TREK Vet Update

Volume 1, Number 1 Fall 2004

Experience with the ESP® Liquid Culture System and the Rapid Culture Identification of *Mycobacterium Avium* subsp. *Paratuberculosis* (MAP)

By William H. Fales, PhD and Thomas J. Reilly, PhD, College of Veterinary Medicine,Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia

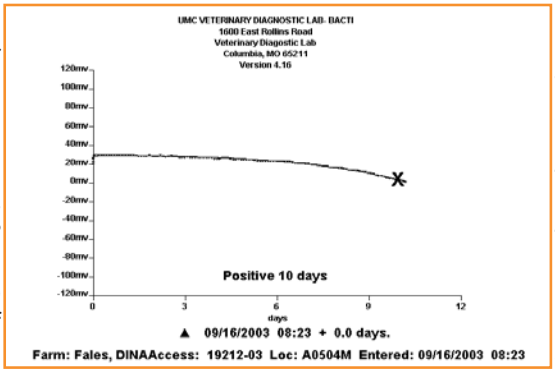
Johne's Culture Results in 10 Days

The traditional method of diagnosing Johne's disease, in ruminants, may take at least 8 weeks and often up to 16 weeks of incubation time when using the standard Herrold's Egg Yolk (HEA) medium. This time delay creates problems for veterinary practioners and clients who want answers as quickly as the laboratory can deliver them in order to make a diagnosis or release newly purchased cattle from quarantine.

To answer this critical need TREK Diagnostic Systems has developed equipment (ESP® Culture System II) and a liquid culture medium (*para*-JEM®) that can indicate the presence of *Mycobacterium avium* subsp *paratuberculosis* (MAP) in cattle feces after 10 days of incubation. Heavily infected cattle typically will demonstrate a positive reaction after 10 days incubation, where as moderate shedders take 25 days and light shedders may take up to 45 days of incubation.

TREK Culture Methods

The fecal sample processing follows the double enrichment technique developed by Dr. Sang Shin at Cornell University and is known worldwide as the Cornell modified method. Briefly, 2 grams of feces are placed in 25 ml of sterile deionized water contained in a sterile conical centrifuge tube. The sample is placed on a mechanical shaker and mixed for 30 minutes. Debris is allowed to settle and the 5 ml of the supernatant fluid is aseptically transferred to 25 ml of ½ strength Brain Heart Infusion Broth (BHI) containing 0.9 % cetylpyridium chloride (Sigma-Aldrich), which is contained in a 50 ml sterile conical centrifuge tube. This step promotes bacterial growth and allows the subsequent killing of vegetative bacterial cells other than MAP after 18-24 hours of incubation at 35°C. The BHI cetylpyridium chloride fecal mixture is centrifuged at 3,000 x g for 30 minutes at the 4° C. The supernatant fluid is aseptically decanted to an autoclavable container in a biohazard hood, and the sedimented button resuspended in 1.5 ml of ½ strength BHI broth mixed with an antimicrobial cocktail (amphotericin B, vancomycin, and nalidixic acid). The sample is reincubated at 35°C for another 18-24 hours which eliminates the presence of spore forming bacteria.



Culture bottles are prepared by adding proprietary TREK *para*-JEM growth reagents, egg yolk and antimicrobials to the sponge containing culture bottles. The sponges are present to provide a solid matrix and surface area for the MAP to grow. After the bottles are prepared and inoculated with 1 ml of the sample, the bottles are vortexed and coupled to a connector and patient demographics are entered into the TREK computer. The computer then indicates the correct positioning of the sample at the TREK ESP unit and the sample placed as indicated.

The TREK ESP liquid culture system monitors the head space pressure and detects the utilization of oxygen which is measured in millivolts (mV). The system is free of radioactivity. Readings of the partial pressure are made every 24 minutes and a curve plotted on the computer screen for visual observation and a print out. When the ESP unit detects sufficient oxygen utilization a positive signal is given by the ESP unit. The ESP and the computer indicate the location of the positive sample.

