

# Assessment of international inter-laboratory comparison tests for the diagnosis of classical swine fever from 1998 until 2007

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## Summary

The inter-laboratory comparison tests for classical swine fever (CSF) laboratory diagnosis organised by the European Community Reference Laboratory for CSF are regularly performed within European Union Member States. The objective of this study was to evaluate the results of the inter-laboratory comparison tests carried out over the last decade, from 1998 until 2007, by using a statistical approach. A set of five or six lyophilised sera was sent to participants. These included sera containing CSF antibodies, sera containing antibodies against ruminant pestiviruses, sera containing CSF virus and negative sera.

This study focused on the results of the diagnostic reference methods for CSF: the neutralisation test for the detection of CSF antibodies (including its interpretation) and virus isolation for the detection of CSF virus. For the detection of CSF antibodies, results were closest to what was expected by the Community Reference Laboratory when only neutralisation tests were performed. The percentage of correct results decreased as soon as the results of CSF antibody enzyme-linked immunosorbent assay were included or when sera with antibodies to ruminant pestiviruses were added to the panel. The results for the detection of CSF antibodies are still valid today, as no additional method has been introduced recently. Regarding CSF virus detection, CSF virus isolation is well established but on the way to being superseded as the reference test by reverse-transcription polymerase chain reaction.

## Keywords

Classical swine fever virus – Cross-neutralisation – Differential diagnosis – Inter-laboratory comparison test – Laboratory diagnosis – Neutralisation test – Pestiviruses – Proficiency test – Quality management – Reference laboratory – Virus isolation.

## Introduction

Classical swine fever (CSF) is a highly contagious disease of pigs which is listed by the World Organisation for Animal Health (OIE). Furthermore, it is also a notifiable disease in European Union (EU) Member States (13, 14, 26).

Although many parts of the world are free of CSF, new introductions causing CSF outbreaks with severe economic losses still occur (12). Therefore, it is important to have a laboratory diagnostic system set up in every country in order to diagnose a possible CSF introduction as fast as possible (7, 8). Within the EU, each Member State has appointed a National CSF Reference Laboratory and there is a Community Reference Laboratory (CRL) (situated in

Germany at the Institute for Virology of Hanover Veterinary School) appointed by the EU Commission (14). Independent from these appointments there are several OIE Reference Laboratories and Collaborating Centres worldwide appointed by the OIE.

Quality management systems are becoming more and more important for all diagnostic laboratories, in particular for those dealing with notifiable diseases (1). A crucial element in the validation of diagnostic tests is participation in inter-laboratory comparison tests, also known as 'proficiency tests' or 'ring-trials'. The objective is that all laboratories obtain the same result when investigating the same sample (17).

The CRL for CSF started to organise inter-laboratory comparison tests in 1985 and they have been conducted annually ever since. Currently, the system is under revision, in order to fulfil recent accreditation demands.

In 2002, the EU Commission published a Diagnostic Manual for CSF which advised when to use which diagnostic test (15). The approved reference methods for CSF virus detection and the detection of CSF antibodies are virus isolation on cell culture and the virus neutralisation test, respectively.

This study will focus on the inter-laboratory comparison tests for CSF organised by the CRL from the last decade, specifically on the reference methods virus isolation (14) and the neutralisation test (22). The aim of this study was to compare the results of inter-laboratory comparison tests on an objective basis. A novel and simple statistical approach from behavioural science was used (3).

The development of reliable test systems for genome detection, i.e. reverse-transcription polymerase chain reaction (RT-PCR), over the last decade, led to amendments in the structure of the inter-laboratory comparison tests (25). From 2004 a separate inter-laboratory comparison test was conducted on the use of RT-PCR, using different samples from those used in the tests presented here. The results of this test give data for evaluation in a separate publication.

## Materials and methods

### Characterisation of sera

Porcine sera were produced in the high containment facilities of the CRL for CSF in accordance with the German Animal Welfare Act. After lyophilisation, a set of five or six duplicate samples was sent out to participants and the results were requested after three months. As of 2004, results were requested after only four weeks so that

the test conditions more closely resembled field conditions, in which a result is due as soon as possible.

