



# VMRD

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## NEWSLETTER

## OCTOBER 2005

### Introducing **ELISAWare™**

By Ethan Adams, Editor in Chief, Marketing Manager, System Analyst

#### *HOW ELISAWARE™ CAME TO BE*

For many years VMRD customers have been requesting a software program that would support our ELISA assays. The development of such software always seemed to be a monumental, if not impossible, effort for a small, private company. At a certain point, however, I realized that we were wasting a lot of time, and creating a lot of error potential, by entering optical density (OD) values by hand. Not only did our customers need software, *we* needed software! This epiphany resulted in several weeks of furious code-writing on my part, the product of which was my first Microsoft® Windows® program. This creation designated “E-Z Reader,” only supported one model of reader and did nothing other than to retrieve and display ODs and export them into Microsoft® Excel®. Nevertheless, it gained something of a following in VMRD’s laboratories that persists to this day. The experience whetted my appetite for programming and I have devoured numerous books and produced several programs in the five intervening years since E-Z Reader’s debut in our labs.

With E-Z Reader’s modest success, an ELISA program no longer seemed completely out of reach. Nevertheless the constraints of my responsibilities at VMRD did not permit sufficient time to develop the software myself. My predecessor as marketing manager began the project using outsourced software engineers and I served in an advisory capacity. Development was severely hampered by language barriers and lack of continuity as programmer after programmer tackled the project. When I became marketing manager, I found that in addition to these problems, we had an identity crisis. Up to that point we had been calling the program “MyLab”—some customers may recall seeing that name in our 2003 catalog—a name that was already in use by several other companies. After much brainstorming and trademark searching I arrived, for better or for worse, at the name “**ELISAWare.**” The identity crisis was solved, but my new duties as marketing manager allowed me less time for programming than ever. Outsourced development had proved so chaotic that I abandoned it and the **ELISAWare™** project ground to a standstill.

Public release of **ELISAWare™** appeared indefinitely postponed until I met an excellent programmer named Eric Foryan. Instead of being half a world of iffy Internet connections away, Eric lived about four minutes from

VMRD, and he was enthusiastic to work on the project. It was a new and refreshing experience to sit down face to face with a programmer, explain what needed to happen, and see evidence of cognition manifest in head nods instead of in instant messages in broken English. Currently working on a computer science degree at Washington State University, Eric shares my user-centric philosophy of software development. We believe that software should be self-explanatory, easy to use, and bug free. We believe that stability takes precedence over an abundance of features. We believe that the computer should work for the user, not the reverse.

As Eric became increasingly familiar with the program he became more and more concerned about the existing code. I had been pushing for rapid development, so he prefaced his request to rewrite with a number of apologies. He did not realize that he was preaching to the choir; I too had serious misgivings. After careful consideration we made a strategic decision to take the time to rewrite. It took some doing and it took some time, but the resulting program is *so* much better. I am sure that **ELISAWare™** users will appreciate the stable and robust program that Eric’s rewrite produced. Very little of the outsourced code remains, so I am proud to say that this program is made in the USA. I thank Eric for his hard work, conscientiousness, and all the overtime he put in to meet deadlines.



Eric: Programmer / Superman

#### *WHAT ELISAWARE™ DOES*

The essential function of **ELISAWare™** is to retrieve data from a microplate absorbance reader, display them to the user, validate the assay, calculate qualitative results from the data, display these to the user, store sample identifications and results, and report these to the user.

Data acquisition is supported for a number of readers from four different manufacturers as noted in the center bar on page two. We are not sure just how many different readers will work with the reader drivers in **ELISAWare™**, but based on customer input we believe that most readers used by our customers will be supported. If your reader is not supported we encourage you to let us know as we are fully capable of adding additional reader drivers and will do so based on demand. Please give us a call at 800-222-8673 or e-mail your reader brand and model to [vmrd@vmrd.com](mailto:vmrd@vmrd.com).

**ELISAWare™** presently supports all of VMRD's ELISA test kits. As we bring new test kits to market, we will offer **ELISAWare™** upgrades to accommodate them. For reasons having to do with stability, complexity, and liability, we chose to build the test validation and calculation into the program rather than to provide some means for the user to enter validation and calculation formulas. This of course means that other manufacturer's assays are not supported for validation and calculation. The software will retrieve ODs from any supported plate reader for any assay, but it will only perform validation and calculation on VMRD's assays. Users may retrieve ODs for any assay by choosing the "Generic" test protocol.

