training at an academic institution or through continuing education or specialized literature.

A number of limitations exist in the data compiled from survey responses. In this study, not all large-animal diplomates of ACVIM responded; the response rate was 33.4%, similar to that for other horse-related surveys.<sup>7-10</sup> Inaccuracy in this study may be due to the fact that diplomates more familiar with PCR are more likely to fill out the questionnaire. In order to prevent this possible response bias, we grouped diplomates based on their working situation (i.e., academia, referral hospital, or field practice). We believe that diplomates working in academia have greater opportunities for exposure to new technologies through their own work, research, or educational seminars. The majority of the respondents worked either in academia (55%) or at a referral hospital (27%), while only 18% worked in a field practice setting. This distribution is to be expected for a large animal-specialty core. However, the lack of information from the 185 non-respondents prevents us from

drawing any correspondence between the working situations of the respondents and the overall ACVIM directory of large-animal diplomates.

With the exception of a single respondent, all the diplomates surveyed reported some familiarity with PCR technology. However, almost half were unfamiliar with the difference between conventional and real-time PCR. Real-time PCR is based on the same principle as conventional PCR, but the detection of PCR products relies on the detection of a fluorescent signal and not on the use of gel electrophoresis and DNA staining, as in conventional PCR. The principles of the two PCR assays can be reviewed by means of a computer-aided learning application online at Johns Hopkins University <[http://pathology2.jhu.edu/molec/techniques\\_main.cfm](http://pathology2.jhu.edu/molec/techniques_main.cfm)> or in recently published veterinary review articles.<sup>5,6,11</sup> Real-time PCR represents today's platform for routine molecular diagnostics and will most likely come to replace all conventional assays. More important than understanding the technology is being aware of the advantages of real-time PCR over conventional PCR, including quantitative quality control of the input-target DNA, elimination of post-amplification steps (i.e., absence of PCR product carry-over and false positive PCR

**Table 2: Responses to a survey on PCR by large-animal diplomates of the ACVIM**

	Samples Used for PCR*	Common Pathogens Tested	PCR Limitations
Academia** (N=50, 55%)	42 blood (84%)	29 <i>Streptococcus equi</i> (58%)	18 costs (36%)
	34 nasal swabs (68%)	21 equine herpesvirus 1 and/or 4 (42%)	9 turnaround time (18%)
	40 feces (80%)	7 equine influenza virus (14%)	10 false negative results (20%)
	26 others*** (52%)	14 <i>Rhodococcus equi</i> (28%)	14 false positive results (28%)
		28 <i>Neorickettsia risticii</i> (56%)	
		28 <i>Lawsonia intracellularis</i> (56%)	
		15 <i>Sarcocystis neurona</i> (30%)	
		4 others (8%)	
Referral hospital** (N=25, 27%)	17 blood (68%)	17 <i>Streptococcus equi</i> (68%)	8 costs (32%)
	12 nasal swabs (48%)	7 equine herpesvirus 1 and/or 4 (28%)	4 turnaround time (12%)
	19 feces (76%)	3 equine influenza virus (12%)	8 false negative results (32%)
	11 others*** (44%)	10 <i>Rhodococcus equi</i> (40%)	9 false positive results (36%)
		6 <i>Neorickettsia risticii</i> (24%)	
		10 <i>Lawsonia intracellularis</i> (40%)	
		8 <i>Sarcocystis neurona</i> (32%)	
		8 others (32%)	
Field Practice** (N=16, 18%)	11 blood (69%)	11 <i>Streptococcus equi</i> (69%)	4 costs (25%)
	10 nasal swabs (63%)	6 equine herpesvirus 1 and/or 4 (38%)	3 turnaround time (19%)
	11 feces (69%)	3 equine influenza virus (19%)	5 false negative results (31%)
	1 other*** (6%)	2 <i>Rhodococcus equi</i> (13%)	5 false positive results (31%)
		2 <i>Neorickettsia risticii</i> (13%)	
		2 <i>Lawsonia intracellularis</i> (13%)	
		3 <i>Sarcocystis neurona</i> (19%)	
		4 others (25%)	
Total (N=91)	70 blood (77%)	57 <i>Streptococcus equi</i> (63%)	30 costs (33%)
	56 nasal swabs (62%)	34 equine herpesvirus 1 and/or 4 (37%)	16 turnaround time (18%)
	70 feces (77%)	13 equine influenza virus (14%)	23 false negative results (25%)
	38 others*** (42%)	26 <i>Rhodococcus equi</i> (29%)	28 false positive results (31%)
		36 <i>Neorickettsia risticii</i> (40%)	
		40 <i>Lawsonia intracellularis</i> (44%)	
		26 <i>Sarcocystis neurona</i> (29%)	
		16 others (18%)	

\*Refers to the specific questions asked in the questionnaire (see Appendix).

