*Rev. sci. tech. Off. int. Epiz.*, 2012, **31** (3), 997-1010

# Evaluation of classical swine fever virus antibody detection assays with an emphasis on the differentiation of infected from vaccinated animals

S. Schroeder [1]*, T. von Rosen [2, 3], S. Blome [4], W. Loeffen [5], A. Haegeman [6], F. Koenen [6] & Å. Uttenthal [2]

(1) Community Reference Laboratory for Classical Swine Fever Virus, Institute for Virology, University of Veterinary Medicine, Buenteweg 17, D-30559 Hanover, Germany
(2) National Veterinary Institute, Technical University of Denmark, Lindholm, DK-4771 Kalvehave, Denmark
(3) Pathobiology Section, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlaegevej 88, 1., DK-1870 Frederiksberg C, Denmark
(4) Institute of Diagnostic Virology, Friedrich-Loeffler-Institute, Südufer 10, D-17493 Greifswald-Insel Riems, Germany
(5) Department of Virology, Central Veterinary Institute of Wageningen UR, P.O. Box 65, NL-8200 AB Lelystad, the Netherlands
(6) Veterinary and Agrochemical Research Centre (VAR-CODA-CERVA), Groeselenberg 99, B-1180 Ukkel, Belgium
*Corresponding author: sabine.schroeder@tiho-hannover.de

**Summary**

The aim of this study was to evaluate the general characteristics of commercially available enzyme-linked immunosorbent assays (ELISAs) to detect antibody against classical swine fever (CSF), as well as to assess their potential use as accompanying marker tests able to differentiate infected from vaccinated animals (DIVA).

The Chekit* CSF-Sero and the HerdChek* CSFV Ab, both of which detect antibodies against the E2 protein of classical swine fever virus (CSFV), had the highest sensitivity. Both tests were practicable and showed good reproducibility. Comparable sensitivity was shown by the Chekit* CSF-Marker, an $E^{rns}$ ELISA. However, this test does not allow differentiation between antibodies directed against ruminant pestiviruses and those against CSFV. Therefore, it is not suitable for use with the chimeric marker vaccines tested.

The PrioCHECK® CSFV $E^{rns}$ was the only ELISA suitable for use in DIVA with marker vaccines containing $E^{rns}$ proteins from ruminant pestiviruses. However, this test was less sensitive and selective than the E2-ELISAs and cannot be recommended.

**Keywords**

Antibody ELISA – Classical swine fever – Differentiation of infected from vaccinated animals – DIVA – Marker test – Sensitivity – Specificity.

## Introduction

Classical swine fever (CSF) is one of the most important epizootic diseases of pigs and wild boar, and it can cause major economic losses in pig production (7). Within the European Union (EU) the disease has been eradicated in domestic pigs with the help of vaccination campaigns in endemic areas, followed by a strict stamping-out policy. Despite the fact that preventive vaccination is prohibited, EU regulations allow the use of emergency vaccinations

when based on an approved plan (10). For emergency vaccination conventional modified live vaccines or marker vaccines may be applied. The advantages of the currently available conventional vaccines are the early onset of immunity and full protection against vertical transmission. Unfortunately, with the use of these vaccines, it is impossible to differentiate infected from vaccinated animals (i.e. to perform DIVA diagnostics) by serological testing. This problem can be overcome with the use of marker vaccines. So far, the only commercially available marker vaccines are subunit vaccines based on the E2 glycoprotein of classical swine fever virus (CSFV). Unfortunately, these vaccines are less protective and the immune response is delayed, when compared with conventional vaccines (3, 5, 27). A new generation of marker vaccine candidates based on chimeric pestiviruses has been developed in order to combine the DIVA strategy with good efficacy (1). Chimeric pestiviruses constructed over recent years consist of CSFV or border disease virus (BDV) E2-encoding sequences inserted into a bovine viral diarrhoea virus (BVDV) backbone (24, 25); alternatively, BVDV or BDV sequences may be inserted into a CSFV backbone (28, 31).

The DIVA principle can only be implemented with the use of a marker test to accompany the vaccine. This test must be able to detect antibodies induced by CSFV infection, even in the presence of vaccine-derived antibodies, in order to detect or rule out CSFV infection in a vaccinated population. Enzyme-linked immunosorbent assays (ELISAs) for the detection of antibodies directed against the E2, $E^{rns}$ or NS3 protein of CSFV are commercially available. The E2-ELISAs are used in the field as conventional screening tests for the detection of CSFV infection on a herd basis (11). The $E^{rns}$ ELISAs were developed as accompanying marker tests for E2 subunit vaccines. Current EU guidelines suggest using these tests only for herd-level testing of domestic pigs, not for individual animals or feral pigs (12, 13). Depending on the construction of the DIVA vaccines, E2, $E^{rns}$ and NS3 ELISAs can all potentially be used as marker ELISAs.

The aim of this study was to evaluate the general characteristics of the commercially available ELISAs and assess their potential as DIVA diagnostic tools. Within a collaborative survey, seven commercially available CSFV antibody ELISAs were compared, including two new or improved ELISAs: the LDL Pigtype® CSFV Ab and the PrioCHECK® CSFV $E^{rns}$. Together with the ELISA kits, 41 randomly allocated serum samples were distributed to the ring trial partners. All participants added positive samples from animal experiments and negative field samples. Altogether more than 13,000 reactions were conducted. The World Organisation for Animal Health (OIE) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* was used to provide guidelines for validation of the tests and determination of assay performance characteristics (33).

# Materials and methods

## Partners in the collaborative survey and evaluation of classical swine fever virus antibody enzyme-linked immunosorbent assays

The collaborative survey was organised by the Community Reference Laboratory (CRL) for CSFV, Hanover, Germany. The four other institutes participating in this study were: the National Reference Laboratories for CSFV at the Central Veterinary Institute of Wageningen UR (CVI) in Lelystad, the Netherlands; the Veterinary and Agrochemical Research Centre (VAR-CODA-CERVA) in Brussels, Belgium; the National Veterinary Institute at the Technical University of Denmark (DTU), in Lindholm, Denmark; the Friedrich-Loeffler-Institute (FLI) on the island of Riems, Germany.

Seven ELISA test kits designed to detect antibodies against CSFV were included in the collaborative survey. Four of them detect antibodies against the CSFV E2 glycoprotein: Chekit* CSF-Sero (IDEXX, Laboratories B.V. Schiphol-Rijk, the Netherlands); HerdChek* CSFV Ab (IDEXX); Pigtype® CSFV Ab (Labor Diagnostik, Leipzig, GmbH Leipzig, Germany); PrioCHECK® CSFV 2.0 (Prionics, Lelystad B.V., the Netherlands). The Serelisa® HCV Ab Mono Blocking (Synbiotics Europe, Lyons, France) detects antibodies to the CSFV NS3 protein. Two kits detect antibodies to the $E^{rns}$ glycoprotein: Chekit* CSF-Marker (IDEXX) and PrioCHECK® CSFV $E^{rns}$ (Prionics). The ELISAs are listed in Table I, together with their abbreviated names used in this study, their suppliers and the types of antibody they detect. All ELISA kits were used according to the manufacturer's instructions. If incubation periods during the day and overnight were both possible, the overnight incubation was carried out.

## Serum samples used in the collaborative survey (section A)

For the collaborative study, 41 serum samples were selected by the CRL, anonymised and distributed to the participants together with the ELISA kits. The serum samples were derived from pigs vaccinated and/or experimentally infected with attenuated live vaccines, commercially available or experimental DIVA vaccines, different CSFV strains, and ruminant pestivirus strains (Table II). In addition, samples free of pestivirus antibodies were included. The animal experiments that produced these samples have been published elsewhere; the references are listed in Table III. The samples were obtained at different times post infection or vaccination to obtain a broad spectrum of different antibody titres. The samples from CSFV-infected animals were produced with various strains to reflect genetic groups from different regions of the world.