At this point the sample is removed from the ESP unit, vortexed, and a 0.1 ml withdrawn and mixed with mycohold (Wescor). Then the sample is stained for acid fast bacilli (AFB) using either the ZN (Zeihl-Neelsen) stain or the modified cold Kinyoun method and examined microscopically.

A 1.0 ml sample is also withdrawn and placed in a labeled microcentrifuge tube and processed for the detection of MAP by PCR.

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UMC Upgrades ESP Capacity

There has been a real void in Johne's culture due to the length of time to recover the slow growing microorganism. The TREK ESP unit with a short 10 day to 45 day turn around time was most attractive to our laboratory. The veterinary practitioners have been delighted with the rapid turn around time and the system has lent itself well to the Johne's free herd certification. In fact, the UMC-VMDL is upgrading its equipment from a unit with 128 sample capacity to a 384. In like manner, the Missouri Department of Agriculture has also purchased a 384 unit. The TREK ESP Liquid Culture System has been remarkably trouble free and when a problem has developed technical support was quick to solve the problem by phone or by dispatching a field service engineer.

It is critical to have a steady electrical power supply and the use of a power surge regulator. The incubator must be placed in an area free of drafts and ventilation openings. The sales and technical representatives are very good at assisting in installation and site selection in order to promote trouble free results.

ESP Achieves USDA Check-Test Certification

The TREK ESP liquid culture system has met the USDA-APHIS-NVSL-VS Johne's certification check test and a certificate hangs on the laboratory wall by the unit.

The system has worked well in our hands. An occasional antimicrobial resistant bacteria or yeast may show up in the culture and indicate a positive growth curve. These curves are usually atypical from a MAP curve. If the AF stain is free of AFB, additional antimicrobial solution is added to the culture bottle and the sample reincubated. This often will solve the problem and allow the completion of the culture time and often the recovery of AFB.

Equipment Decision

The time delay of 16 weeks was too long for clients and veterinary practitioners to wait for results. Cattle with diarrhea and wasting were either ignored or sent to slaughter without a diagnosis. The health of cattle, both beef and dairy, and the concerns of Crohn's disease (an inflammatory bowel disease of humans) possibly being linked to MAP has prompted an interest in the control of Johne's disease. In order to expedite test results and assist in disease detection and herd certification, the TREK ESP unit is most beneficial to the laboratory and demand has required an upgrade from the 128 to the 384 unit.

The system provides an excellent screening device and coupled with PCR confirmation offers an excellent method for the detection of MAP in cattle fecal samples.

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Sensititre® Veterinary-Specific Plates

Standard Veterinary Identification Plates

GNID	Gram-Negative Identification, 10/box
GPID	Gram-Positive Identification, 10/box

Veterinary-Specific JustOne® Strips

JO-EYE	Breakpoint -- Eye Isolates, 12/box

Standard Veterinary MIC Susceptibility Plates

CMV1ABPF	Bovine/Porcine One Isolate MIC Plate, 10/box
CMV1AGNF	NARMS Gram-Negative MIC Plate, 10/box
CMV1AGPF	NARMS *Enterococcus* MIC Plate, 10/box
CMV1AMAF	Mastitis Two Isolate MIC Plate, 10/box
CMV1BAVF	Avian One Isolate MIC Plate, 10/box
CMV1BURF	Urinary Two Isolate MIC Plate, 10/box
CMV2ECOF	Companion/Equine One Isolate MIC Plate, 10/box

Custom Veterinary Susceptibility Plates

Custom susceptibility plates are designed to meet the specific needs of animal testing. A choice of over 40 veterinary-specific drugs is available. (See product catalog on www.trekds.com for a complete listing.) Custom plates are available in minimum order quantities of 500 plates (packaged 10 per box). Larger quantity discounts are available.

