PrioCHECK® CSFV Ab
ELISA for in vitro detection of antibodies against Classical Swine Fever Virus in serum and plasma of pigs

5 plate kit for 440 samples
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Version 1.0_e

Component 4
Antigen (lyophilized)
(Reconstitute and dilute before use)
One vial contains 1.5 ml lyophilized Antigen. Shelf life of reconstituted antigen: until expiry date at -20°C.

Component 5
Demineralized Water
One vial contains 10 ml Demineralized Water.

Component 6
Washing Fluid (200x)
(Reconstitute and dilute before use).
One vial contains 60 ml Washing Fluid. Shelf life of washing solution: 1 week at 22±3°C.

Component 7
Reference Serum 1 (Ready-to-use)
One vial contains 1.5 ml Reference Serum 1 (positive control).

Component 8
Reference Serum 2 (Ready-to-use)
One vial contains 1.5 ml Reference Serum 2 (weak positive control).

Component 9
Reference Serum 3 (Ready-to-use)
One vial contains 1.5 ml Reference Serum 3 (negative control).

Component 10
Reference Serum 4 (Ready-to-use)
One vial contains 1.5 ml Reference Serum 4 (negative control).

Component 11
Chromogen (TMB) Substrate (Ready-to-use)
One vial contains 60 ml Chromogen (TMB) Substrate.

Component 12
Stop Solution (Ready-to-use)
One bottle contains 60 ml Stop Solution.

Additional Kit Contents:
- 5 plate sealers
- Package Insert
- Certificate of Analysis

Introduction

The virus of Classical Swine Fever (CSF, also known as hog cholera) is a member of the genus pestivirus (together with Bovine Viral Diarrhoea Virus and Border Disease Virus) in the family Flaviviridae. CSF is a contagious viral disease of pigs that is notifiable (OIE list A) and outbreaks cause severe economic losses in the pig-producing countries. A rapid detection of an infected pig is essential to prevent the virus to spread and to control the disease.

The PrioCHECK® CSFV Ab detects antibodies against high-, moderate- and low virulent CSFV-strains early after infection. Detection of antibodies elicited by low virulent CSFV strains is of paramount importance to trace subclinical CSFV infections.

The PrioCHECK® CSFV Ab combines a convenient and simple test procedure with high sensitivity and specificity. The present design enables easy application for automated ELISA systems (e.g. robots). The PrioCHECK® CSFV Ab is suitable for large-scale screening.

Test Principle

The key reagents, used in the PrioCHECK® CSFV Ab, are monoclonal antibodies (mAb’s) that are directed against different epitopes on the envelope protein E2 (GP-55) of CSFV. The PrioCHECK® CSFV Ab uses the mAb’s to measure the binding of CSFV antibodies in the test sample directed against the E2-protein of CSFV. Test sample, Conjugate and Antigen are dispensed to the wells of a Test Plate and incubated at room temperature (22±3°C). Subsequently, the plates are washed and the Chromogen (TMB) Substrate is dispensed to all wells of the Test Plate. After incubation at 22±3°C the color development is stopped. Co for development measured optically at a wavelength of 450 nm shows the presence of antibodies directed against Classical Swine Fever Virus.

Kit Components

Store kit at 5±3°C until expiry date. See kit label for actual expiry date. The shelf life of diluted, opened or reconstituted components is noted below, when appropriate. Chemical hazard data are available in section “Safety Regulations and R&S Statements” (Appendix II).

Component 1
Test Plate
Five Test Plates are delivered in bags which contain a dessicant sachet.

Component 2
Conjugate (30x)
(30x concentrated, dilute before use)
One vial contains 1.5 ml Conjugate. Diluted conjugate is not stable, prepare just before use.

Component 3
Dilution Buffer (Ready-to-use)
One vial contains 60 ml Dilution Buffer.

Additional Material Required

General:
Laboratory equipment according to national safety regulations.

Analysis of Results:
Plate Reader e.g. Multiscan EX or equivalent. The reader has to have an appropriate filter set to read the plates at 450 nm.

Optional:
Plate washer e.g. Tecan EIA Tray Washer or equivalent.

Test Procedure

Precautions
National guidelines for working with animal samples must be strictly followed. The PrioCHECK® CSFV Ab must be performed in laboratories suited for this purpose. Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated.

Chemical hazard data are available in section “Safety Regulations and R&S Statements” (Appendix II).

Notes
To achieve optimal results with the PrioCHECK® CSFV Ab, the following aspects must be considered:

- The Test Procedure protocol must be strictly followed.
- All reagents of the kit must be equilibrated to room temperature (22±3°C) before use.
- Pipette tips have to be changed for every pipetting step.
- Separate solution reservoirs must be used for each reagent.
- Kit components must not be used after their expiry date or if changes in their appearance are observed.
- Kit components of different kit lot numbers must not be used together.
- Diluted conjugate or water of equal quality must be used for the test.

SOLUTIONS TO BE MADE IN ADVANCE

Conjugate dilution
Dilute Conjugate (30x) (Component 2) 1/30 in Dilution Buffer (Component 3); e.g. for one plate prepare 6 ml (add 0.2 ml Conjugate (30x) to 5.8 ml of Dilution Buffer).

