Enferplex Bovine BCV/BRSV

01D16 (465 Tests)

Test for the *in vitro* detection of antibodies to *Bovine Coronavirus* and *Bovine Respiratory Syncytial* virus.

For *in vitro* veterinary diagnostic and research use only

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1.0 General Information

Bovine Coronavirus

Bovine coronaviruses (BCoV) are members of the Coronaviridae family in the Nidovirus order, subgroup 2a, which are enveloped, single stranded, positive sense RNA viruses with shaped projections on the surface, (4). BCoV infects the upper and lower respiratory tract and intestine. It sheds in both feces and nasal secretions (5). Bovine CoV is involved in the etiology of 3 distinct clinical syndromes in cattle: calf diarrhea, winter dysentery with hemorrhagic diarrhea in adults, and respiratory infections in cattle of various ages including the bovine respiratory disease complex or shipping fever of feed lot cattle, (3). Economic losses are the primary concern of newborn or adult BCoV infection, as the morbidity rates are high and may reach up to 100% of the herd. In dairy herds suffering a BCoV outbreak milk production may not return to normal for several weeks, or even during that lactation period, resulting in significant losses for the milk industry (6).

Bovine Respiratory Syncytial virus

Bovine Respiratory syncytial virus (BRSV) is an enveloped, non-segmented, negative stranded RNA virus. This virus is classified within the genus Pneumovirus of the family Paramyxoviridae, (1). The virus is transmitted by aerosols and infects respiratory tract mucosal cells. The clinical signs of BRSV infection are associated with pulmonary localisation of the virus which is mainly in the cranio-ventral lobes of the lung, (2). BRSV infection is widespread and has major economic impact and is regarded as one of the most important causes of respiratory tract disease, especially in young calves. An infection can cause respiratory distress, fever, anorexia, pulmonary and subcutaneous emphysema and can lead to secondary bacterial pneumonia and death, (3).

2.0 Intended Use

The Enferplex Bovine BCV/BRSV assay is a semi-quantitative luminescent (emission) immunological method for the detection of various antibodies in bovine serum, individual and bulk milk. The Enferplex Bovine BCV/BRSV kit is intended for *in vitro* veterinary diagnostic use.

3.0 Principle of the Procedure

The Enferplex Bovine BCV/BRSV assay is a semi-quantitative enzyme immunoassay based on the sequential addition of bovine serum, individual or bulk milk to a multiple antigen coated plate, followed by antibody-enzyme conjugate and a chemiluminescent substrate.
Upon incubation of the test sample in the multiple antigen coated well, antibodies specific to the various diseases form complexes with the immobilized antigens.
This step is followed by a wash step with 1X Wash Buffer solution, and then Sheep anti-bovine sera labelled with HRPO (Horseradish peroxidase) is added, forming an antigen-antibody-conjugate-peroxidase complex. Next, unbound conjugate is washed away and a chemiluminescent substrate is used to generate the light signal and the image is captured. The image is analysed and data reduced to determine sample status in the appropriate Enferplex Macro.

4.0 Reagents

Reagent pack 01D16 contains sufficient material for 465 tests. The reagent pack is stored at 2-8°C.
Note the storage conditions for individual components.

	Component	Function	Quantity	Storage Requirement
1.	BCV/BRSV Antibody Capture Plate	Plate used for antibody capture	5 x 96-well plates	2-8°C in sealed foil pouch
2.	20X Wash Buffer	Washes off unbound antibody/reagent	1 x 500ml of 20X concentrate solution (contains 0.05% Proclin 300® & 0.02%	2-8°C

			Protectol BN ®)	
3.	BMD Sample Diluent	Buffer for dilution of serum, or milk	2 x 500ml working strength solution (contains 0.05% Proclin 300® & 0.02% Protectol BN ®)	2-8°C
4.	BMD Conjugate Diluent	Conjugate diluent for dilution of the BMD Concentrate Conjugate	1 x 500ml of working strength solution (contains 0.05% Proclin 300® & 0.02% Protectol BN ®)	2-8°C
5.	Bovine Concentrate Conjugate	Undiluted Sheep anti-bovine IgG antibody	1 x microvial containing 0.1ml	2-8°C
6.	Multi-Lite A	Chemiluminescent substrate for peroxidise when combined with Multi-Lite B	1 x bottle containing 15ml solution	+15°C to 30°C (Room Temperature, RT)
7.	Multi-Lite B	Chemiluminescent substrate for peroxidase when combined with Multi-Lite A	1 x bottle containing 15ml solution	+15°C to 30°C
8.	BCV/BRSV Negative Control	Non-reactive with antibody capture plate, used as a control	1 x microvial containing 0.1ml	-15°C -to -25°C
9.	BCV/BRSV Positive Control	Non-infectious, Reactive with antibody capture plate, used as a control	1 x microvial containing 0.1ml	-15°C -to -25°C

