

SERELISA[®] HCV Ag Mono Indirect

KIT FOR THE DETECTION FOR THE DETECTION OF HOG CHOLERA VIRUS (HCV) ANTIGEN IN SWINE (INDIVIDUAL)

INDIRECT IMMUNOENZYMATIC TECHNIQUE

192 single well reactions

I. PRINCIPLE OF THE TEST

The SERELISA[®] HCV Ag kit uses a single well indirect immunoenzymatic technique for the detection of an antigen (p125 non structural protein), which possesses at least one epitope common to all strains of Hog Cholera Virus. This screening test is performed directly on whole blood, serum, plasma, leukocytes or tissue extracts, allowing the identification of infected swine at early stage of infection. There are four steps:

1. The controls and samples are placed in wells sensitised with anti-HCV [p125] monoclonal antibodies (Mab). Viral protein present in the sample binds to the specific sites.

2. After a wash step to eliminate the non-associated fractions, an anti-HCV [p125] rabbit antiserum is added. It binds to the antigen previously bound to the solid phase.

3. Following a second wash step, the addition of a goat anti-rabbit Ig/peroxidase conjugate allows the revelation of the rabbit anti-HCV [p125] antiserum forming the following complex: (Mab) - (Ag HCV [p125]) - (Rabbit Ig anti-HCV [p125]) - (Goat Ab anti-rabbit Ig / peroxidase)

4. After a third wash step, the enzyme coupled to the conjugate is revealed by the addition of a substrate which transforms it into a coloured product. The optical densities are recorded and used to determine the presence or absence of the antigen as a function of threshold values obtained through the use of the positive control.

Note: Due to the fact that p125 antigen can be detected during an acute BVD-BD infection, it is advisable to confirm any positive result obtained in this screening test using a specific method for HCV detection (virus isolation and/or immunofluorescence).

II. KIT COMPOSITION AND CONSERVATION

REAGENT NATURE	RECONSTITUTION AND CONSERVATION
2 microplates containing 12 strips of 8 wells sensitised with anti-HCV [p125] monoclonal antibodies	Use within 3 months after opening of the sachet which must be closed after use.
Conjugate: goat anti-rabbit Ig antiserum peroxidase conjugate, (concentrated 10X) (CJ)	Dilute 10 times in the conjugate diluent and use within 24 hrs after dilution.
Anti-HCV [p125] rabbit antiserum, (AS) (concentrated 10X)	To be diluted 10 times in the CD diluent and used within 24 hours after dilution.
Buffered peroxidase substrate (PS)	Ready-to-use.
Negative control (N)	Ready-to-use.
Positive control (P)	Ready-to-use.
Blood sample diluent (SD)	Ready-to-use.
Wash solution (W) (10X concentrated)	Dilute 10 times in distilled or demineralised water. Use within 48 hrs after dilution.
Conjugate and antiserum diluent (CD)	Ready-to-use.
Stop solution (S)	Ready-to-use.
Adhesive films	6 films

Note: Kit and diluted reagents should be stored at + 5°C ± 3°C and used as mentioned above.

III. MATERIALS AND REAGENTS REQUIRED (NOT SUPPLIED)

- Distilled or demineralised water.
- Adjustable or set pipettes to measure and deliver between 0 to 1000 µl. Measurement deviation must be ≤ 10% for volumes ≤10 µl and ≤ 5% for all other volumes.
- Graduated cylinders (100 ml and 1000 ml).
- Manual, automatic or semi-automatic washing device for microtitration plates.
- Microplate reader, fitted with filters for bichromatic reading at 450 and 630 nm. It is also possible to use a monochromatic reader fitted with a 450 nm filter.
- Incubator at +37°C ± 3°C.

IV. PRECAUTIONS FOR USE

The quality of the results depends on the respect of good laboratory practices and the procedure (see paragraph VI).

1. Do not mix or associate reagents from kits with different batch numbers
2. Do not use reagents after the expiry date.
3. Place all reagents at laboratory temperature for at least 1 hour prior to use.
4. Handle all reagents and samples as biohazardous material.
5. Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
6. Never pipette by mouth.
7. Avoid inter sample contamination during sample collection, storage or transport. Use separate disposable pipette tips for each sample.
8. Avoid contamination of the substrate solution with metallic ions, oxidizing agents or detergents. Make sure that all containers are clean. Do not use the same container or the same pipette tip for the conjugate and the substrate.
9. It is recommended to dispose reagents and contaminated material according to the applicable regulations. The safety data sheets for the product are available upon request.

Risk phrases:

- R35: Causes severe burns.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S30: Never add water to the product.
S45: In case of accident or if you feel unwell, seek medical advice immediately.