\*\*Refers to the working situation of the diplomates.

\*\*\*Includes cerebrospinal fluid, transtracheal wash/bronchoalveolar lavage fluid, organ tissue, guttural pouch lavage, urine, purulent aspirate, and culture isolate.

product generation), short turnaround time, standardized PCR protocols, and high reproducibility and reliability.<sup>3</sup> It is not surprising that almost 80% of diplomates working in academia were familiar with real-time PCR, since this technology is rapidly emerging in the research setting. Although general information on PCR is available in

specialized journals and textbooks focusing on molecular technology or diagnostics, this information is not routinely accessible to practicing veterinarians. The majority of survey respondents emphasized the need for information (82%) and continuing education (88%) on molecular diagnostics. This topic, with associated clinical applications, should be

addressed more often in both veterinary journals and clinical textbooks.

Ninety-two percent of the respondents regularly used PCR for the detection of equine pathogens, and 88% commonly submitted their samples to university/state veterinary laboratories. Many academic and state veterinary institutions are involved in the establishment and validation of PCR assays for specific pathogens. They often act as reference laboratories, which explains why they are commonly used by diplomates for molecular analyses. Further, commercial laboratories offering molecular diagnostics are rare, and they routinely offer detection services for only a small selection of equine pathogens, such as *S. equi* and West Nile virus. Blood, nasal swabs, and feces were the three most commonly submitted equine specimens for PCR analysis of *S. equi*, *L. intracellularis*, *N. risticii*, equine herpesvirus 1/4, *R. equi*, *S. neurona*, and equine influenza virus. The fact that blood samples are commonly used for molecular diagnostics is surprising, given that the majority of respiratory, enteric, and neurologic pathogens are not detectable in peripheral blood.<sup>6</sup> Submission of the wrong sample type is, in our opinion, one of the most common mistakes leading to false negative results. The clinician should be aware of the pathogenesis of each organism and select samples appropriate for its detection. For example, nasopharyngeal swabs are very reliable for detecting viral (equine herpesvirus 1/4, equine influenza virus) and bacterial (*S. equi*) pathogens invading the upper respiratory tract, while pulmonary fluid samples (transtracheal, bronchoalveolar) are the sample of choice for pathogens invading the lower respiratory tract (*R. equi*, equine herpesvirus 1/4, equine influenza virus). Feces are the sample of choice to detect *L. intracellularis* and *N. risticii*; however, the last can also be detected in peripheral blood. Although highly sensitive real-time PCR methods have been developed to detect viral and protozoal genomes in the cerebrospinal fluid (CSF) of neurological patients, these methods are often of limited value in the routine diagnosis of these diseases, because viremia can be very short-lived or because the pathogen has no affinity to the cells of the CSF. Consequently, pathogens are usually no longer detectable at the onset of systemic or central nervous system signs. In contrast, PCR testing of neural tissue has been shown to be useful for post-mortem diagnosis. There are no limits as to what sample type can be submitted for molecular detection; however, the clinician should understand the unknown reliability of such testing when the sample type has not been validated. When in doubt, veterinarians should consult with a diagnostician at a molecular diagnostic laboratory.

Respondents reported costs associated with molecular diagnostics as the most common limitation of PCR. Although molecular diagnostic tests have traditionally been two to three times as costly as cultural or serologic assays, the ease with which some molecular tests can now be performed and the rapid results generated by these methods can lead to more timely diagnosis and thus translate into overall savings. For example, a rapid PCR test method may replace labor-intensive cell-culture methods previously used to detect viruses. Cost savings may be realized where rapid diagnosis obviates the need for other diagnostic procedures, limits unnecessary empirical

antimicrobial therapy, and shortens hospital stays in expensive isolation facilities. Earlier detection of infectious agents may also limit the spread of contagious pathogens to healthy horses and increase the overall quality of the veterinary service.

Respondents also expressed concerns about the unreliability of PCR because the procedure may generate either false positive or false negative results. One challenge in using nucleic-acid testing methods is avoiding false-positive tests due to amplicon or nucleic acid contamination of control and test specimens. Such results are generally avoided by means of strict workflow practices and the use of fully automated nucleic extraction workstations and closed-tube systems. The equine practitioner, at the point of specimen collection, must follow meticulous quality-control measures as well. On the other side, false negative results may occur when the wrong sample is submitted, the specimen is of poor quality, or substances are present that inhibit nucleic acid amplification. For PCR to gain better acceptance in the veterinary community, the quality and partial standardization of molecular testing needs to be improved, including laboratory requirements for certification and proficiency surveys evaluated by an objective third party. In veterinary medicine, the American Association of Veterinary Laboratory Diagnosticians (AAVLD) seeks to establish uniform diagnostic techniques and accepted guidelines for the improvement of diagnostic laboratory standards. Standardization is necessary to allow comparison among laboratories. The standard should include a definition of the sample type to be analyzed, sample preparation and PCR amplification, and reporting of quantitative results. For molecular microbiology testing, validation data should be available to detail both analytical and diagnostic specificity and sensitivity for every assay offered. This information is often only available for assays that have been published in peer-reviewed journals.

