

## CHAPTER 3.8.2.

# CAPRINE ARTHRITIS/ENCEPHALITIS & MAEDI-VISNA

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### SUMMARY

Caprine arthritis/encephalitis (CAE) and maedi-visna (MV) are persistent lentivirus infections of goats and sheep. They are often grouped together as the small ruminant lentiviruses (SRLVs). Maedi-visna is also known as ovine progressive pneumonia (OPP). Phylogenetic analyses comparing nucleotide sequences of MV virus (MVV) and CAE virus (CAEV) have demonstrated that these are closely related lentiviruses. One source of CAEV and MVV transmission is colostrum and milk. The source of horizontal transmission in the absence of lactation remains unknown; however, faeces and lung fluids are known to harbour infectious virus. Ovine lentiviruses have been identified in most of the sheep-rearing countries of the world, with the notable exceptions of Australia and New Zealand. The distribution of CAEV is highest in industrialised countries, and seems to have coincided with the international movement of European breeds of dairy goats. Clinical and subclinical MV and CAE are associated with progressive, mononuclear cell inflammatory lesions in the lungs, joints, udder and central nervous system. Indurative mastitis is common in both host species, and its economic significance may be underestimated. Laboured breathing associated with emaciation caused by progressive pneumonitis is the predominant feature in clinically affected sheep, whereas polyarthritis is the main clinical sign in goats. However, most lentivirus-infected sheep and goats are largely asymptomatic, but remain persistent carriers of virus and are capable of transmitting infection via colostrum or milk and respiratory secretions. The most practical and reliable approach to confirming a diagnosis of MV or CAE is a combination of serology and clinical evaluation. Although serology represents the most cost-effective method of diagnosing infection in persistently infected, clinically normal animals, it should be understood that testing errors can occur. The frequency of error depends on several factors including but not limited to: 1) the assay format, 2) the homology between the strain of virus used in the assay and the strains of virus present in the tested populations, and 3) the viral antigen used in the assay.

**Identification of the agent:** Virus isolation can be attempted from live clinical or subclinical cases by co-cultivating peripheral blood or milk leukocytes with appropriate ovine or caprine cell cultures, such as choroid plexus (MVV) or synovial membrane (CAEV) cells. Virus isolation is very specific but has variable sensitivities. Following necropsy, virus isolation is most readily accomplished by establishing explant cultures of affected tissues, e.g. lung, choroid plexus, synovial membrane or udder. Also, alveolar macrophages may be obtained from the lung at post-mortem and co-cultivated with susceptible cells. The cytopathic effects are characteristic, consisting of the appearance of refractile stellate cells and syncytia. The presence of MVV or CAEV can be confirmed by immunolabelling methods and electron microscopy.

**Nucleic acid recognition methods:** Many standard and a few quantitative polymerase chain reaction (PCR) assays for detecting MV and CAE provirus have been described and are used routinely in many laboratories for the rapid detection, quantitation, and identification of the small ruminant lentivirus strains. Cloning and/or sequencing of PCR products is the most direct method to confirm the specificity of PCR results.

**Serological tests:** Most infected sheep and goats possess detectable specific antibodies that can be assayed by a number of different serological tests. The two most commonly used are the agar gel immunodiffusion test and the enzyme-linked immunosorbent assay (ELISA). Western blot analysis and radio-immunoprecipitation are also performed, but only in specialised laboratories. A milk antibody assay may be appropriate in dairy goat herds. The time required for seroconversion

following infection can be relatively prolonged and unpredictable, being measured in months rather than in weeks. However, after seroconversion, the antibody response usually persists and antibody-positive sheep and goats are regarded as virus carriers.

