

# VMRD

PO Box 502 ❖ Pullman, WA 99163 ❖ USA

Phone: (800) 222-8673  
(509) 334-5815  
FAX: (509) 332-5356  
E-mail: [order@vmrd.com](mailto:order@vmrd.com)  
[techserve@vmrd.com](mailto:techserve@vmrd.com)  
Web: [www.vmrd.com](http://www.vmrd.com)

## NEWSLETTER

## SPRING 2009

### BLV Update

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

Many of our customers have considered and some have asked about published sensitivity and specificity data for our **Bovine Leukemia Virus Antibody Test Kit, ELISA (Cat. No. 284; 1 Plate and 284-5; 5 Plate)**. We publish our field validation data as required and reviewed by CVB-USDA. The idea is to make sure that companies use third party field validation data for published specificity and sensitivity information rather than in-house data which might look more enticing to the buyer.

The field validation data for our kit contained no false positives among the 280 BLV antibody negative samples tested in 3 different laboratories. This led to jubilation, albeit temporary, on our part because we could (or had to) legitimately claim 100% specificity for the kit. Temporary because about the time we had the sense to think it through we also had fielded a few technical service calls which indicated that some customers had encountered samples that were borderline positive with the ELISA and AGID negative.

If there's one thing we should have learned in 25+ years of manufacturing immunodiagnostic tests, it's "there's no such thing as 100% specificity, or sensitivity for that matter." Well, you could get something like 95% and round it up to 100%, or 51% and round up to 100%. Or you could get lucky like we did with a specific sample set that had no false positives. But in the real world there is no 100% sensitivity or specificity. We have encountered false positives with this assay subsequent to the field validations and have modified our literature with the following proviso about our 100% specificity claim:

"[100% specificity is] based on a specific sample set. However, no diagnostic test kit is always 100% specific on all sample populations. Since market introduction of our BLV kit, occasional false positives have been encountered. We therefore advise all users that when BLV prevalence is low, positive samples should be confirmed by some other method, particularly where valuable animals may be involved and/or when BLV status is used as the single criterion for disposition of animals. Whenever import restrictions do not prohibit it, VMRD will provide reference assay service for positives of high-value animals or for positives in low-prevalence

situations. We are not capable of testing large numbers of samples, and therefore cannot provide this reference assay service for all positives found."

Since the realization of this problem, we have expended considerable research effort trying to improve the specificity of this assay. In fact we have spent more time and money post introduction on this assay than on any other we have every produced. We continue to do so and have been able to make substantive progress with the current format. We are thankful for the patience and help provided by our customers, especially, in providing problem samples for analysis. The best way for us to make improvements is to work with the problem samples.



In doing so, we have been able to determine that anti-mouse IgG antibodies in bovine sera account for the majority of false positive reactions. For those not familiar with the assay configuration, the gp51 antigen is captured with monoclonal mouse anti-gp51 antibody.

In a recent analysis of data from one laboratory where 2682 samples were tested by the ELISA as it currently exists, the specificity was 97.5% against AGID as the referent assay. There were 57 samples that were determined to be "false positives." Of these we were able to test 43 with an improved iteration of the ELISA which registered positive on only 4. Extrapolating and assuming all other factors would remain the same, this would have improved the specificity in that sample set to 99.9%.

We realize, as I'm sure others do, that there are no other USDA licensed BLV antibody test kits in ELISA format available for sale the United States at this time. We are

working towards licensure of the improved BLV ELISA as quickly as possible. Our current kit is a good assay with only very rare occurrence of “false positives,” but thanks to the cooperation of our many customers and the hard work of our technicians we have an opportunity to turn a very good kit into an excellent one. We would like to extend a “thank you” to everybody that has assisted us by providing the troublesome samples found in the field.