In **ELISAWare™**, Sample IDs are entered directly on a 96-position grid representing the microplate. To prevent confusion of results, calculated results will not be displayed until sample IDs have been entered. In most cases, sample IDs can be copied from a spreadsheet and pasted into the **ELISAWare™** sample ID grid. Samples can be separated into groups such as by farm, vet or client. These groups can be used to report results from a single plate, a single run or any result in any run within a specified date range. Because reporting over a date range requires that the group name used be identical in each run, **ELISAWare™** keeps a registry of group names and automatically suggests matching group names as the user enters data.

Once **ELISAWare™** retrieves ODs it performs validation on the results, verifying that the controls are performing within the ranges specified by the kit insert. If test verification fails, **ELISAWare™** reports the reason and displays the ODs but does not calculate results. If the validation failure was caused by something such as mislabeling a well as a control when it was actually a sample, the user can re-designate the well as a sample and **ELISAWare™** will automatically re-verify and calculate results if validation passes—without the necessity of re-reading the plate. In fact, the position of all the controls, blanks, and samples on the plate, or even the assay, can be changed and **ELISAWare™** will re-verify and re-calculate with each change without the necessity of re-reading the plate. Another of **ELISAWare's™** handy features is the tooltip that displays the OD and calculated result when a user hovers the cursor over a sample ID, and displays the sample ID and calculated result when a user hovers the cursor over the OD.

Storing, aggregating, and displaying data is the ultimate purpose of **ELISAWare™**. These functions necessitate some sort of database. We chose a Microsoft® Access® database for its speed, prevalence, and reliability. Advanced users may find that they can re-locate this database, or even use multiple databases—and that the database is password protected to

preserve data integrity. Ordinary users will be content with storing their runs in the database and using **ELISAWare's™** built-in reporting features. These features include the ability to generate separate reports for each group on a plate, each group in a run, or for a group across multiple runs and multiple tests. There are both client reports, which merely list sample, test date, and results, and analytical reports intended largely for internal laboratory use. These latter additionally show ODs, %I or S/P, test used, read date, etc. While we based these report layouts on customer input and our own experience, we expect that, as **ELISAWare™** is used in the field, customers will help us fine tune them for maximum usefulness. We look forward to your input.

From the perspective of displaying report data, we wanted **ELISAWare™** to be as flexible as possible. It is frustrating when reports are only useful for printing; we thought it would

be great if one could copy results out of a report and paste them into Microsoft® Word® or Excel®. It is annoying when you cannot get back into the software to check something because a report is open. We thought it would be convenient to have reports open in a separate window. A one-size-fits-all approach to report screen resolutions and font sizes always leaves some users squinting or leaning back for a more distant view. We thought it would be nice if **ELISAWare's™** reporting engine had the flexibility to adapt to different screen resolutions and font-size preferences. Sometime during all this thinking we realized that an Internet browser satisfied all of these requirements. We gave it a try and the more we used it, the better we liked it. We were careful to make the reports as adaptable to different browsers as possible, so whether you prefer Internet Explorer®, Firefox™, Opera™, or Netscape®, **ELISAWare™** will display a readable and printable report in your

default browser. Furthermore, since the reports are composed of simple HTML, the standard markup language of the Internet, they have great potential for importation and manipulation in many different text-editing, word-processing, and spreadsheet programs.

That concludes our tour of **ELISAWare™**. We think that the hard work that we have invested has resulted in a good piece of software. However, the opportunity to make it *great* software lies in your hands. There is no way that we as programmers and assay developers can think of every desirable feature or catch every bug. We encourage you to give us your input on this software—not only by reporting bugs, but by telling us how you *wish* the software worked. We developed it; we can change it; and we are eager to make it useful to you! ❖

*For more about ELISAWare™, visit: [www.vmr.com/elisaware](http://www.vmr.com/elisaware).*

## ELISAWare™

### Microplate Absorbance Reader Support:

- Bio-Tek ELx 800/808
- BioWhittaker: Kinetic-QCL 10 710
- LabSystems/Multiscan/Thermo (most models)
- Molecular Devices ThermoMax (and some others)

### Test Kit Support:

- *Anaplasma* cELISA
- *Babesia caballi* cELISA
- *Babesia equi* cELISA
- BTV cELISA
- BLV ELISA
- *Brucella* ELISA
- CAEV cELISA
- EIAV ELISA
- MCF cELISA
- *Neospora caninum* cELISA

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## See you at the AAVLD/USAHA Conference!