Four types of sera were produced, as follows:

a) Classical swine fever antibody-positive sera from pigs which had recovered from CSF were collected on different days post infection. The panels consisted of sera with high titres ( $> 2 \log_{10} \text{ND}_{50}$ ), sera with low titres ( $1.4\text{-}2 \log_{10} \text{ND}_{50}$ ) and sera with borderline or cut-off titres ( $1\text{-}1.3 \log_{10} \text{ND}_{50}$ ). According to EU legislation, a CSF antibody titre of  $1 \log \text{ND}_{50}$  is considered CSF positive (14, 15).

b) Sera from pigs inoculated with other pestiviruses, either with bovine viral diarrhoea virus (BVDV) of cattle or border disease virus (BDV) of sheep or non-CSF (ruminant) pestiviruses from pigs, were also produced and included in the trial. This is of special importance for differential diagnosis as there is cross-reaction of neutralising antibodies against different pestiviruses (5, 6). A serum with a high non-CSF pestivirus antibody titre can give a low CSF antibody titre due to cross-neutralisation. If only a neutralisation test for CSF is carried out a false positive result may occur (24). According to the Diagnostic Manual, a threefold titre difference allows discrimination between CSF and other pestiviral infections (15).

c) Sera containing CSF virus were collected in the early phase of infection and from pigs which were moribund from CSF, mostly with an acute course of CSF infection. Although organ samples or leukocyte preparations are the sample of choice under field conditions, serum was used in the inter-laboratory comparison test because it is easier to distribute and transport, participants receive equal material and a fair evaluation is possible. Field conditions, such as organ samples in different stages of decay, were not prepared, because a fair evaluation is hard to perform.

d) Negative sera from pigs which had no contact with any pestivirus were included each year.

### Classical swine fever virus isolates for the production of sera

Classical swine fever virus isolates used to inoculate the pigs were mostly CSF field virus isolates from EU Member States, e.g. CSF0123 isolated from domestic pigs during the 1995 CSF epidemic in Germany; CSF0277 isolated from domestic pigs during the CSF epidemic in 1997 which spread through Germany, the Netherlands, Belgium, Italy and Spain; and CSF0634 isolated in 2001 from domestic pigs and also found in the wild boar population in Germany.

In addition, a clone of CSF reference virus strain 'Alfort 187' (CSF0941) was used for the production of sera.

Each CSF virus isolate used for the production of reference material is registered in the database at the CRL for CSF (11, 18, 19) and most CSF virus isolates have been characterised by clinical studies (16).

Pestivirus antibody-positive porcine sera have also been characterised previously (24).

An overview of the characterisation of sera, CSF virus isolates and pestivirus isolates used for inoculation of the animals, and the degree of difficulty in diagnosing the serum, is given in Table I.

## Participants

Between 1998 and 2003 the countries participating in the tests were: all the EU Member States, some candidate countries for EU accession (Poland, Czech Republic, Slovak Republic, Hungary, Romania) and three third countries (Norway, Switzerland, United States of America).

In 2004, with the enlargement of the EU, changes occurred. More laboratories participated and reference methods for CSF diagnosis were set up in more laboratories. By 2007 more third countries had joined and a total of 32 laboratories were participating, all using the reference methods.

## Rating system

The results of laboratories which performed the virus isolation and neutralisation reference tests were evaluated from 1998 to 2007. From 1999 onwards the result for the detection of CSF antibodies had to be evaluated after performing differential diagnosis for BVDV and BDV. From 2004, due to the enlargement of the EU, the interpretation of CSF antibodies of all participants was evaluated, regardless of which methods were used. In 2007 results were again evaluated only from those participants who had used the virus isolation and neutralisation tests.

A scoring system was used to evaluate the results, taking into account how difficult each serum was to diagnose; see Table I.

One serum (0002/16) with a low CSF antibody titre was included in five consecutive years in order to give the laboratories a chance to follow up their sensitivity. One serum (0077/21) with a low antibody titre was included in 1998 and again in three consecutive years from 2004 until 2006. Another serum (0007/21) was included in 1999 and again in 2004.

Two ruminant pestivirus antibody-positive sera were included twice (Serum 0015/77 in 2000 and 2003; Serum 0925/34 in 2001 and 2002; see Table I) to give the laboratories a chance to follow up their specificity.

Exactly the same panel of sera was never distributed twice.

## Statistical analysis

For each of the samples the following information is available:

- a)  $A_i$  – minimum score attainable for sample  $i$  (false test result)
- b)  $B_i$  – maximum score reachable for sample  $i$  (correct test result)
- c)  $S_i$  – score achieved for sample  $i$  (according to the actual test result)
- d) As one further score for comparison, we consider the random score which represents the mean score when test results are chosen randomly:  $Z_i = (A_i + B_i) / 2$ , random score for sample  $i$ .