**Table I**
**Enzyme-linked immunosorbent assays used in this study and their characteristics**

| Abbreviated name | Chekit E2 | HerdChek E2 | Pigtype E2 | PrioCHECK E2 | Serelisa NS3 | Chekit $E^{rns}$ | PrioCHECK $E^{rns}$ |
|---|---|---|---|---|---|---|---|
| Full name | Chekit* CSF-Sero | HerdChek* CSFV Ab | Pigtype® CSFV Ab | PrioCHECK® CSFV Ab 2.0 | Serelisa® HCV Ab Mono Blocking | Chekit* CSF-Marker | PrioCHECK® CSFV $E^{rns}$ |
| Producer/supplier | IDEXX Laboratories | IDEXX Laboratories | Labor Diagnostik Leipzig | Prionics Lelystad B.V. | Synbiotics Europe | IDEXX Laboratories | Prionics Lelystad B.V. |
| Antibodies detected | E2 | E2 | E2 | E2 | NS3 | $E^{rns}$ | $E^{rns}$ |
| Solid plates/strip plates | Both | Both | Both | Both | Both | Both | Both |
| No. of steps | 3 | 3 | 3 | 3 | 3 | 4 | 4 |
| Dilution of samples | Yes | Yes | No | Yes | Yes | Yes | No |
| Day and/or overnight incubation | Both | Both | Both | Day | Overnight | Day | Day |
| Incubation time (short protocol) | 135 min | 160 min | 130 min | 110 min | 15.5 h | 150 min | 135 min |
| Incubation at room temperature | Yes | Yes | Yes | No, 37°C | Yes | No, 37°C | No, 37°C |
| Reagents that have to be prepared | Wash buffer | Wash buffer | Wash buffer, conjugate | Wash buffer, conjugate | Wash buffer, conjugate | Wash buffer, conjugate, sample diluent | Wash buffer, conjugate dilution buffer, antigen |
| Filter wave-length in nm | 450 | 450 | 450 | 450 | 450 | 405 | 450 |
| Clear instructions | Yes | Yes | Yes | Yes | Yes | No | No |

For all samples, the titres of neutralising antibodies against the homologous virus and against CSFV strain Alfort 187 (4), as well as antibody titres against BVDV-1 strain NADL (23), BVDV-2 strain Munich-2 (2), and BDV strain Moredun (29), were determined in neutralisation tests (NT), which are considered the standard approach (17; the NT data on CSFV Alfort and the homologous viruses are shown in Table II).

## Serum samples added by the partners (section B)

Each of the participating laboratories included between 49 and 54 samples from their national pig herds (a total of 253 samples), which were supposed to be negative for CSFV antibodies, but with an unknown status for the presence of antibodies against ruminant pestiviruses. In addition, approximately 100 well-characterised samples from vaccination and/or infection experiments were tested separately by each partner to ensure wide coverage of different serum samples (Table IV).

## Experimental set-up

For all ELISA kits the following characteristics were calculated as described below: validity, reproducibility, and the percentages of correctly identified positive (sensitivity) and negative samples (specificity). In addition, the practicability of the kits was assessed. Each serum sample was tested in duplicate using all ELISA kits. For ELISA kits that classify results as doubtful, the doubtful results were counted as positive. This was necessary in order to be able to compare the results of different ELISAs and also to reflect use in the field, where positive and doubtful results are usually confirmed using a second test (often NT).

The validity of the ELISA kits was calculated using the number of ELISA plates with valid results as a percentage of all plates tested: percentage validity = (valid plates × 100)/tested plates. Valid results were achieved when the results for the standard sera were within the acceptance criteria.

During an outbreak of CSF it is sometimes necessary to perform an ELISA under emergency field conditions. Hence, the practical aspects of handling the different CSFV antibody ELISA kits were evaluated, on the basis of the test characteristics (Table I). Practical aspects of particular interest were how long it took to perform the test, how simple it was to use, and whether or not it had a high sample throughput without special equipment.

The reproducibility, i.e. the inter-laboratory repeatability, was calculated from the results of the 41 samples used in

**Table II**

**Serum samples distributed to the ring trial partners (section A)**

Strain identification (ID) and genotype designation are in accordance with the CSFV database at the European Central Reference Laboratory

| ID No. | Vaccine strain name | gt | Virus strain name and ID | gt | dpv/dpi | NT Alf187 | NT homologue |
|---|---|---|---|---|---|---|---|
| 16 | n.a. | n.a. | CSFV 759/Rus CSF0695 | 1.1 | 14 | 30 | 30 |
| 17 | CSFV Riemser C-strain[a] | 1.1 | n.a. | n.a. | 28 | 120 | 640 |
| 21 | CSFV Riemser C-strain | 1.1 | n.a. | n.a. | 42 | 240 | 640 |
| 10 | CSFV Riemser C-strain | 1.1 | CSFV Behring CSF0927 | 1.1 | 58/30 | > 640 | > 640 |
| 30 | CSFV GPE$^{-}$[a] | 1.1 | n.a. | 1.1 | 35+42[b] | 480 | 480 |
| 15 | CSFV GPE$^{-}$ | 1.1 | CSFV Uelzen CSF0634 | 2.3 | 43/27 | > 640 | > 640 |
| 22 | n.a. | 1.3 | CSFV Guatamala CSF0650 | 1.3 | 27 | 240 | 640 |
| 28 | CSFV BAYOVAC®[c] | 1.2 | n.a. | n.a. | 14[b] | 7.5 | 7.5 (Alf187) |
| 9 | CSFV BAYOVAC® | 1.2 | n.a. | n.a. | 377 | > 640 | > 640 (Alf187) |
| 38 | CSFV BAYOVAC® | 1.2 | CSFV Paderborn CSF0277 | 2.1 | 60/45 | > 640 | > 640 (Alf187) |
| 7 | n.a. | n.a. | CSFV Paderborn CSF0277 | 2.1 | 21 | 30 | 480 |
| 33 | n.a. | n.a. | CSFV Paderborn CSF0277 | 2.1 | 42 | 80 | 640 |
| 6 | n.a. | n.a. | CSFV Uelzen CSF0621 | 2.3 | 41 | 240 | > 640 |
| 35 | n.a. | n.a. | CSFV Spreda CSF0854 | 2.3 | 27 | < 5 | 15 |
| 14 | n.a. | n.a. | CSFV Uelzen CSF0634 | 2.3 | 23 | 10 | 60 |
| 12 | n.a. | n.a. | CSFV Uelzen CSF0634 | 2.3 | 23 | 20 | 640 |
| 13 | n.a. | n.a. | CSFV Uelzen CSF0634 | 2.3 | 28 | 20 | 640 |
| 2 | n.a. | n.a. | CSFV Kanagawa CSF0309 | 3.4 | 24 | 30 | 320 |
| 1 | CSFV FLc9[d] | 1.1 | n.a. | n.a. | 35[b] | < 5 | n.d. |
| 37 | CSFV FLc9 | 1.1 | n.a. | n.a. | 93 | 10 | n.d. |
| 26 | CSFV FLc9 | 1.1 | CSFV Paderborn CSF0277 | 2.1 | 74/49 | 480 | 320 |
| 29 | CSFV FLc11[e] | 1.1 | n.a. | n.a. | 21[b] | 15 | 480 |
| 36 | CSFV FLc11 | 1.1 | n.a. | n.a. | 93 | 640 | > 640 |
| 39 | CSFV FLc11 | 1.1 | CSFV Bresia CSF0905 | 1.2 | 56/42 | > 640 | > 640 |
| 11 | CSFV pRiemsABCgif [f] | 1.1 | n.a. | n.a. | 43 | < 5 | 160 (BDV Gifhorn) |
| 18 | CSFV pRiemsABCgif | 1.1 | n.a. | n.a. | 71 | 20 | 640 (BDV Gifhorn) |
| 23 | CSFV pRiemsABCgif | 1.1 | n.a. | n.a. | 71 | 30 | > 640 (BDV Gifhorn) |
| 4 | CSFV pRiemsABCgif | 1.1 | CSFV Kozlov CSF0382 | 1.1 | 36/13 | 480 | > 640 (Kozlov) |
| 42 | n.a. | n.a. | BVDV NADL | 1 | 69 | < 5 | 15 |
| 43 | n.a. | n.a. | BVDV NADL | 1 | 69 | 5 | 15 |
| 40 | n.a. | n.a. | BVDV CS-8644 | 2 | 44 | < 5 | n.d. |
| 20 | n.a. | n.a. | BVDV CS-8644 | 2 | 72 | 7.5 | n.d. |
| 32 | BVDV CP7_E2alf[g] | 1 | n.a. | n.a. | 28 | 40 | 40 (Alfort187) |
| 41 | BVDV CP7_E2alf | 1 | n.a. | n.a. | 35 | 320 | 320 (Alfort187) |
| 8 | BVDV CP7_E2alf | 1 | CSFV Kozlov CSF0382 | 1.1 | 36/13 | > 640 | > 640 (Kozlov) |
| 5 | BVDV CP7_E2gif[h] | 1 | n.a. | n.a. | 21[b] | < 5 | 30 (BDV Gifhorn) |
| 34 | BVDV CP7_E2gif | 1 | CSFV Eystrup CSF0910 | 1.1 | 43/15 | 120 | > 640 (Eystrup) |
| 27 | n.a. | n.a. | BDV 137/4 | 1 | 44 | < 5 | n.d. |
| 25 | n.a. | n.a. | BDV 137/4 | 1 | 44 | 7.5 | n.d. |
| 31 | n.a. | n.a. | Negative serum | n.a. | n.a. | < 5 | n.d. |
| 24 | n.a. | n.a. | Negative serum | n.a. | n.a. | < 5 | n.d. |