We hope you attend the 48th Annual Conference of American Association of Veterinary Laboratory Diagnosticians (AAVLD) and the 109th Annual Meeting of Animal Health Association (USAHA) in Hershey, PA. The conference will be held from Thursday, November 3rd until Wednesday, November 9th at the Hershey Lodge and Convention Center. Exhibits will be held in the Blue Room on Saturday, Nov. 5th, from 12:00 noon to 5 pm, and Sunday, Nov. 6th from 8:00 am until 5:00 pm, and again on Monday Nov. 7th from 8:00 am through 1:00 pm. Make sure to visit us in the Blue Room at booth 26, next to the refreshments, for a catalog, fun prizes, useful gifts and to **try out ELISAWare™!** ❖

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## Obtaining Quantitative Data Using VMRD's cELISAs

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

It is often useful to know the titer of antibody in a serum for comparison with sera taken earlier or later from the same animal or for other purposes. The cELISAs made by VMRD recommend using undiluted sera and the results are scored as positive or negative. These assays are adjusted to detect low as well as high amounts of antibody. Therefore, moderate amounts of specific antibody will cause maximal % inhibition preventing using the % inhibition to differentiate sera with moderate and high antibody. Of course, sera with low amounts of antibody that cause % inhibitions near the cutoff have less antibody than sera with higher % inhibitions. However, sera with similar % inhibitions near maximal for a particular cELISA can have very different amounts of antibody. These cELISAs can easily be turned into very quantitative tests by evaluating serial dilutions of a serum. The endpoint can be determined for each dilution by scoring each dilution tested as positive or negative based on the % inhibition cutoff recommended for the kit being used.

For instance, using the CAEV cELISA to test dilutions of 1:10, 1:100, 1:1000 and 1:10,000 of an infected goat's serum results in a >60% inhibition at 1:10, 1:100 and 1:1000 dilutions with the 1:10,000 dilution causing <35% inhibition. Since the recommended cutoff for a negative test in the CAEV cELISA is 35% or less, then the titer of the serum is 1:1000 in this example. A similar procedure can be used to obtain antibody titers using the *Anaplasma*, *Neospora*, *Babesia equi*, *Babesia caballi* and Bluetongue virus cELISAs.

One issue to keep in mind when determining antibody titers using these cELISAs is that antibody is being measured to a single epitope present on a single protein. This is because a positive assay for antibody in all these tests depends on the antibody in the serum of interest inhibiting the binding of a monoclonal antibody. The monoclonal antibodies are directed to an epitope on a protein or glycoprotein of the organisms for which antibody is being used. Detecting antibody to a single epitope is not a problem since the monoclonal antibodies in each test were selected because sera from infected animals inhibited their binding. This is the basis of the usefulness of these cELISA's to detect specific antibody in infected animals. ❖

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## Heifers, Hippies and Cowboys

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

In 1971 miniskirts were the latest cause of insanity among college boys, gasoline was 36 cents per gallon (\$1.45 in today's dollars) and I was a junior in veterinary school at Washington State University. In that year a fortunate few veterinary students spent a week or two at a southern Idaho feedlot "helping" calve out several hundred heifers. The attending veterinarian knew that catching the heifers early in labor was important and that those veterinary students unaccustomed to cattle ranching would jump at the chance to get some experience at not sleeping. So, he asked the WSU Veterinary School for volunteers. Ignorance being the primary prerequisite for the job, I quickly volunteered.

In the early '70s there were three or four veterinary students at WSU who did not fit the mold. Some of us had long hair, by 1971 standards, and some of us were even unshaven. I had long hair, a beard and was venturing into that bastion of conservatism, southern Idaho. When we arrived at the feedlot we met the veterinarian. He was a young cowboy in his mid-to-late 20s. I thought to myself, "This could be rough." He probably thought to himself, "Oh boy." He did a double take of me as this was, I supposed, his first close encounter with a "hippie". I don't think he even had his signature mustache at the time. He didn't say anything about mine, but then it was barely visible.

