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NEWSLETTER

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Fraternity

By Joseph Adams, Assistant Comptroller



On the left is my brother, Ethan Adams, now Chief Operations Officer of VMRD, Inc. That's me on the right. My dad started VMRD in our basement in 1980. By 1982, when this picture was taken, VMRD was a fledgling company that had just graduated to its first official location. In the new location, Ethan no longer had opportunity to answer the phone and baby-talk with the customers. But that was only temporary; now, in our state-of-the-art facility where VMRD has been since 1999, Ethan is on the phone with our customers every day. Today Ethan and I are back to eating lunch together; in July I joined the company in the accounting department. I am excited to be a part of this vibrant family business, where we work together to provide our customers with high-quality products and down-home service. ❖



VMRD's EIA Positive Control

By Ethan Adams, COO

Since the release of our EIA ELISA early this year we have sent out many kits for evaluation. Most laboratories have been very satisfied with the performance of the kit and many have adopted it. A few laboratories expressed dissatisfaction with the performance of the kit on positive samples. This was initially perplexing to us, but further investigation revealed that these laboratories did not have ready access to positive field samples and the only positive sample included in their evaluation was the assay's positive control. Once we were informed of this, the reason for their dissatisfaction became readily apparent. By design the positive control is calibrated to produce weak color development that is not typical of a positive sample.

In VMRD's EIA ELISA, the positive control is a cutoff control; samples producing a lower optical density (OD) than the positive control are negative while those producing an equal or higher OD are positive. While there are many ways to calculate a cutoff in an ELISA assay, we chose to use a positive cutoff control because it so readily facilitates visual interpretation of the assay. Many small labs cannot justify the expense of a microplate absorbance reader and need the ability to read the assay by eye. With a cutoff control they can call any well that appears darker than the positive control positive. While this is not nearly as precise as the use of a microplate spectrophotometer, it is quite adequate for the job, especially given the broad dynamic range of our EIA ELISA.

In establishing the cutoff for a serological assay, it is necessary to balance sensitivity with specificity. This involves finding the negative samples that give the highest background signal as well as the positive samples that give the lowest specific signal and finding a happy medium between the two. The more samples that can be tested the fewer unpleasant surprises arise in the field. In the case of the EIA ELISA, we tested over 1600 AGID negative horses and as many positive samples as we could gather. Of particular interest to us among the latter were the numerous weak-positive samples that we have collected in our many years of making our EIA AGID. In the largest set of negative

samples (over 1000 samples), the highest OD that we encountered was 0.07 and the average OD was 0.04. In one set of 50 positive samples, which contained some notoriously weak AGID positives, the lowest positive that we encountered had an OD greater than 0.30 and the average positive OD was over 1.10. Other positive sample sets produced similar results. From this data we concluded that our positive control should be calibrated to produce an OD between 0.10 and 0.20. This cutoff results in excellent differentiation of negative samples while providing excellent sensitivity.

Producing the positive control for the EIA ELISA was a simple matter of diluting a positive sample sufficiently to produce an OD falling in the desired range. Its OD, generally between 0.10 and 0.20, is not especially impressive for a true positive sample, but it must be understood that the kit positive control is not representative of true positive samples. Rather it is a serum artificially diluted to give a certain OD that is much lower than that typical of positive samples encountered in the field, yet significantly higher than the OD of negative samples. Thus judging the assay based on its cutoff positive control does not give the investigator an accurate picture of the performance of the assay.

We recognize that positive samples are difficult to acquire for some laboratories, and we want our customers to be able to see how our assay performs on positive samples. Furthermore, we understand that laboratory quality managers are increasingly interested in obtaining positive reference sera for EIA that are from a single, consistent, and large supply. To encompass these needs, VMRD plans to offer a strong, a medium, and a weak positive reference serum for EIA. These sera will be obtained from large pools, aliquoted to minimize freeze-thawing, and preserved for long shelf life. Although we cannot fully anticipate demand, we believe that the pools are large enough to last for many years. We expect that these three new products will be available in early 2007. We welcome any input with respect to aliquot size.

We heartily encourage anyone who evaluated our EIA ELISA and was disappointed with the intensity of the positive control to re-evaluate it using these new reference sera. Try your toughest AGID weak positive samples, your most petulant check set samples, and the weakest positives you have found with other EIA ELISA assays. Titer them if you wish. You will not be disappointed! ❖

John Wesley Black

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

I first met John Wesley Black in 1985. I was in Middle Tennessee, where John had just begun his own testing laboratory after spending about 20 years in veterinary

diagnostic virology. He called his new company American BioResearch, or ABR. The business performs virology-related services, consulting, reagent manufacture, and virus and cell seed production.

