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NEWSLETTER

APRIL 2006

EIAV ELISA Licensed!

Catalog Numbers 290-1 & 290-5

VMRD has developed an Equine Infectious Anemia Virus ELISA that was recently USDA licensed.

Our assay compares very favorably to the other commercially-available EIAV ELISAs and achieved 100% sensitivity and specificity in field testing. Total incubation time is 35 minutes and the plates can be read by eye as well as with a microplate absorbance reader. The kit contains no Thimerosal and produces no hazardous waste. We are presently offering a stripwell 1-plate kit and a stripwell 5-plate kit. ❖



A Tail of Flagella

By Ethan Adams, Director of Marketing

In 1995, when we first tested the *Clostridium chauvoei* antiserum that serves as the raw material for our *C. chauvoei* conjugate, vehement expostulations proceeded from our darkened microscopy room that are probably not printable in this newsletter. The source of this angst was the fluorescence of not only rod-shaped *C. chauvoei* bacteria but also what appeared to be spirochetes. As you can see from Figures 1 & 2, there is a certain resemblance between the spiral structure that our conjugate labeled and a typical spirochete. We theorized that the *C. chauvoei* culture against which we had raised our antiserum (and from which we had made our slides) had been contaminated with a spirochete, resulting in the development of antibody not only to *C. chauvoei* but also to the mystery spirochete. Though we were somewhat chagrined by this development we decided that, since spirochetes are easy to differentiate from rods, the antibody would still be useful for detecting *C. chauvoei*. We conjugated the antibody, noted the apparent snafu on our Certificate of Analysis, and sold the conjugate.



Figure 1
Spirochete?



Figure 2
Borrelia burgdorferi

Fast-forward a decade to 2005 when a gentleman named Peter Wragg at Veterinary Laboratory Agencies in the UK observed what we had thought was cross-reactivity.

Being better informed than we, Peter knew that *Clostridium chauvoei* is peritrichous (has flagella uniformly distributed over its cell wall). Peter realized that what we had thought to be spirochetes were actually quite probably detached flagella. He was kind enough to share some photomicrographs of our conjugate staining a fresh culture of *C. chauvoei* that clearly show the flagella attached (Figure 3).

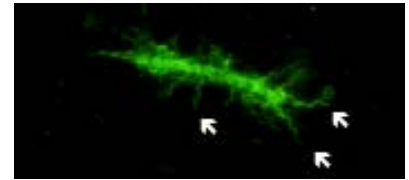


Figure 3
Fresh culture of *C. chauvoei*

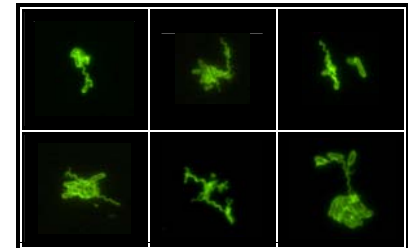


Figure 4

Following Peter's revelation, we applied our conjugate to quite a few wells of our *C. chauvoei* slides, which are made with our killed *C. chauvoei* culture, and photographed a number of spiral structures (Fig.4). We agree with Peter's opinion of these structures and have modified our Certificate of Analysis accordingly.

We thank Peter Wragg for pointing out our error and kindly sharing his photomicrographs. We also thank Veterinary Laboratory Agencies for permitting the use of Peter's findings and photos in this newsletter. ❖

See You at Both AVM Conferences!

VMRD will be attending the 12th Annual Meeting of the Heartland Chapter of the Association of Veterinary Microbiologists in Reynoldsburg Ohio, as well as the National AVM meeting in Gatlinburg, Tennessee. The AVM Heartland Chapter meeting convenes on the Ohio Department of Agriculture's campus from April 28-29, 2006. The National meeting will be held August 17-20, 2006 at the Park Vista Hotel in the beautiful Smoky Mountains of Tennessee. We will be exhibiting new products, including our ELISAWare™ software and our recently licensed EIAV ELISA, at both meetings. If you attend the AVM-Heartland meeting be sure to stop by the Broomfield Administration Building to pick up a VMRD catalog and some useful loot, or just to chat and let us know how we can better meet your lab's needs. ❖

Technical Services Inquiries

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

Ehrlichia canis –

Question: Why is the IFA positive using VMRD *E. canis* substrate slides while with the same sample I get a negative reaction with an *E. canis* lateral flow device?