**Requirements for vaccines:** There are no vaccines available.

## A. INTRODUCTION

Caprine arthritis/encephalitis (CAE) of goats and maedi-visna (MV) of sheep are persistent virus infections caused by closely related lentiviruses (Minguijón *et al.*, 2015; Peterhans *et al.*, 2004). Maedi-visna is also known as ovine progressive pneumonia (OPP). Sheep can be experimentally infected with CAE and goats can be experimentally infected with MV. In addition, phylogenetic analyses comparing nucleotide sequences of MV virus (MVV) and CAE virus (CAEV) show clear indications of the existence and epidemiological importance of cross-species transmission between sheep and goats without demonstrating clearly that one virus has emerged from the other (Shah *et al.*, 2004a; 2004b). MV and CAE are characterised by lifelong persistence of the causal agent in host monocytes and macrophages, and a variable length of time between infection and induction of a serologically detectable antiviral antibody response. Most infected sheep and goats do not exhibit clinical disease, but remain persistently infected and are capable of transmitting virus (Adams *et al.*, 1983; Crawford *et al.*, 1980).

Maedi-visna is an Icelandic name that describes two of the clinical syndromes recognised in MV virus (MVV)-infected sheep. 'Maedi' means 'laboured breathing' and describes the disease associated with a progressive interstitial pneumonitis, and 'visna' means 'shrinkage' or 'wasting', the signs associated with a paralysing meningoencephalitis. Whereas progressive lung disease is the primary finding with MVV infection, chronic polyarthritis, with synovitis and bursitis is the primary clinical outcome of CAEV infection. Encephalitis occurs primarily in kids aged between 2 and 6 months following CAEV infection, but careful differential diagnoses need to be conducted to rule out other syndromes or infections in kids. Indurative mastitis occurs in both syndromes. The lungs of sheep affected by MV do not collapse when removed from the thorax and often retain the impression of the ribs. The lungs and lymph nodes increase in weight (up to 2–3 times the normal weight). The lesions are uniformly distributed throughout the lungs, which are uniformly discoloured or mottled grey-brown in colour and of a firm texture. Diagnosis of CAEV and MVV induced respiratory disease was reviewed by Chakraborty *et al.* (2014). Udders affected by MV are diffusely indurated and associated lymph nodes may be enlarged.

When MV or CAE is the suspected cause of clinical disease, confirmation of the diagnosis can be achieved by a combination of clinical evaluation, detection and identification of the viruses, or by serology and, when necessary, histological examination of appropriate tissues collected at necropsy. Important tissues to examine include lung for progressive interstitial pneumonitis, brain and spinal cord for meningoencephalitis, udder for indurative mastitis, affected joints and synovium for arthritis, and kidney for vasculitis (Crawford & Adams, 1981). The nature of the inflammatory reaction in each site is similar, consisting of an interstitial, mononuclear cell reaction, sometimes with large aggregates of lymphoid cells and follicle formation.

## B. DIAGNOSTIC TECHNIQUES

*Table 1. Test methods available for the diagnosis of caprine arthritis/encephalitis and maedi-visna and their purpose*

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
<b>Identification of the agent</b>						
Virus isolation	–	–	–	+	–	–
Antigen detection	–	–	–	+	–	–
PCR	+	+	++	++	++	–

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of immune response						
AGID	+	+++	++	+++	+++	+
CFT	-	-	-	-	-	-
ELISA	+++	+++	+++	+	+++	+
VN	-	-	-	-	-	+++
IFAT	-	+	-	-	+	-

Key: +++ = recommended for this purpose; ++ recommended but has limitations;  
+ = suitable in very limited circumstances; - = not appropriate for this purpose.

PCR = polymerase chain reaction; AGID = agar gel immunodiffusion; CFT = complement fixation test;  
ELISA = enzyme-linked immunosorbent assay; VN = virus neutralisation; IFAT = indirect fluorescent antibody test.

## 1. Identification of the agent

Isolation and characterisation of MVV or CAEV would not normally be attempted for routine diagnostic purposes. Due to the persistent nature of these infections, the establishment of a positive antibody status is sufficient for the identification of virus carriers. However, due to a late seroconversion after infection, negative serology may occur in recently infected animals.

There are two approaches to the isolation of MVV and CAEV: one for use with the live animal, and the second for use with necropsy tissues.