John's laboratory in Tennessee, a cinderblock building in the countryside, had been a small grocery store before he converted it into a lab. He couldn't purify the well water (too much sulfur and iron) to make medium, so he concocted an elaborate PVC gutter system that collected rainwater into a holding tank. I don't think he knew it, but right then I knew we would be long-time friends: VMRD's first laboratory shaker/rocker was made from a wiper motor out of a '58 Chevy and our first roller bottle apparatus was a modified GE clothes dryer.

We discussed collaborations, but John was wary since I was from North of the extrapolated Mason-Dixon Line. Later I revealed that my dad's side of the family had come from East Tennessee. Then, most importantly, I showed him a copy of my great-great-great grandfather's honorable discharge papers from the Confederate Army. Suddenly, I was virtual family and all kinds of business could be transacted on a hand shake alone. After years of working together, we agreed that VMRD would purchase ABR when John was ready to retire so that he could devote more time to his many interests.



John had more interests than time. He was a devoted husband/father, Boy Scout leader, gardener, Mason, ham radio operator, Civil War—War Between the States to us Southerners—historian and re-enactment buff, small arms collector and aficionado, supreme story teller (most often perpetrated by the telephone), skilled public speaker, scientist, genuine “bad-boy” Hog-Davidson rider, and of late, photographer and collector of the Leica sort extraordinaire. He could expound at length on nearly any subject with credible, and often overwhelming, expertise.

John considered himself a nerd as he was growing up. Once early in his ham radio days at a boys' military academy, he went up to the roof to tend to his antenna. As he crawled out of the trap door to the roof he came face to rump with a young lady sunbathing in the buff. Not prepared for such an encounter, John fainted dead away and promptly fell down the stairs. Once awake, he

skedaddled as fast as his feet could carry him. He never knew whom he had seen, mostly because he didn't see the ordinarily recognizable parts. Since it was a boys' school, and it definitely was not a boy on the roof, John assumed she was a professor's daughter. He spent weeks in deathly fear of her father's discovery and certain punishment. And, of course, every woman he encountered from that point forward looked different to him somehow.

I learned a great deal from and about him. Though not an evangelist, John was religious and could accurately articulate the essentials of the Christian faith. If you were his friend, he let you know it – and he wasn't afraid to tell you the things he felt you needed to hear, whether you liked it or not. He was politically conservative and independent. Though generous, he thought everyone should pull his own weight, and he taught by example. He knew how to work hard.

His scientific achievements included, but were not limited to: The original design of the equine infectious anemia agar gel-immunodiffusion test; isolation and development of one of the major canine parvovirus vaccine strains; and major contribution to the understanding of feline infectious peritonitis serotypes and disease. He was considered one of the world's foremost experts in detection of BVDV in industrial biological materials using classical virological methodology. Anyone involved in classical animal diagnostic virology knew John Black. He was always available for needed consultations and was a prime source of accurate information. Even though he didn't have a PhD, many PhDs, myself included, sought his advice.

He had unique ways of expressing himself, affectionately known to us as, "Johnisms." For example, if he was depressed about something he would say that he felt "lower than a snake belly in a wagon track." If something was VERY unusual, unlikely or unpredictable he would call it "rare as chicken lips." If something was exceptionally good he would declare it "fine as frog hair."

In May of this year John was diagnosed with inoperable and untreatable pancreatic cancer. VMRD purchased ABR from him and his devoted wife, Pat Black, on June 30, 2006. John understood what he was up against and faced it bravely, and at peace with the Lord. He crossed over the river into Paradise on July 20, 2006. He was a dear friend of mine and many of us miss him very much. ❖

Can cELISAs be Used With Different Animal Species?

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

cELISAs sold by VMRD are licensed for use in defined species. For instance, the test for antibody to *Anaplasma* is approved for use with bovine serum samples. The cELISA will detect antibody to *Anaplasma* in serum from other animals including sheep and goats¹. Further, the test

should detect antibody to *Anaplasma* in serum from any animal species because sufficient quantities of antibody of the appropriate specificity will inhibit the binding of labeled mouse monoclonal antibody in the test and cause a positive result. The reason that the *Anaplasma* cELISA is not approved for use with sera from sheep and goats and other animals such as wild ruminants is that the available data is insufficient to determine the appropriate cutoff value in order to resolve positives and negatives. The cutoff for the bovine serum cELISA is 30% inhibition (i.e. samples inhibiting the binding of the labeled monoclonal antibody greater than or equal to 30% are positive; samples with less than 30% inhibition are negative).