Antigen
Reconstitute the lyophilized Antigen (Component 4) with 1.5 ml Demineralized Water (Component 5). The reconstituted antigen should be aliquoted (12 aliquots for a maximum number of 12 test runs). Avoid multiple freezing and thawing. Aliquotted antigen can be stored in small vials at -20°C until expiry date. Dilute reconstituted antigen 1:30 in Dilution Buffer (Component 3); e.g. for one plate prepare 6 ml (add 0.2 ml reconstituted antigen to 5.8 ml of Dilution Buffer). Can be stored up to 30 minutes at 22±3°C.

Washing solution
The Washing Fluid (200x) (Component 6) must be diluted 1/200 in demineralized water and is sufficient for a final volume of 12 liters of washing solution. Washing solution can be stored 1 week at 22±3°C.

Remark: Inadequate washing of the Test Plate may result in high background. The use of automatic equipment is preferable (not necessary) over washing by multichannel pipettes or over submerging the Test Plate in the washing solution. For all methods it is not necessary to soak the Test Plate in between washings. A minimal volume of 200 µl per well is necessary for adequate washing of the plates.

1The designation E1 for the envelope protein (GP-55) of CSFV has been tentatively changed into E2 by the International Committee for Taxonomy of Viruses (Australia, September, 1995)
INCUBATION OF SERUM, CONJUGATE AND ANTIGEN

1.1 Dispense 50 µl of Reference Serum 1 (Component 7) to wells A1 and B1 of the Test Plate (Component 1).
1.2 Dispense 50 µl of Reference Serum 2 (Component 8) to wells C1 and D1.
1.3 Dispense 50 µl of Reference Serum 3 (Component 9) to wells E1 and F1.
1.4 Dispense 50 µl of Reference Serum 4 (Component 10) to wells G1 and H1.
1.5 Dispense 50 µl test sample to one (single test) or two (duplicate test) adjacent wells of the Test Plate.
1.6 Dispense 50 µl diluted conjugate to all wells.
1.7 Dispense 50 µl diluted antigen to all wells.
1.8 Seal the Test Plate with a plate sealer.
1.9 Shake the plate gently.
1.10 Incubate the Test Plate for 90±5 minutes at 22±3°C.

INCUBATION WITH CHROMOGEN (TMB) SUBSTRATE

2.1 Empty the Test Plate and wash the plate 6 times with 200 to 300 µl washing solution. Tap the plate firmly after the last wash cycle.
2.2 Dispense 100 µl of the Chromogen (TMB) Substrate (Component 11) to all wells.
2.3 Incubate the plate 15 - 20 minutes at 22±3°C.
2.4 Add 100 µl of the Stop Solution (Component 12) to all wells.
2.5 Mix the content of the wells of the plate.

Note: Start the addition of Stop Solution 15 - 20 minutes after the first well was filled with Chromogen (TMB) Substrate. Add the Stop Solution in the same order and the same place as the Chromogen (TMB) Substrate was dispensed.

READING OF THE TEST AND CALCULATING THE RESULTS

3.1 Measure the optical density (OD) of the wells at 450 nm within 15 minutes after color development has stopped.
3.2 Calculate the mean OD$_{450}$ value of Reference Serum 1 (wells A1 and B1 = OD$_{450}$ blank).
3.3 Calculate the corrected OD$_{450}$ of Reference Sera 2, 3 and 4 and of the test samples by subtracting the OD$_{450}$ blank.
3.4 Calculate the percent inhibition (PI) of Reference Sera 2 and 3 of the test samples according to the formula below.

\[
\text{PI} = \frac{100 - \frac{\text{corrected OD}_{450} \text{ test sample}}{\text{corrected OD}_{450} \text{ Reference Serum 4}}} {\times 100}
\]

RESULT INTERPRETATION

Validation criteria

4.1 The mean OD$_{450}$ of Reference Serum 1 (wells A1 and B1 = OD$_{450}$ blank) must be <0.250.
4.2 The corrected mean OD$_{450}$ of Reference Serum 4 must be >1.000.
4.3 The percent inhibition of Reference Serum 2 must be >50%.
4.4 The percent inhibition of Reference Serum 3 must be >50%.
4.5 Not meeting these criteria is reason to discard the results of that specific test run.

Note: If the OD$_{450}$ of a test sample is higher than the OD$_{450}$ of Reference Serum 4, the percent inhibition can be interpreted as 0%. If the corrected mean OD$_{450}$ of Reference Serum 4 is below 1.000 possibly the Chromogen (TMB) Substrate is too cold. In that case prewarm the solution to 22±3°C or incubate up to 30 minutes.

Interpretation of the percent inhibition

\[
\text{PI} = \begin{cases} 
<30\% & \text{(negative)} \\
31\% - 50\% & \text{(inconclusive)} \\
>50\% & \text{(positive)} 
\end{cases}
\]

CSFV-specific antibodies are absent in the test sample.

The test sample should be retested with the PrioCHECK® CSFV Ab. If the PI is again 31%- 50%, test the sample in a neutralization test.

We do recommend retesting sera with an inconclusive or even positive test result. Inconclusive and positive test results should be confirmed in a virus neutralization test for CSFV.

Appendix I

Notice

This manual is believed to be complete and accurate at the time of publication. In no event shall Prionics AG be liable for incidental or consequential damage in connection with or arising from the use of this manual.

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Appendix II

Safety Regulations and R&S Statements

National Safety Regulations must be strictly followed.

R&S Statements

Component 1

Test Plate

Hazard Code: This product is not classified according to EU regulations.

Component 2

Conjugate (30x)

Hazard Code: This product is not classified according to EU regulations.

Component 3

Dilution Buffer (Ready-to-use)

Hazard Code: This product is not classified according to EU regulations.

Appendix III

References


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