Alf187: Alfort187
BDV: border disease virus
BVDV: bovine viral diarrhoea virus
CSFV: classical swine fever virus
dpi: days post infection
dpv: days post vaccination
gt: genotype
n.a.: not applicable
n.d.: not done
NT: virus neutralisation test
a) a vaccine based on a live attenuated virus
b) pooled samples
c) a vaccine based on an E2 subunit
k–o: chimeric differentiation of infection from vaccination (DIVA) vaccines constructed as follows:
d) classical swine fever virus (CSFV) backbone and E2 from bovine viral diarrhoea virus (BVDV)-2
e) CSFV backbone and $E^{rns}$ from BVDV-2
f) CSFV backbone and E2-ABC domain from border disease virus (BDV)
g) BVDV-1 backbone and E2 from CSFV
h) BVDV-1 backbone and E2 from BDV

the collaborative survey (section A). The reproducibility of each test is given as the percentage of the 41 samples that achieved the same result in that test in all laboratories (including only valid results): percentage reproducibility = number of samples × number of ELISA test runs (using the same kit in different laboratories) with the same results × 100/number of samples × total number of ELISA test runs (using the same kit in different laboratories).

The percentage of correctly identified positive results for each ELISA kit was calculated for different categories of samples, i.e. different viruses or vaccines, separately for section A as well as for section B and, in addition, for all samples in the collaborative survey independent of their category. The percentage of correctly identified negative results for each ELISA kit was calculated in the same way. Correct positive and negative results depend

**Table III**
**Percentages of correctly identified positives (pos.) and negatives (neg.) calculated for samples distributed within an inter-laboratory comparison test (section A)**

| Category | No. of samples | dpv | dpi | Criterion | Chekit E2 | Herd-chek E2 | Pigtype E2 | Prio-CHECK E2 | Serelisa NS3 | Chekit $E^{rns}$ | PrioCHECK $E^{rns}$ | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CSFV (different strains) | 10 | n.a. | 14–42 | % pos. | 95 | 90 | 74 | 77 | 52 | 90 | 68 | |
| BVDV-1 NADL, BVDV-2 CS-8644, BDV 137/4 | 6 | n.a. | 44–72 | % neg. | 92 | 97 | 89 | 100 | 17 | 0 | 54 | (16, 30, 32) |
| C-strain, $GPE^-$ | 3 | 28–42 | n.a. | % pos. | 100 | 93 | 93 | 100 | 17 | 87 | 42 | (18, 19) |
| C-strain + Behring*; $GPE^-$ + VI3837/38 | 2 | 58; 43 | 30; 27 | % pos. | 100 | 100 | 100 | 100 | 38 | 100 | 88 | (18, 19) |
| E2 subunit | 2 | 14; 377 | n.a. | % pos. | 100 | 90 | 60 | 100 | | | | (3) |
| | | | | % neg. | | | | | 100 | 100 | 100 | |
| E2 subunit + V1240/97 | 1 | 60 | 45 | % pos. | 100 | 100 | 100 | 100 | 100 | 100 | 75 | (3) |
| CP7_E2alf | 2 | 28; 35 | n.a. | % pos. | 100 | 100 | 10 | 100 | | | | (25) |
| | | | | % neg. | | | | | 100 | 0 | 100 | |
| CP7_E2alf + Kozlov | 1 | 36 | 13 | % pos. | 100 | 100 | 100 | 100 | 100 | 100 | 75 | (25) |
| Flc11 | 2 | 21; 93 | n.a. | % pos. | 100 | 90 | 90 | 100 | 50 | | | (28) |
| | | | | % neg. | | | | | | 0 | 75 | |
| Flc11 + Brescia | 1 | 56 | 42 | % pos. | 100 | 100 | 100 | 100 | 100 | 100 | 25 | (28) |
| CP7_E2gif | 1 | 21 | n.a. | % neg. | 75 | 100 | 100 | 100 | 100 | 0 | 100 | (24) |
| CP7_E2gif + Eystrup | 1 | 43 | 15 | % pos. | 100 | 100 | 100 | 100 | 100 | 100 | 25 | (24) |
| pRiems_ ABCgif | 3 | 43–71 | n.a. | % pos. | | | | | 67 | 47 | 33 | (31) |
| | | | | % neg. | 58 | 60 | 40 | 44 | | | | |
| pRiems_ABCgif + Kozlov | 1 | 36 | 13 | % pos. | 100 | 100 | 100 | 100 | 0 | 100 | 75 | (31) |
| Flc9 | 2 | 35; 93 | n.a. | % pos. | | | | | 12 | 60 | 88 | (28) |
| | | | | % neg. | 100 | 90 | 100 | 100 | | | | |
| Flc9 + V1240/97 | 1 | 74 | 49 | % pos. | 100 | 100 | 100 | 100 | 75 | 100 | 50 | (28) |
| Negative samples | 2 | n.a. | n.a. | % neg. | 100 | 100 | 90 | 100 | 100 | 100 | 100 | |
| Summary, all samples: | | | | | | | | | | | | |
| Samples with CSFV antibodies | | | | % pos. | 98 | 94 | 86 | 91 | 52 | 85 | 59 | |
| Samples with antibodies against BVDV-1, BVDV-2, BDV | | | | % neg. | 83 | 87 | 79 | 86 | 44 | 0 | 70 | |
| Samples with no pestivirus antibodies | | | | % neg. | 100 | 100 | 90 | 100 | 100 | 100 | 100 | |

dpi: days post infection
dpv: days post vaccination
n.a.: not applicable
*challenge infections were added with a + sign
% pos: sensitivity
% neg: specificity
BDV: border disease virus
BVDV: bovine viral diarrhoea virus
CSFV: classical swine fever virus

on the specifications of the test and the virus or vaccine used. Given that the samples did not represent a realistic distribution in the field, and that the same samples were tested several times in different laboratories, these percentages cannot be regarded as absolute estimates of diagnostic sensitivity and specificity, but can be used for comparative purposes. If false positives are the result of cross-reactivity with antibodies against ruminant pestiviruses, this reflects in particular a lack of selectivity.

