



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Interlaboratory comparison of Porcine circovirus-2 indirect immunofluorescent antibody test and enzyme-linked immunosorbent assay results on experimentally infected pigs

Citation for published version:

Patterson, AR, Johnson, JK, Ramamoorthy, S, Opriessnig, T, Hesse, RA, Murtaugh, MP, Puvanendiran, S, Pogradichniy, RM, Erickson, GA, Carman, S, Hause, B & Meng, X-J 2011, 'Interlaboratory comparison of Porcine circovirus-2 indirect immunofluorescent antibody test and enzyme-linked immunosorbent assay results on experimentally infected pigs' *Journal of Veterinary Diagnostic Investigation*, vol. 23, no. 2, pp. 206-212. DOI: 10.1177/104063871102300203

Digital Object Identifier (DOI):

[10.1177/104063871102300203](https://doi.org/10.1177/104063871102300203)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Journal of Veterinary Diagnostic Investigation

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Journal of Veterinary Diagnostic Investigation

<http://vdi.sagepub.com/>

Interlaboratory Comparison of *Porcine Circovirus-2* Indirect Immunofluorescent Antibody Test and Enzyme-Linked Immunosorbent Assay Results on Experimentally Infected Pigs

Abby R. Patterson, John K. Johnson, Sheela Ramamoorthy, Richard A. Hesse, Michael P. Murtaugh, Sumathy Puvanendiran, Roman M. Pogradichny, Gene A. Erickson, Susy Carman, Ben Hause, Xiang-Jin Meng and Tanja Opriessnig

J VET Diagn Invest 2011 23: 206

DOI: 10.1177/104063871102300203

The online version of this article can be found at:

<http://vdi.sagepub.com/content/23/2/206>

Published by:



<http://www.sagepublications.com>

On behalf of:



Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc.

Additional services and information for *Journal of Veterinary Diagnostic Investigation* can be found at:

Email Alerts: <http://vdi.sagepub.com/cgi/alerts>

Subscriptions: <http://vdi.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.com/journalsPermissions.nav>

>> [Version of Record](#) - Mar 1, 2011

[What is This?](#)

Interlaboratory comparison of *Porcine circovirus-2* indirect immunofluorescent antibody test and enzyme-linked immunosorbent assay results on experimentally infected pigs

Abby R. Patterson, John K. Johnson, Sheela Ramamoorthy, Richard A. Hesse, Michael P. Murtaugh, Sumathy Puvanendiran, Roman M. Pogradichniy, Gene A. Erickson, Susy Carman, Ben Hause, Xiang-Jin Meng, Tanja Opriessnig¹

Abstract. A blinded interlaboratory assessment of the diagnostic agreement and accuracy of serologic tests for routine detection of antibodies against *Porcine circovirus-2* (PCV-2), including indirect fluorescent antibody tests (IFATs) and enzyme-linked immunosorbent assays (ELISAs) was conducted in 7 North American laboratories. Serum samples were collected weekly, on trial days 0, 7, 14, 21, 28, 35, 42, and 49, from the following groups of animals: 1) negative controls ($n = 7$), 2) PCV-2a ($n = 8$), 3) PCV-2b ($n = 8$), 4) PCV-1 ($n = 8$), 5) PCV-2 vaccine A ($n = 8$; Ingelvac[®] CircoFLEX[™]), 6) PCV-2 vaccine B ($n = 8$; Circumvent[®] PCV2), and 7) PCV-2 vaccine C ($n = 8$; Suvaxyn[®] PCV2 One Dose). Results from each laboratory were analyzed by kappa and receiver operating characteristic (ROC) analysis. Kappa analysis indicated that, by trial day 49, IFATs had almost perfect agreement, in-house ELISAs had fair to almost perfect agreement, and commercially available anti-PCV-2 immunoglobulin G ELISAs (I or S) had moderate to substantial agreement. From trial days 14–49, the area under the ROC curve for the 2 laboratories that offered IFATs, the 4 laboratories that offered in-house ELISAs, and the 3 laboratories that used commercially available ELISAs ranged from 0.94 to 1.00, 0.72 to 1.00, and 0.95 to 1.00, respectively. However, test sensitivities varied based on laboratory-specific cutoffs that were used to dichotomize test results.

Key words: Enzyme-linked immunosorbent assay; indirect fluorescent antibody test; laboratory comparison; pigs; *Porcine circovirus-2*; serology.