The diagnostic findings for a set of  $n$  samples (e.g. all samples tested by a given laboratory) can now be summarised by the quantities:

$$S = \sum_{i=1}^n S_i \quad \text{Total score achieved}$$

$$B = \sum_{i=1}^n B_i \quad \text{Maximum total score}$$

$$Z = \sum_{i=1}^n Z_i \quad \text{Random total score}$$

To compare the results between different laboratories and/or between different inter-laboratory comparison tests more easily we expressed the total score in percent ( $P$ ). One hundred percent means that the maximum total score has been achieved ( $S = B$ ). Zero percent indicates that the test results were obtained randomly ( $S = Z$ ).  $P$  has been calculated as follows:

$$P = \frac{S - Z}{B - Z} \times 100\%$$

The statistical method presented here (3) for assessing inter-laboratory comparison tests has been used previously to evaluate the national inter-laboratory comparison tests for CSF diagnosis from Germany (10).

Table I

**Characterisation of sera used in the international inter-laboratory comparison tests for the diagnosis of classical swine fever (1998 to 2007) and the points system used in the assessment of these tests**

The maximum and minimum points varied in accordance with how difficult a serum was to diagnose: correct test results for sera that were classified as being easy to diagnose were awarded a maximum of 1 point, but correct results for sera that were deemed difficult to diagnose were awarded a maximum of 3 points

Year	Serum no.	Serum identification/days post infection	Characteristics	Degree of diagnostic difficulty	Homologous virus*	Maximum points (correct result)	Points for a doubtful result	Minimum points (false result)	Random points
1998	1	Negative	Negative	--		1	-2	-3	-1
	2	0077/21	CSF ab pos.	+	CSF0941	2	-1	-2	0
	3	0075/14	CSF virus pos.	+/-	CSF0277	3	0	-1	1
	4	0079/32	CSF ab pos.	++	CSF0123	1	-2	-3	-1
	5	0473/17	CSF ab pos.	++	CSF0102	1	-2	-3	-1
1999	6	0002/16	CSF ab pos.	+/-	CSF0123	3	0	-1	1
	7	0007/21	CSF ab pos.	++	CSF0123	1	-2	-3	-1
	8	Negative	Negative	--		1	-2	-3	-1
	9	0093/15	CSF virus pos.	++	CSF0123	1	-2	-3	-1
	10	Field serum	BVD/BD ab pos.	+/-	Unknown BVD field virus	3	0	-1	1
2000	11	Negative	Negative	--		1	-2	-3	-1
	12	0006/21	CSF ab pos.	+	CSF0123	2	-1	-2	0
	13	0015/77	BVD/BD ab pos.	-	BDV 'Frijters'/ BVDV 'Salza'	2	-1	-2	0
	14	0002/16	CSF ab pos.	+/-	CSF0123	3	0	-1	1
	15	Pesti	BVD/BD ab pos.	-	BDV 'Averon'/ BVDV 'NADL'	2	-1	-2	0
	16	0934/43	CSF ab pos.	++	CSF0123	1	-2	-3	-1
2001	17	0053/26	CSF ab pos.	+	CSF0573	2	-1	-2	0
	18	0046/20	CSF ab pos.	+/-	CSF0634	3	0	-1	1
	19	0037/16	CSF virus pos.	++	CSF0277	1	-2	-3	-1
	20	0002/16	CSF ab pos.	+/-	CSF0123	3	0	-1	1
	21	Negative	Negative	--		1	-2	-3	-1
	22	0925/34	BVD/BD ab pos.	-	BDV 'Frijters'	2	-1	-2	0
2002	23	0054/17	CSF ab pos.	+	CSF0277	2	-1	-2	0
	24	Negative	Negative	--		1	-2	-3	-1
	25	0925/34	BVD/BD ab pos.	-	BDV 'Frijters'	2	-1	-2	0
	26	0002/16	CSF ab pos.	+/-	CSF0123	3	0	-1	1
	27	0082/27	CSF ab pos.	+	CSF0650	2	-1	-2	0
	28	0049/15	CSF virus pos.	++	CSF0634	1	-2	-3	-1
2003	29	0002/16	CSF ab pos.	+/-	CSF0123	3	0	-1	1
	30	0115/26	CSF ab pos.	+	CSF0634	2	-1	-2	0
	31	Negative	Negative	--		1	-2	-3	-1
	32	0087/13	CSF ab pos.	+	CSF0695	2	-1	-2	0
	33	BVD	BVD virus pos.	+/-	BVDV of cell culture origin	3	0	-1	1
	34	0015/77	BVD/BD ab pos.	-	BDV 'Frijters'/ BVDV 'Salza'	2	-1	-2	0
2004	35	0175/24	CSF virus pos.	+	CSF0634	2	-1	-2	0
	36	Negative	Negative	--		1	-2	-3	-1
	37	0059/85	BD ab pos.	-	BDV 'Moredun'	2	-1	-2	0
	38	0077/21	CSF ab pos.	+	CSF0941	2	-1	-2	0
	39	0007/21	CSF ab pos.	++	CSF0123	1	-2	-3	-1
	40	0154/21	CSF ab pos.	+	CSF0634	2	-1	-2	0
2005	41	Negative	Negative	--		1	-2	-3	-1
	42	0216/18	CSF ab pos.	+/-	CSF0634	3	0	-1	1
	43	0115/26	CSF ab pos.	++	CSF0634	1	-2	-3	-1
	44	0051/33	CSF ab pos.	++	CSF0573	1	-2	-3	-1
	45	0077/21	CSF ab pos.	+	CSF0941	2	-1	-2	0
	46	0208/8	CSF virus pos.	++	CSF0905	1	-2	-3	-1
2006	47	0215/23	CSF ab pos.	+/-	CSF0634	3	0	-1	1
	48	180/24	CSF ab pos.	+	CSF0309	2	-1	-2	0
	49	Negative	Negative	--		1	-2	-3	-1
	50	0235/17	CSF ab pos.	+/-	CSF0634	3	0	-1	1
	51	0077/21	CSF ab pos.	+	CSF0941	2	-1	-2	0
	52	0082/27	CSF ab pos.	++	CSF0650	1	-2	-3	-1
2007	53	0155/18	CSF ab pos.	+/-	CSF0634	3	0	-1	1
	54	0143/26	CSF ab pos.	+	CSF0634	2	-1	-2	0
	55	0086/27	CSF ab pos.	++	CSF0695	1	-2	-3	-1
	56	0225/29	CSF ab pos.	++	CSF0375	1	-2	-3	-1
	57	0001/7	CSF virus pos.	++	CSF0382	1	-2	-3	-1
	58	Negative	Negative	--		1	-2	-3	-1