If you would like to be notified when the new format BLV kit is available for commercial release please contact VMRD, Inc. at [vmrd@vmrd.com](mailto:vmrd@vmrd.com). ❖



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## QC Manuals: FAs and Red Ink

By Ethan Adams, COO

Our quality control lab was troubled with a spate of FAs and IFAs that had extreme autofluorescence—or so we thought. Negative cells ranged in color from dull orange to olive-drab with sufficient brightness to make the apple-green specific fluorescence difficult to observe. Particularly puzzling was that this supposed autofluorescence was intermittent in the QC lab and was not happening in our other labs. After much hair-pulling and tinkering, we discovered that it only happened when we used a red lab marker to mark the slides. Further, we discovered that it only happened if the writing in red ink on the frosted edge of the slide was submerged in the soaking buffer. We presume that there is an orange-fluorescing dye in the red ink that rapidly and efficiently stains cells. Apparently, only minute quantities of the dye are necessary to stain the cells as there was no visible leaching of the written ink. We have traditionally used black lab markers to mark slides and have experienced no problems with these, regardless of whether the ink was submerged in the soaking buffer. We report this incident not only to warn our customers against using red lab markers on FA slides, but also to demonstrate the sort of minute factors that can affect any laboratory process. A zany red lab pen put our QC lab in a quandary for several weeks. When things stop working, it is necessary to look for any change in procedure, no matter how seemingly trivial. ❖

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## Is Equine Infectious Anemia (EIA) Still a Threat?

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

EIA is still clearly a threat; however the threat is less than in the 1970s. The overall percentage of EIA positive horses among those tested in the United States decreased from near 4% in 1972 to <0.01% in 2005<sup>1</sup>. Prior to the mid 1990s, 92% of the positive horses were in a “hot zone” (see illustration) of states that included the South (including Texas and Oklahoma) and parts of the

Midwest<sup>1</sup>. Since the mid 1990s there have been clusters of EIA occurring in states outside the hot zone that can be seen on the website: [http://imsprod.aphis.usda.gov/website/eia\\_grouped\\_toc/viewer.htm](http://imsprod.aphis.usda.gov/website/eia_grouped_toc/viewer.htm), which contains complete data and maps on EIA testing in the United States from 1972 to 2007. Notable increases in positive horses outside the hot zone states include those in Nevada in 2003; South Dakota in 2001; Utah in 2000; North Dakota in 1999; Utah, Oregon and Indiana in 1998; South Dakota in 1997; Idaho in 1996; and New York and Michigan in 1995. For 2006, the percent positive horses among those tested varied by state from zero in 27 states to 0.048% in Mississippi where 20 of 41,331 samples were positive. It seems that infected horses in the hot zone states continue transmission and that sometimes transmission occurs in states outside the hot zone. Infections in the states inside and outside the hot zone are due to transmission from infected horses, although the sources of the infected horses are not always known.



A documented outbreak of EIA outside the United States demonstrates the threat of an infected horse. On June 15, 2006, the first case of EIA was reported in the Republic of Ireland<sup>2</sup> and by November 14, 2006, the number of cases in the Republic of Ireland was 26<sup>3</sup>. All the 26 cases appeared to be epidemiologically linked. In August of 2006 the first case of EIA occurred in Northern Ireland and the case was epidemiologically linked to the outbreak in the Republic of Ireland<sup>3</sup>. The source of the outbreak in the Republic of Ireland was thought to be due to the use of an unauthorized veterinary medical product<sup>2</sup>. Regardless of the source of the initial infection with EIA virus, there was transmission to other horses. In this outbreak, the first horse infected was documented to be a threat to other horses.

Transmission of EIA from an infected horse usually occurs by blood transfer to a non-infected horse. Blood transfer and transmission occurs naturally by biting insects such as horseflies and deerflies following interrupted feeding on an infected horse<sup>1</sup>. Blood transfer and transmission also occurs from the use of blood-contaminated needles and equipment. Transmission with other body fluids may

occur. Recent quantification of EIA virus RNA by RT-PCR demonstrated significant RNA copies in nasal, buccal and genital secretions obtained from swabs; however, the quantities were always less than in plasma<sup>4</sup>. Transmission from horses in the carrier-state with low-level viremia is much less probable than from an acute case with high-level viremia. The practical problem in EIA control and in determining the threat from a particular EIA-infected horse is distinguishing whether the infected horse has a high- or low-level viremia. Further, depending on the horse and the duration of infection, periods of low-level viremia are interrupted with episodes of high-level viremia increasing the potential for transmission. ❖