**Table IV**
**Percentages of correctly identified positives (pos.) and negatives (neg.) for classical swine fever virus (CSFV) antibody enzyme-linked immunosorbent assays (ELISAs), calculated from samples added individually by the partners (section B)**

| Category | No. of samples in each category | dpv | dpi | Criterion | Chekit E2 | Herd-Chek E2 | Pigtype E2 | Prio-CHECK E2 | Serelisa NS3 | Chekit $E^{rns}$ | Prio-CHECK $E^{rns}$ | Partner |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CSFV | 24 | n.a. | 14–65 | % pos. | 100 | 100 | 100 | 83 | n.d. | 100 | 58 | CRL |
| BVDV, BD | 6 | n.a. | 34–90 | % neg. | 100 | 100 | 33 | 100 | n.d. | 17 | 83 | CRL |
| C-strain | 20 | 11–49 | n.a. | % pos. | 95 | 70 | 55 | 15 | 25 | 70 | 10 | CODA |
| E2 subunit + challenge[a] | 84 | 14–330 | 21; 42[b] | % pos. | 100 | 100 | 99 | 100 | 9 | 85 | 56 | DTU, CVI |
| CP7_E2alf | 82–128[c] | 7–98 | n.a. | % pos. | 86 | 85 | 72 | 62 | | | | CRL, CODA, FLI |
| | | | | % neg. | | | | | 97 | 29 | 96 | |
| Flc11 + challenge | 15 | 7–28 | 21 | % pos. | 100 | 100 | 100 | 100 | 67 | 100 | 53 | CVI |
| CP7_E2gif + challenge | 16–20[g] | 28 | 3–42 | % pos. | 95 | 60 | Invalid[d] | 62 | 65 | 100 | 33 | DTU |
| pRiems_ ABC gif | 37 | 7–71 | n.a. | % pos. | | | | | 17 | 11 | 5 | CRL |
| | | | | % neg. | 95 | 92 | 63 | 89 | | | | |
| Flc9 + challenge | 33 | 7–28 | 21 | % pos. | 97 | 100 | 76 | 97 | 64 | 100 | 82 | CVI |

a) challenge infections were added with a + sign
b) also contact animals
c) number was different for different tests
d) invalid: the plate used failed to meet the validation criteria
BD: border disease
BVDV: bovine viral diarrhoea virus
CODA: Centrum voor Onderzoek in Diergeneeskunde en Agrochemie (Veterinary and Agrochemical Research Centre)
CRL: Community Reference Laboratory
CVI: Central Veterinary Institute of Wageningen
dpi: days post infection
dpv: days post vaccination
DTU: Technical University of Denmark
FLI: Friedrich-Loeffler-Institute
n.a.: not applicable
n.d.: not done

# Results

## Evaluation of the general characteristics of the enzyme-linked immunosorbent assays

### Validity

Every participating laboratory tested three to five plates for each ELISA kit: all together between 19 and 22 plates were tested for each kit. All the ELISAs had invalid plates in at least one laboratory (Table V). The percentage validity of the test kits ranged from 77.2% for the PrioCHECK E2 to 95.2% for the HerdChek E2.

The reasons for invalidity varied. For the Chekit E2 and Chekit $E^{rns}$, the difference between the optical densities (OD) of the negative and positive controls was too low. For the Pigtype E2, PrioCHECK E2, and PrioCHECK $E^{rns}$, the average OD of the negative controls was too low. For the Serelisa NS3 and PrioCHECK E2 the inhibition percentage of the positive or low positive control was too low, and for the PrioCHECK $E^{rns}$ the inhibition percentage of the low positive control was too high. One plate of the HerdChek E2 ELISA kit displayed no reaction at all. The results from invalid plates were not used for further evaluation.

### Practicability

An overview of the methodical aspects of the ELISAs and the equipment needed is given in Table I. The Pigtype E2, the HerdChek E2 and the Chekit E2 ELISAs achieved the best results for practicability. They were quick and easy to perform, with variable incubation times and clear instructions, and no need for special equipment. The $E^{rns}$ ELISAs were less practicable.

### Reproducibility

The reproducibility of the different ELISAs ranged between 91.4% and 98.4% (Table VI). The calculation did not reflect the number of laboratories with valid results, and these are therefore also indicated in Table VI. The PrioCHECK E2 test showed the best reproducibility, but valid results could be achieved for this test in only three laboratories. The new ELISAs, PrioCHECK $E^{rns}$ and Pigtype E2, showed the lowest reproducibility, but had valid results in four and five laboratories, respectively.

### Percentage of correctly identified positive and negative samples

*Samples distributed to the partners in the collaborative survey (section A, Table II).*
The percentages of correctly identified positives and negatives calculated using all suitable ring trial samples, independent of their origin, are shown in Table III. The best results for E2-ELISAs with CSFV antibody positive samples were obtained using the Chekit E2 ELISA (98.1% correctly identified) and HerdChek E2 (94.0%). The Chekit $E^{rns}$ test showed the highest percentage among the

**Table V**
**Number of valid plates/number of tested plates for every enzyme-linked immunosorbent assay tested in all participating laboratories, and the resulting percentage validity**

| Laboratory | Chekit E2 | HerdChek E2 | Pigtype E2 | PrioCHECK E2 | Serelisa NS3 | Chekit $E^{rns}$ | PrioCHECK $E^{rns}$ |
|---|---|---|---|---|---|---|---|
| CRL | 5/5 | 5/5 | 5/5 | 5/5 | 4/4 | 5/6 | 3/5 |
| FLI | 3/3 | 3/3 | 2/3 | 3/3 | 0/3 | 2/2 | 3/3 |
| DTU | 2/4 | 4/4 | 2/4 | 2/4 | 4/4 | 3/4 | 3/4 |
| CODA | 4/4 | 5/5 | 4/4 | 2/5 | 4/4 | 4/4 | 4/4 |
| CVI | 4/4 | 3/4 | 4/4 | 5/5 | 4/4 | 4/4 | 5/5 |
| Sum | 18/20 | 20/21 | 17/20 | 17/22 | 16/19 | 18/20 | 18/21 |
| % Validity | 90.0 | 95.2 | 85.0 | 77.2 | 84.2 | 90.0 | 85.75 |

CODA: Centrum voor Onderzoek in Diergeneeskunde en Agrochemie (Veterinary and Agrochemical Research Centre)
CRL: Community Reference Laboratory
CVI: Central Veterinary Institute of Wageningen
DTU: Technical University of Denmark
FLI: Friedrich-Loeffler-Institute

**Table VI**
**Reproducibility and specificity of the classical swine fever virus antibody enzyme-linked immunosorbent assay (ELISA) test kits**

| ELISA | No. of laboratories with valid results | % reproducibility | % specificity[a] | SD[b] |
|---|---|---|---|---|
| Chekit E2 | 4 | 95.7 | 93.7 | 0.033 |
| HerdChek E2 | 5 | 94.1 | 99.2 | 0.016 |
| Pigtype E2 | 5 | 92.1 | 92.0 | 0.098 |
| PrioCHECK E2 | 3 | 98.4 | 100.0 | 0.000 |
| Serelisa NS3 | 4 | 95.1 | 92.0 | 0.042 |
| Chekit $E^{rns}$ | 5 | 95.6 | 98.8 | 0.008 |
| PrioCHECK $E^{rns}$ | 4 | 91.4 | 99.5 | 0.010 |

a) specificity given as mean value calculated from the individual results of the five participating laboratories
b) standard deviation of specificity

$E^{rns}$- and NS3-ELISAs. The lowest percentages were obtained for the PrioCHECK $E^{rns}$ and Serelisa NS3 tests, with only 58.7% and 51.8% of positive samples correctly identified, respectively.