A group of bovine samples defined as *Anaplasma* positive or negative by nested PCR followed by hybridization² were used to define a 30% inhibition cut-off which resulted in a sensitivity of 95% and a specificity of 98% (see data at www.vmr.com). Why not use the 30% inhibition cutoff for the other animal species? It is not used because it might not be correct. Sera from defined negative cattle inhibit the binding of labeled monoclonal antibody used in the *Anaplasma* cELISA to some extent (from 0% to <30%). Whether sera from other animals with no antibody to *Anaplasma* (negative sera) always inhibit <30% is not known; they may or may not, but it is an empirical question that has not been examined in sufficient detail. Further, a determination of the ability of each infected animal species to make sufficient amounts of antibody with the appropriate specificity needs to be made in order to evaluate the sensitivity of the cELISA for that species.

The cELISA to detect serum antibody to caprine arthritis-encephalitis virus (CAEV) is licensed for use only for goats. However, in research to see if the CAEV cELISA could detect cross-reacting antibodies in sheep infected with a related virus, ovine progressive pneumonia virus

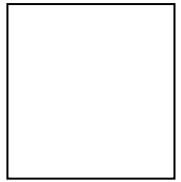
1. Ndung'u LW, et al. Detection of *Anaplasma ovis* infection in goats by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. *J Clin Microbiol.* 33 (3):675-679 1995.
2. Torioni de Echaide S, et al. Detection of cattle naturally infected with *Anaplasma marginale* in a region of endemicity by nested PCR and a competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5. *J Clin Microbiol.* 36:777-782, 1998.
3. Herrmann LM, 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.* 10:267-271, 2003.
4. Herrmann LM, et al. Detection of serum antibodies to ovine progressive pneumonia virus in sheep by using a caprine arthritis-encephalitis virus competitive-inhibition enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol.* 10:862-865, 2003.



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(OPPV), it seemed clear that sera from CAEV negative goats inhibited binding of the labeled CAEV monoclonal antibody more than did than serum from OPPV negative sheep^{3,4}. The % inhibition cutoff for the VMRD CAEV cELISA for goats is 35% to obtain high sensitivity and specificity, whereas in a research study a cutoff of 20.9% inhibition could be used in the CAEV cELISA with sheep sera to detect cross-reacting antibodies to OPPV (4). However, more data is needed to further evaluate the sensitivity for the CAEV cELISA for use in detecting antibody to OPPV in sheep from various geographic regions.

Therefore, cutoff data needs to be obtained with serum from each animal species before using a particular cELISA. On the surface, it appears that obtaining data for determining a cutoff is easy. In fact, it requires considerable effort because a “gold standard” is needed to determine true negative and true positive status for a relatively large number of sera from different locations for use in obtaining a cutoff which results in high specificity and sensitivity. ❖

Seeking Customer Input on Slides

We are seeking input from our customers who use our IFA slides. We plan to convert our ten-well slides over to a twelve well format. We cannot imagine anyone objecting to receiving 20% more wells for the same price, but if you have any concerns about the change please share them with us. We are also considering a uniform diameter for wells—either 4 mm or 7 mm wells. The 7 mm wells require 50 μ l of reagents whereas 4 mm wells require 20 μ l to run. If you have a strong preference for either well diameter please call us at (800) 222-8673, or send an email to vmrd@vmrd.com. ❖

Goodbye to VMRD Customers

By Ann Huffaker, Customer Service Representative

After more than 13 years with VMRD—I was a young thing in my 30s and am now in my 50s—I am leaving to take a position at Washington State University. After educating my husband and three children (two PhDs, one almost-PhD, one JD, two BSs, one BA), it is FINALLY my turn to return to school for an advanced degree. The educational benefits of the new job make it financially attractive to do so. However, it is with great sadness that I bid farewell to my VMRD colleagues and to our VMRD customers. I have enjoyed getting to know you by phone, fax, email, and at AVM and AAVLD meetings. It has been a real pleasure, and I will miss you all. I am confident that you are in good hands with Ethan Adams (technical service), Luke Brown (marketing), Jennifer Miller (customer service) and the rest of the VMRD crew. You will continue to receive the great products, service and support you expect from VMRD. Thank you for the past thirteen years of your business, collaborations and conversations.

P.S. It is comforting that Scott Adams—President of VMRD—has offered to take me back should I change my mind at a future date. ❖