### 1.1. Isolation from the live animal

#### 1.1.1. Maedi-visna virus

The MV provirus DNA is carried in circulating monocytes and tissue macrophages. Virus isolation from the live animal therefore requires the establishment of leukocyte preparations, with aseptic precautions, from peripheral blood or milk during lactation, culturing them together with indicator cells. Sheep choroid plexus (SCP) cells are commonly used for this purpose. These indicator cells can be prepared as primary explant cultures from fetal or new-born virus-free lambs, and their number can be multiplied over three to four passages for storage in liquid nitrogen. The recovered SCP cells are suitable for co-cultivation for up to 10 or 15 passages. Although the cells continue to grow well thereafter, their susceptibility to MVV may become reduced.

Leukocyte preparations can be made from peripheral blood as buffy coats by the centrifugation at 1000 *g* of heparinised, ethylenediamine tetra-acetic acid (EDTA) or citrated samples for 15 minutes. The cells are aspirated, suspended in Hanks' balanced salt solution (HBSS), and further purified by centrifugation at 400 *g* on to a suitable cushion of density medium for 40 minutes. The interface cells are spin-washed once or twice in HBSS at 100 *g* for 10 minutes, and the final cell pellet is resuspended in medium to a concentration of approximately 10<sup>6</sup> cells/ml; cells are generally cultured for 10–12 days in Teflon bags and are then added to a washed monolayer of slightly subconfluent SCP cells in a flask with an area of 25 cm<sup>2</sup>.

Leukocytes can be similarly deposited from milk by centrifugation, when they are spin-washed, resuspended and finally added to SCP monolayer cultures.

These cultures are maintained at 37°C in a 5% CO<sub>2</sub> atmosphere, changing the medium and passaging as necessary. They are examined for evidence of a cytopathic effect (CPE), which is characterised by the appearance of refractile stellate cells with dendritic processes accompanied by the formation of syncytia. The cultures should be maintained for several weeks before being discarded as uninfected. Once a CPE is suspected, cover-slip cultures should be prepared. These are fixed, and evidence of viral antigen is sought by immunolabelling, usually by means of indirect fluorescent antibody or by the use of indirect immunoperoxidase methods. In addition, the cells of any suspect monolayers are deposited by centrifugation, and preparations are made for the identification of any characteristic lentivirus particles by transmission electron microscopy. Reverse transcriptase in the supernatant of the cell culture is indicative of the presence of retroviruses.

### 1.1.2. Caprine arthritis/encephalitis virus

The same principles that apply to the isolation of MVV also apply to the isolation of CAEV. CAEV was originally isolated by explantation of synovial membrane from an arthritic goat (Crawford & Adams, 1981). With live CAEV-infected goats, peripheral blood, milk, and possibly joint fluid aspirate represent the most suitable specimens from which leukocyte preparations can be established. Goat synovial membrane (GSM) cells are suitable indicator cells. If a CPE is suspected, tests for detection of viral antigen should be carried out, as described above.

## 1.2. Isolation from necropsy tissues

### 1.2.1. Caprine arthritis/encephalitis virus and maedi-visna virus

Samples of suspect tissues, collected as fresh as possible, such as lung, synovial membranes, udder, etc., are collected aseptically into sterile HBSS or cell culture medium and minced finely in a Petri dish using scalpel blades. Individual fragments are collected by Pasteur pipette and transferred to flasks of 25 cm<sup>2</sup>, approximately 20–30 fragments per flask, and a drop of growth medium is placed carefully on each. The flasks are then incubated at 37°C in a humid 5% CO<sub>2</sub> atmosphere, and left undisturbed for a few days to allow the individual explants to adhere to the plastic. Fresh medium can be added with care, after which rafts of cells will gradually grow out from the fragments. When there is sufficient cell out-growth, the cultures are trypsin dispersed to allow the development of cell monolayers. These can be examined for CPE, and any suspected virus growth is confirmed in the same way as for the co-cultivations.

Adherent macrophage cultures are easy to establish from lung-rinse material (post-mortem broncho-alveolar lavage) and can be tested for virus production by serology, electron microscopy, or reverse transcriptase assay within 1–2 weeks. Virus isolations can be done by co-cultivation of macrophages and SCP or GSM cells as described for leukocytes above.