Introduction

Porcine circovirus-2 (PCV-2; family *Circoviridae*; genus *Circovirus*) is a small, nonenveloped, single-stranded circular DNA virus.¹⁹ Since the first reports of PCV-2-associated disease in 1996,⁶ PCV-2 has been related to a number of disease manifestations in

swine, including enteritis, respiratory disease, systemic infections, porcine dermatitis and nephropathy syndrome, and reproductive problems.¹³

Several vaccines were introduced into the United States market in 2006^{1–3} and have proven to be highly effective.⁷ Whereas, most herds are seropositive for PCV-2 either because of natural infection⁹ or vaccination, commercial serologic assays, including the indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA), are widely used. Common uses include determination of the PCV-2 serologic status of herds (specific pathogen-free herds and research studies), cross-sectional serologic surveys to determine the prevalence and levels of anti-PCV-2 antibodies for optimal timing of vaccination, and assessing vaccination compliance. Although the IFAT and ELISA have advantages over standard cultivation methods, including rapid detection of antibodies in a wide range of samples,²¹ the IFAT is not automated and has a subjective end point, whereas the ELISA is automated and has an objective end point.

In a previous study, 20 serum samples taken from both experimentally and naturally infected pigs were analyzed by 5 different laboratories by using IFAT

From the Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA (Patterson, Johnson, Ramamoorthy, Opriessnig), the Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS (Hesse), the Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN (Murtaugh, Puvanendiran), the School of Veterinary Medicine, Department of Comparative Pathobiology, Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN (Pogradichniy), the North Carolina Veterinary Diagnostic Laboratory, Raleigh, NC (Erickson), the Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada (Carman), Newport Laboratories, Worthington, MN (Hause), and the Department of Biomedical Sciences and Pathobiology, Center of Molecular Medicine and Infectious Diseases, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA (Meng).

¹Corresponding Author: Tanja Opriessnig, Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011. tanjaopr@iastate.edu

Table 1. Summary of enzyme-linked immunosorbent assay (ELISA) protocols and indirect fluorescent antibody tests (IFAT) used by each of the 7 diagnostic laboratories.*

Assay	Laboratory	Protocol	Assay use	Reporting format
In-house ELISA	1	Adapted from previously described protocol ¹²	Commercial	S/P ratio
	3	Indirect ELISA by using plates coated with an ORF2 gene amplified from a PCV-2b isolate and anti-pig peroxidase HRP conjugate at a 1:2,000 dilution	Research	S/P ratio
	4	ELISA by using plates coated with N-truncated ORF2 gene from a PCV-2b isolate and anti-pig peroxidase-HRP conjugate at a 1:5,000 dilution	Research	S/P ratio
	5	Adapted from previously described protocol ¹²	Commercial	S/P ratio
I-ELISA	1	Manufacturer's protocol ^f	Research	OD value
S-ELISA	1	Manufacturer's protocol ^g	Research	S/N ratio
	6	Manufacturer's protocol ^g	Commercial	S/N ratio
	7	Manufacturer's protocol ^g	Commercial	S/N ratio
IFAT	1	Previously described protocol ¹⁵	Commercial	Titer
	2	Previously described protocol ⁷	Commercial	Titer
	5	Adapted from previously described protocol ¹⁵	Commercial	Positive or negative

* Commercial = routine use in diagnostic laboratory setting; S/P = sample to positive; ORF2 = open reading frame 2; PCV-2 = *Porcine circovirus-2*; Research = modified use for individual studies; HRP = horseradish peroxidase; I-ELISA = Ingezim^h; OD = optical density; S-ELISA = Serelisa^g; S/N = sample to negative.

and/or immunoperoxidase monolayer assay (IPMA).¹¹ Analysis of results indicated that there was a wide variation of anti-PCV-2 antibody detection and that use of different fixatives (paraformaldehyde, acetone, or ethyl alcohol) and fixation methods may be the cause for the different results.¹¹ No statistical analysis was performed in that trial. In addition, other studies have been published that compared newly developed serologic tests with either an IFAT^{12,16,17,20} or an IPMA.¹⁰ In a 2008 study in which 2 commercially available ELISAs and 1 in-house ELISA were compared, it was found that the assays were able to detect both PCV-2a and PCV-2b, and that all assays had high diagnostic accuracies based on receiver operating characteristic (ROC) analysis.¹⁵ To the authors' knowledge, an interlaboratory comparison between currently offered in-house and commercial ELISAs and IFATs has not been conducted to date. The objective of the current study was to evaluate the agreement and diagnostic accuracy of PCV-2 serologic assays from 7 diagnostic laboratories using known positive and negative samples from experimentally infected specific pathogen-free conventional pigs.

Materials and methods

Diagnostic laboratories

Each of the 7 participating laboratories (coded 1–7) voluntarily participated in the research project. Each laboratory was asked to perform a currently available PCV-2 IFAT and/or ELISA by using laboratory-specific protocols (Table 1). The samples were coded with a numerical system that had no association with the original treatment system.