The highest percentages for E2-ELISAs with samples containing antibodies to ruminant pestiviruses (selectivity) were obtained by HerdChek E2 and PrioCHECK E2 ELISAs. The Chekit $E^{rns}$ test (0% selectivity) was clearly unable to differentiate between antibodies against the $E^{rns}$ protein of CSFV and those of ruminant pestiviruses. According to the producer's test manual the assay is specific for pestiviruses and not for CSFV.

### *Negative samples (section B)*

The percentages of correctly identified negative samples were calculated using samples included by the partners that were obtained from their national pig herds (section B). The percentages were determined for the individual partners and then given as mean values together with the standard deviations (SD; Table VI). The best results were achieved by the PrioCHECK E2, PrioCHECK $E^{rns}$, HerdChek E2 and Chekit $E^{rns}$, with percentages close to 100%.

## Samples from animals infected with classical swine fever virus

The E2-ELISAs Chekit E2 and HerdChek E2 showed the highest percentages of correctly identified positives with samples from CSFV-infected pigs in sections A and B (Tables III and IV). The other E2-ELISAs, Pigtype E2 and PrioCHECK E2, showed problems in detecting samples from animals infected with CSFV genotype 3.4 strain Kanagawa, or did not recognise all CSFV samples with low NT titres (between 1:< 5 and 1:20) against CSFV Alfort 187. The result of the $E^{rns}$ ELISA Chekit $E^{rns}$ was comparable to those of the E2-ELISAs. The lowest percentages of correctly identified positives were shown by the Serelisa NS3 and PrioCHECK $E^{rns}$ tests. With both these ELISAs, false-negative results were obtained when testing sera from animals infected with the older reference strains 759/Ru, Brescia, Eystrup or Guatemala HC/#4409, or those vaccinated with C-strain or $GPE^{-}$. These viruses and vaccine strains belong to the genetic groups 1.1, 1.2 and 1.3.

## Samples from animals infected with bovine viral diarrhoea virus and border disease virus

The highest percentages of correctly identified CSFV-negative samples that contained antibodies against ruminant pestiviruses were achieved by the PrioCHECK E2 and HerdChek E2 tests (Tables III and IV). The NS3 and $E^{rns}$ ELISAs were less selective.

## Samples from animals vaccinated with conventional vaccines

The percentages of correctly identified positive samples from animals that had been vaccinated with GPE⁻ or C-strain, and from those that had undergone vaccination and challenge infection, are also shown in Table III. Most results were comparable to the percentages obtained with samples from CSFV-infected animals. However, the NS3-ELISA Serelisa NS3 detected only 16.7% and 37.5% of these samples, respectively. Samples from GPE⁻ vaccinated and GPE⁻ vaccinated and subsequently challenged animals were not detected at all by this test. On the other hand, the Chekit E2 and PrioCHECK E2 ELISAs detected all of these samples.

All tests showed lower percentages with samples from animals vaccinated with the C-strain in section B (Table IV) in comparison to section A. The reason is that most of the samples were obtained shortly after vaccination. Despite this fact, the Chekit E2 ELISA achieved very good results. The PrioCHECK E2 ELISA was very insensitive when testing these sera.

## Differentiation between infection and vaccination using the enzyme-linked immunosorbent assays

### E2 subunit vaccine

The NS3 and $E^{rns}$ ELISAs could be used as discriminatory ELISAs for animals vaccinated with an E2 subunit vaccine. The percentage of correctly identified negatives was 100% for these tests (Table III). The Chekit $E^{rns}$ achieved the best results after challenge infection. The percentage of correctly identified positives was lower for section B (Table IV) compared with section A and with samples from CSFV-infected animals in general. This is probably because serum samples from contact animals and samples obtained before day 21 post challenge infection were also included in section B.

### CP7_E2alf

The backbone of the new generation live marker vaccine candidate CP7_E2alf is the BVDV-1 strain CP7. The E2 protein of this strain was exchanged with the E2 protein of the CSFV strain Alfort 187. Therefore, the NS3 and $E^{rns}$ ELISAs could theoretically be used as discriminatory ELISAs. Two samples distributed to the partners in the collaborative survey were identified correctly as negative by the Serelisa NS3 and the PrioCHECK $E^{rns}$ tests, while the Chekit $E^{rns}$ test found both samples to be positive (Table III). One serum sample taken from an animal after challenge infection was positive in all laboratories with the Serelisa NS3 and in three out of four laboratories with the PrioCHECK $E^{rns}$ test. For samples from animals vaccinated with CP7_E2alf (section B), the percentage of samples correctly identified as negative was between 96% and 97% for these two tests (Table IV).

### Flc11

For the vaccine candidate Flc11 (CSFV C-strain backbone with $E^{rns}$ protein from BVDV-2), the $E^{rns}$ tests could theoretically be used as discriminatory ELISAs. Two serum samples from animals vaccinated with this construct were tested and should have been negative in the $E^{rns}$ tests. However, the PrioCHECK $E^{rns}$ was positive for one of these two samples in two out of four laboratories (Table III). The Chekit $E^{rns}$ test was positive for both samples in all laboratories. One serum sample taken from an animal after challenge infection was correctly identified as positive in only one out of four laboratories with the PrioCHECK $E^{rns}$ test. With 15 more samples, taken after vaccination and challenge infection, tested by CVI, the percentage of correctly identified positives was 53.3% for the PrioCHECK $E^{rns}$ test (Table IV).

### CP7_E2gif

All the ELISAs tested, the E2-, $E^{rns}$- and NS3-ELISAs, could theoretically be used as marker tests for the modified live vaccine candidate CP7_E2gif (BVDV-1 strain CP7 with the E2 protein of BDV strain Gifhorn).

With one available sample taken after vaccination, the ELISAs were correctly negative in all laboratories, with the exception of the Chekit E2 (one out of four positive) and the Chekit $E^{rns}$ ELISAs (all five positive; Table III). The sample taken after challenge infection was positive with all ELISA kits in all laboratories, except with the PrioCHECK $E^{rns}$ (three out of four negative). Additionally, 20 samples from animals vaccinated with CP7_E2gif and subsequently challenge infected with CSFV strain Eystrup on day 28 after vaccination were tested (section B). The percentages of correctly identified positives ranged from 33.3% to 100% (Table IV). It should be noted that some samples were obtained shortly after challenge infection, when high antibody titres would not be expected.

### pRiems_ABCgif

For the new generation live marker vaccine candidate pRiems_ABCgif (the ABC domain of the E2 protein of CSFV vaccine strain 'C-Riems' exchanged with the same domain of BDV strain Gifhorn), E2-ELISAs targeting the ABC domain could theoretically be used as discriminatory ELISAs. The prerequisite is that these ELISAs differentiate between BDV E2 and CSFV E2 antibodies. The four E2-ELISAs tested only achieved percentages between 40% and 60% for correctly identified negative samples with this construct; three samples without challenge were distributed in the comparison test (Table III). Two samples taken on day 71 after vaccination with pRiems_ABCgif,

which were positive in most of the laboratories, showed high NT titres against the homologous BDV strain and low titres against CSFV Alfort 187. All E2-ELISAs were able to detect a sample from an animal vaccinated and subsequently challenged.

With the 37 samples from animals vaccinated with pRiems_ABCgif in section B, the selectivity of the E2-ELISAs was considerably higher (Table IV). This is probably because a large number of samples taken shortly after vaccination were included.