## 1.3. Nucleic acid recognition methods

Nucleic acid recognition methods may be used for the detection, quantitation, and identification of MV and CAE proviral DNA using the standard polymerase chain reaction (PCR) followed by Southern blotting, *in situ* hybridisation, or cloning and/or sequencing of the PCR product (Alvarez *et al.*, 2006; Herrmann-Hoesing *et al.*, 2007; Johnson *et al.*, 1992). Standard PCR techniques for the detection of MV and CAE proviral DNA in cells and tissues are routinely used in many laboratories and are generally used as supplemental tests for determining infection status of those animals that cannot be definitively diagnosed by serology (Deandres *et al.*, 2005). Real-time or quantitative PCR techniques are used in some laboratories and these tests, in addition to determining infection status, also quantify the amount of MV or CAE provirus in an animal (Alvarez *et al.*, 2006; De Regge & Cay, 2013; Herrmann-Hoesing *et al.*, 2007). Furthermore, molecular techniques such as PCR, cloning and sequencing also provide knowledge on a country's or region's specific MV and CAE strains, which may influence which serological assay and corresponding MV or CAE antigen to use. Phylogenetic analyses of MV and CAE proviral DNAs from SRLV strains throughout the world have suggested that in some areas, MV may have naturally infected goats, and CAE may have naturally infected sheep (Shah *et al.*, 2004a; 2004b). Recently, loop-mediated isothermal amplification (LAMP) was applied to the detection of CAE provirus (Balbin *et al.*, 2014). LAMP uses 4–6 primers that amplify 6–8 regions of the target gene (Notomi *et al.*, 2000). In the future, molecular diagnostic tests along with phylogenetic analyses of MV and CAE provirus may be used to track transmission.

An important issue in the use of PCR is specificity. Because of the possibility of amplifying unrelated sequences from the host's genomic DNA (false positives), the amplified product should be checked by either hybridisation, restriction endonuclease digestion patterns, or sequencing. Sequencing provides the best proof of specificity in the validation of PCR-based tests and is recommended by the WOA. Sensitivity of PCR-based tests can be improved by the use of nested PCR, but specificity of the nested PCR test should be checked using hybridisation, restriction endonuclease digestion patterns, or sequencing methods.

## 2. Serological tests

Ovine and caprine lentivirus infections are frequently persistent, so antibody detection is a valuable serological tool for identifying virus carriers. The close antigenic relationship between MVV and CAEV does not extend to detection of heterologous antibody in some serological assays (Knowles *et al.*, 1994).

The assays most commonly used to serologically diagnose the presence of a small ruminant lentivirus infection are agar gel immunodiffusion (AGID) and the enzyme-linked immunosorbent assay (ELISA). AGID was first developed and reported in 1973 (Terpstra & De Boer, 1973), and the ELISA was first developed and reported in 1982 (Houwers *et al.*, 1982). The AGID is specific, reproducible and simple to perform, but experience is required for reading the results. The ELISA is economical, quantitative and can be automated, thus making it useful for screening large numbers of sera. The sensitivity and specificity of both the AGID assay and ELISA depend upon the virus strain used in the assay, viral antigen preparation, and the standard of comparison assay. Western blot analysis and/or radio-immunoprecipitation are the standards of comparison used to assess sensitivity and specificity of new AGID tests and ELISAs.

### 2.1. Agar gel immunodiffusion

There are two MV and CAE viral antigens of major importance in routine serology, a viral surface envelope glycoprotein commonly referred to as SU or gp135, and a nucleocapsid protein referred to as CA or p28. These are both conserved in an antigen preparation consisting of medium harvested from infected cell cultures and concentrated approximately 50-fold by dialysis against polyethylene glycol. As an example the WLC-1 strain of MV virus is commonly used in the AGID assay in the United States (Cutlip *et al.*, 1977)<sup>1</sup> and a Canadian MV field strain is used for AGID tests in Canada (Simard & Briscoe, 1990b).