V. SAMPLES

The test is performed directly on whole blood drawn with an anticoagulant, serum, plasma diluted 1:2, as well as on leukocytes and tissue extracts.

Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Serum, plasma or whole blood drawn with an anticoagulant *	max. 7 days	Yes	No
Leucocytes**	max. 48h	Yes	No
Tissue extracts**	max. 48h	Yes	No

* *Standard sample*

** *The technique for the preparation of leukocytes is described at the end of the leaflet.*

*** *blood clot, lymphoid organs (spleen, lymph nodes, intestine) : the technique for the tissue extract preparation is described at the end of the leaflet.*

VI. PROCEDURE

Strictly comply with the procedure indicated below. Use negative and positive controls in duplicate for each test run, for each plate.

A. PRELIMINARY STEPS

Carefully set up the distribution and identification of controls and samples.

B. TEST PROCEDURE

I - CONTROL AND SAMPLE DISTRIBUTION

1. Control distribution:

Controls are ready-to-use.

After shaking the vials, add 100 µl of negative control (N) to wells A1 and A2, and 100 µl of positive control (P) to wells B1 and B2.

2. Sample distribution:

Serum, plasma and whole blood samples are tested after a dilution 1:2 performed directly in the well, using 100 µl per well: distribute 50 µl of the sample diluent (SD) and add 50 µl of the sample to be tested.

Leukocytes, tissue extracts: distribute 100 µl of the supernatant from the tissue extract without any further dilution.

Samples may be tested individually or in duplicate.

Strips should always be placed on the frame so that both washer and reader can be used.

Cover the wells with adhesive film, cut to the necessary length by the number of strips used.

Mix by gentle shaking the plate manually or by using a plate agitator.

3. Incubation of the plate

Incubate the plate 2 hours ± 5 min. at + 37°C ± 3°C or overnight (14-18 hours) at + 20°C ± 5°C.

WASHING:

Wash buffer: dilute the concentrated washing solution (W) 1:10 in distilled or demineralised water.

Carefully remove the adhesive film and wash 4 times.

Caution: blood provokes the degradation of the substrate. It is essential to control the cleanliness of the wells before adding the buffered substrate and if necessary, to perform two or three additional washes with distilled or demineralised water until all residual trace of blood on the walls or edges of the wells have been eliminated.

II – ADDITION OF ANTISERUM

1. Preparation of antiserum:

Dilute the antiserum (AS) 1:10 with the diluent CD. Quantity of diluted AS required for each strip is 1ml : 100 µl of concentrated anti-serum (AS) plus 0.9 ml of conjugate diluent (CD).

2. Distribution of antiserum:

Add 100 µl of diluted antiserum to all the wells and cover with a new adhesive film.

3. Incubation of antiserum:

Incubate 1 hour ± 5 min at +37°C ± 3°C.

WASHING:

Carefully remove the adhesive film and wash 4 times.

III – ADDITION OF CONJUGATE

1. Preparation of conjugate:

Prepare the conjugate solution by diluting the concentrate (CJ) 1:10 in the conjugate diluent (CD). (1 ml are needed for one strip, meaning 100 µl of CJ diluted in 0.9 ml of CD).

2. Distribution of conjugate:

Add 100 µl of diluted conjugate to all the wells and cover with a new piece of adhesive film.

3. Incubation of conjugate:

Incubate for 1 hour ± 5 min at + 37°C ± 3°C

WASHING:

Carefully remove the adhesive film and wash 4 times.

IV – REVELATION

1. Addition of the substrate:

Add 100 µl of peroxidase buffered substrate (PS) per well. Do not cover with adhesive film at this stage. Mix by gentle shaking the plate manually or by using a plate agitator to ensure correct mixing.

2. Incubation of substrate:

30 min. ± 5 min. at laboratory temperature (+ 20°C ± 5°C), shielded from light.

3. Addition of Stop Solution:

Add 50 µl of stop solution (S) per well.

Mix by gentle shaking the plate manually or by using a plate agitator. Make sure that no bubbles occur in the wells.

4. Measure of the optical density:

Measure the optical density (OD) bichromatically at 450 and 630 nm or monochromatically at 450 nm (in the yellow band).

Reading bichromatically is strongly recommended. Should a monochromatic reader be used, ensure the cleanliness of the bottom of the wells prior to reading.

VII. TEST VALIDATION

The results of each test run are valid if:

- the OD obtained with the positive control (P) is ≥ 0.300 , and

- the OD of the negative control (N) is $< 70\%$ of the OD P

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

Two methods for the calculation and interpretation are possible:

First Method: INDEX CALCULATION

For each sample:

$$\text{Sample index} = 0.5 \times (\overline{\text{OD sample}} - \overline{\text{OD P}})$$

$\overline{\text{OD}}$ = Average of the sample optical densities if the test is performed with duplicate samples.