CSF ab pos.: classical swine fever antibody positive

BVD/BD ab pos.: ruminant pestivirus antibody positive

CSF virus pos.: classical swine fever virus positive

BVDV: bovine viral diarrhoea virus

BDV: border disease virus

- negative serum; medium

-- negative serum; easy

+ positive serum; medium

++ positive serum; easy

+/- positive serum; difficult

\* The homologous virus is the virus isolate the pig was inoculated with

## Results

The P achieved for each serum by all participating laboratories during all years are listed in Table II. From a total of 58 sera distributed during all 10 years, 14 sera (24%) scored a P of 100%. Of these 14 sera, 6 sera (43%) were negative sera.

The average P for each year for all sera and all participating laboratories is also shown in Table II. The average P was mostly above 70%. Only twice was the P of all laboratories below 70%: in 1999 (66%) and in 2006 (47%). There was a decrease of P from 81% in 1998 to 66% in 1999. Until 2003, the P increased slowly to 84%. From 2004 until 2006 P varied between 76% and 47%. In 2007 P increased to 92%.

The results of the sera included several times are listed in Table III. Serum 0002/16 was included from 1999 until 2003. The first year this serum was included, the average P for all participating laboratories was 89%. During the following years P was considerably lower. Serum 0077/21 scored a P of 100% in 1998, but six years later when it was included again it had fallen to 59%, and in the two years that followed the P was consistently below 70%. Serum 0007/21 scored a P of 100% in 1999 and five years later P decreased to 79%.

Pestivirus antibody-positive serum 0925/34 was included in two consecutive years, 2001 and 2002. In 2002, P was 14% lower than the previous year. Serum 0015/77 was included in 2000 and 2003. Here P increased by 4%.

The distribution of the total scores of the 140 laboratories that participated in the tests is shown in Figure 1. Scores below 50% rarely occurred. Looking at the number of laboratories in each category of P, 50 out of 140 laboratories (35%) achieved a P of 100%. This indicates that there are only a few laboratories scoring unsatisfactory results (four laboratories scored less than 0%). Evaluation here was limited to the years 1998 to 2003 because during these years the participants and the methods used were more constant. Later there was much more variation of participants and methods applied.

### Classical swine fever virus detection

A total of seven CSF virus positive sera and one BVDV positive serum were included. When the difficulty in diagnosing these CSF virus positive sera was classified as difficult or medium, P was < 30%. When these sera were characterised as easy to diagnose, P was well above 75%. The BVDV positive serum was detected correctly by all participants (P 100%).

## Discussion

The inter-laboratory comparison tests for diagnosis of CSF conducted by the CRL are a valuable tool for quality assurance of CSF diagnostic laboratories. The increasing number of participating laboratories and the increasing number of laboratories which are able to perform the reference tests are encouraging. For statistical evaluation, the following points have to be considered:

- the sera chosen represented acute difficulties and field problems in CSF diagnosis and these difficulties varied over the 10 years
- the system was developed long before accreditation became common and before standards for the performance of inter-laboratory comparison tests were available from the International Organization for Standardization
- the objective of an inter-laboratory comparison test is not necessarily to obtain 100% correct results, because difficult sera are included on purpose. This should give the laboratory a chance to find their individual detection limit.