It is important to recognise that the sensitivity of the AGID test for detecting anti-CAEV antibody is dependent on both the virus strain and the viral antigen used (Adams & Gorham, 1986; Knowles *et al.*, 1994). It was demonstrated that an AGID test with CAEV gp135 afforded greater sensitivity than an AGID test with CAEV p28 (Adams & Gorham, 1986). Also, it was shown that when compared with radio-immunoprecipitation, the sensitivity of the AGID test for anti-CAEV antibody was 35% greater using CAE virus antigen over using MV virus antigen (Knowles *et al.*, 1994). The most likely explanation for this difference in sensitivity between the CAE and MV virus antigen for the detection of anti-CAEV antibody is that although the radio-immunoprecipitation assay requires only the binding of a single epitope by antibody to obtain a positive result, precipitation in an agar gel requires multiple epitope-antibody interactions. Although the MV and CAE viruses have 73–74.4% nucleotide sequence identity in the envelope gene, this amount of identity may not be sufficient to produce sufficient antibody to CAE and MV mutually common epitopes resulting in undetectable antibody/antigen precipitin lines using MV virus antigen. When the appropriate antigen is used, the AGID test performance is high. When compared with immunoprecipitation, the AGID for the detection of anti-CAEV antibody, if CAEV antigen was used, had 92% sensitivity and 100% specificity (Knowles *et al.*, 1994). In addition, the AGID for detection of anti-MVV antibody, if MVV antigen was used, had 99.3 and 99.4% sensitivity and specificity, respectively.

In adult persistently MVV-infected sheep and CAEV-infected goats, the predominant immunoprecipitating antibody response is directed against gp135 antigen. An anti-p28 response is usually present at a lower titre than the anti-gp135 response in persistently infected adult small

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<sup>1</sup> This virus has been distributed by Dr Howard Lehmkuhl, National Animal Disease Center, United States Department of Agriculture, P.O. Box 70, Ames, Iowa, USA.

ruminants using immunoprecipitation. In some CAEV-infected goats there is evidence to suggest that an anti-gp135 antibody response is produced, in the absence of an anti-p28 response and vice versa, in a proportion of individuals (Rimstad *et al.*, 1994). Hence, for validation of a test, standard sera producing both anti-gp135 and anti-p28 precipitin lines are required.

The gel medium is 0.7–1% agarose in 0.05 M Tris buffer, pH 7.2, with 8.0% NaCl. The test is conveniently performed in plastic Petri dishes, or in 10 cm<sup>2</sup> plastic trays. The pattern and size of the wells will determine the number of sera tested per plate. Various well patterns can be adopted, but a hexagonal arrangement with a central well is usual: for example, a pattern with alternating large (5 mm in diameter) and small (3 mm in diameter) peripheral wells, 2 mm apart and 2 mm from a central antigen well that is 3 mm in diameter. The large peripheral wells are used for test sera and the small ones for standard sera. A weak positive control must be included in each test. The plates are incubated overnight at 20–25°C in a humid chamber, and then examined for precipitin lines. Plates may be incubated at 2–8°C for another 24 hours to enhance the precipitin lines.

An important consideration is the need for experienced personnel to interpret the AGID. Interpretation of AGID results is dependent on the antigen used. Examples of AGIDs with different antigen preparations and a guide for interpretation of the results can be found in Adams *et al.*, 1983.

## 2.2. Enzyme-linked immunosorbent assay

Currently, there are over 30 different ELISAs reported for the detection of anti-MVV or anti-CAEV antibodies in the sera of sheep or goats, respectively (Deandres *et al.*, 2005). Most of these ELISAs are indirect ELISAs (I-ELISA) although there are three reported competitive ELISAs (C-ELISA) using monoclonal antibodies (Herrmann *et al.*, 2003; Houwers & Schaake, 1987). Half of I-ELISAs use purified whole virus preparations for antigen whereas the other half use recombinant protein and/or synthetic peptide antigens. A few of the I-ELISAs have shown high sensitivity and specificity against a standard of comparison, western blot analysis or radio-immunoprecipitation (Rosati *et al.*, 1994; Saman *et al.*, 1999). When compared with radio-immunoprecipitation, one C-ELISA has shown high sensitivity and specificity both in sheep and goats in the USA suggesting that this one test can be used for both MVV and CAEV US surveillance (Herrmann *et al.*, 2003). ELISAs have been used for several years in some European countries in control and eradication schemes for MVV in sheep and CAEV in goats (Motha & Ralston, 1994; Pépin *et al.*, 1998), AGID is useful to confirm positive ELISA results due to its high specificity.