5.0 Materials and Equipment required but not provided

- Microplate Incubator/shaker thermostated at 37°C ± 2°C and capable of shaking at 900rpm
- Quansys Biosciences Q-View Imager
- Device for the delivery and aspiration of wash solution
- High quality deionised, distilled, or reverse osmosis water
- Microplate Cover Seals
- Precision Micropipettes and Multichannel micropipettes of appropriate volume and disposable tips
- Reagent dispensing trays
- Glass containers for dilution of the concentrate conjugate
- Glass or polypropylene containers for dilution of other reagents
- Polypropylene tubes/plates for dilution of the samples

6.0 Warnings and Precautions

6.1 The reagents are solely for *in vitro* veterinary diagnostic use on bovine serum, bulk and individual milk samples.

6.2 Please refer to the manufacturer's safety data sheets and the product labelling for information on potentially hazardous components.

6.3 Do not perform the test in the presence of reactive vapours (acids, alkalis, aldehydes) or dust, which could alter the enzymatic activity of the conjugate.

6.4 Use perfectly washed glassware, rinsed in distilled/deionised water or preferably disposable material.

6.5 Use a new pipette tip for each sample.

6.6 Do not modify the test procedure or substitute reagents from other manufacturers.

6.7 Do not use the reagents beyond the stated expiry date and do not intermix components from different kit lots. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.

6.8 Use separate dispensing trays for each reagent used in the assay. Avoid cross contamination of the Multi-Lite solution with the diluted conjugate solution. Do not pour unused Multi-Lite solution back into the Multi-Lite bottles.

6.9 Do not allow plates to sit for more than 3 minutes between wash steps and the addition of reagents.

6.10 Do not expose the substrate to strong light or oxidizing agents.

6.11 Allow the reagents to adjust to room temperature (RT) (+18°C to 30°C), for approximately 30 minutes before use. Immediately after use, return all reagents to their appropriate storage conditions.

6.12 All reagents must be prepared in either clean glass, or polypropylene bottles. Care must be taken to avoid cross contamination of reagents.

6.13 Washing of the wells is an essential step of the procedure; ensure that all wells are completely filled, then completely emptied. Do not adjust the recommended washing steps. Inadequate washing can give incorrect results.

7.0 Health and Safety Information

The 20X Wash Buffer, BCV/BRSV Positive and Negative Control must be handled with care. Please note hazard identified on individual container label. The 20X Wash Buffer, BCV/BRSV Positive and Negative Control contains 2-methyl-2H-isothiazol-3-one, which is classified as per EC Directive EC 1272/2008 Skin. Sens 1 – H317. The following are the appropriate Hazard (H) and Precautionary (P) Statements.

H317 May cause an allergic skin reaction
P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
P272 Contaminated work clothing should not be allowed out of the workplace
P302 & P352 IF ON SKIN: Wash with plenty of soap and water
P321 Specific treatment (see on this label)
P333 & P313 If skin irritation or rash occurs: Get medical advice/attention.
P363 Wash contaminated clothing before reuse.

The BMD Sample Diluent and BMD Conjugate Diluent must be handled with care. Please note hazard identified on individual container label. The BMD Sample Diluent and BMD Conjugate Diluent contains Donor Goat serum which is classified as per EC Directive No. 1272/2008 [CLP] Resp. Sens. 1 H334. The following are the appropriate Hazard (H) and Precautionary (P) Statements.

H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Precautionary statements (CLP)
P261 - Avoid breathing vapours, mist, or spray.
P272 - Contaminated work clothing should not be allowed out of the workplace.
P280 - Wear protective gloves, protective clothing, and eye protection.
P284 - [In case of inadequate ventilation] wear respiratory protection.