### Flc9

The percentage of correctly identified negatives was found to be 90% for the HerdChek E2 and 100% for the other three E2-ELISAs with two samples taken after vaccination with the Flc9 vaccine candidate (CSFV C-strain backbone with E2 protein of BVDV-2). After challenge infection, all E2-ELISAs were able to detect this sample as positive (Table III). With 33 additional samples from animals vaccinated with Flc9 and infected subsequently with different strains on days 7, 14 and 28 post vaccination (section B), the results could be stated more precisely. The Chekit E2 and the PrioCHECK E2 showed 97.0% correctly identified positives, the HerdChek E2 100% and the Pigtype 75.8% (Table IV).

# Discussion and conclusion

## General characteristics of the classical swine fever virus antibody enzyme-linked immunosorbent assays

Within the collaborative survey the validity of the ELISAs was in general low to moderate. Every ELISA kit had one or more invalid plates, sometimes in as many as three of the five participating laboratories. The validity needs to be improved if the tests are to be used for routine purposes, to avoid the need to retest a large number of plates. It was observed that tests that were routinely used in a laboratory had fewer invalid plates in that particular laboratory. The practicability of the tests may have also influenced the validity in this study. Two tests that were quick and easy to perform, the HerdChek and the Chekit Sero, also achieved the best results in the validity evaluation. However, other factors such as the stability of the manufacturers' control sera may have influenced the validity. Technical difficulties were the cause of invalidity and false-positive or false-negative results with the Chekit $E^{rns}$ in a previous study (13). In that study it was found that most of the technical difficulties could be avoided by using automatic plate shakers and plate washers, which thereby decreased the practicability.

The practicability of the tests is important for the application of CSFV antibody ELISAs in the field. While all the E2-ELISAs were quick and easy to perform, the NS3-ELISA needs a long incubation time and the $E^{rns}$ ELISAs required preparation of many solutions, of which the stock was often limited.

The results for correctly identified positive samples indicated that the Chekit E2 and the HerdChek E2 had the highest sensitivity among the E2-ELISAs, whereas the PrioCHECK E2 and the Pigtype E2 were less sensitive. Among the $E^{rns}$- and NS3-ELISAs the Chekit $E^{rns}$ was the most sensitive test. Its percentage of correctly identified positives, especially with sera from animals infected with CSFV, was comparable to those of the Chekit E2 and HerdChek E2 ELISAs. This result is in accordance with former studies (13, 15).

In the EU Diagnostic Manual (11) it is stated that ELISAs must be able to detect as positive all serum samples from convalescent pigs that are obtained after 21 days post infection (dpi). When the percentage of correctly identified positives was calculated using only the samples obtained after 21 dpi, no significant difference was seen when compared with the values obtained from the calculations based on all samples (data not shown).

In experiments that involve challenge infection after vaccination, it is important to note that, depending on the success of vaccination, only minor propagation of challenge virus may occur. Therefore, a reduced antibody response may be seen against the immunogenic proteins that are present on the challenge virus but not on the vaccine. As a consequence, the ELISAs may remain negative (6). This may not cause major problems in control and eradication programmes, because former studies have shown that successfully immunised animals are fully protected against clinical signs of CSFV infection, viraemia and shedding of the challenge virus (20, 21, 22, 25).

Based on the number of correctly identified negative samples in a random field population, the PrioCHECK E2, PrioCHECK $E^{rns}$, HerdChek E2 and Chekit $E^{rns}$ seemed to have the highest specificity. However, the PrioCHECK $E^{rns}$ and Chekit $E^{rns}$ tests lacked selectivity as a result of cross-reactivity with antibodies against ruminant pestiviruses. The same outcome was observed in an evaluation study of the Chekit $E^{rns}$ test performed in 2003 (13). This study concluded that the Chekit $E^{rns}$ ELISA could be used as a discriminatory test on a herd basis for animals vaccinated with an E2 subunit vaccine. Nevertheless, it was not recommended that this test be used in regions with a high prevalence of BVDV and BDV antibodies. Therefore, this test is not expected to be used in combination with recombinant pestivirus vaccines.

## Differentiation between infection and vaccination using the classical swine fever virus antibody enzyme-linked immunosorbent assays

The requirements for DIVA antibody ELISAs, especially ELISAs based on $E^{rns}$-differentiation, are higher than those for E2-ELISAs because detection of CSFV infection after vaccination with a DIVA vaccine depends entirely on the discriminatory ELISA. No confirmatory assays are available. Generally, NTs are used to confirm positive results with E2-ELISAs. However, it is not possible to perform NT after vaccination with vaccines containing antigenic domains of CSFV E2. Therefore, false-positive results with $E^{rns}$ ELISAs caused by cross-reactivity with the DIVA vaccine itself or infection with other pestiviruses cannot be excluded. In addition, the detection of genomic material from recombinant DIVA vaccines in blood samples is only possible shortly after vaccination, or not at all, using real-time reverse transcriptase polymerase chain reaction (RT-PCR) (20, 22, 24, 26).

For E2 subunit vaccines, $E^{rns}$- and NS3-ELISAs could potentially be used as DIVA tests. With samples from animals vaccinated with E2 subunit vaccines and subsequently challenged, as well as with samples from CSFV-infected animals, the Chekit $E^{rns}$ test was shown to be much more sensitive than the PrioCHECK $E^{rns}$ and the Serelisa NS3. The Chekit $E^{rns}$ ELISA and the precursor of the PrioCHECK $E^{rns}$ ELISA, the Ceditest CSFV-$E^{rns}$ ELISA, have already been evaluated with E2 subunit vaccines in other studies (13, 14), with similar results. When using these tests in the field, it must be borne in mind that the Chekit $E^{rns}$ ELISA cannot differentiate between antibodies directed against CSFV and those against ruminant pestiviruses.

For the chimeric pestivirus constructs containing $E^{rns}$ proteins of ruminant pestiviruses (CP7_E2alf and FLc11), only the $E^{rns}$ ELISAs could potentially be used as DIVA tests. For CP7_E2gif, $E^{rns}$- as well as E2-ELISAs could be used (see below with regard to E2-ELISAs).

It is known that antigenic epitopes of the NS3 protein are conserved among pestiviruses (8, 9). Therefore, for the Serelisa NS3 test, differentiation between BVDV and CSFV antibodies and potential utility as a DIVA ELISA for CP7_E2alf or CP7_E2gif marker vaccines was not expected. Negative results for animals vaccinated with CP7_E2alf or CP7_E2gif probably occurred as a result of the low sensitivity of this test and not on account of the good selectivity.

Preliminary trials (13), as well as this study, showed that the Chekit $E^{rns}$ ELISA is not specific for CSFV and therefore cannot be used as a DIVA test for chimeric pestiviruses. Hence, the PrioCHECK $E^{rns}$ ELISA is the only candidate for use as an accompanying test for the CP7_E2alf and Flc11 vaccines. However, in the present study the percentages of correctly identified negatives (in samples containing antibodies to ruminant pestiviruses) and positives were low. Former studies with these vaccines were conducted with the Ceditest CSFV-$E^{rns}$ ELISA, a precursor of the PrioCHECK $E^{rns}$ ELISA, which is no longer available (20, 22, 25). A lack of sensitivity was also described for this ELISA (13, 28).