Study samples

Four hundred and forty serum samples from a previous trial were used for the present research project. Briefly, 3-week-old conventional pigs purchased from a PCV-2-negative herd were randomly divided into 7 groups and inoculated or vaccinated at trial day 0 as follows: 1) negative controls ($n = 7$), 2) PCV-2a (inoculated with ISU-40895; GenBank accession no. AF264042; $n = 8$), 3) PCV-2b (inoculated with NC-16845; GenBank accession no. EU340258, $n = 8$), 4) PCV-1 (inoculated with a well-characterized PCV-1 clone²; $n = 8$), 5) vaccine A^a ($n = 8$), 6) vaccine B^b ($n = 8$), and 7) vaccine C^c ($n = 8$). All the pigs were bled at weekly intervals until trial day 49. The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee.

Sample preparation and submission

After blood collection, the samples were centrifuged at $1,700 \times g$ for 10 min. The serum samples from all pigs from trial days 0–49 ($n = 440$ total samples) were each divided into 4-ml aliquots and stored at -80°C . Before shipment to the various diagnostic laboratories, each sample was further divided into seven 50- μl aliquots and assigned an individual identification number to which all laboratories were blinded. Coded samples were packaged with ice packs for overnight shipment to the various diagnostic laboratories.

Definition of positive and negative samples

A correctly identified positive sample ($n = 280$) was defined as a sample obtained from pigs in the PCV-2-infected groups (PCV-2a, PCV-2b) and the PCV-2-vaccinated groups (vaccines A–C; $n = 40$) on trial days 7, 14, 21, 28, 35, 42, and 49. A correctly identified negative sample ($n = 160$) was defined as a sample obtained from any pig on trial day 0 ($n = 55$ independent observations) and from pigs in the negative control group and pigs

Table 2. Percentage of correctly identified *Porcine circovirus-2* antibody negative (specificity) or positive (sensitivity) samples by different serologic assays used in 7 diagnostic laboratories by assay use (commercial or research).*

Assay use	Assay	Laboratory	Specificity		Sensitivity on different trial days						Total	
			Independent	Dependent†	7	14	21	28	35	42		49
Commercial	In-house	1	55/55 ^{a,‡}	105/105	0/40 ^a	18/40 ^a	37/40 ^a	38/40 ^a	40/40 ^a	40/40 ^a	40/40 ^a	213/280
		5	53/55 ^a	103/105	4/40 ^a	33/40 ^b	36/40 ^a	38/40 ^a	40/40 ^a	40/40 ^a	40/40 ^a	231/280
	S-ELISA	6	55/55 ^a	105/105	4/40 ^a	12/40 ^a	5/40 ^b	12/40 ^b	16/40 ^b	26/40 ^b	30/40 ^b	105/280
		7§	51/51 ^a	96/96	0/40 ^a	11/40 ^a	2/40 ^b	12/40 ^b	19/40 ^b	8/39 ^c	18/40 ^c	70/279
	IFAT	1	55/55 ^a	105/105	0/40 ^a	38/40 ^b	40/40 ^a	37/40 ^a	40/40 ^a	40/40 ^a	39/40 ^{ab}	234/280
2		55/55 ^a	104/105	0/40 ^a	36/40 ^b	39/40 ^a	40/40 ^a	40/40 ^a	40/40 ^a	40/40 ^a	235/280	
5		55/55 ^a	82/105	1/40 ^a	15/40 ^a	24/40 ^c	24/40 ^b	28/40 ^b	40/40 ^a	40/40 ^a	172/280	
Research	In-house	3	38/55 ^a	97/105	7/40 ^a	6/40 ^{ab}	16/40 ^a	14/40 ^a	19/40 ^a	22/40 ^a	26/40 ^{ab}	110/280
		4	55/55 ^b	105/105	0/40 ^b	0/40 ^a	6/40 ^b	14/40 ^a	15/40 ^a	19/40 ^a	21/40 ^a	75/280
	I-ELISA	1	55/55 ^b	105/105	1/40 ^{ab}	13/40 ^b	26/40 ^c	30/40 ^b	34/40 ^b	35/40 ^b	34/40 ^b	173/280
		1	55/55 ^b	105/105	2/40 ^{ab}	9/40 ^b	1/40 ^b	11/40 ^a	16/40 ^a	17/40 ^a	23/40 ^a	79/280

* ELISA = enzyme-linked immunosorbent assay; S-ELISA = Serelisa[®]; IFAT = indirect fluorescent antibody test; I-ELISA = Ingezim.[®]

† $n = 15$ pigs over time; McNemar test is based on independent assumption of observations and was not done.

‡ Assays not sharing common superscripts (^{a,b,c}) within each of the assay use groups are significantly different in classification probability (sensitivity by day or specificity).

