

SERELISA[®] BVD p80 Ag Mono Indirect

**KIT FOR THE DETECTION OF BVD/MD/BD VIRUS ANTIGEN
IN INDIVIDUAL SAMPLES OF IMMUNOTOLERANT
PERSISTENTLY INFECTED (I.P.I.) RUMINANTS**

INDIRECT IMMUNOENZYMATIC TECHNIQUE

192 single well reactions

I. PRINCIPLE OF THE TEST

The SERELISA[®] BVD p80 Ag Mono Indirect kit uses a single well indirect immunoenzymatic technique for the detection of an antigen (p80/125 non structural protein), which possesses an epitope common to all cytopathogenic and non-cytopathogenic strains of BVD/MD/BD virus. The test is performed directly on serum, plasma or whole blood samples, allowing the identification of immunotolerant persistently infected animals. This test can also be performed on leukocytes and tissue extracts. There are four steps:

1. The controls and samples under test are placed directly in wells sensitised with anti-BVD/MD/BD [p80/125] monoclonal antibodies. Viral protein present in the sample bind to the specific sites.
2. After a wash step to eliminate the non-associated fractions, an anti-BVD/MD/BD [p80/125] 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-BVD/MD/BD [p80/125] antiserum forming the following complex:
(Mab) - (Ag BVD/MD/BD [p80/125]) - (Rabbit Ig anti-BVD/MD/BD [p80/125]) - (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 value obtained through the use of the positive control.

II. KIT COMPOSITION AND CONSERVATION

REAGENT NATURE	RECONSTITUTION AND CONSERVATION
2 microplates containing 12 strips of 8 wells sensitised with anti-BVD/MD/BD [p80/125] 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-BVD/MD/BD [p80/125] 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.
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 serum, plasma or whole blood samples. It can also be performed on leukocytes and tissue extracts. Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Bovine serum, plasma or whole blood drawn with an anticoagulant *	max. 7 days	Yes	No
Whole blood from sheep and goat species	max. 7 days	Yes	No
Leucocytes**	max. 48h	Yes	No
Tissue extracts**	max. 48h	Yes	No

* Standard sample for animal older than 6 months

** As pellets

NB :

- **For animals less than 6 months of age:** the reaction is performed on leukocytes or blood clot extracts (Techniques for the preparation of leukocytes and clot extracts are described at the end of the leaflet).

- **Tissue extracts:** 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: distribute 100 µl of the suspension obtained from the leukocyte pellet without any further dilution.

- 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.

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.

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.

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 optical density (OD) obtained with the positive control is ≥ 0.300 , and
- the optical density (OD) obtained with the negative control is $< 0.50 \times OD P$.

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

Two methods for the calculation and interpretation are possible:

First Method: INDEX CALCULATION

For each sample:

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

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

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

Any SERUM or PLASMA sample presenting an index $\geq -0.15 \times \overline{\text{OD}} \text{ P}$ is considered as positive.

Any SERUM or PLASMA sample presenting an index $< -0.3 \times \overline{\text{OD}} \text{ P}$ is considered as negative.

Any BLOOD sample presenting an index $\geq -0.1 \times \overline{\text{OD}} \text{ P}$ is considered as positive.

Any BLOOD sample presenting an index $< -0.25 \times \overline{\text{OD}} \text{ P}$ is considered as negative.

Any LEUKOCYTE or TISSUE EXTRACT sample presenting an index $\geq 0.1 \times \overline{\text{OD}} \text{ P}$ is considered positive.

Any LEUKOCYTE or TISSUE EXTRACT sample presenting an index $< -0.1 \times \overline{\text{OD}} \text{ P}$ is considered negative.

Doubtful zone:

Any SERUM or PLASMA sample that presents an index situated in the DOUBTFUL ZONE comprised between $-0.15 \times \overline{\text{OD}} \text{ P}$ and $-0.3 \times \overline{\text{OD}} \text{ P}$ should be considered as doubtful. An additional control should be performed either with the clot extract from the same sample or on a sample collected from the same animal at a later date.

Any BLOOD sample that presents an index situated in the DOUBTFUL ZONE comprised between $-0.1 \times \overline{\text{OD}} \text{ P}$ and $-0.25 \times \overline{\text{OD}} \text{ P}$ should be considered as doubtful. An additional control should be performed on the leukocyte preparation of the same sample or on a sample collected from the same animal at a later date.

Any LEUKOCYTE or TISSUE EXTRACT sample that presents an index situated in the DOUBTFUL ZONE comprised between $0.1 \times \overline{\text{OD}} \text{ P}$ and $-0.1 \times \overline{\text{OD}} \text{ P}$ should be considered as doubtful. An additional control should be performed on a leukocyte preparation from the same animal at a later date.

Positive samples:

Any positive result is considered indicative of a persistent viremic animal; two positive results (3-4 weeks apart) are needed to confirm that an animal is persistently infected with a BVD/MD/BD virus.

Note: A positive result may be obtained from a transient viremic animal (and potentially from a recently vaccinated animal with a modified live BVD/MD/BD virus vaccine).

Remark:

- In IPI animals less than six months old, colostral antibody residues in whole blood samples can lead to doubtful or even negative results. In this case, it is necessary to perform the test on LEUKOCYTE or CLOT EXTRACT samples.

- Antibodies present in serum or plasma can lead to doubtful or even negative results in infected animals with mucosal disease. In this case, it is recommended to perform the test on LEUKOCYTE or CLOT EXTRACT samples.

Second Method: ANALYSIS OF OPTICAL DENSITIES

- Calculate the OD cut-offs (OD CO 40, OD CO 50, OD CO 70, OD CO 80 and OD CO 120) corresponding to 40%, 50%, 70%, 80% and 120% of the optical density of the positive control (OD P).

$$\begin{aligned} \text{OD CO 40} &= 0.40 \times \overline{\text{OD}} \text{ P} \\ \text{OD CO 50} &= 0.50 \times \overline{\text{OD}} \text{ P} \\ \text{OD CO 70} &= 0.70 \times \overline{\text{OD}} \text{ P} \\ \text{OD CO 80} &= 0.80 \times \overline{\text{OD}} \text{ P} \\ \text{OD CO 120} &= 1.20 \times \overline{\text{OD}} \text{ P} \end{aligned}$$

- Compare each of the ODs obtained for the serum or plasma samples to that of the OD CO 40 and the OD CO 70

- Compare each of the ODs obtained for the blood samples to that of the OD CO 50 and the OD CO 80.

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

Result interpretation:

	OD CO 40	OD CO 70	Sample OD
Serum / plasma	-	+/-	+
	OD CO 50	OD CO 80	Sample OD
Blood	-	+/-	+
	OD CO 80	OD CO 120	Sample OD
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 BVD p80 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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