The E2-ELISAs are potential DIVA tests for the chimeric pestivirus constructs that contain antigenic domains of the E2 protein of ruminant pestiviruses (pRiems_ABCgif, Flc9 and CP7E2_gif). After vaccination with these constructs and subsequent CSFV challenge infection, the Chekit E2 ELISA showed the highest percentage of correctly identified positives. Compared with the $E^{rns}$ ELISAs, the E2 ELISAs showed, in general, higher percentages of correctly identified negatives for samples containing antibodies to ruminant pestiviruses (see above). However, for all the E2-ELISAs tested, cross-reactivity was observed with antibodies directed against BDV-E2 Gifhorn in samples obtained 71 days after vaccination with pRiems_ABCgif. Even though the ELISAs showed a higher selectivity when testing additional samples from animals vaccinated with pRiems_ABCgif and CP7_E2gif, the use of the E2-ELISAs tested as DIVA ELISAs for chimeric pestiviruses with E2 proteins of BDV-strain Gifhorn is questionable. Studies by Wehrle *et al.* (31) and an inter-laboratory evaluation within the framework of an EU project (CSF vaccine and wild boar, A. Meindl-Böhmer, personal communication) also showed increasing ELISA values, until they scored positive after a longer period post vaccination with pRiems_ABCgif. However, it must be noted that CP7_E2gif and pRiems_ABCgif contain different parts of the BDV Gifhorn E2 protein. Further analyses are necessary to validate the E2-ELISAs for these chimeric vaccine candidates, and to determine their suitability for testing on a herd level and in individual animals.

The commercially available E2-ELISAs Chekit E2 and HerdChek E2 proved to be sensitive tests, with the Chekit E2 slightly more sensitive and the HerdChek E2 more specific. Furthermore, both tests showed good practicability and reproducibility, making them currently the most promising DIVA ELISA candidates for marker vaccines containing the E2 protein of BVDV.

The $E^{rns}$ ELISA Chekit $E^{rns}$ achieved a percentage of correctly identified positives that was comparable to the two E2-ELISAs mentioned above. The reproducibility of this test was also good. It can be used as a possible DIVA ELISA for E2 subunit vaccines. However, it must be remembered that this test is not specific for CSFV and that it will therefore not be an appropriate DIVA test for chimeric vaccines that contain the $E^{rns}$ of a ruminant pestivirus.

The PrioCHECK E$^{rns}$ ELISA is the only test suitable for use as a DIVA ELISA for the chimeric pestivirus vaccines CP7_E2alf and FLc11. Nevertheless, it was shown that this test has low sensitivity and selectivity and it therefore cannot be recommended in its current version. Further studies are planned within an EU project (CSFV_goDIVA) to provide more data regarding the safety and efficacy of the CP7_E2alf vaccine for market authorisation. In this context additional information on the accompanying ELISA will be provided.

## Acknowledgements

The research leading to these results has received funding from the European Community's Sixth and Seventh Framework Programmes (FP6-2004-Food-3-A, FP7/2007-2013) under grant agreement n° FOOD-CT-2006-016236 and n°227003 CP-FP, respectively. The authors thank the following companies for supplying test kits at reduced prices for this collaborative survey: Labor Diagnostik Leipzig GmbH, Prionics Lelystad B.V., Synbiotics Europe, and IDEXX Laboratories B.V. In addition they thank Andreas Fröhlich from FLI in Wusterhausen for his valuable comments.

# Évaluation des épreuves de détection des anticorps dirigés contre le virus de la peste porcine classique et plus particulièrement de leur intégration potentielle dans une stratégie DIVA (différenciation des animaux infectés et des animaux vaccinés)

S. Schroeder, T. von Rosen, S. Blome, W. Loeffen, A. Haegeman, F. Koenen & A. Uttenthal

**Résumé**

Les auteurs présentent les résultats d'une étude conduite en vue d'évaluer les caractéristiques générales de plusieurs épreuves immuno-enzymatiques (ELISA) disponibles dans le commerce pour la détection des anticorps dirigés contre le virus de la peste porcine classique et de déterminer le potentiel de ces épreuves en tant que tests marqueurs compagnons dans une stratégie DIVA (différencier les animaux infectés des animaux vaccinés).

Les épreuves les plus sensibles étaient les tests Chekit* CSF-Sero et HerdChek* CSFV Ab pour la détection des anticorps dirigés contre la protéine E2 du virus de la peste porcine classique. Elles étaient d'une réalisation aisée et affichaient un bon taux de reproductibilité. L'épreuve Chekit* CSF-Marker, une ELISA utilisant les protéines E$^{rns}$, a fait preuve d'une sensibilité comparable. En revanche, ce dernier test ne permettait pas de différencier les anticorps dirigés contre les pestivirus de ruminants de ceux dirigés contre le virus de la peste porcine classique. Par conséquent, il n'était pas adapté pour accompagner les vaccins marqués chimériques utilisés dans l'étude.

L'épreuve PrioCHECK® CSFV E$^{rns}$ était la seule ELISA adaptée à une stratégie DIVA avec des vaccins marqués contenant des protéines E$^{rns}$ de pestivirus de ruminants. Néanmoins, cette épreuve présentant une sensibilité et une spécificité moindres que les ELISA-E2, son utilisation n'est pas recommandée.

**Mots-clés**

Différenciation des animaux infectés et des animaux vaccinés – DIVA – Épreuve ELISA de détection des anticorps – Peste porcine classique – Sensibilité – Spécificité – Test marqueur.

# Evaluación de las pruebas de detección de anticuerpos contra el virus de la peste porcina clásica, poniendo el acento en la discriminación entre animales infectados y vacunados

S. Schroeder, T. von Rosen, S. Blome, W. Loeffen, A. Haegeman, F. Koenen & A. Uttenthal

**Resumen**

Los autores describen un estudio destinado a evaluar las características generales de los ensayos inmunoenzimáticos (ELISA) disponibles en el mercado para detectar anticuerpos contra la peste porcina clásica (PPC), y a valorar además su posible uso como prueba marcadora complementaria para distinguir entre animales infectados y vacunados.

La mayor sensibilidad la ofrecieron dos pruebas de detección de anticuerpos contra la proteína E2 del virus de la PPC: la *Chekit® CSF-Sero* y la *HerdChek®* CSFV Ab. Ambas eran practicables y ofrecían un buen nivel de reproductibilidad. Parecida sensibilidad se obtuvo con la prueba *Chekit® CSF-Marker*, que es un ELISA de detección de proteínas $E^{rns}$. Pero esta técnica no permite distinguir entre anticuerpos contra pestivirus de rumiantes y anticuerpos contra el virus de la PPC, por lo que su uso no es compatible con el de las eventuales vacunas marcadoras quiméricas ensayadas.

La prueba *PrioCHECK® CSFV* $E^{rns}$ resultó ser el único ELISA adecuado para discriminar entre animales infectados y animales inmunizados con vacunas marcadoras que contienen proteínas $E^{rns}$ de pestivirus de rumiantes. Esta prueba, sin embargo, resultó menos sensible y selectiva que los ELISA-E2, por lo que no cabe recomendarla.

**Palabras clave**

Discriminación entre animales infectados y vacunados – ELISA de detección de anticuerpos – Especificidad – Peste porcina clásica – Prueba marcadora – Sensibilidad.

## References

1. Beer M., Reimann I., Hoffmann B. & Depner K. (2007). – Novel marker vaccines against classical swine fever. *Vaccine*, **25** (30), 5665–5670.

2. Beer M., Wolf G. & Kaaden O.R. (2002). – Phylogenetic analysis of the 5-untranslated region of German BVDV type II isolates. *J. vet. Med., B*, **49** (1), 43–47.

3. Bouma A., de Smit A.J., de Kluijver E.P., Terpstra C. & Moormann R.J. (1999). – Efficacy and stability of a subunit vaccine based on glycoprotein E2 of classical swine fever virus. *Vet. Microbiol.*, **66** (2), 101–114.

4. Dahle J. & Liess B. (1995). – Comparative study with cloned classical swine fever virus strains ALFORT and GLENTORF: clinical, pathological, virological and serological findings in weaner pigs. *Wien. tierärztl. Monatsschr.*, **82**, 232–238.