$\overline{\text{OD P}}$ = Average of the positive control optical densities.

Any blood sample presenting an index ≥ 0 is considered **positive**.

Any blood sample presenting an index $< -0.15 \times \overline{\text{OD P}}$ is considered **negative**.

Any tissue extract, leukocyte, plasma or serum sample presenting an index $\geq 0.1 \times \overline{\text{OD P}}$ is considered **positive**.

Any tissue extract, leukocyte, plasma or serum sample presenting an index $< -0.1 \times \overline{\text{OD P}}$ is considered **negative**.

Doubtful zone:

Any blood sample showing an index value situated in the DOUBTFUL ZONE (comprised between 0 and $-0.15 \times \overline{\text{OD P}}$) should be considered as doubtful. A new test can be performed on leukocytes obtained from the same sample.

Any serum, plasma, tissue extract or leukocyte sample showing an index comprised between $0.1 \times \overline{\text{OD P}}$ and $-0.1 \times \overline{\text{OD P}}$ should be considered as **doubtful**.

Positive and doubtful samples: Any positive or doubtful result is considered indicative of an animal acutely infected with a pestivirus. It is recommended to confirm the result using a method specific for HCV detection (virus isolation and/or immunofluorescence).

Second Metho : ANALYSIS OF OPTICAL DENSITIES

Calculate the OD cut-offs (OD CO x) corresponding to x% of the optical density of the positive control ($\overline{\text{OD P}}$).

OD CO 70 for blood samples, OD CO 120 and OD CO 80 for tissue extract, leukocyte and serum samples.

- Compare each of the ODs obtained for the blood samples to that of the OD CO 70 and the OD P.

- Compare each of the ODs obtained for the serum, plasma, leukocyte or tissue extract samples to that of the OD CO 80 and the OD CO 120.

$$\text{OD CO 70} = 0.70 \times \overline{\text{OD P}}$$

$$\text{OD CO 80} = 0.80 \times \overline{\text{OD P}}$$

$$\text{OD CO 120} = 1.20 \times \overline{\text{OD P}}$$

Result interpretation:

		OD CO 70	$\overline{\text{OD P}}$	Sample OD
Blood	-	+/-		+

		OD CO 80	OD CO 120	Sample OD
Serum, plasma, Tissue extracts, leukocytes	-	+/-		+

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LEUKOCYTE PREPARATION TECHNIQUE FROM WHOLE BLOOD

Materials:

- Blood drawn with an anticoagulant (preferably heparin) not previously frozen.
- 5 ml hemolysis tube
- 5 ml pipette
- Vortex mixer
- Centrifuge
- Hemolysis buffer: NH₄ Cl: 16.6 g
NaH CO₃: 2.0 g
diNa EDTA: 0.185 g
qsp 1000 ml distilled or
demineralised H₂O
pH 7.2 - 7.4

Store at + 5°C ± 3°C.

Method:

- Add 1 volume (2 ml) of hemolysis buffer to the hemolysis tube.
- Add 1 equal volume (2 ml) of blood sample.
- Mix well with the vortex mixer
- Incubate 5-15 minutes at room temperature in order to obtain complete lysis of the red blood cells.
- Centrifuge for 15 minutes at 1000 g.
- Eliminate the supernatant by emptying the tube and gently tapping the tube on absorbent paper.
- Re-suspend the leukocyte pellet by agitation with the vortex mixer in 200 µl of sample diluent (SD).
- Use 100 µl of the leukocyte suspension without further dilution to perform the test.

Reference:

Mignon B., Waxweiler S., Thiry E., Boulanger D., Dubuisson J. and Pastoret P.P.
1992 J. Virol. Methods, 40 ; 85-94.

TECHNIQUE FOR THE PREPARATION OF TISSUE EXTRACTS

Materials:

- Tissues:
 - blood clots (dry tubes) after exudation of the serum
 - organs: spleen, lymph nodes, intestine, lung (preferably choose a lymphoid organ)
- 5 ml hemolysis tube
- Scissors or scalpel
- Vortex
- Centrifuge
- *Sample diluent (SD) provided in the SERELISA® HCV
Ag Mono Indirect kit*

Method:

- Blood clots: discard the serum and add 1 ml of sample diluent (SD) furnished in the kit.
- Organs:
 - Isolate 0.5 to 1.0 cm³ of tissue.
 - Cut up into small pieces (using scissors, or scalpel).
 - Add 1 ml of sample diluent (SD) furnished in the kit.
- Homogenise (vortex).
- Incubate 30 minutes at room temperature (+20°C ± 5°C) preferably with shaking.
- Centrifuge for 15 minutes at 1000 g.
- Recover the supernatant.
- Add 100 µl of the recovered supernatant without any further dilution directly into the well.

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