P302+P352 - IF ON SKIN: Wash with plenty of water.
P304+P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P321 - Specific treatment (see section 4 on this SDS).
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P342+P311 - If experiencing respiratory symptoms: Call a POISON CENTER or doctor.
P362+P364 - Take off contaminated clothing and wash it before reuse.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

Safety Data Sheets are available upon request.

8.0 Preparation of Reagents

Component	Method	Storage of Prepared Reagents
Working Strength Wash Buffer (1X) Requires: 1. 20X Wash Buffer 2. Deionised or distilled water	**1.** Dilute 1-part 20X Wash Buffer in 19 parts deionised or distilled water. **2.** Mix thoroughly, e.g. For 4L of working strength 1X Wash Buffer, add 200ml 20X Wash Buffer to 3800ml of water	1 month at RT or at 2-8°C
Working Strength Conjugate Requires: 1. BMD Conjugate Diluent 2. Bovine Concentrate Conjugate	**1.** Prepare only the required volume for the number of tests to be carried out. **2.** 400µl of working strength conjugate is required for 8 wells. **3.** 5ml of working strength conjugate is required for 1 plate. **4.** Dilute the Bovine Concentrate Conjugate 1:25000 in BMD Conjugate Diluent, e.g. to 75ml of BMD Conjugate Diluent, add 3µl of Bovine Concentrate Conjugate. **5.** Mix by inversion. Invert a minimum of 8 times	Prepare 15-20mins before use
Multi-Lite Solution Requires: 1. Multi-Lite A 2. Multi-Lite B	**1.** Prepare only the required volume for the number of tests to be carried out. **2.** 400µl of Multi-Lite Solution is required for 8 wells. 5ml of Multi-Lite Solution is required for 1 plate. **3.** Add equal parts of Multi-Lite A to Multi-Lite B in either a clean glass or plastic disposable vessel. For example, add 1ml of Multi-Lite A to 1ml of Multi-Lite B. **4.** Mix by inversion twice.	Prepare 15-20mins before use and store in the dark
Working Strength BCV/BRSV Negative Control Requires: 1. BCV/BRSV Negative Control 2. BMD Sample Diluent	**1.** Prepare only the required volume for the number of tests to be carried out. **2.** 50µl of working strength BCV/BRSV Negative Control is required per plate. **3.** Dilute BCV/BRSV Negative Control 1:300 in the BMD Sample Diluent, e.g. to 1.2ml of BMD Sample Diluent, add 4µl of BCV/BRSV Negative Control. **4.** Mix by inversion a minimum of 2 times.	Store at 2-8°C and use within 8hrs of preparation
Working Strength BCV/BRSV Positive Control Requires:	**1.** Prepare only the required volume for the number of tests to be carried out. **2.** 50µl of working strength BCV/BRSV Positive Control is required per plate.	Store at 2-8°C and use within 8hrs of preparation

1. BCV/BRSV Positive Control 2. BMD Sample Diluent	**3.** Dilute BCV/BRSV Positive Control 1:300 in the BMD Sample Diluent, e.g. to 1.2ml of BMD Sample Diluent, add 4µl of BCV/BRSV Positive Control. **4.** Mix By inversion a minimum of 2 times.	

9.0 Sample & Control Preparation

Bring all specimens to room temperature prior to testing. All samples and controls must be added to the BCV/BRSV antibody capture plate at approximately the same time, therefore use of a transfer/master plate is recommended to add samples and controls to first, and then transfer to the BCV/BRSV antibody capture plate.

9.1 Controls

9.1.1 Add 50µl of BMD Sample Diluent to A1 of the test plate.

9.1.2 Add 50µl of BCV/BRSV Negative Control to B1 of the test plate.

9.1.3 Add 50µl of BCV/BRSV Positive Control to C1 of the test plate.

9.2 Serum or Plasma Samples

9.2.1 Fresh, refrigerated, or previously frozen serum can be tested. Icteric, lipemic, haemolysed, heat treated and contaminated sera may cause erroneous results.

9.2.2 If specimens are not immediately tested, they should be refrigerated at 2-8°C. For storage periods greater than 24 hours, freeze the serum at -20°C or below.