5. De Smit A.J., Bouma A., van Gennip H.G., de Kluijver E.P. & Moormann R.J. (2001). – Chimeric (marker) C-strain viruses induce clinical protection against virulent classical swine fever virus (CSFV) and reduce transmission of CSFV between vaccinated pigs. *Vaccine*, **19** (11–12), 1467–1476.

6. Depner K.R., Bouma A., Koenen F., Klinkenberg D., Lange E., de Smit H. & Vanderhallen H. (2001). – Classical swine fever (CSF) marker vaccine. Trial II. Challenge study in pregnant sows. *Vet Microbiol.*, **83** (2), 107–120.

7. Edwards S., Fukusho A., Lefèvre P.C., Lipowski A., Pejsak Z., Roehe P. & Westergaard J. (2000). – Classical swine fever: the global situation. *Vet. Microbiol.*, **73** (2–3), 103–119.

8. Edwards S., Moennig V. & Wensvoort G. (1991). – The development of an international reference panel of monoclonal antibodies for the differentiation of hog cholera virus from other pestiviruses. *Vet. Microbiol.*, **29** (2), 101–108.

9. Edwards S., Sands J.J. & Harkness J.W. (1989). – The application of monoclonal antibody panels to characterize pestivirus isolates from ruminants in Great Britain. *Arch. Virol.*, **102** (3–4), 197–206.

10. European Union (EU) (2001). – Council Directive 2001/89/EC of 23 October 2001 on Community measures for the control of classical swine fever. *Off. J. Eur. Communities*, **L316**, 5–35.

11. European Union (EU) (2002). – Commission Decision of 1 February 2002 approving a Diagnostic Manual establishing diagnostic procedures, sampling methods and criteria for evaluation of the laboratory tests for the confirmation of classical swine fever (2002/106/EC). *Off. J. Eur. Communities*, **L039**, 71–88.

12. European Union (EU) (2003). – Commission decision of 5 December 2003 amending Decision 2002/106/EC as regards the establishment of a classical swine fever discriminatory test. *Off. J. Eur. Communities*, **L324**, 55–56.

13. European Union (EU) (2003). – Report on the evaluation of a new classical swine fever discriminatory test (2003/265/EC) (SANCO 10809/2003). European Commission, Directorate-General for Health and Consumer Protection, Brussels, 1–150.

14. Floegel-Niesmann G. (2001). – Classical swine fever (CSF) marker vaccine. Trial III: evaluation of discriminatory ELISAs. *Vet. Microbiol.*, **83** (2), 121–136.

15. Floegel-Niesmann G. (2003). – Marker vaccines and companion diagnostic tests for classical swine fever. *Dev. Biol. (Basel)*, **114**, 185–191.

16. Gutekunst D.E. & Malmquist W.A. (1963). – Separation of a soluble antigen and infectious particles of bovine viral diarrhea viruses and their relationship to hog cholera. *Can. J. comp. Med. vet. Sci.*, **27** (5), 121–123.

17. Hyera J.M., Liess B. & Frey H.R. (1987). – A direct neutralizing peroxidase-linked antibody assay for detection and titration of antibodies to bovine viral diarrhoea virus. *Zentralbl. Veterinärmed., B*, **34** (3), 227–239.

18. Ishikawa K., Nagai H., Katayama K., Tsutsui M., Tanabayashi K., Takeuchi K., Hishiyama M., Saitoh A., Takagi M. & Gotoh K. (1995). – Comparison of the entire nucleotide and deduced amino acid sequences of the attenuated hog cholera vaccine strain GPE– and the wild-type parental strain ALD. *Arch. Virol.*, **140** (8), 1385–1391.

19. Kaden V., Schurig U. & Steyer H. (2001). – Oral immunization of pigs against classical swine fever. Course of the disease and virus transmission after simultaneous vaccination and infection. *Acta virol.*, **45** (1), 23–29.

20. Koenig P., Hoffmann B., Depner K.R., Reimann I., Teifke J.P. & Beer M. (2007). – Detection of classical swine fever vaccine virus in blood and tissue samples of pigs vaccinated either with a conventional C-strain vaccine or a modified live marker vaccine. *Vet. Microbiol.*, **120** (3–4), 343–351.

21. Koenig P., Lange E., Reimann I. & Beer M. (2007). – CP7_E2alf: a safe and efficient marker vaccine strain for oral immunisation of wild boar against classical swine fever virus (CSFV). *Vaccine*, **25** (17), 3391–3399.

22. Leifer I., Lange E., Reimann I., Blome S., Juanolac S., Duranc J.P. & Beer M. (2009). – Modified live marker vaccine candidate CP7 E2alf provides early onset of protection against lethal challenge infection with classical swine fever virus after both intramuscular and oral immunization. *Vaccine*, **27** (47), 6522–6529.

23. Müller T., Depner K.R., Burow J., Ahl R., Conraths F.J. & Moennig V. (1997). – Comparison of different BVD virus strains for their use in the differential diagnosis of classical swine fever: an attempt to standardize neutralization tests. *Dtsch. tierärztl. Wochenschr.*, **104** (3), 91–96.

24. Rasmussen T.B., Uttenthal A., Reimann I., Nielsen J., Depner K. & Beer M. (2007). – Virulence, immunogenicity and vaccine properties of a novel chimeric pestivirus. *J. gen. Virol.*, **88** (2), 481–486.

25. Reimann I., Depner K., Trapp S. & Beer M. (2004). – An avirulent chimeric pestivirus with altered cell tropism protects pigs against lethal infection with classical swine fever virus. *Virology*, **322** (1), 143–157.

26. Tignon M., Kulcsár G., Haegeman A., Barna T., Fábián K., Lévai R., van der Stede Y., Farsang A., Vrancken R., Belák K. & Koenen F. (2010). – Classical swine fever: comparison of oronasal immunisation with CP7E2alf marker and C-strain vaccines in domestic pigs. *Vet. Microbiol.*, **142** (1–2), 59–68.

27. Uttenthal A., Le Potier M.F., Romero L., De Mia G.M. & Floegel-Niesmann G. (2001). – Classical swine fever (CSF) marker vaccine. Trial I. Challenge studies in weaner pigs. *Vet Microbiol.*, **83** (2), 85–106.

28. Van Gennip H.G., van Rijn P.A., Widjojoatmodjo M.N., de Smit A.J. & Moormann R.J. (2000). – Chimeric classical swine fever viruses containing envelope protein E(RNS) or E2 of bovine viral diarrhoea virus protect pigs against challenge with CSFV and induce a distinguishable antibody response. *Vaccine*, **19** (4–5), 447–459.

29. Vantsis J.T., Barlow R.M., Fraser J. & Mould D.L. (1976). – Experiments in border disease. VIII. Propagation and properties of a cytopathic virus. *J. comp. Pathol.*, **86** (1), 111–120.

30. Vilcek S., Nettleton P.F., Paton D.J. & Belák S. (1997). – Molecular characterization of ovine pestiviruses. *J. gen. Virol.*, **78** (4), 725–735.

31. Wehrle F., Renzullo S., Faust A., Beer M., Kaden V. & Hofmann M.A. (2007). – Chimeric pestiviruses: candidates for live-attenuated classical swine fever marker vaccines. *J. gen. Virol.*, **88** (8), 2247–2258.

32. Wolfmeyer A., Wolf G., Beer M., Strube W., Hehnen H.R., Schmeer N. & Kaaden O.R. (1997). – Genomic (5′UTR) and serological differences among German BVDV field isolates. *Acta virol.*, **142** (10), 2049–2057.

33. World Organisation for Animal Health (OIE) (2008). – Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Vol. I. OIE, Paris, 34–45.