### Evaluation of classical swine fever antibody detection

By 1998, when this study started, the inter-laboratory comparison tests for CSF were already being conducted (and had been for many years) and consequently the standard was high. Adjustments to field situations at that time led to a decrease in the average P. In 1998, only the results of virus isolation and the virus neutralisation test were evaluated. In 1999 differential diagnosis of BVD and BD for the detection of CSF antibodies became important (24). Consequently, sera containing these kind of ruminant pestivirus antibodies were included in the panels and neutralisation tests for different pestiviruses were performed. The result had to be interpreted by the laboratories. This can explain the decrease of correct results from 1998 to 1999. In one year, differential diagnostic systems were set up in most laboratories and in 2000 P increased again.

Another explanation for varying results could be the use of CSF0902, also known as 'Alfort 187', as the standard virus for the neutralisation test. It is used by most laboratories but not by all. CSF0902 has not occurred in the field in EU Member States recently. Therefore, its continuing use as standard virus is under discussion (17). Recent CSF virus isolates from outbreaks in EU Member States belonged to genotype 2. CSF virus isolates of genotype 2.3 were also present in the wild boar population in an outbreak in Germany, posing a risk for domestic pigs (23). On the other hand CSF0902 is well adapted to cell culture and easy to diagnose as laboratory contaminant (if suspected)

**Table II**  
**The combined scores of all participating laboratories for each serum tested**

The overall average score for the year of all laboratories for all sera, expressed as a percentage, is indicated with an asterisk

Year	Serum no.	Serum identification/days post infection	Degree of diagnostic difficulty	Maximum points available	Actual points scored	Random points	Percentage	Number of participating laboratories
1998	1	Negative	--	17	13	-17	88	17
	2	0077/21	+	34	34	0	100	
	3	0075/14	+/-	51	24	17	21	
	4	0079/32	++	17	17	-17	100	
	5	0473/17	++	17	15	-17	94	
		<b>Total</b>		<b>136</b>	<b>103</b>	<b>-34</b>	<b>81*</b>	
1999	6	0002/16	+/-	57	53	19	89	19
	7	0007/21	++	19	19	-19	100	
	8	Negative	--	19	19	-19	100	
	9	0093/15	++	19	15	-19	89	
	10	Field serum	+/-	57	0	19	-50	
		<b>Total</b>		<b>171</b>	<b>106</b>	<b>-19</b>	<b>66*</b>	
2000	11	Negative	--	27	27	-27	100	27
	12	0006/21	+	54	50	0	93	
	13	0015/77	+	54	25	0	46	
	14	0002/16	+/-	81	59	27	59	
	15	pesti	-	54	26	0	48	
	16	0934/43	++	27	21	-27	89	
		<b>Total</b>		<b>297</b>	<b>208</b>	<b>-27</b>	<b>73*</b>	
2001	17	0053/26	+	50	40	0	80	25
	18	0046/20	+/-	75	47	25	44	
	19	0037/16	++	25	20	-25	90	
	20	0002/16	+/-	75	50	25	50	
	21	Negative	--	25	22	-25	94	
	22	0925/34	-	50	38	0	76	
		<b>Total</b>		<b>300</b>	<b>217</b>	<b>0</b>	<b>72*</b>	
2002	23	0054/17	+	52	45	0	87	26
	24	Negative	--	26	22	-26	92	
	25	0925/34	-	52	32	0	62	
	26	0002/16	+/-	78	61	26	67	
	27	0082/27	+	52	48	0	92	
	28	0049/15	++	26	14	-26	77	
		<b>Total</b>		<b>286</b>	<b>222</b>	<b>-26</b>	<b>79*</b>	
2003	29	0002/16	+/-	78	57	26	60	26
	30	0115/26	+	52	49	0	94	
	31	Negative	--	26	26	-26	100	
	32	0087/13	+	52	52	0	100	
	33	BVD	+/-	78	78	26	100	
	34	0015/77	+	52	26	0	50	
		<b>Total</b>		<b>338</b>	<b>288</b>	<b>26</b>	<b>84*</b>	
2004	35	0175/24	+	56	16	0	29	28 VI <sup>(a)</sup>
	36	Negative	--	33	33	-33	100	
	37	0059/85	+	66	54	0	82	
	38	0077/21	+	66	39	0	59	
	39	0007/21	++	33	19	-33	79	
	40	0154/21	+	66	45	0	68	
		<b>Total</b>		<b>320</b>	<b>206</b>	<b>-66</b>	<b>70*</b>	
2005	41	Negative	--	36	36	-36	100	29 VI <sup>(a)</sup>
	42	0216/18	+/-	108	52	36	22	
	43	0115/26	++	36	32	-36	94	
	44	0051/33	++	36	32	-36	94	
	45	0077/21	+	72	32	0	44	
	46	0208/8	++	29	29	-29	100	
		<b>Total</b>		<b>317</b>	<b>213</b>	<b>-108</b>	<b>76*</b>	
2006	47	0215/23	+/-	111	50	37	18	37
	48	180/24	+	74	40	0	54	
	49	Negative	--	37	21	-37	78	
	50	0235/17	+/-	111	24	37	-18	
	51	0077/21	+	74	47	0	64	
	52	0082/27	++	37	25	-37	84	
		<b>Total</b>		<b>444</b>	<b>207</b>	<b>0</b>	<b>47*</b>	
2007	53	0155/18	+/-	96	72	32	63	32
	54	0143/26	+	64	60	0	94	
	55	0086/27	++	32	28	-32	94	
	56	0225/29	++	32	32	-32	100	
	57	0001/7	++	32	32	-32	100	
	58	Negative	--	32	32	-32	100	
		<b>Total</b>		<b>288</b>	<b>256</b>	<b>-96</b>	<b>92*</b>	