§ Only 426 samples were tested by laboratory 7.

infected with PCV-1 ($n = 15$ dependent observations) on trial days 7, 14, 21, 28, 35, 42, and 49.

Statistics

Differences in sensitivity and specificity among assays were tested for significance by the pairwise McNemar test, with Bonferroni correction for multiple comparisons. A kappa statistic was calculated for paired tests by using dichotomized data. For tests in which results are identified as positive, negative, or suspect, the suspect samples were considered negative. Values for kappa range from -1 to 1 , in which -1 indicates perfect disagreement, 0 equals agreement no better than expected by chance, and 1 equals perfect agreement.¹⁸ The following arbitrary standards for the strength of agreement as described by Landis and Koch⁸ were used: ≤ 0 = poor, 0.01 – 0.2 = slight, 0.21 – 0.4 = fair, 0.41 – 0.60 = moderate, 0.61 – 0.80 = substantial, and 0.81 – 1 = almost perfect. Statistical analysis was performed by using JMP software.⁴ Diagnostic accuracy of each assay was also evaluated by using ROC analysis. This method results in area under the ROC curve (AUC) values that range from 0.5 to 1 : 0.5 indicates that the test is not discriminating between positive and negative animals, and 1 indicates perfect discrimination.²² All ROC calculations were performed on continuous data with infection status as the indicator of true infection status by using MedCalc.⁵ Specifically, samples from pigs in the PCV-2a, PCV-2b, vaccine A, vaccine B, and vaccine C groups were considered “true positive”; negative controls and PCV-1 were considered “true negative” samples.

Results

The percentage of correctly identified negative and positive samples for all assays is provided in Table 2. Kappa values for all assays on trial days 14 and 49 samples are summarized in Tables 3 (commercial

setting) and 4 (research setting). The percentage of correctly identified test results for trial day 49 on PCV-2–infected animals, PCV-2–vaccinated animals, and PCV-2–negative animals for each assay appear in Figure 1. Results of ROC analysis on trial days 14–49 are summarized in Table 5. The sensitivity and specificity generated through ROC analysis by using recommended cutoffs for each assay at trial days 14 and 49 are presented in Table 6.

Enzyme-linked immunosorbent assays

In-house ELISAs. The overall specificity was 100% ($55/55$ correctly identified negative samples) for laboratories 1 and 4, 69.1% ($38/55$ correctly identified negative samples) for laboratory 3, and 96.4% ($53/55$ correctly identified negative samples) for laboratory 5 (Table 2). The overall sensitivity was 76.1% ($213/280$ correctly identified positive samples) for laboratory 1, 39.3% ($110/280$ correctly identified positive samples) for laboratory 3, 26.8% ($75/280$ correctly identified positive samples) for laboratory 4, and 82.5% ($231/280$ correctly identified positive samples) for laboratory 5. It is of interest to note that the assays with sensitivities of 76.1 – 82.5% (laboratories 1 and 5) were used for commercial diagnostic purposes, and those with sensitivities of 26.8 – 39.3% (laboratories 3 and 4) were used for research purposes. The commercially used assays had improved sensitivity at an early trial day (45% and 82.5% ; trial day 14) compared with assays used for research purposes (0% and 15% ; trial day 14; Table 2). The agreement on results was almost perfect for laboratories 1 and 5 (Table 3) and moderate for laboratories 3 and 4 (Table 4).

Table 3. Overall measure of statistical agreement (kappa statistic ± standard error) trial days 14 and 49 among laboratories (commercial assay use).*

Trial day	Assay	Laboratory	In-house ELISA	IFAT			S-ELISA	
			Laboratory 5	Laboratory 1	Laboratory 2	Laboratory 5	Laboratory 6	Laboratory 7
14	In-house ELISA	1	0.42 ± 0.10	0.34 ± 0.10	0.38 ± 0.09	0.29 ± 0.14	0.01 ± 0.13	-0.07 ± 0.12
		5		0.81 ± 0.08	0.77 ± 0.09	0.19 ± 0.11	0.25 ± 0.09	0.27 ± 0.08
	IFAT	1		0.80 ± 0.09	0.12 ± 0.10	0.19 ± 0.08	0.19 ± 0.06	
		2			0.04 ± 0.10	0.24 ± 0.07	0.20 ± 0.07	
		5				-0.16 ± 0.11	-0.15 ± 0.12	
	S-ELISA	6					0.61 ± 0.13	
49	In-house ELISA	1	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.90 ± 0.07	0.62 ± 0.10	0.25 ± 0.07
		5		1.00 ± 0.00	1.00 ± 0.00	0.90 ± 0.07	0.62 ± 0.10	0.25 ± 0.07
	IFAT	1		1.00 ± 0.00	0.90 ± 0.07	0.62 ± 0.10	0.27 ± 0.08	
		2			0.90 ± 0.07	0.62 ± 0.10	0.25 ± 0.07	
		5				0.54 ± 0.10	0.21 ± 0.07	
	S-ELISA	6					0.48 ± 0.10	

* ELISA = enzyme-linked immunosorbent assay; IFAT = indirect fluorescent antibody test; S-ELISA = Serelisa.^g The following arbitrary standards for the strength of agreement previously described⁸ were used: ≤0 = poor, 0.01–0.2 = slight, 0.21–0.4 = fair, 0.41–0.60 = moderate, 0.61–0.80 = substantial, and 0.81–1 = almost perfect.