Answer: IFA has broader reactivity than the lateral flow assay. The lateral flow assay detects antibody to *E. canis* only. The IFA will detect antibody to *E. ewingii* and *E. chaffeensis* as well and perhaps to other *Ehrlichia spp.* yet to be identified.

Clostridium novyi species reclassification –

Question: Does VMRD's *C. novyi* Direct FA conjugate (Catalog Nos. 210-16-CN & 210-17-CN) react with *C. haemolyticum*?

Answer: We have recently learned that *Clostridium novyi* and *Clostridium haemolyticum* have been reclassified as very similar, if not the same organism. Thus, our *C. novyi* (B) direct FA conjugate will react with both *C. novyi* and *C. haemolyticum* organisms.

Anaplasma marginale-*Anaplasma phagocytophilum* cross-reactivity –

Question: A recent paper out of Switzerland [Dreher *et al.*] documents serologic cross-reactivity between *A. marginale* and *A. phagocytophilum* using VMRD's cELISA and IFA for the aforementioned agents, respectively. What is VMRD's position with respect to the meaning of data derived from either assay?

Answer: From the time that VMRD began marketing the cELISA for detection of antibody to *Anaplasma* in cattle it was known and we readily acknowledged in our literature and product insert that it was designed to detect antibody to *Anaplasma spp.* not *A. marginale* only. This was based on work previously published about MSP-5, major surface protein-5 of *Anaplasma spp.* by Visser *et al.* The species specifically mentioned at the time were *A. marginale*, *A. centrale* and *A. ovis*. Furthermore, the cELISA has been shown to detect antibody to *A. ovis* in goat sera [Ndung'u *et al.*]. Thus it is not surprising to find that another species, *A. phagocytophilum*, which has recently been reclassified from an *Ehrlichia* to an *Anaplasma spp.*, would also induce antibody capable of inhibiting binding of the monoclonal antibody used in the cELISA, thus giving a positive result. Therefore, as indicated from the beginning, VMRD's cELISA is a genus specific test for *Anaplasma*.

Dreher, U.M., *et al.* Serologic Cross-Reactivity between *Anaplasma marginale* and *Anaplasma phagocytophilum*. *Clinical and Diagnostic Laboratory Immunology* 12(10):1177-1183 (Oct. 2005).

Ndung'u, L.W., *et al.* Detection of *Anaplasma ovis* infection in goats by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* 33(3): 675-679 (Mar. 1995).

Visser, E.S., *et al.* 1992. The *Anaplasma marginale* msp5 gene encodes a 19-kilodalton protein conserved in all recognized *Anaplasma* species. *Infect. Immun.* 60:5139-5144 (1992).

All of the samples tested by the two assays in the Swiss paper were from experimental infections. Nevertheless, the authors bring up an important point that serological results must be carefully considered when cross-reactions are possible. In my opinion, that would be always. Without clinical evaluation and epidemiological considerations, serology has limited value. Where *Anaplasma spp.* are concerned, by far the most prominent problem in cattle is *A. marginale*. Its differentiation from *A. phagocytophilum* infection, if necessary and, if clinical inquiry does not reveal the difference, may require molecular analysis of the agents involved to make the diagnosis.

Bovine Herpesvirus 1-Bovine Herpesvirus 5 cross-reactivity –

Question: Do any of VMRD's BHV-1 monoclonal antibodies react with BHV-5?