a) In 2004 and 2005, virus isolation was performed by fewer laboratories  
 - negative serum; medium  
 -- negative serum; easy

+ positive serum; medium  
 ++ positive serum; easy  
 +/- positive serum; difficult



**Table III**  
**Results of sera included several times in the distributed panels**

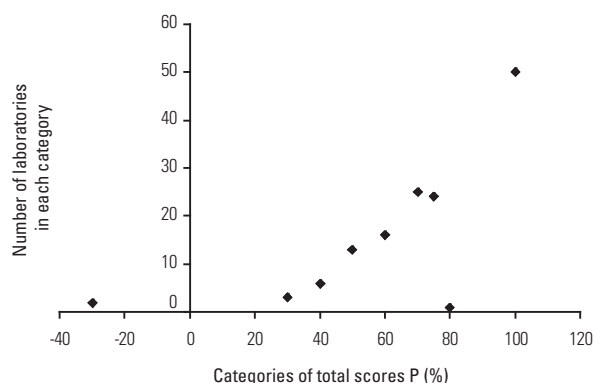
Serum 0002/16 had a low classical swine fever antibody titre and was classified as difficult to diagnose. Serum 0077/21 was classified as medium and serum 0007/21 was classified as easy. Serum 0925/34 and 0015/77 had an antibody titre against other pestiviruses and were classified as medium

Serum	Year	Percentage (P)
0002/16	1999	89
	2000	59
	2001	50
	2002	67
	2003	60
0077/21	1998	100
	2004	59
	2005	44
	2006	64
0007/21	1999	100
	2004	79
0925/34	2001	76
	2002	62
0015/77	2000	46
	2003	50

by genetic typing. Recent field viruses are a more heterogeneous material to handle (17).

In 2004 the purpose of the inter-laboratory comparison test was to imitate real time conditions and the time for sending back the results was shortened. In addition, more laboratories participated and the number of EU Member States increased. The decrease of P to 47% in 2006 seems to be an exception. On investigation, it emerged that several laboratories had not interpreted the results as requested or they relied too much on an enzyme-linked immunosorbent assay (ELISA) result. Commercially available CSF antibody ELISAs are not as sensitive or as specific as the neutralisation test (17). Consequently, they give doubtful or false negative results in sera with low CSF antibody titres and in sera with antibody titres to other pestiviruses. It is very important, therefore, to have the neutralisation test available to verify doubtful CSF antibody ELISA results. Furthermore, it should not be forgotten that if a fresh CSF infection is suspected with low CSF antibody titres in the samples, the neutralisation test is the method of choice. CSF antibody ELISAs are not able to replace the neutralisation test completely.

The sera scoring a P of -18% and +18% both had a borderline CSF antibody titre against CSF0902. In this case the use of a recent field strain for the performance of the neutralisation test could have been favourable, as mentioned above.



**Fig.1**  
**The distribution of the total scores of the 140 laboratories that participated in the inter-laboratory comparison tests for classical swine fever diagnosis, 1998 to 2003**

By 2007, most participants had begun to use the prescribed reference methods. Consequently, only those methods were evaluated and P increased to the best results in 10 years and the result of 2006 should not be overemphasised.