Commercially produced ELISAs. The overall specificity was 100% for all commercially produced ELISAs (laboratories 1 [55/55], 6 [55/55], and 7 [53/53]; Table 2). The overall sensitivity was 61.8% (Ingezim,^f or I-ELISA) and 28.2% (Serelisa,^g or S-ELISA) with 173 and 79, respectively, of 280 correctly identified positive samples for laboratory 1; 37.5% (105/280 correctly identified positive samples) for laboratory 6; and 25.1.0% (70/279 correctly identified positive samples) for laboratory 7. Regardless of purpose, S-ELISAs appeared to perform very similarly (Table 2). By trial day 49, the agreement on results was moderate for laboratories 6 and 7 with S-ELISA (Table 3). Similarly, laboratory 1 (S-ELISA and I-ELISA) had moderate agreement on trial day 49 (Table 4).

Indirect fluorescent antibody tests

The overall specificity was 100% (55/55 correctly identified negative samples) for laboratories 1, 2, and

5 (Table 2). The overall sensitivity was 83.6% (234/280 correctly identified positive samples) for laboratory 1, 83.9% (235/280 correctly identified positive samples) for laboratory 2, and 61.4% (172/280 correctly identified positive samples) for laboratory 5 (Table 2). By trial day 49, the 3 IFATs had almost perfect agreements, which ranged from 0.90 ± 0.07 to 1.00 ± 0.00 (Table 3).

Discussion

To reflect the actual outcomes (positive or negative for anti-PCV-2 antibodies) obtained by 7 North American laboratories, kappa analysis was used in the current study to compare dichotomous test data. Conversely, ROC analysis, which has become an increasingly valuable tool for comparison of diagnostic tests,^{4,5,22} uses information on the sensitivity and specificity across the entire range of possible cutoffs and can provide an optimized cutoff, which maxi-

Table 4. Overall measure of statistical agreement (kappa statistic ± standard error) on trial days 14 and 49 among laboratories (research assay use).*

Trial day	Assay	Laboratory	In-house ELISA	S-ELISA	I-ELISA
			Laboratory 4	Laboratory 1	Laboratory 1
14	In-house ELISA	3	Not done†	0.27 ± 0.17	0.28 ± 0.15
		4		Not done†	Not done†
49	S-ELISA	1		0.10 ± 0.14	
		3	0.78 ± 0.08	0.49 ± 0.12	0.60 ± 0.10
	In-house ELISA	4		0.38 ± 0.09	0.55 ± 0.01
		S-ELISA	1		

* ELISA = enzyme-linked immunosorbent assay; S-ELISA = Serelisa^g; I-ELISA = Ingezim.^f The following arbitrary standards for the strength of agreement previously described⁸ were used: ≤0 = poor, 0.01–0.2 = slight, 0.21–0.4 = fair, 0.41–0.60 = moderate, 0.61–0.80 = substantial, and 0.81–1 = almost perfect.

† Laboratories 1 and 3 were not compared with laboratory 4 on trial day 14, because laboratory 4 had no positive samples.