1. [http://nahms.aphis.usda.gov/equine/equine05/equine05\\_infosheet\\_eia.pdf](http://nahms.aphis.usda.gov/equine/equine05/equine05_infosheet_eia.pdf)
2. Reynolds, DI. Equine infectious anaemia in Ireland. *Veterinary Record* 159:187, 2006.
3. Menzies F, Patterson T. Description of the first case of equine infectious anaemia in Northern Ireland. *Veterinary Record* 159:753-754, 2006.
4. Quinlivan M, Cook RF, Cullinane A. Real-time quantitative RT-PCR and PCR assays for a novel European field isolate of equine infectious anaemia virus based sequence determination of the gag gene. *Veterinary Record* 160:611-618, 2007.



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## VMRD Lab Tips: Beware of Bio-Tek Bottom Wash

By Ethan Adams, COO

We all know that hog wash is to be eschewed, but we have recently encountered another type of wash that should be avoided as it can cause needless headaches—and possibly invalid results—with some of our ELISA assays. We have always enjoyed working with Bio-Tek Instruments, Inc. They make good equipment, their people are easy to work with, and they have good technical support. We have recently discovered, though, that many of their plate washers have a feature that, if used, is deleterious to the performance of our assays. This feature is called “bottom wash.” When bottom wash is enabled, a wash cycle consists of the machine’s dispensing a small amount of wash in the bottom of the wells, aspirating it out, and finally completely filling and aspirating the wells. Because of the way our plates are coated, the use of bottom wash effectively doubles the amount of washing that the antigen-antibody complex receives. In some of our assays, this does not matter too much, but in some, for instance the Anaplasma cELISA, bottom wash deprives the assay of much of its ability to differentiate positives from negatives. As a rule our washing recommendations should be strictly observed. More washing is not better. Less washing is not better. When we recommend three washes, you can be sure that we have tried one, two, three,

four, and five washes, and three worked the best. Doubtless there are applications where the Bio-Tek bottom wash feature is a great thing, but for VMRD assays, we highly recommend that bottom wash be turned off. ❖

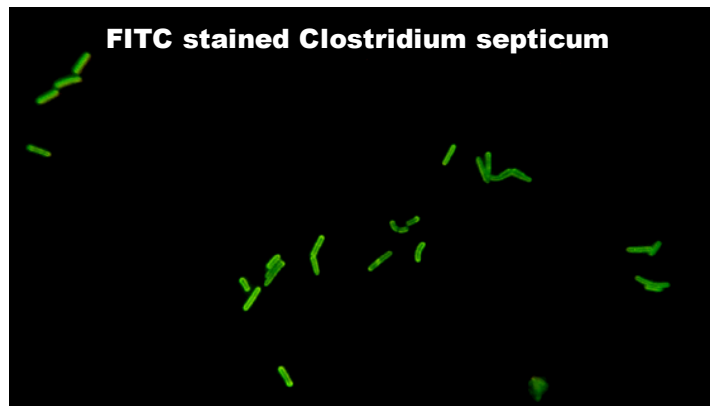
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## Adventures with Clostridia

By Theresa Adams, Research Technician

We have received several claims that the VMRD Clostridium septicum direct FA conjugate does not bind certain cultures of Clostridium thought to be C. septicum. To investigate these claims, we obtained one of the putative C. septicum isolates. The stench it produced in anaerobic culture supported its identity as a Clostridium species, and the swarming behavior on agar reported by our customer suggested C. septicum. A Gram stain showed Gram-positive rods with some spore formation. This culture did not react with our C. septicum direct FA conjugate. It also did not react with our C. chauvoei, C. novyi, or C. sordellii direct FA conjugates. All that could be seen by FA microscopy were faint outlines of long rods. We were prepared to develop a new C. septicum conjugate that would react with this ornery culture but first we had to be certain of its identity. DNA analysis by PCR amplified a segment of the 16S ribosomal RNA gene. The closest sequence match—homology was 100%—in GenBank was C. fallax. An earlier clue suggesting that the sample was not C. septicum was that chopped meat broth turned partially black instead of pink after 48 hours of growth<sup>1</sup>. The species name, fallax, meaning “deceptive” in Latin<sup>2</sup>, was most appropriate for this sample, which misled even the best bacteriologists to believe that it was C. septicum. ❖