Answer: Yes. The table below shows the results of IFA tests against our isolates of BHV-1 and BHV-5. Only F2 does not react with BHV-5. L6G reacts only with BHV-5 and not BHV-1. ❖

Ab	Description	Reacts with:	
		BHV-1	BHV-5
D9E7	BHV-1 (gB - gI)	+	+
H2	BHV-1 (gB - gI)	+	+
G2	BHV-1 (gC - gIII)	+	+
F2	BHV-1 (gC - gIII)	+	-
1B8-F11	BHV-1 (gD - gIV)	+	+
L6G	BHV-5 (gC)	-	+

Interpreting Antibody Responses to EIAV Proteins that Vary

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

Antigenic variation occurs in a number of infectious organisms and in all persistent infections that have been carefully investigated. Antigenic variation is a result of changes in the protein sequence of agents which are caused by changes in the nucleic acid sequence that determine which amino acids are used to make the protein. Changes in nucleic acid sequence are caused by different mechanisms in different agents.

In lentiviruses—including equine infectious anemia virus (EIAV), caprine arthritis and encephalitis virus (CAEV), ovine progressive pneumonia virus (OPPV), feline immunodeficiency virus (FIV), human immunodeficiency virus (HIV) and others—amino acid changes occur in all

the proteins the virus makes. These changes occur when a DNA copy is made from viral RNA after a cell is infected because about one in 5000 nucleotides used to make the DNA is wrong. This is not corrected because the reverse transcriptase (RNA-dependent DNA polymerase) which catalyses the reaction does not have the proof-reading capacity attributed to other DNA polymerases. Therefore, one or two random mutations occur each time a DNA copy of the approximately 8000 nucleotide viral RNA is made. During virus production, the mutated DNA copy is integrated into the infected cell's genome and then RNA and subsequently protein are made. Some of this new RNA is packaged into progeny virus which carries the mutations to newly infected cells to repeat the cycle. Since the mutations are random, amino acid changes occur in every protein that the virus makes.

Why then does the envelope protein gp90 of EIAV have more mutations when the sequence is actually determined than do other proteins like p26? The answer is selection. In this case, the selective pressures that determine which mutant viruses survive are the immune response of the host and the survivability of the mutants. Examples that affect virus survivability are that some mutations are lethal, some may infect cells better or worse, and some may replicate faster or slower. Rapid changes in gp90 are best explained by the host immune response, in particular, neutralizing antibody. Neutralizing antibody is made to gp90 of the infecting virus and prevents infection of new cells by any virus with the same gp90. Therefore, for the continued infection which occurs with EIAV, viruses which have mutations in gp90 occur which cannot be recognized by existing neutralizing antibody in the horse. Only those gp90 mutants can infect new cells.

Since neutralizing antibody does not bind to p26 which is an internal capsid protein of the virus, what is the explanation for amino acid changes in p26 which can be as high as 12%? One explanation is selection by cytotoxic T lymphocytes (CTL) which recognize and kill infected cells which present p26 epitopes bound to MHC class I molecules on their surface. Once these CTL are present, there is a selective advantage for mutant viruses which have amino acid changes in the recognized epitopes. Another explanation is selection based on mutations that affect survivability that were listed in the previous paragraph.

If antigenic variation occurs in p26, how can protein derived from a single recombinant bacterium used in the VMRD AGID and cELISA tests detect antibody in the serum of horses infected with viruses with amino acid changes in p26? First, the maximum p26 amino acid

Chung, C., *et al.* Evaluation of high functional avidity CTL to Gag epitope clusters in EIAV carrier horses. *Virology* 342:228-239 (2005).
 Coggins, L., *et al.* Diagnosis of equine infectious anemia by immunodiffusion test. *Am. J. Vet. Res.* 33:11-18 (1972).
 Howe, L., *et al.* Equine infectious anemia virus envelope evolution *in vivo* during persistent infection progressively increases resistance to *in vitro* serum antibody neutralization as a dominant phenotype. *J. Virol.* 76:10588-10597 (2002).
 Zhang, W., *et al.* Natural variation of equine infectious anemia virus Gag protein cytotoxic T lymphocyte epitopes. *Virology* 261:242-252 (1999).

difference described is 12%, so shared epitopes remain and/or mutated epitopes cross-react. Second, other common epitopes are present and stable in p26 because mutations in these regions do not result in viable virus or competitive virus. Such common epitopes are likely located in regions of the protein required for function. In addition, there are common epitopes in functional regions of gp90. These explanations may account for the initial observation that p26 derived from a single virus strain could be used to detect serum antibody from horses infected with laboratory and field strains. ❖

