Enferplex Goat/Sheep Multi-Disease 5D

01H12 (465 Tests)

Test for the *in vitro* detection of antibodies to *Caprine Arthritis-encephalitis Virus*, *Maedi Visna Virus*, *Corynebacterium pseudotuberculosis, & Mycobacterium paratuberculosis*

For *in vitro* veterinary diagnostic use only

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1.0 General Information

Caprine arthritis-encephalitis virus (CAEV) causes an economically important viral disease of goats. Many infections are subclinical, but animals can develop progressive, untreatable disease syndromes, including polyarthritis in adults and encephalomyelitis in kids, chronic proliferative pneumonia, and indurative mastitis, resulting in decreased milk production. The distribution of CAEV is worldwide and the spread often seems to coincide with the international movement of dairy goats.

Maedi-Visna virus (MVV) causes an economically important viral disease of sheep. Again many infections are subclinical, but a significant proportion of animals develop progressive, untreatable disease syndromes including chronic proliferative pneumonia with dyspnoea (maedi) or wasting and neurologic signs (visna). Both maedi and visna can be fatal. In additional MVV causes indurative mastitis and polyarthritis. Additional economic costs may include marketing and export restrictions.

CAEV and MVV are members of the genus *Lentivirus* in the family Retroviridae. Phylogenetic analyses have demonstrated that CAEV and MVV are closely related and these two viruses share many molecular and pathogenic features, and are often considered together as the small ruminant lentiviruses (SRLV). They are mainly transmitted from infected dams to their kids by the ingestion of virus-containing colostrum or milk, or by respiratory transmission, and this usually occurs early in life. The SRLVs have the ability to evade the defense mechanisms of their natural host, thus causing persistent infection. Most animals develop antibody responses, though seroconversion can be delayed for several weeks or months. Antibodies usually persist during the course of the disease, though may decline in the clinical stages of the disease. In order to control the spread of the virus, the viral carriers have to be detected and eradicated as no vaccine or treatment is yet available.

Caseous Lymphadenitis (CLA) is a chronic infectious disease of sheep and goats that is caused by the bacterium *Corynebacterium pseudotuberculosis*. This organism belongs to a family of related bacteria, several of which are pathogenic for man and/or animals. Economic losses due to CLA are caused by the disease due to absecessation in the head and neck region, the lungs and in some cases, systemically. It can affect breeding stock making them no longer marketable and carcasses can be condemned or devalued at slaughter due to internal or superficial abscesses. The CLA bacteria can exist in the environment for long periods of time. The primary mode of infection is direct contact with pus or the secretion from abscesses that contain *C. pseudotuberculosis* bacteria. Does and ewes can transmit CLA to kids and lambs if abscess occur in the mammary tissues. Current treatment of diseased animals is limited. Monitoring of disease status is compounded by the fact that animals may be infected without showing obvious clinical symptoms.

Johne's disease or paratuberculosis in ruminants, including sheep and goats, is caused by *Mycobacterium avium* subspecies *paratuberculosis*, an acid-fast bacterium. It may survive for extended periods in the environment in soil, water and manure and it is resistant to many common disinfectants. The age of onset of clinical disease tends to be younger in sheep and goats then in cattle. Sheep and goats present with chronic weight loss as the primary clinical sign of Johne's disease. Hypoproteinemia with intermandibular edema and wool break and poor fleece condition have been reported in paratuberculous sheep and anorexia, depression or clumping of feces may be present in the end stages of the disease in goats. Infection is transmitted primarily by faeces from infected animals.

2.0 Intended Use

The Enferplex Goat/Sheep Multi-Disease 5D assay is a semi-quantitative luminescent (emission) immunological method for the detection of various antibodies in caprine and ovine serum, plasma, or milk. The Enferplex Goat/Sheep Multi-Disease 5D kit is intended for *in vitro* veterinary diagnostic use.

3.0 Principle of the Procedure

The Enferplex Goat/Sheep Multi-Disease 5D assay is a semi-quantitative enzyme immunoassay based on the sequential addition of caprine or ovine serum, plasma, or 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 Rabbit anti-sheep sera labelled with HRPO (Horseradish perxoidase) 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 01H12 contains sufficient material for 465 tests. The reagent pack is stored at 2-8°C. Note the storage conditions for individual components. Note GSMD: Goat/Sheep Multi-Disease.

	Component	Function	Quantity	Storage Requirement
1.	GSMD 5D 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	500ml of 20X concentrate solution (contains 0.15% Proclin 950® & 0.02% Protectol BN ®)	2-8°C
3.	GSMD Sample Diluent	Buffer for dilution of serum, plasma or milk	1 x1L working strength solution (contains 0.15% Proclin 950® & 0.02% Protectol BN ®)	2-8°C
4.	GSMD Conjugate Diluent	Conjugate diluent for dilution of the Concentrate Conjugate	1 x 500ml of working strength solution (contains 0.15% Proclin 950® & 0.02% Protectol BN ®)	2-8°C
5.	Sheep Concentrate Conjugate	Undiluted Rabbit anti-sheep IgG antibody	1 x microvial containing 0.1ml	2-8°C
6.	Multi-Lite A	Chemiluminescent substrate for peroxidase when combined with Multi-Lite B	1 bottle containing 15ml solution	RT
7.	Multi-Lite B	Chemiluminescent substrate for peroxidase when combined with Multi-Lite A	1 bottle containing 15ml solution	RT
8.	GSMD 5D Negative Control	Non-reactive with antibody capture plate, used as a control	1 microvial containing 0.1ml	-20°C
9.	GSMD 5D Positive Control	Non-infectious, Reactive with antibody capture plate, used as a control	1 x microvial containing 0.1ml	-20°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 conjugates
- 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 caprine or ovine serum, plasma, and milk samples. For professional use only.