Whole-virus antigen preparations are produced by differential centrifugation of supernatants from infected cell cultures and detergent treatment of purified virus, and are coated on microplates (Dawson *et al.*, 1982; Simard & Briscoe, 1990a; Zanoni *et al.*, 1994). Whole-virus preparations should contain both gp135 and p28. Recombinant antigens or synthetic peptides are usually produced from whole or partial segments of the gag or envelope genes and may be used in combination (Power *et al.*, 1995; Rosati *et al.*, 1994; Saman *et al.*, 1999). Thus, recombinant gag or envelope gene products fused with glutathione S-transferase fusion protein antigen that have been produced in *Escherichia coli* provide a consistent source of antigen for international distribution and standardisation.

The ELISA technique is also applicable to colostrum or milk, and some studies have evaluated paired serum and milk samples. Because colostrum and milk are sources of CAEV transmission, the testing of milk samples for anti-CAEV or anti-MVV antibody would not provide timely information for the prevention of transmission, especially to offspring from the immediate gestation.

The ELISA is performed at room temperature (~25°C) and is easy to perform in laboratories that have the necessary equipment (microplate reader) and reagents. It is convenient for large-scale screening, as it is a reliable and quantitative technique for demonstrating small ruminant lentiviruses (SRLVs) antibodies in sheep and goats. It requires relatively pure antigen. One disadvantage of several ELISAs is that many have not been validated against a standard of comparison such as western blot analysis or radio-immunoprecipitation. The test method should be validated in accordance with Chapter 1.1.6 *Validation of diagnostic assays for infectious diseases of terrestrial animals* using a standard of comparison such as western blot analysis or radio-immunoprecipitation. To date, only one ELISA has met these testing standards (Zanoni *et al.*, 1994).

For I-ELISA, wells of the microplate are coated with antigen. Diluted serum samples are added to the wells and react to antigens bound to the solid support. Unbound material is removed by washing after a suitable incubation period. Conjugate (e.g. horseradish-peroxidase-labelled anti-ruminant Ig) reacts with specific antibodies bound to the antigen. Unreacted conjugate is removed by washing after a suitable incubation period. Enzyme substrate is added. The rate of conversion of substrate is proportional to the amount of bound antibodies. The reaction is terminated after a suitable time and the amount of colour development is measured spectrophotometrically. A disadvantage of the I-ELISA is that test sera typically need to be diluted 1/50 or greater in order to lower the number of false positives.

Specific MAbs have been used in a C-ELISA for SRLVs to capture gp135 or p28 as antigen (Frevereiro *et al.*, 1999; Herrmann *et al.*, 2003; Houwers & Schaake, 1987; Ozyoruk *et al.*, 2001). C-ELISA overcomes the problem of antigen purity, as the specificity of this test depends on the MAb epitope. For C-ELISA, sample sera containing anti-SRLV antibodies inhibit binding of enzyme-labelled MAb to SRLV antigen coated on the plastic wells. Binding of the enzyme-labelled MAb conjugate is detected by the addition of enzyme substrate and quantified by subsequent colour product development. Strong colour development indicates little or no blockage of enzyme-labelled MAb binding and therefore the absence of SRLV antibodies in sample sera. In contrast, weak colour development due to the inhibition of the enzyme-labelled MAb binding to the antigen on the solid phase indicates the presence of SRLV antibodies in sample sera. The format of the C-ELISA requires that serum antibodies must bind to or bind in close proximity to the specific MAb epitope.

### 2.2.1. Materials and reagents

Microtitre plates with 96 flat-bottomed wells, freshly coated or previously coated with SRLV antigen; microplate reader (equipped with 405, 450, 490 and 620 nm filters); 37°C humidified incubator; 1-, 8- and 12-channel pipettes with disposable plastic tips; microplate shaker (optional); fridge; freezer.