9.2.3 Specimens containing precipitate may yield inconsistent test results and such specimens must be clarified prior to testing.

9.2.4 Ensure the serum sample is mixed thoroughly before addition to the BMD Sample Diluent.

9.2.5 The samples are prepared to a 1:300 dilution by adding for example 4µl of the serum to 1.2ml of the BMD Sample Diluent.

9.2.6 Mix the prepared sample by inversion or by pipetting up and down a minimum of 2 times.

9.3 Bulk and individual Milk Samples

9.3.1 Whole milk samples can be used after centrifugation for 15 minutes at 2000 x g or left to stand if refrigerated (2-8°C). No pre-treatment is needed for defatted milk.

9.3.2 If specimens are not immediately tested, they should be refrigerated at 2-8°C. For storage periods greater than 24 hours, freeze the milk at -20°C or below.

9.3.3 Bulk milk samples are prepared to a 1:5 dilution by adding for example 18µl of the bulk milk to 90µl of the BMD Sample Diluent.

9.3.4 Mix the prepared sample by inversion or by pipetting up and down a minimum of 2 times.

9.3.5 Individual milk samples are prepared to a 1:10 dilution by adding for example 10µl of the individual milk to 100µl of the BMD Sample Diluent.

9.3.6 Mix the prepared sample by inversion or by pipetting up and down a minimum of 2 times.

10.0 Test Protocol

10.1 All samples are tested in singlicate. Refer to 13.0 for recommended plate layout.

10.2 Remove the BCV/BRSV antibody capture plate from the protective packaging. For each single plate, transfer 50µl of the controls and samples into the wells of the BCV/BRSV antibody capture plate.

10.3 Cover the microplate with a microplate cover seal.

10.4 Incubate the microplate, shaking, for 60 minutes at 37 ± 2°C.

10.5 Remove the microplate cover seal, and wash the wells 6 times with 200/250µl of 1X wash buffer.

10.6 Dry by inversion on absorbent paper.

10.7 Add 50µl of working strength conjugate to each well. Cover with a microplate cover seal.

10.8 Incubate the microplate, shaking, for 60 minutes at 37 ± 2°C.

10.9 Remove the microplate cover seal, and wash the wells 6 times with 200/250µl of 1X wash buffer.

10.10 Dry by inversion on absorbent paper.

10.11 Add 50µl of the substrate solution to each well of the microplate. Immediately read the plate on the Q-View Imager set at 220 seconds exposure time.

11.0 Results

11.1 Validation of Test Performance

Each plate must be considered separately when calculating and interpreting results of the assay. The control results must be validated before the sample results can be interpreted. The criteria for the Buffer, BCV/BRSV Negative Control and BCV/BRSV Positive Control are all contained within the appropriate 'Enferplex BCV/BRSV Macro' provided and the results are calculated automatically.

11.2 Acceptable Range of Control Results

If the criteria for the controls are not met, the assay is invalid and must be repeated.

11.3 Interpretation of Results

Negative Result

Samples giving a 'Negative' result in the macro are considered non-reactive in the BCV/BRSV assay.

Positive Result

Samples giving a 'Positive' result in the macro are considered reactive in the BCV/BRSV assay.

12.0 Limitations of the Procedure

As with any biological test, this test may give a false positive or a false negative result owing to local conditions. A test should be interpreted in the context of all available clinical, historical, and epidemiological information relevant to the animal(s) under test.

A negative result with a semi-qualitative immunological method does not preclude the possibility of infection with *Bovine Coronavirus* or *Bovine Respiratory Syncytial Virus*.

Any change or modification of the procedure might affect the results. *Enfer Scientific accepts no responsibility for any loss or damage, howsoever caused, arising out of the interpretation of test results.*

13.0 Recommended Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Buffer	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
B	NC	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
C	PC	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
D	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
E	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
F	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
G	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
H	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93

Buffer = Sample Diluent
NC = Negative Control
PC = Positive Control
S = Test Samples in singlicate

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Appendix 1

Spot Number	Antigen
Spot 1	Blank
Spot 2	BCV Haem
Spot 3	BCV N
Spot 4	BCV S1
Spot 5	BRSV-1
Spot 6	BRSV-B
Spot 7	BRSV tF1
Spot 8	BRSV Nuc