Sorting Numeric Sample IDs in ELISAWare

By Ethan Adams, System Analyst

Several customers have communicated to us their annoyance with the way our ELISAWare microplate reading software sorts numeric sample IDs in reports. ELISAWare is designed to sort sample IDs alphabetically. Computers effectively sort from left to right when they sort alphabetically, whereas they effectively sort from right to left when they sort numerically. This leads to sorting such as the following: 1, 10, 11, 12 . . . 2, 20, 21, 22 . . . 3, 30, 31, etc. We concur that this is highly annoying and hope to eliminate the problem in a future version of ELISAWare. However, one of our customers, Pilar Gunter at Kansas State University, came up with a simple and easy workaround. We would like to share this solution with you for your use in the interim until a new version of ELISAWare is available. An alphabetic sort of numbers can mimic a numeric sort if place-holding zeroes are inserted before the number. The number of place-holding zeroes to use is determined by the maximum number of digits in the

Problem	Solution
Sample Name	Sample Name
1	01
10	02
14	03
17	04
2	10
20	14
22	17
23	20
24	22
3	23
30	24
31	30
32	31
33	32
34	33
4	34
40	40
41	41
42	42

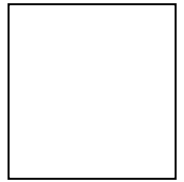
largest number in the set of numbers to be sorted. For instance, if the largest number that will be used is 873, which has 3 digits, then type the numbers 1-9 as 001, 002, 003 . . . 009 and the numbers 10-99 as 010, 011, 012 . . . 099. Of course the numbers 100-873 may be typed normally. As a second example, if the largest number anticipated is 91, then a zero only needs to be inserted before the numbers 1-9. We thank Pilar for sharing her solution to this problem and hope that others find it useful. ❖



VMRD, Inc.

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THE VET'S WIFE'S REFRIGERATOR

Contributed by Baxter Black, DVM

A scream from the kitchen. The thud of a faint.
She sighs and arises and walks with restraint.
Her neighbor lays peaceful, eyes fixed in a stare
She's passed out in front of the new Frigidaire.
She looks at the rack with eggs in its keep
Winking up at her's the eye of a sheep.
There's a bottle of PenStrep near the Swanson's Pot Pies
And down in the crisper's a bagful of flies.
The butter tray's filled with test tubes of blood
Marked, "E.I.A. samples, from Tucker's old stud."
High on the shelf near a platter of cheese
Is a knotted, but leaking, obscene plastic sleeve.

Fecal containers are stacked, side by side,
With yesterday's piece of chicken, home fried.
The freezer's a dither of guts, lungs and spleens
Scattered amongst the Birds Eye green beans.
Her home's a museum of animal parts.
Lymphomatous lymph nodes, selenium hearts.
Enough tissue samples to hold up a bridge
But why do they always end up in the fridge?
But she doesn't worry or turn up her nose,
She's the wife of a vet, it's the life that she chose.
But maybe he'd worry at lunch if he knew
He might just be dining on Whirl-Pack stew!

Baxter Black, cowboy poet / DVM, can be found on the web at www.baxterblack.com. He can also be reached by E-mail at headcowboy@baxterblack.com or by a good old fashioned phone call at (800)654-2550. He resides in Benson AZ and writes a weekly column entitled "ON THE EDGE OF COMMON SENSE."



K99+ Pilitest—Correct Media is Critical

Users of our *Escherichia coli* Antigen Test Kit (K99 Pilitest catalog nos. 299-10 & 299-25) should be aware that certain growth media discourage the development of K99 pili on *Escherichia coli* organisms. If the pili are not present, of course, our test cannot detect them. ATCC recommends 18 Trypticase soy agar and we find that this media produces good growth and formation of K99 pili. We suggest that cultures to be tested for K99 pili be grown on this media, or on media that you have validated in your own laboratory as suitable for this purpose. ❖
