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A SENSITIVE, SPECIFIC, PATENTED ELISA FOR DETECTING ANTIBODIES TO MAEDI VISNA VIRUS (MVV) IN SHEEP OR CAPRINE ARTHRITIS ENCEPHALITIS VIRUS IN GOATS

ELITEST MVV/CAEV ELISA is an indirect ELISA for detecting antibodies to Maedi Visna virus (MVV) of sheep and Caprine Arthritis encephalitis virus (CAEV) of goats. **ELITEST** was developed by a collaborative effort between laboratories in the UK, Spain, Italy and Belgium. The ELISA test can be used in serum, plasma, or milk.

Kit presentation: It comes as a kit of 5 plates (which will test a maximum of 460 samples) and is in a format where a whole plate can be used, or strips of 8 wells can be used individually. All necessary reagents are supplied with the kit. The laboratory will need deionised water, pipettes for dilution and filling the wells, bottles for making up wash solutions and an ELISA reader with filters capable of reading at wavelengths of 450 and 595 nm.

Test principle: ELITEST has a number of unique features, some of which are patented. The microwell plates are coated with a combination of the major core protein p25 of MVV produced in *Escherichia coli* and a peptide derived from the immunodominant region of the viral transmembrane protein gp46. The peptide carries an N-terminal biotin residue and is complexed with streptavidin prior to being coated. Putting a combination of defined antigens onto the plate in such a way that they are fully accessible to antibody is technically difficult and this method has been patented. The specific combination of antigens, from the core and the envelope of the virus gives **ELITEST** unique sensitivity, particularly for detecting animals early in the course of infection and during the later or clinical phases of the infection when antibody responses to core antigens decline. Using defined antigens, recombinant p25 and a synthetic peptide, means that the test is highly standardised and reproducibility of results within and between kits is very good. The way the plates are prepared means that test sera can be diluted 1 in 500, thus ensuring that non-specific reactions which are quite commonly due to 'sticky' sheep and goat sera are minimised.

Performance characteristics:

From our studies, the overall sensitivity and specificity of **ELITEST** in sheep is 99.4% and 99.3% respectively. Similar results were obtained for CAEV in goats. This compares with a sensitivity and specificity of 76% and 99.8% obtained respectively with the Agar Gel Immunodiffusion Test. We have found that **ELITEST** can detect seroconversion from 14 to 51 days post experimental infection and consistently detects seroconversion earlier than the AGID test. Intraplate coefficient of variation is 5.5 – 7.5% and interpolate variation is 3.1% in our studies.

The performance of **ELITEST** has been compared by the Dutch Animal Health Service with that of other commercially available ELISA tests and found to be superior by almost all measures (Brinkhof & van Maanen, 2007). It was concluded that **ELITEST** was the method of choice for serodiagnosis of small ruminant lentiviruses.

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Why use our ELITEST MVV/CAEV ELISA?

Independent trial of **ELITEST**: An independent trial has been carried out by the Dutch Animal Health Service between **ELITEST**, other commercially available ELISAs, an ELISA produced by the Dutch government Laboratory in Lelystad (not commercially available) and the AGID test. The tests were compared for sensitivity, specificity, ability to detect clinically affected sheep, ability to detect seroconversion, usability, and reproducibility. The key findings were:

- **ELITEST** is more sensitive than earlier than the AGID test – 99.4% versus 76%
- **ELITEST** picks up infection than other ELISAs and the AGID test – from 2 weeks after infection compared with 6 – 8 weeks for the other ELISAs and about 16 weeks for the AGID test
- **ELITEST** had the best detection limit (geometric mean titre 1 in 25) compared to the other ELISAs for MVV, and had a detection limit for CAEV of GMT = 8
- **ELITEST** detects clinically affected animals which are missed by the other ELISAs – this is because the antibody to the core protein of MVV declines when the animal is clinically affected, but our test detects antibody to the envelope. Because of this **ELITEST** shows the best agreement with the AGID-positive samples
- **ELITEST** detected MVV infection in all animals of a group of 4 experimentally infected sheep by 46 days post infection, whereas 50% had still not become positive by AGID test at 81 days post infection. In a group of 14 goats **ELITEST** detected CAEV infection in all animals by 55 days post infection whereas 42% had not become positive by AGID test at 191 days post infection
- **ELITEST** can be used on milk

