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Caprine Arthritis-Encephalitis Virus Antibody Test Kit, cELISA

The study of CAEV has a long history at VMRD. Dr. Scott Adams, President of VMRD, was a member of the team that initially isolated CAEV and characterized the disease in the late 1970s and early 1980s.

VMRD's competitive enzyme-linked immunosorbent assay (cELISA) detects antibodies to caprine arthritis encephalitis virus (CAEV) in goat sera. Our CAEV cELISA test utilizes a proprietary xeno-monoclonal antibody derived by fusion of goat splenocytes and mouse myeloma cells which has excellent characteristics for use in cELISA. This antibody is conjugated to horeseradish peroxidase and is used to compete with serum antibodies for antigen bound to the microtiter plate.

Most indirect ELISA systems presently in use lack specificity to varying degrees. False positive reactions are particularly undesirable in goats of high commercial value. VMRD's competitive ELISA assay for CAEV antibody detection eliminates most of these non-specific reactions.

Several validation studies, in addition to the one summarized here, have confirmed the superior quality of VMRD's CAEV cELISA test kit.

**VMRD's CAEV cELISA Is
Now USDA Licensed and
May Be Purchased Within
the USA!**

caprine samples		CAEV AGID and IP		
		+	-	Sum
VMRD	+	165	1	166
CAEV	-	0	250	250
cELISA	Sum	165	251	416

Sensitivity: 100% • Specificity: 99.6% §

Field Testing Data, 2003.

Overview of the VMRD CAEV Kit Procedure

1	Place 50 µl of samples and controls into wells of Antigen Plate
2	Incubate 60 minutes at room temperature
3	Wash all wells three times with Wash Solution
4	Add 50 µl of Conjugate to all wells
5	Incubate Conjugate 30 minutes at room temperature
6	Wash all wells three times with Wash Solution
7	Add 50 µl of Substrate Solution to all wells
8	Incubate 20 minutes at room temperature
9	Add 50 µl of Stop Solution to all wells
10	Read at 620-650 nm

Samples causing <35% inhibition are negative. Samples causing ≥ 35% inhibition are positive.

Formula for calculating % inhibition:
%I = 100 - [(Sample OD/Mean Negative OD) x 100]

The mean OD of the Negative Controls must be ≥ 0.300. The mean of the Positive Controls must yield a percent inhibition ≥ 35%.

About Caprine Arthritis Encephalitis

Caprine Arthritis Encephalitis (CAE) is one of the most important diseases of goats worldwide. Two manifestations of disease occur: Encephalitis in young kids 2 to 4 months of age, and arthritis in adult goats. CAE is caused by a lentivirus closely related to North American isolates of Ovine Progressive Pneumonia Virus (OPPV). The infection persists for life and antibodies to Caprine Arthritis Encephalitis Virus (CAEV) can be detected using, among others, AGID, ELISA and IFA procedures. The major mode of transmission is from doe to kid through milk and colostrum.

Caprine Arthritis-Encephalitis Virus Antibody Test Kit, cELISA					
Format	Species	Sample	Sensitivity	Specificity	Assay Time
cELISA	caprine	serum	100%	99.6%	2 hours

USDA Licensed Product

Catalog No.	Configuration	Tests
289-2	2 stripwell plates	184
289-5	5 stripwell plates	460

Herrmann, L.M., et al. Competitive-inhibition enzyme-linked immunosorbent assay for detection of serum antibodies to caprine arthritis-encephalitis virus: Diagnostic tool for successful eradication. *Clin. Diagn. Lab. Immunol.* 10(2):267-271 (2003). [This publication documents that the cELISA was more sensitive than immunoprecipitation for detecting antibody in sera of experimentally-infected goats.]

CAEV: “The Rest of the Story”

By D. Scott Adams, D.V.M., Ph.D., President and CEO

To the Irish March 17 was St. Patrick’s Day, but for me it was a major milestone for VMRD and for me personally. We received a USDA product license for our Caprine Arthritis-Encephalitis Virus Antibody Test Kit, cELISA, the culmination of many years of careful research and development. This assay in my opinion is the best serodiagnostic test in the world today for CAEV infection of goats.