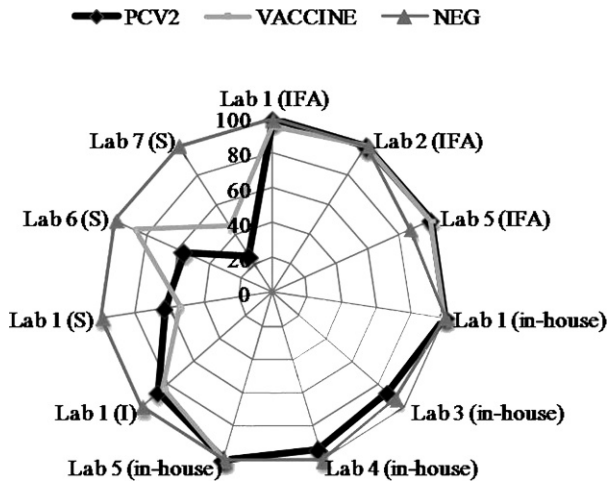


Figure 1. Percentage of correctly identified test results, which correspond to classification based on established cutoffs on trial day 49 from 7 different diagnostic laboratories in the United States. The data are divided into 3 main categories: PCV2, VACCINE, and NEG. A correctly identified *Porcine circovirus-2* (PCV-2) sample was defined as a sample from a pig infected with PCV-2 (combined data from PCV-2a and PCV-2b groups). VACCINE samples were from vaccinated animals (combined data from vaccine A, B, and C groups), and NEG samples were from negative animals (combined data from *Porcine circovirus-1* and negative controls). Assays are organized by submitting laboratory and type of assay; indirect fluorescent antibody tests (IFA), in-house enzyme-linked immunosorbent assays (ELISA), and commercially available (Serelisa[®] [S] or Ingezim[®] [I]) ELISAs.

mizes the sensitivity and specificity based on the continuous data.⁴ Therefore, the discrepancy noted in the present study between the results of ROC and kappa analyses on the commercial and in-house ELISAs was because of the selection of the cutoff values for data dichotomization. This concept is clearly evident in Table 4, in which sensitivities differ among laboratories that had similar high AUC values. Because this concept is not applicable for comparing the results obtained from diagnostic laboratories, only sensitivities and specificities generated at laboratory-specific cutoffs were used in the present study, and AUC values, although provided, were not compared among laboratories.

When the assays were compared by using kappa analysis, all 3 laboratories that offered IFATs had almost perfect agreement by trial day 49. The IFATs also had almost perfect agreement with the in-house ELISAs from laboratories 1 and 5. However, these data were generated by experimentally inoculating naïve animals with PCV-2, and ancillary data from one of the participating laboratories indicated that agreement between ELISA and IFAT at early trial days may not be as high in piglets with passively acquired maternal antibodies (R. Hesse, personal communication, 2009). Based on previous experimen-

Table 5. Area under the receiver operating curve values for each of the assays on different trial days.

Assay use	Assay*	Laboratory†							Trial day‡												
		1	5	6	7	1	2	3	4	1	1	1	1	1	1	1	1	1			
Commercial	In-house ELISA	1	0.94 (0.84; 0.98)	0.99 (0.93; 1.00)	0.99 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)		
		5	0.95 (0.86; 0.99)	0.89 (0.78; 0.96)	0.95 (0.86; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	
		6	0.96 (0.87; 0.99)	0.95 (0.86; 0.99)	0.95 (0.86; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	0.96 (0.87; 0.99)	
	Research	IFAT	1	0.96 (0.87; 0.99)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	
			2	0.98 (0.89; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)	1.00 (0.9; 1.00)
		In-house ELISA	3	0.94 (0.85; 0.99)	0.99 (0.91; 1.00)	0.99 (0.91; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)
			4	0.75 (0.61; 0.86)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)	0.84 (0.71; 0.92)
S-ELISA	1	0.72 (0.58; 0.83)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)	0.86 (0.74; 0.94)		
	1	0.97 (0.89; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)		
I-ELISA	1	0.96 (0.87; 0.99)	0.99 (0.93; 1.00)	0.99 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)	1.00 (0.93; 1.00)		

* ELISA = enzyme-linked immunosorbent assay; S-ELISA = Serelisa[®]; I-ELISA = Ingezim[®]; IFAT = indirect fluorescent antibody test.

† Data from laboratory 1 have been previously published.¹⁴ Data from the IFAT from laboratory 5 were not included because continuous data were not provided.

‡ Numbers in parentheses are 95% confidence intervals; receiver operating characteristic analysis was performed by denoting PCV-2a, vaccine A, vaccine B, and vaccine C pigs as “true positive” and negative controls, and PCV-1 as “true negative” animals. Area under the receiver operating curve values range from 0.5 to 1, where 1 indicates that, when using a given sample, the diagnostic test would perfectly discriminate between PCV-2-positive and PCV-2-negative pigs, and 0.5 indicates that there is no discrimination between groups.

Table 6. Recommended cutoffs and associated sensitivity and specificity for trial days 14 and 49 for each assay.*†