For more information on Sensititre Veterinary-Specific Plates, contact TREK Customer Service at 800-871-8909 or via e-mail at info@trekds.com

TREK Opens Doors for SWIN™ Customer Training at Headquarters

By Steve Shemo, Supervisor of Technical Service and Amy Miskov, Technical Service Specialist

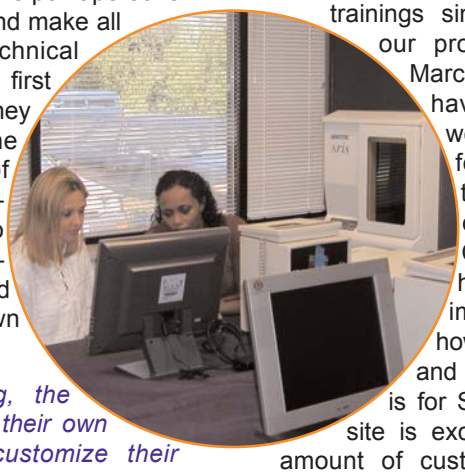
This spring we unveiled a brand new training program at TREK headquarters in Cleveland, Ohio. In the past, the Technical Service group typically traveled to customer sites to perform training on new software or equipment. This spring, we decided to bring customers to Cleveland for two days of intense SWIN training. Bringing customers to Cleveland offers several unique advantages compared to customer site training:

Training is operated without distraction – No phones, no pages, no emergencies to pull lab technologists away from training.

More thorough training – Our system becomes their system. Spending two days of intense training with our customers instills strong confidence in the customers' own abilities to run SWIN.

Better customer support – Less time on the road means that we can now use that extra time for increased phone support to our customers.

The customer becomes the product champion – The customers who come to Cleveland for training are the point persons from their lab and make all calls into Technical Service for the first six months. They become the champions of their SWIN system and learn to do basic troubleshooting and teach their own employees.



During training, the customer uses their own computer to customize their system -- This computer is then shipped directly to the customer immediately after training is completed.

Our SWIN Training Room holds up to six people. There are two ARIS® 2X and AutoReader units, and two SensiTouch® units in the room. A giant screen in front of the training room provides customers easy to see real-time training provided by one of our Technical Service Specialists.

We have completed nearly 20 trainings since we began our program in mid-March. The trainings have gone very well with positive feedback from the participating customers. Customers have been impressed with how user friendly and intuitive SWIN is for Sensititre. Each site is excited about the amount of customization that specifically fits the needs of their lab. The customers also enjoy leaving their work environment for training and coming to a new place where they can focus on training.

It is wonderful to be able to show customers the energy within TREK, our great facility, and to have the opportunity to bond with our customers face-to-face!

A Customer's Perspective on TREK's In-House Training Program

By Lisa Willis, Murray State University, Breathitt Veterinary Center, Hopkinsville, KY

The lab at the Breathitt Veterinary Center at Murray State University in Hopkinsville, KY is a full service veterinary diagnostic lab. Our bacteriology section encompasses all aspects of microbiology to include routine culturing, anaerobic cultures, mycology, molecular, Johne's, poultry surveillance, etc. We have a fairly high volume of testing, running between 200-300 sensitivites a month (in addition to being the only lab for the state of KY to run Johne's culturing).

Our lab has been using the TREK Sensititre® system since 1994. Our equipment was in need of upgrading and it only made sense to stay with a system that we knew and liked. We waited until SWIN™ was available to upgrade.

The in-house training I received at TREK was great. Amy Miskov was our trainer and she really knew the system well and provided great training for us, from actually hooking up the computer to the ARIS® 2X and SensiTouch®, to all the customizations we would need for our specific lab. The hands-on training was great, and with it being in Cleveland, rather than our own busy lab, there were no distractions. When I returned from my training and the computer arrived, it was immediately put into use. We had a few glitches with our interface to VisuLab, but other than that, it was very easy to use and very easy to show my co-workers how to use as well. The software people were very quick about helping work out the problem. QC was also a breeze!

