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Anaplasma Antibody Test Kit, cELISA

VMRD's *Anaplasma* Antibody Test Kit is a competitive enzyme-linked immunosorbent assay (cELISA) for the detection of antibodies specific for *Anaplasma* in bovine serum samples. It is intended to provide results which will give guidance about the presence of *Anaplasma* infection in bovine species. Sensitivity and specificity are more than four-fold better than the complement fixation test (CFT) which was the former gold standard test. In the study presented in the sensitivity and specificity table on this page, CFT was able to detect only ~20% of positive samples in three independent laboratories.

Our *Anaplasma* cELISA is a breakthrough in diagnosis of anaplasmosis in persistently-infected animals and is recommended by the OIE. It will detect antibodies to *Anaplasma marginale*, *Anaplasma ovis*, and *Anaplasma centrale*. The kit is available in 2-plate and 5-plate formats; both formats use breakaway stripwells.

		Nested PCR		
		+	-	Sum
VMRD <i>Anaplasma</i> cELISA	+	91	1	92
	-	5	40	45
	Sum	96	41	137
Sensitivity: 95% • Specificity: 98% §				

In-house data submitted to USDA in support of licensure, February 1998.



About Anaplasmosis

Anaplasmosis is a non-contagious, arthropod-borne parasitic disease of ruminants that results in significant economic losses to the cattle industry. The disease in cattle is caused by *Anaplasma marginale*, recently classified in geno-group II of Ehrlichiae. *Anaplasma marginale* is an intra-erythrocytic parasite that causes severe anemia, abortion, weight loss, jaundice and death. Diagnosis of the acute disease is based upon clinical signs, anemia and finding of *Anaplasma* inclusion bodies in erythrocytes. Animals surviving the acute phase become lifelong carriers. Ticks transmit the infection from carriers to naive cattle, which develop clinical disease. Cycles of rickettsemia in carriers fluctuate between 10^{2.5} and 10⁷ infected erythrocytes per ml, levels generally undetectable by Giemsa staining. Carriers can be identified by detection of serum antibodies to *A. marginale*.

**VMRD's *Anaplasma* cELISA
Is Setting a New Standard In the
Diagnosis of Anaplasmosis**

Overview of the VMRD *Anaplasma* Kit Procedure

- 1 Place 70 µl of samples & controls into wells of Adsorption Plate
- 2 Incubate 30 minutes at room temperature
- 3 Transfer 50 µl of samples & controls into wells of Antigen Plate
- 4 Incubate 60 minutes at room temperature
- 5 Wash all wells two times with Wash Solution
- 6 Add 50 µl of Conjugate to all wells
- 7 Incubate Conjugate 20 minutes at room temperature
- 8 Wash all wells four times with Wash Solution
- 9 Add 50 µl of Substrate Solution to all wells
- 10 Incubate 20 minutes at room temperature
- 11 Add 50 µl of Stop Solution to all wells
- 12 Read at 620-650 nm

Samples causing < 30% inhibition are negative. Samples causing ≥ 30% inhibition are positive.

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

The mean OD of the Negative Controls must range from 0.40 to 2.10. The mean of the Positive Controls must yield a percent inhibition ≥ 30%.

Anaplasma Antibody Test Kit, cELISA

Format	Species	Sample	Sensitivity	Specificity	Assay Time
cELISA	cattle	serum	95%	98%	145 minutes

USDA Licensed Product

Catalog No.	Configuration	Tests
282-2	2 stripwell plates	182
282-5	5 stripwell plates	455

Cattle Are a Major Reservoir of *Anaplasma marginale*

By Travis C. McGuire, D.V.M., Ph.D., Director of Research

Cattle persistently infected with *Anaplasma marginale* are a major reservoir for transmission of anaplasmosis. This is because a main mode of transmission is by ticks, yet the ticks involved in transmission do not transmit the organism to their progeny through the eggs. This lack of transovarial transmission means that new generations of ticks must acquire infection by feeding on an *A. marginale*-infected host. Once an immature tick stage acquires infection, it is transmitted to subsequent developmental stages, including the adult stage. Any infected stage that feeds on cattle can transmit the infection during feeding.

Uninfected ticks can acquire *A. marginale* infection by feeding on cattle which have very low numbers of infected erythrocytes. In fact, the number of infected erythrocytes can be 10 to 10,000 times less than can be detected by microscopic examination of stained blood smears. Once the tick is infected from small amounts of organisms obtained by feeding on persistently-infected cattle, organisms replicate to high levels in the tick's salivary gland and can be transmitted to uninfected cattle during feeding.

Other Relevant Publications:

Knowles D, Torioni de Echaide S, Palmer G, McGuire T, Stiller D, McElwain T.: Antibody against an *Anaplasma marginale* MSP5 epitope common to tick and erythrocyte stages identifies persistently infected cattle. J Clin Microbiol. 1996 Sep;34(9):2225-30.

Torioni de Echaide S, Knowles DP, McGuire TC, Palmer GH, Suarez CE, McElwain TF.: Detection of cattle naturally infected with *Anaplasma marginale* in a region of endemicity by nested PCR and a competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5. J Clin Microbiol. 1998 Mar;36(3):777-82.

Herrero MV, Perez E, Goff WL, Torioni de Echaide S, Knowles DP, McElwain TF, Alvarez V, Alvarez A, Buening GM.: Prospective study for the detection of *Anaplasma marginale* Theiler, 1911 (Rickettsiales: Anaplasmataceae) in Costa Rica. Ann N Y Acad Sci. 1998 Jun 29;849:226-33.

Based on this information, ticks cannot be a long-term reservoir for *A. marginale*. However, cattle are infected with *A. marginale* for life and can serve as a continual source of infection for tick populations which can then transmit to uninfected cattle. The information that cattle are a major reservoir of *A. marginale* provides a rationale for testing herds in regions where anaplasmosis occurs and removing infected cattle from these herds. Since antibody is continuously present in *A. marginale*-infected cattle, testing for antibody is the easiest and most economical way to identify persistently-infected cattle for treatment with long-acting oxytetracycline or culling.

REFERENCES:

Stich RW, Kocan KM, Palmer GH, Ewing SA, Hair JA, Barron SJ. Transstadial and attempted transovarial transmission of *Anaplasma marginale* by *Dermacentor variabilis*. Am J Vet Res. 50:1377-1380, 1989.

Eriks IS, Stiller D, Palmer GH. Impact of persistent *Anaplasma marginale* rickettsemia on tick infection and transmission. J Clin Microbiol. 31:2091-206, 1993.