Assay use	Assay	Laboratory	Recommended cutoff	Trial day 14‡		Trial day 49‡	
				Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Commercial	In-house ELISA	1	≥0.20	42.5 (27.1; 59.1)	100.0 (78.0; 100.0)	100.0 (91.1; 100.0)	100.0 (78.0; 100.0)
		5	≥0.4	82.5 (67.2; 92.6)	100.0 (78.0; 100.0)	100.0 (91.1; 100.0)	100.0 (78.0–100.0)
	S-ELISA	6	≤0.15	30.0 (16.6; 46.5)	100.0 (78.0; 100.0)	75.0 (58.8; 87.3)	100.0 (78.0–100.0)
		7	≤0.15	25.0 (12.7; 41.2)	100.0 (76.7; 100.0)	45.0 (29.3; 61.5)	100.0 (76.7; 100.0)
	IFAT	1	≥0.90§	95.0 (83.0; 99.2)	100.0 (78.0; 100.0)	97.5 (86.8; 99.6)	100.0 (78.0; 100.0)
		2	≥1.3	90.0 (76.3; 97.1)	93.3 (68.0; 98.9)	100.0 (91.1; 100.0)	100.0 (78.0; 100.0)
		3	>0.3	0.0 (0.0; 8.9)	100.0 (78.0; 100.0)	52.5 (36.1; 68.5)	97.5 (86.8; 99.6)
Research	In-house ELISA	4	≥0.4	0.0 (0.0; 8.9)	100.0 (78.0; 100.0)	0.0 (0.0; 8.9)	100.0 (78.0; 100.0)
		1	≤0.15	25.0 (12.7; 41.2)	100.0 (78.0; 100.0)	60.0 (43.3; 75.1)	100.0 (78.0; 100.0)
	S-ELISA	1	≥0.33¶	27.5 (14.6; 43.9)	100.0 (78.0; 100.0)	82.5 (67.2; 92.6)	100.0 (78.0–100.0)
		1					

* ELISA = enzyme-linked immunosorbent assay; S-ELISA = Serelisa®; IFAT = indirect fluorescent antibody test; I-ELISA = Ingezim.†

† Data from laboratory 5 (IFAT) was not included because continuous data were not provided.

‡ Numbers in parentheses are 95% confidence intervals; sensitivity and specificity were generated by using receiver operating characteristic analysis.

§ Corresponds to a cutoff of ≥1:8.

|| Corresponds to a cutoff of ≥1:20.

¶ Plate-specific cutoff was determined by the average optical density value of the positive control wells multiplied by 0.3; the number represents an average of plate-specific cutoffs when using the experimental study data.

tal studies,¹⁴ there were likely consistently high levels of antibodies present at this point, which enhanced uniformity in test results in comparison with samples taken from animals shortly after PCV-2 infection as evident in the kappa values (poor to almost perfect) obtained on trial day 14.

In comparison with the ELISAs, kappa analysis revealed differences among the laboratories. Specifically, the commercially available S-ELISAs from laboratories 6 and 7 had only moderate agreement at trial day 49. Reasons for the interlaboratory variability of the commercial assays in the present study need to be further elucidated but may include variability among technicians because the laboratories were using the same commercially available kits according to the manufacturers' directions. Among in-house ELISAs, the sensitivity varied based on the main purpose (76.1–82.5% for commercial purpose and 26.8–39.3% for research purpose) for which the assay was designed and being used. Reasons for the interlaboratory variability of the in-house assays may have been because of differences in strain of virus used in the coating of the plates, the length of the PCV-2 capsid protein used in the assay (laboratories 1 and 5 used an intact capsid, whereas laboratory 4 used a truncated capsid), antibody specificity, selection of cutoff value, or variability between technicians. In addition, laboratories designated as research (laboratories 3 and 4) were routinely modifying assays for individual studies; therefore, the conditions used in the current study do not necessarily predict future performance.

Based on data from trial day 14, the IFATs in laboratories 1 and 2, and the in-house ELISA used in laboratory 5 detected the highest number of positive samples. Therefore, the use of one of these assays may result in a higher likelihood of early disease detection when monitoring negative herds. However, before trial day 14, few samples (0, 0, and 4, respectively) were correctly identified as being positive by these 3 assays, which indicates that the use of serology for routine monitoring of a PCV-2–negative population may be of limited use for detection of early infections (Table 2).

These results demonstrate that, whereas differences among laboratories do exist, in-house ELISAs (excluding in-house ELISAs categorized for research use) and IFATs had high diagnostic accuracy based on kappa analysis. Commercial ELISAs used in the current study had fair to substantial agreement with the in-house ELISAs.

Acknowledgements

The authors thank Paul Thomas and Jeremy Johnson for assistance with animal work and Boehringer Ingelheim

Vetmedica Inc., for funding of this study. The authors also thank Dr. Chong Wang for statistical advice.

Sources and manufacturers

- a. Ingelvac[®] CircoFLEX[™], Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO.
- b. Circuvent[®] PCV, Intervet Inc., Millsboro, DE.
- c. Suvaxyn[®] PCV2 One Dose, Fort Dodge Animal Health, Fort Dodge, IA.
- d. JMP[®] version 6.0.0, SAS Institute Inc., Cary, NC.
- e. MedCalc[®] Software version 9.1.0.1, Mariakerke, Belgium.
- f. Ingezim Circovirus IgG, Ingenasa, Madrid, Spain.
- g. Serelisa[®] PCV2 Ab Mono Blocking, Synbiotics Europe SAS, Lyon, France.