Serum 0002/16, which has a low CSF antibody titre, was included in five consecutive years, and the best P result was obtained the first time it was included, when there was still little emphasis on differential diagnosis for BVDV and BDV. Antibodies produced from CSF virus isolates belonging to genotype 2.3 cause more cross-reactions to ruminant pestiviruses than antibodies produced from CSF virus isolates and reference strains belonging to genotype 1 (unpublished data). Furthermore, the decrease of P in the following years can be explained by the increased use of CSF antibody ELISAs which scored the serum doubtful (17). This becomes even more evident when looking at serum 0077/21. In 1998, when only the reference methods were used, the total P for all participating laboratories was 100%. This result was not achieved again in any of the years in which it was subsequently used, i.e. 2004, 2005 and 2006. Similar results were obtained with serum 0007/21.

It can be concluded that the best results in the inter-laboratory comparison test for CSF were obtained when only the reference method neutralisation test was performed. The neutralisation test is still the most reliable method for CSF antibody detection. CSF diagnostic laboratories should trust in the CSF antibody titres they obtain in the neutralisation test and not get puzzled by the results of commercial ELISAs. However, the neutralisation test is less suitable for mass diagnostics. The use of CSF antibody ELISAs is a vital element in the laboratory diagnosis of CSF in the framework of monitoring and surveillance programmes. A reduced sensitivity of CSF antibody ELISAs can be overcome by an appropriate sample size and sample choice in accordance with

statistical calculations as, for example, given in the Diagnostic Manual (15).

The tasks of a CSF Reference Laboratory, as defined by EU directive 2001/89, also include providing assistance in the interpretation of laboratory results for epidemiological investigations (14), which is more than is requested by ISO17025 (the principal standard used by testing and calibration laboratories [1]). In the future it might be worth sending out samples with an artificial case history to help in interpreting the results obtained.

Although the first data used in the evaluation of the inter-laboratory comparison tests might be regarded as historical, all results concerning the detection of CSF antibodies are still valid today. No method that could supersede the neutralisation test as the reference method has been introduced since these tests took place.

### Classical swine fever virus detection

Sera containing CSF virus were easy to detect by virus isolation. Different porcine cell lines are available for this method (20). Only sera with a rather low CSF virus titre caused difficulties. However, it is not easy to offer a serum with a consistently low CSF virus titre after the process of lyophilisation and shipping. Organ samples are the samples of choice in the field, because the amount of virus in organ samples is higher than in serum samples. But for the purpose here the results on serum samples can also be interpreted, because a laboratory which is able to detect CSF virus in serum has appropriate facilities for cell culture for CSF virus growth and suitable antibodies for staining the cell culture (a certain number of laboratories still do not have these). Otherwise it would not be possible to obtain any indication of a laboratory's ability to detect CSF virus detection.

The serum with an unknown BVDV, which grew well in porcine cells, was diagnosed correctly as non-CSF (P of 100%). It can be concluded that virus isolation is a well established method and false results due to the use of non-specific methods, detecting pestiviruses only and not differentiating between CSF virus and BVDV, are unlikely. In addition, the development of RT-PCR during the decade described here was fast, starting with pestivirus-specific conventional RT-PCR and gel electrophoresis. Today, real-time RT-PCR using CSF-specific primers and probes is already well established. Consequently, RT-PCR is on the way to superseding virus isolation as a reference method (2, 9, 21). Negative sera from pigs which never had contact with any pestivirus achieved most scores of P 100%. False positive results in CSF diagnosis are very unlikely.

In general, laboratory diagnosis of CSF is taken seriously, as indicated by the increasing number of participants from EU Member States, candidate countries and third countries. The majority of Reference Laboratories are now accredited to ISO 17025. The documentation required by accreditation also helps troubleshooting when errors occur. However, laboratories which scored unsatisfactory results can obtain new reference material from the CRL, participate in a workshop at the CRL or even send a scientist or technician to the CRL for a longer stay. All these possibilities have been offered and used regularly over the years and have contributed to the success of CSF diagnosis.

For the future the following amendments to the inter-laboratory comparison test could be useful:

- an artificial case history to help interpret laboratory results in epidemiological investigations could help a national CSF Reference Laboratory meet its responsibilities for inter-laboratory comparison tests, as requested by EU directive (14). This should also include the results of RT-PCR;
- a request for results in a shorter period of time to imitate the time pressures of field conditions;
- evaluation of the correlation of errors in the inter-laboratory comparison test with the field experience of the laboratory in CSF diagnosis.

However, any so far unknown problem of CSF diagnosis which might occur will be taken into account.