Everyone from TREK was great. During the training, we were given plenty of time to ask questions and "play" with the system. The "playtime" gave us an opportunity to see just how SWIN functions in the lab on a daily basis.

My trip to TREK was well worth it!

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Begin your TREK into the World of *para*-JEM®

By Sara Allen, M.S., Senior Research Microbiologist

TREK proudly offers the ESP® *para*-JEM® kit to the world of veterinary diagnostics. The *para*-JEM kit has been designed specifically for the detection of *Mycobacterium paratuberculosis*, the causative agent of Johne's Disease. Testing for the disease is a very valuable tool for herd management as it aids in identification of infected animals and monitors the progress of control efforts. Currently TREK's ESP technology is being used successfully to assist in this effort in over twenty veterinary labs throughout the U.S. and the world.

When a lab selects the ESP system for their Johne's diagnostic testing, we present a multi-faceted approach for them to learn how to operate the system. First, the lab receives site-personalized, hands-on training for proficiency of the instrument and components utilized within the system, this inclues comprehensive sessions on software navigation and graph interpretation.

Second, there is a question and answer session and group quiz to help initiate inquiries that may not have been raised during the training. Throughout the trainings a wide variety of practical questions have been presented by some very thorough customers. The range covers not only those specific to the test but extends to the ergonomics of sample processing, logistics of tracking samples, and PCR confirmation. Brainstorming sessions with the lab most often generate a helpful hint for all *para*-JEM users. (See sidebar below.)

Third, customers are provided with an ESP *para*-JEM reference guide for questions that may arise subsequent to training. The reference guide contains procedures for processing and inoculating samples, the *para*-JEM technical insert, and software shortcut sheets.

Fourth, labs may contact TREK Technical Support, the Department of Research and Development, or other *para*-JEM users (who are very willing to share their experiences) for information.

With all these resources available more and more labs are excitedly beginning their TREK into the world of Johne's, won't you join them?

For more information, please contact TREK Technical Suport at 800-871-8909 ext. 189 or e-mail us at info@trekds.com.

Visit us at AAVLD for more information on *para*-JEM!
Booth #1
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para-JEM Helpful Hints

As more labs join forces with TREK in the fight against Johne's Disease, ideas are generated to help streamline the system. Some of our *para*-JEM users have thoughtfully shared their ideas with us:

"Supplements may be combined and *para*-JEM bottles may be supplemented with one addition. It's a time saver."

"Supplement bottles the day before inoculation and store them in the refrigerator. All you have to do is pull them out the next day and go!"

"We used to make up our own antibiotics for the decontamination process but now we use TREK's *para*-JEM AS for that. It is simple and saves time."

"Archive disks may be burned onto a CD to save on floppy disks. The files will need to be returned to a floppy to be read on the computer but the disks can then be reused."

If your site has a helpful hint that other users could benefit from, please contact us.
We always enjoy hearing from our customers.

Sensititre® Products: Establishing New Standards for Aquatic and Campylobacter Susceptibility Test Procedures

By Jenny Lorbach, Sensititre Product Manager and Scott Killian, Laboratory Services Manager

With the advent of continued concerns regarding increasing resistance issues in the environment, the NCCLS Veterinary Antimicrobial Susceptibility Test Committee has recently established standards for broth microdilution testing for Aquatic and Campylobacter isolates. Sensititre custom MIC products, frozen and dry form, were used to establish both of these standard microbroth dilution tests.

Aquaculture

Frozen reference plates (NCCLS M7) are considered the "Gold Standard" for MIC testing in any research project. However, it is not always feasible for laboratories to utilize frozen plates. In order to validate the performance of the established QC ranges for *E.coli* (ATCC 25922) and *A. salmonicida* subsp. *Salmonicida* (ATCC 33658), it was determined that a 100 isolate study by scientists at FDA-CVM would commence comparing frozen plate methodology to Sensititre dry microbroth dilution plates. Up until this point, disk diffusion techniques had been used to determine qualitative susceptibility results.