If one types the acronym “CAEV” on the search line of PubMed, 251 citations appear going back to 1980. Many of them describe diagnostic tests for CAEV serology. So why do I think VMRD’s new cELISA is the best? It has to do with the long history of the research organizations and people involved in the development of this assay. If one types in names of people I know who were directly involved in development of this assay and CAEV, a total of 89 citations appear. The point is that those involved in the development of this assay have played a major role in what is known and published about CAE.

Tim Crawford started the CAE project in the early 1970s when he was alerted to a rheumatoid arthritis-like disease in a herd of Toggenberg goats by the owner, a savvy physician’s wife named Betty Gustafson. Linda Cork, under Tim’s tutelage and whose committee included such distinguished scientists as Bill Hadlow and John Gorham, worked on leukoencephalitis which occurred among young goats in the same herd. It was thought to be viral because lesions could be produced with a filtrate injected intracerebrally. However, no virus was isolated.

The other clinical presentation in the index herd was chronic arthritis. It was not necessarily thought that the two conditions were related. Tim obtained grants from the Kroc Foundation and NIH to try to determine the etiology of the arthritis in part because it was clinically and histologically quite similar to rheumatoid arthritis. I was his graduate student on the project and Travis McGuire was a member of my graduate committee.

After multiple explants of goat synovial membranes from affected animals, syncytia were observed in a flask of cells taken from a Toggenberg Buck named Victory (G63). Cytopathic effect and indirect immunofluorescence indicated that we had isolated a viral agent. Reverse transcriptase assays performed by Phil Cheevers, buoyant density and electron microscopy confirmed that it was a

retrovirus. Paula Klevjer-Andersen worked out the in-vitro biology of the isolate. The isolate was called 79-63 virus. In vivo experiments with the isolate confirmed Koch’s Postulates. The pathogenesis and immune responses of experimentally-induced disease were worked out. &

The clinical disease was characterized and an agar gel immunodiffusion assay was developed to determine seroprevalence. Morbidity of clinical arthritis in adult goats was much higher than the leukoencephalitis of kid goats so we changed the name to CAE. All of this happened before 1981.

Scrolling forward to 2001-2003, two publications came out of Don Knowles’ laboratory describing reactivity of monoclonal antibodies to the gp135 surface envelope glycoprotein (SU) of CAEV isolate 79-63 (CAEV-63) and use of VMRD’s cELISA which is based upon two of those monoclonal antibodies. The cELISA’s performance was compared to gp135/90 SU/TM protein immunoprecipitation (IP) which is considered the gold standard for detection of antibodies to CAEV viral antigens. Two hundred goat sera collected from the United States were tested by IP and the cELISA. Sensitivity and specificity of the cELISA against IP were 100% and 96.4%, respectively. To analyze the possibility that the cELISA was more sensitive and not less specific than IP, sequential sera from eight experimentally-infected goats were tested by both assays. The cELISA detected antibody in seven of the eight goats before IP, indicating that it is more sensitive than IP.

A field study was carried out by three independent university-associated veterinary diagnostic laboratories located in the eastern, central and western areas of the United States. Four hundred sixteen samples obtained by the participating laboratories were tested by these laboratories with the cELISA and compared to results from a USDA-licensed agar gel immunodiffusion (AGID). Discrepancies were refereed by IP.

Against AGID, the cELISA had 14 false negatives and 20 false positives. Twenty-four of these samples had sufficient volume for IP analysis. Immunoprecipitation results agreed with the cELISA on 23 of the samples. The single sample agreeing with AGID was a negative, but remember that the cELISA was more sensitive than IP on the experimental infections.

A panel of 50 samples (24 positive and 26 negative) was also tested by the laboratories to determine tester interdependency. All three laboratories scored 100% correct results, indicating the field robustness of the cELISA.

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 7. Herrmann LM, Cheevers WP, McGuire TC, Adams DS, Hutton MM, Gavin WG, Knowles DP. Competitive-inhibition enzyme-linked immunosorbent assay for detection of serum antibodies to caprine arthritis-encephalitis virus: diagnostic tool for successful eradication. *Clin Diagn Lab Immunol.* 2003 Mar;10(2):267-71.