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## Évaluation des tests comparatifs inter-laboratoires de niveau international réalisés entre 1998 et 2007 pour le diagnostic de la peste porcine classique

G. Floegel-Niesmann, C. Staubach, S. Blome & V. Moennig

### Résumé

Des tests comparatifs inter-laboratoires portant sur les épreuves de diagnostic de la peste porcine classique (PPC), organisés par le Laboratoire de référence de la Communauté européenne pour cette maladie sont régulièrement conduits au sein des États membres de l'Union européenne. La présente étude visait à évaluer les résultats des tests comparatifs réalisés pendant la décennie de 1998 à 2007, en les soumettant à une analyse statistique. Pour ce faire, les laboratoires participants ont reçu un pool de cinq ou six sérums lyophilisés. Les pools contenaient des sérums possédant des anticorps dirigés contre la PPC, des sérums possédant des anticorps dirigés contre des pestivirus de ruminants, des sérums contenant le virus de la PPC et des sérums négatifs.

L'étude s'est principalement intéressée aux résultats des méthodes de référence pour le diagnostic de la PPC : l'épreuve de neutralisation pour la détection des anticorps dirigés contre la PPC (y compris l'interprétation des résultats) et l'isolement viral. En ce qui concerne les méthodes de détection des anticorps, les résultats les plus proches de ceux attendus par le Laboratoire communautaire de référence étaient ceux obtenus en recourant uniquement à la neutralisation. Le pourcentage de résultats corrects diminuait lorsqu'on ajoutait ceux obtenus au moyen de l'épreuve immuno-enzymatique pour la détection d'anticorps, ou lorsqu'on ajoutait au panel de sérums ceux contenant des anticorps dirigés contre les pestivirus de ruminants. Les résultats d'évaluation des méthodes de détection des anticorps dirigés contre la PPC sont encore valables aujourd'hui car aucune autre méthode n'a été introduite récemment. Concernant les méthodes de détection du virus de la PPC, la méthode d'isolement viral est bien établie mais sera bientôt remplacée par l'amplification en chaîne par polymérase couplée à une transcription inverse en tant qu'épreuve de référence.

### Mots-clés

Diagnostic de laboratoire – Diagnostic différentiel – Essai d'aptitude – Gestion de la qualité – Isolement viral – Laboratoire de référence – Neutralisation croisée – Pestivirus – Test comparatif inter-laboratoires – Test de neutralisation – Virus de la peste porcine classique.



## Evaluación de pruebas internacionales de comparación entre laboratorios con respecto al diagnóstico de la peste porcina clásica entre 1998 y 2007

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### Resumen

Dentro de los Estados Miembros de la Unión Europea se llevan a cabo periódicamente pruebas organizadas por el Laboratorio comunitario de referencia para la peste porcina clásica (PPC) para evaluar y comparar entre sí

el desempeño de los laboratorios en el diagnóstico de la enfermedad. Los autores describen un estudio destinado a valorar los resultados de las pruebas de comparación entre laboratorios realizadas en el último decenio, entre 1998 y 2007, utilizando para ello un planteamiento estadístico. Los participantes en el estudio recibieron una batería de cinco o seis sueros liofilizados, entre ellos: sueros con anticuerpos contra la PPC, sueros con anticuerpos contra pestivirus de rumiantes, sueros con virus de la PPC y sueros negativos.

El análisis se concentró en los métodos de referencia para el diagnóstico de la PPC, a saber: la prueba de neutralización para detectar anticuerpos contra la enfermedad (lo que incluye su interpretación) y el aislamiento del virus de la PPC. Por lo que respecta a la detección de anticuerpos, los resultados más próximos a lo esperado por el Laboratorio comunitario de referencia se obtuvieron al realizar únicamente pruebas de neutralización. El porcentaje de resultados correctos bajaba en cuanto se incluían los resultados del ensayo inmunoenzimático de detección de anticuerpos anti-PPC o en cuanto se añadían a la batería sueros con anticuerpos contra pestivirus de rumiantes. Los resultados relativos a la detección de anticuerpos anti-PPC siguen siendo válidos hoy en día, pues en fechas recientes no se ha introducido ningún otro método. Por lo que respecta a la detección de virus de la PPC, aunque el aislamiento del virus es un método ya tradicional, se empieza a ver desplazado, como prueba de referencia, por la reacción en cadena de la polimerasa (PCR) acoplada a transcripción inversa.

#### Palabras clave

Aislamiento de virus – Diagnóstico diferencial – Diagnóstico de laboratorio – Evaluación de la capacidad – Gestión de calidad – Laboratorio de referencia – Neutralización cruzada – Prueba de comparación entre laboratorios – Prueba de neutralización – Pestivirus – Virus de la peste porcina clásica.



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