Campylobacter

In the past, laboratories performing susceptibility testing on *Campylobacter* spp. have used labor-intensive agar dilution as the only standardized method. Recently, laboratories have been attempting to use microbroth dilution as an alternative method for testing these isolates but needed a standard test methodology to validate its use. With this in mind, research scientists at FDA-CVM, JMI Laboratories and TREK Diagnostics established a broth dilution test methodology and validated its performance as an alternative standard method. Utilizing the validated microbroth dilution method, the FDA-CVM sponsored a NCCLS M23 study to establish quality control ranges for several appropriate antimicrobials using *Campylobacter jejuni* (ATCC 33560).

We have continued to work with scientists at JMI Laboratories and FDA-CVM to validate both frozen and dried broth microdilution methods by testing wild-type clinical strains. The data was overwhelmingly favorable and showed substantial equivalency to the frozen reference microbroth dilution method, thus allowing laboratories to utilize Sensititre dried panels when testing these isolates.

Both sets of data for Aquaculture and Campylocbacter were presented at the recent June NCCLS Veterinary Sub Committee meeting and approved as alternative standard methods. The approved method for Aquaculture will be included in the M49 document, Methods for Dilution Susceptibility Testing of Bacteria Isolated from Aquatic Animals, in February 2005. The approved method for Campylobacter will be included in the M31-A performance standards for antimicrobial disk susceptibility testing for bacteria isolates from animals.

Immediate Benefits

Implementation of Sensititre dry microbroth dilution plates for testing Aquatic and Campylobacter isolates in the laboratory provides these immediate benefits:

- *Extended 24 month shelf life from date of manufacture*
- *Ability to incubate plates according to NCCLS recommendations for different Aquatic and Campylobacter isolates*
- *On-scale QC ranges for all antimicrobics*
- *Extended dilution ranges to monitor shifts in resistance*
- *Decreased shipping costs compared to frozen MIC plates*
- *Easy-to-read endpoints*

Choice for Most Surveillance Studies

Due to their consistent quality and excellent performance, Sensititre products have been utilized by global surveillance programs and are now setting new standards for veterinary research projects worldwide. *For more information, or to request a copy of the data presented, please contact us at 800-871-8909.*

Visit us at AAVLD for more information on Aquatic and Campylobacter Susceptibility Test Procedures!
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Available Fall 2004
New Standard MIC Plates for Aquatic and Campylobacter

New standard MIC plate for Aquatic isolates (Part No. Aquatic) will contain the following antimicrobics:	*New standard MIC plate for Campylobacter jejuni/coli (Part No. Campy) isolates will contain the following antimicrobics:*
Enrofloxacin	*Azithromycin*
Ampicillin	*Ciprofloxacin*
Oxytetracycline	*Erythromycin*
Erythromycin	*Gentamicin*
Florfenicol	*Tetracycline*
Flumequine	*Florfenicol*
Ormetoprim/Sulfadimethoxine	*Nalidaxic acid*
Oxolinic acid	*Telithromycin*
Gentamicin	*Clindamycin*
Trimethoprim/Sulfamethoxazole	

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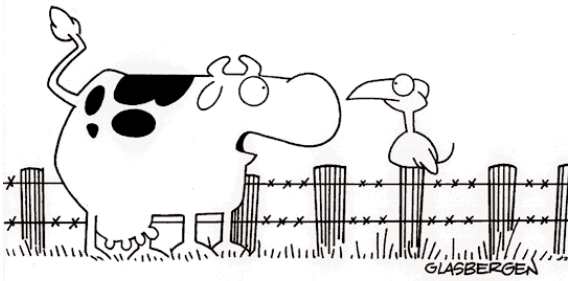
"I finally figured out why cats spend so much time licking their paws—they taste GREAT! Try one!"



"It's true, I did jump over the moon.
I had waaaaay too much coffee that day!"

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