References

1. Fachinger V, Bischoff R, Jedidia SB, et al.: 2008, The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. *Vaccine* 26:1488–1499.
2. Fenaux M, Opriessnig T, Halbur PG, Meng XJ: 2003, Immunogenicity and pathogenicity of chimeric infectious DNA clones of pathogenic porcine circovirus type 2 (PCV2) and nonpathogenic PCV1 in weanling pigs. *J Virol* 77:11232–11243.
3. Fort M, Sibila M, Allepuz A, et al.: 2008, Porcine circovirus type 2 (PCV2) vaccination of conventional pigs prevents viremia against PCV2 isolates of different genotypes and geographic origins. *Vaccine* 26:1063–1071.
4. Gardner IA, Greiner M: 2006, Receiver-operating characteristic curves and likelihood ratios: improvements over traditional methods for the evaluation and application of veterinary clinical pathology tests. *Vet Clin Pathol* 35:8–17.
5. Hanley J, McNeil B: 1983, A method of comparing the areas under the receiver operating characteristic curves derived from the same cases. *Radiology* 148:839–843.
6. Harding J, Clark E: 1997, Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). *Swine Health Prod* 5:201–203.
7. Horlen KP, Dritz SS, Nietfeld JC, et al.: 2008, A field evaluation of mortality rate and growth performance in pigs vaccinated against porcine circovirus type 2. *J Am Vet Med Assoc* 232:906–912.
8. Landis J, Koch G: 1977, The measurement of observer agreement for categorical data. *Biometrics* 33:159–174.
9. Laroche R, Magar R, D'Allaire S: 2003, Comparative serologic and virologic study of commercial swine herds with and without postweaning multisystemic wasting syndrome. *Can J Vet Res* 67:114–120.
10. Liu C, Ihara T, Nunoya T, Ueda S: 2004, Development of an ELISA based on the baculovirus-expressed capsid protein of porcine circovirus type 2 as antigen. *J Vet Med Sci* 66:237–242.
11. McNair I, Marshall M, McNeilly F, et al.: 2004, Interlaboratory testing of porcine sera for antibodies to porcine circovirus type 2. *J Vet Diagn Invest* 16:164–166.
12. Nawagitgul P, Harms PA, Morozov I, et al.: 2002, Modified indirect porcine circovirus (PCV) type 2-based and recombinant capsid protein (ORF2)-based enzyme-linked immunosorbent assays for detection of antibodies to PCV. *Clin Diagn Lab Immunol* 9:33–40.
13. Opriessnig T, Meng XJ, Halbur PG: 2007, Porcine circovirus type 2 associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. *J Vet Diagn Invest* 19:591–615.
14. Opriessnig T, Patterson AR, Elsener J, et al.: 2008, Influence of maternal antibodies on efficacy of porcine circovirus type 2 (PCV2) vaccination to protect pigs from experimental infection with PCV2. *Clin Vaccine Immunol* 15:397–401.
15. Patterson AR, Johnson J, Ramamoorthy S, et al.: 2008, Comparison of three enzyme-linked immunosorbent assays to detect porcine circovirus-2 (PCV-2)-specific antibodies after vaccination or inoculation of pigs with distinct PCV-1 or PCV-2 isolates. *J Vet Diagn Invest* 20:744–751.
16. Pogranichnyy RM, Yoon KJ, Harms PA, et al.: 2000, Characterization of immune response of young pigs to porcine circovirus type 2 infection. *Viral Immunol* 13:143–153.
17. Shang SB, Li YF, Guo JQ, et al.: 2007, Development and validation of a recombinant capsid protein-based ELISA for detection of antibody to porcine circovirus type 2. *Res Vet Sci* 84:150–157.
18. Sim J, Wright C: 2005, The kappa statistic in reliability studies: use, interpretation, and sample size requirements. *Phys Ther* 85:257–268.
19. Tischer I, Gelderblom H, Vettermann W, Koch MA: 1982, A very small porcine virus with circular single-stranded DNA. *Nature* 295:64–66.
20. Wu P-C, Chein M-S, Tseng Y-Y, et al.: 2008, Expression of the porcine circovirus type 2 capsid protein subunits and application to an indirect ELISA. *J Biotechnol* 133:58–64.
21. Yolken RH, Viscidi R: 1989, Enzyme immunoassay and radioimmunoassay, *In*: Diagnostic procedures for viral, rickettsial and chlamydial infections, ed. Schmidt NJ, Emmons RW, 6th ed., pp. 157–178. American Public Health Association, Washington, DC.
22. Zweig M, Campbell G: 1993, Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 39:562–557.