Positive and negative control sera; conjugate (e.g. ruminant anti-immunoglobulin labelled with peroxidase); tenfold concentration of diluent (e.g. phosphate buffered saline/Tween); distilled water; 10× wash solution; substrate or chromogen (e.g. ABTS [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] or TMB [3,3',5,5'-tetramethylbenzidine]); stop solution (e.g. detergent, sulfuric acid).

### 2.2.2. Indirect ELISA: test procedure

- i) Dilute the serum samples, including control sera, to the appropriate dilution (e.g. 1/20) and distribute 0.1–0.2 ml per well (in duplicate if biphasic ELISA). Control sera are positive and negative sera provided by the manufacturer and an internal positive reference serum from the laboratory in order to compare the titres between different tests.
- ii) Cover the plate with a lid and incubate at room temperature or 37°C for 30–90 minutes. Empty the contents and wash three times in washing solution at room temperature.
- iii) Add the appropriate dilution of freshly prepared conjugate to the wells (0.1 ml per well). Cover each plate and incubate as in step ii. Wash again three times.
- iv) Add 0.1 ml of freshly prepared or ready-to-use chromogen substrate solution to each well (e.g. ABTS in citrate phosphate buffer, pH 5.0, and 30% H<sub>2</sub>O<sub>2</sub> solution [0.1 µl/ml]).
- v) Shake the plate; after incubation, stop the reaction by adding stopping solution to each well (e.g. 0.1 ml sulphuric acid).
- vi) Read the absorbance of each well with the microplate reader at 405 nm (ABTS) or 450–620 nm (TMB). The absorbance values will be used to calculate the results.
- vii) Interpretation of the results

For commercial kits, interpretations and validation criteria are provided with the kit.

Interpretation criteria should be developed and validated for the individual procedures and reagents used in the laboratory. The following is given as an example:

Calculate the mean absorbance (Ab) of the sample serum and of the positive (Ab<sub>pos</sub>) and negative (Ab<sub>neg</sub>) control sera, and for each serum, calculate the percentage:

$$\frac{Ab - Ab_{neg}}{Ab_{pos} - Ab_{neg}} \times 100$$

if a test sample has mean absorbance of <30% it is classed as negative, 30-40% is classed as doubtful, and >40% as positive.

### 2.2.3. Competitive ELISA: test procedure

- i) Add 0.05 ml of undiluted serum and positive/negative controls to antigen-coated plate.
- ii) Incubate for 1 hour at room temperature.
- iii) Empty the plate and wash the plate three with diluted wash solution.
- iv) Add 0.05 ml of diluted antibody-peroxidase conjugate to each well. Mix well and incubate for 30 minutes at room temperature.
- v) After the 30-minute incubation, empty the plate and repeat the washing procedure described in step iii.
- vi) Add 0.05 ml of substrate solution (ex: TMB) to each well. Mix and cover plate with aluminium foil. Incubate for 20 minutes at room temperature. Do not empty wells.
- vii) Add 0.05 ml of stop solution to each well. Mix. Do not empty wells.
- viii) Immediately after adding the stop solution, the plate should be read on a plate reader (620, 630 or 650 nm).
- ix) Interpretation of results

Interpretation criteria should be developed and validated for the individual procedures and reagents used in the laboratory. The following is given as an example:

Calculation:  $100 - [(Sample\ Ab \times 100) / (Mean\ negative\ control\ Ab)] = \% \text{ inhibition}$ .

For goats, if a test sample causes >33.2% inhibition, it is positive; if a test sample causes <33.2% inhibition, it is negative. For sheep, if a test sample causes >20.9% inhibition, it is positive; if a test sample causes <20.9% inhibition, it is negative.

## C. REQUIREMENTS FOR VACCINES

There are no vaccines available.

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**NB:** At the time of publication (2017) there were no WOA Reference Laboratories for caprine arthritis/encephalitis & maedi-visna (please consult the WOA Web site for the current list: <https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).

**NB:** CAPRINE ARTHRITIS/ENCEPHALITIS FIRST ADOPTED IN 1990; MAEDI-VISNA FIRST ADOPTED 1989.  
MOST RECENT UPDATES ADOPTED IN 2017.