Real-time PCR represents today's platform for routine molecular diagnostics and will most likely replace all conventional assays in the future. Veterinarians should be aware of the advantages of real-time PCR over conventional PCR. Educating veterinarians about currently available molecular diagnostic tests such as real-time PCR is especially important in anticipation of the introduction of new pathogen-detection assays. The addition of more specific didactic training about molecular diagnostics, both in veterinary schools and in continuing-education courses, may help to alleviate some of the deficiencies and ensure that tomorrow's molecular diagnostics are used appropriately in practice.

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#### NOTE

- a Microsoft Corp., Redmond, WA  
<[www.microsoft.com](http://www.microsoft.com)>.

## REFERENCES

- 1 Mullis KB, Faloona F. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol* 155:335–344, 1987.
- 2 Wolk D, Mitchell S, Patel R. Principles of molecular microbiology testing methods. *Infect Dis Clin N Am* 15:1157–1204, 2001.
- 3 Lutz H, Leutenegger C, Hofmann-Lehmann R. The role of polymerase chain reaction and its newer developments in feline medicine. *J Feline Med Surg* 1:89–100, 1999.
- 4 Wilhelm J, Pingoud A. Real-time polymerase chain reaction. *ChemBioChem* 4:1120–1128, 2003.
- 5 Webster K. Demystifying diagnostic testing: non-culture techniques for large animal infectious disease. *In Practice* 26:325–330, 2004.
- 6 Pusterla N, Madigan JE, Leutenegger CM. Real-time PCR: a novel molecular diagnostic tool for equine infectious diseases. *J Vet Intern Med* 20:3–12, 2006.
- 7 Traub-Dargatz JL, Salman MD, Voss JL. Medical problems of adult horses, as ranked by equine practitioners. *J Am Vet Med Assoc* 198:1745–177, 1991.
- 8 Savage CJ, Traub-Dargatz JL, Mumford EL. Survey of the large animal diplomates of the American College of Veterinary Internal Medicine regarding percutaneous lung biopsies in the horse. *J Vet Intern Med* 12:456–464, 1998.
- 9 Shuster R, Traub-Dargatz J, Baxter G. Survey of diplomates of the American College of Veterinary Internal Medicine and the American College of Veterinary Surgeons regarding clinical aspects and treatment of endotoxemia in horses. *J Am Vet Med Assoc* 210:87–92, 1997.
- 10 Southwood LL, Baxter GM, Hutchison JM, Shuster R. Survey of diplomates of the American College of Veterinary Surgeons regarding postoperative intra-abdominal adhesion formation in horses undergoing abdominal surgery. *J Am Vet Med Assoc* 211:1573–1576, 1997.
- 11 Yeary TJ, Kapil S. A primer on diagnostic virology: direct and molecular-based detection of viral pathogens. *Comp Cont Educ Pract* 26:730–739, 2004.

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## APPENDIX: PCR SURVEY

What is your clinical working situation?

1. Field Practice
2. Referral equine hospital
3. Academia
4. Other

Are you familiar with the diagnostic assay PCR (polymerase chain reaction)?

1. Yes
2. No

Do you know the difference between conventional and real-time PCR?

1. Yes
2. No

Is enough information available to the equine practitioner regarding PCR?

1. Yes
2. No

In your opinion is there a need to educate veterinarians in the field of molecular diagnostics?

1. Yes
2. No

What is your experience with PCR?

1. I do not use it
2. I use it occasionally
3. I am a regular user

Where do you send your samples for PCR diagnostic?

1. Commercial veterinary laboratory
2. Commercial human laboratory
3. University/State laboratory

What sample types do you commonly use for testing?

1. Blood
2. Nasopharyngeal swab
3. Feces
4. Others, such as . . .

Please list pathogen(s) for which you commonly use PCR as a diagnostic tool.

1. *Streptococcus equi* (strangles)
2. Equine herpesvirus 1 and/or 4
3. Equine influenza virus
4. *Rhodococcus equi*

5. *Neorickettsia (Ehrlichia) risticii*

6. *Lawsonia intracellularis*

7. *Sarcocystis neurona*

8. Others such as . . .

What are the limitations of PCR in your experience?

1. Costs of assay

2. Turnaround time

3. Unreliable due to false negative results

4. Unreliable due to false positive results

5. Others such as . . .