1. Sneath, Peter H. A.. “Bergey’s Manual of Systematic Bacteriology, Volume 2.” Maryland: Williams & Wilkin, 1986. Page 1188
2. Ibid. Page 1167

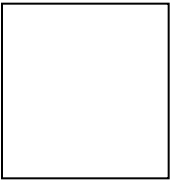


**Invitation for a Lab Tip:** Got a Lab Tip? We invite you submit any tricks of the trade that you are using in your laboratory. If your tip is chosen for publication in the VMRD Newsletter you will be rewarded for your advice with a tin of the world famous Cougar Gold Cheese! Submit your tip to: [luke@vmrd.com](mailto:luke@vmrd.com).



**VMRD, Inc.**

PO Box 502  
Pullman, WA 99163



## Baxter On The Back



### PEOPLE ARE FUNNY CRITTERS

Contributed by Baxter Black, DVM

There's

Apple pie bakers and crooked bookmakers  
 Blondes and brunettes and birthday forgetters  
 Chicken fry lovers and blue-eyed soul brothers  
 Drinkers and boozers and winners and losers  
 Elephant trainers and tireless campaigners  
 Fixers and menders and paper clip benders  
 Goers and stayers and pinochle players  
 Handkerchief users and tissue abusers  
 Interstate bikers and wilderness hikers  
 Joggers and addicts and handball fanatics  
 Kissers and tellers and friends of the fellers  
 Lovers and fighters and fingernail biters  
 Mayonnaise dippers and Miracle Whippers  
 Newspaper readers and drivers and speeders  
 Overweight hookers and magazine lookers  
 People with answers and bottomless dancers  
 Quivering flunkers and basketball dunkers  
 Readers and thinkers and double scotch drinkers  
 Soda straw manglers and bar napkin stranglers  
 Teasers and cryers and high rollin' fliers  
 Uncles and sisters and passive resisters  
 Virtuous girlies and sillies and squirrelies  
 Weirdos and sickies and five dollar quickies  
 Xylophone pickers and popsicle lickens  
 Yawners and nappers and one handicappers  
 Zippy old timers and lunatic rhymers...

people are funny critters

### PEOPLE ARE FUNNY CRITTERS CHAPTER TWO

There's

Artichoke peelers and ex-Pittsburgh Steelers  
 Building erectors and true genuflectors  
 Chewbacca rooters and hard eight crap shooters  
 Down in the dumpsters, measlers and mumpsters  
 Electrical wizards with spark in their gizzards  
 Flakey fast talkers and wild turkey stalkers  
 Garbanzo bean eaters and chronic repeaters  
 Happy go luckers, goosers and duckers  
 Illusive bill payers, watchers and players  
 Jugglers and punters and cardiac shunters  
 Krackle Korn crunchers and martini lunchers  
 Lookingbill spotters and 3-gaited trotters  
 Mare ridin' mothers and ol' Tommy Smothers  
 Nuisance creators and foul catfish baiters  
 Oliver Twisters and squabblin' sisters  
 Pulitzer writers and persimmon biters  
 Quacky ol' doctors and potion concoctors  
 Right handed wipers, plumbers and pipers  
 Silver tongued devils and men on the level  
 Tit clingin' babies and wine drinkin' maybes  
 Unfulfilled madiens with hearts heavy laden  
 Vocal exclaimers and name droppin' namers  
 Wild parachuters and 5-buckle booters  
 X rated peekers and Kiowa Creekers  
 Yacht racin' crazies and barn sour lazies  
 Zany 'don't throw its!' and maniac poets

people are funny critters

*Baxter Black, cowboy poet / DVM, can be found on the web at [www.baxterblack.com](http://www.baxterblack.com). He can also be reached by E-mail at [headcowboy@baxterblack.com](mailto:headcowboy@baxterblack.com) or by a good old fashioned phone call at (800)654-2550*