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 conjugates.

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 (active ingredient 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, GSMD 5D Positive and Negative Control GSMD Sample and Conjugate diluents must be handled with care. Please note hazard identified on individual container label. The 20X Wash Buffer, GSMD 5D Positive and Negative Control GSMD Sample and Conjugate diluents 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.

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. GSMD Conjugate Diluent 2. Sheep 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 Sheep Concentrate Conjugate at 1:20000 in GSMD Conjugate Diluent. **5.** Mix by inversion. Invert a minimum 8 times, e.g. to 60ml of GSMD Conjugate Diluent, 3µl of the Sheep Concentrate Conjugate.	Prepare 15-20 mins 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 1 part of Multi-Lite A to 1 part of Multi-Lite B in either a clean glass or plastic vessel. For example, add 1ml of Multi-Lite A to 1ml of Multi-Lite B. **4.** Mix by inversion.	Prepare 15-20 mins before use and store in the dark
Working Strength GSMD 5D Negative Control Requires: 1. GSMD 5D Negative Control 2. GSMD Sample Diluent	**1.** Prepare only the required volume for the number of tests to be carried out. **2.** 50µl of working strength GSMD 5D Negative Control is required per plate. **3.** Dilute GSMD 5D Negative Control 1:150 in the GSMD Sample Diluent. **4.** Mix by inversion, e.g. to 1.5ml of GSMD Sample Diluent, add 10µl of GSMD 5D Negative Control.	Store at 2-8°C and use within 8hrs of preparation
Working Strength GSMD 5D Positive Control Requires: 1. GSMD 5D Positive Control 2. GSMD Sample Diluent	**1.** Prepare only the required volume for the number of tests to be carried out. **2.** 50µl of working strength GSMD 5D Positive Control is required per plate. **3.** Dilute GSMD 5D Positive Control 1:150 in the GSMD Sample Diluent. **4.** Mix By inversion, e.g. to 1.5ml of GSMD Sample Diluent, add 10µl of GSMD 5D Positive Control.	Store at 2-8°C and use within 8hrs of preparation

9.0 Sample & Control Preparation

Bring all specimens to room temperature prior to testing. All samples and controls must be added to the GSMD 5D 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 GSMD 5D antibody capture plate.

9.1 Controls

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

9.1.2 Add 50µl of GSMD 5D Negative Control to B1 of the test plate.

9.1.3 Add 50µl of GSMD 5D Positive Control to C1 of the test plate.

9.2 Serum or Plasma Samples

9.2.1 Fresh, refrigerated, or previously frozen serum or plasma 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/plasma 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/plasma sample is mixed thoroughly before addition to the GSMD Sample Diluent.

9.2.5 The samples are prepared to a 1:150 dilution by adding for example 10µl of the serum/plasma to 1.5ml of the GSMD Sample Diluent.

9.2.6 Mix the prepared sample.

9.3 Bulk or 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 100µl of the bulk milk to 500µl of the GSMD Sample Diluent.

9.3.4 Mix the prepared sample by inversion.

9.3.5 Individual milk samples are prepared to a 1:50 dilution by adding for example 10µl of the individual milk to 500µl of the GSMD Sample Diluent.

9.3.6 Mix the prepared sample by inversion.

10.0 Test Protocol

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

10.2 Remove the GSMD 5D antibody capture plate from the protective packaging. For each single plate, transfer 50µl of the controls and samples into the wells of the GSMD 5D 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 the working strength conjugate to each well. Cover with a microplate cover seal.

10.8 Incubate the microplate, shaking, for 30 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.

11.0Results

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, GSMD 5D Negative Control, and GSMD 5D Positive Control are all contained within the appropriate 'Enferplex 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 GSMD 5D assay.

Inconclusive Result

Samples giving an 'Inconclusive' result in the macro are considered suspect animals in the GSMD 5D assay, with results falling within 15% of the respective antigen threshold (a ratio of between 0.85 and 1.15). It is recommended that these samples be re-tested and confirmed before deciding on the result.

Positive Result

Samples giving a 'Positive' result in the macro are considered reactive in the GSMD 5D 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-quantitative immunological method does not preclude the possibility of infection with *Caprine Arthritis Encephalitis Virus*, *Maedi Visna Virus*, *and Corynebacterium pseudotuberculosis & Mycobacterium paratuberculosis.* 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
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	p25
Spot 3	TM1c
Spot 4	PLD
Spot 5	CP40
Spot 6	ParaTB