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APPLICATIONS

Using ELITEST for accreditation schemes: The Dutch Animal Health Service routinely use **ELITEST** in their accreditation scheme to establish flocks which are 'free' of MVV infection (95% confidence of less than 2% prevalence). In accreditation schemes it is vitally important that false positives are avoided as loss of accredited status can have very significant implications for the farmer. In order to reduce the problem presented by occasional false positive results (which are a feature of all sensitive ELISAs) they use a higher cut-off (approximately 0.7 OD units) than that which is used for maximum sensitivity (approximately 0.25 OD units). This is consistent with OIE recommendations on the use of ELISAs to maximise sensitivity (for diagnostic testing) or to maximise specificity (for accreditation schemes).

False positive results can be minimised further by using a rigorous re-testing protocol. In this protocol each positive serum is re-tested in duplicate to eliminate testing artefacts. If both tests are positive and more than 10% above the cut-off then the animal is regarded as infected. If both are negative, then the animal is regarded as not infected. If the duplicate tests give discrepant results (one positive, one negative) then a further re-test is done until both tests are consistently either positive or negative. If after this scheme the animal is still positive, and especially if it is within 10% above the cut-off, then the animal should be re-bled (to eliminate sample artefacts, e.g. 'sticky' sera, problems with sample quality) ideally within 7 days of the first sample being taken, and the serum sample tested in duplicate as above. It is important to re-bleed within 7 days to avoid animals going negative due to levels of antibody declining (the literature shows that this can happen). We emphasise this re-testing scheme should only be needed in accreditation schemes when occasional positives appear and you have to be certain they are genuine positives or false positives.

Using ELITEST on pooled sera: The Dutch AHS and the Scottish Agricultural College have investigated the possibility of pooling sera to minimise the cost of testing for MVV/CAEV. The Dutch have found that it is possible to use pools of 5 sera when testing flocks of low MVV prevalence, as you would find in accreditation schemes. This can reduce the cost of testing by 40 – 50%. The relative sensitivity of **ELITEST** is slightly reduced, to about 95%, when using pooled sera in this way. Pooling is not economically feasible in flocks with a seroprevalence of more than about 2 – 5%.

Using ELITEST on milk: The Italian laboratory in Pisa (Pr Francesco Tolari and colleagues) have done the most work using **ELITEST** on single and pooled milks. They have shown that, using paired serum and milk samples from the same animal, that the sensitivity and specificity of **ELITEST** is similar in serum and milk. Further, it is possible to detect infection in pools of about 5 milks. The sample preparation protocol for milk (colostrum has not been fully evaluated) is simple in that no de-fatting or other pre-treatment is necessary and the milk is simply diluted 1 in 50 in the diluent used for serum. The ability to use milk to diagnose infection may be particularly useful in milking flocks, or when the farmer has to take the sample himself.

Who uses ELITEST?: As a result of the comprehensive trials described above the Dutch AHS use **ELITEST** for their MVV/CAEV control schemes. In the UK, the Scottish Agricultural College uses **ELITEST** in their MVV/CAEV Accreditation Scheme for eradicating infection from flocks

which have become infected. In the future they plan to use **ELITEST** for the whole scheme. In Spain, the regional government in Aragon uses **ELITEST** to survey flocks as part of a control scheme. In Norway and Sweden **ELITEST** is used in control schemes also. **ELITEST** is currently being used in Belgium, Austria and Finland, and has been used in Italy, Germany, Eire, Greece, Cyprus and Tanzania.

References:

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