In the week or two I spent there I learned a few things: From a couple of Basques, Augustine and Jacinto, I learned how to say "abra la puerta y cierre la puerta" and "mucho trabajo, poco dinero." Which is almost the extent of my printable Spanish vocabulary, but quite applicable to graveyard duty watching heifers in the feedlot pens where we cut out the "close" ones and ran them into the maternity pens so that intervention could be timely if needed. We chased heifers (no additional experience needed for vet students), opened and closed gates, and were paid less than little—nothing in fact—but we had a great time with the Basques.

I not only had opportunity to help with many obstetrical problems and to assist with several caesarean sections but I also had a chance to perform a caesarean section myself under the direction of the cowboy veterinarian. In that setting, which I later repeated scores of times in large animal practice, I learned that sterility is a relative ideal. Contrary to what I was taught in school, during farm surgery sterility is constantly being compromised and redefined by the athleticism of the patient, success of restraint and availability of airborne manure, dust, hay, straw and sometimes the stock dog. In any case, the heifer and calf survived both the surgery and the "sterile technique" thanks to Pfizer Animal Health. When I left southern Idaho, I returned home to Pullman, Washington triumphant as one of the few in my class who had actually performed a real caesarean on a real cow.

I learned other things while working with the cowboy veterinarian. He was gracious and an able mentor. Of course, I picked up practical things about cows and veterinary medicine from him, but I also picked up lessons on respect. Instead of making it rough for me, he demonstrated himself to be open-

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## Heifers, Hippiers and Cowboys

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mindful without necessarily agreeing with anything I said. He was genuinely liberal but not politically so . . . and had ample common sense. He talked a lot. In fact he did most of the talking so, fortunately, I had little opportunity to put my foot in my mouth. He was obviously a great story teller and incredibly funny. Years later I saw him on TV once at someone's house. Now I read and look forward to his weekly missive, *On the Edge of Common Sense*, in the *AgJournal* out of La Junta, Colorado. The byline reads, "By Baxter Black." ❖

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## Veterinary Diagnostic Voice Mail

Contributed by Baxter Black, DVM

*Hello. You have reached the automated voice mail of Triple A, Aardvarks Are Us, All Creatures Great and Small Veterinary Clinic, Animal Health Supply, Grooming, Boarding and Training, and Counseling Center. If you have a credit card limit of no less than five thousand dollars please press one, if not, please hold.*

[1] Thank you. If your problem concerns a pet – including dogs, cats, small rodents, reptiles, cockatiels, highway accidents and other creatures where cost is no object . . . please press one. If you have livestock whose value is dependent on a fickle, unpredictable, often cruel market BUT you have a good job in town, a wife with a job, federal disaster insurance or land and farm equipment that can be sued as collateral . . . please press two.

[2] If you have a poultry problem . . . press one for the Campbell's® soup buyer. If you are a pork producer . . . press two for counseling and hysteria prevention. If your problem concerns cattle . . . press three.

[3] If the condition is serious enough (over \$500) and you can bring the animal to the clinic . . . press one.

[1] If the condition is not life threatening or you do not have a stock trailer . . . press two.

[2] If you have already been treating this animal yourself for weeks . . . press one.

[1] If the animal is ambulatory . . . press one. If the animal is recumbent . . . press two. If the animal is comatose . . . press three.

[2] If the animal has been down for less than two days . . . press one.

[1] If the animal is still eating and drinking . . . press one. If the animal is not eating but still has a detectable pulse . . . press two.

[2] We have not reached the critical stage in this automated voice mail Diagnostic Situation Prognosis Assessment Device. Your prognosis is: Poor to partly cloudy – estimated cost \$112.00. Add \$5 for weekend and after hours – satisfaction barometer minus 3.

If you would like to have the veterinarian make a house call . . . press one. If you want to kiss it off and bite the bullet, press two for Johansen's Hide and Tallow.

[2] Have a nice day.

*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.*

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## EIAV ELISA License Expected Soon

VMRD has developed an excellent Equine Infectious Anemia Virus ELISA for which we hope to receive a USDA license soon. 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. We plan to offer a stripwell 1-plate and a stripwell 5-plate kit, but are also seeking licensure for a stripwell 2-plate kit should there be demand for such. Now, instead of asking if we have an EIAV ELISA yet, you can ask whether product numbers 290-1, 290-2, or 290-5 have received a USDA license yet! ❖