

Using TEGO Devices to Preg Test Cows

Enoch Bergman DVM, Swans Veterinary Services



The domestication of cattle has been one of the critical steps in the history of both civilization and human development. Access to a reliable source of protein and milk products allowed us to spend less time worrying about our next meal and more on making the world a better place. From the beginning, working out whether or not we and the animals we lived with were pregnant has been both a fascination and a financial imperative to human kind. Over time, advances have been made which allow us to reliably ascertain a diagnosis, and new options, tailored to differing circumstances continue to assist veterinarians to service their client's needs.

Trans-rectal palpation began relatively recently, having been described, developed, and implemented in the early 1800's. The speed and accuracy of this cow side test has made it the gold standard of pregnancy diagnosis. Detecting non pregnant animals is a profitable exercise for beef and dairy producers. Further, in most instances, the earlier the diagnosis, the greater the return on investment. Ultrasonography has allowed veterinarians to diagnose very early pregnancies with less manipulation of the reproductive tract, arguably increasing sensitivities for early pregnancies with less risk of iatrogenic loss.

Since the mid 1900's researchers have worked towards producing serum, urine, or milk based assays to diagnose pregnancy. Assays focusing on Pregnancy Associated Glycoproteins (PAG's) have recently become available to Australian veterinarians. Domenic Dell'Osa, a veterinarian working for IDEXX Laboratories, recently published an article describing the assay, in the March 2013 Australian Cattle Vets Journal. Capable of diagnosing pregnancies down to 28 days of gestation, the assay may hold some real value to Australian beef and dairy production systems. Dominic discussed IDEXX's in house validation data comparing both serum and plasma results to ultrasound confirmation.

IDEXX Validation Data	Ultrasound Positive	Ultrasound Negative
Serum Test Positive	706	29
Serum Test Negative	5	441

Sensitivity = 99.3% (95% CI: 98.3%-99.7%)

Specificity = 93.8% (95% CI: 91.2%-95.7%)

IDEXX Validation Data	Ultrasound Positive	Ultrasound Negative
Plasma Test Positive	635	28
Plasma Test Negative	6	535

Sensitivity = 99.1% (95% CI: 97.9%-99.6%)

Specificity = 95.1% (95% CI: 93.0%-96.6%)

had long been interested in the impending release of this assay. I felt strongly that maintaining veterinary involvement in the diagnosis of reproductive performance significantly underpins the sustainability of Australian beef and dairy production systems. The PAG assay provides another option for veterinarians and will suit some circumstances. My goal was to attempt to maintain veterinary oversight in the implementation of this new test. Further, I was interested to see if I could simplify the collection procedure for blood based samples, allowing producers to collect and submit their own samples through their veterinarian for analysis.

Our BVDV diagnostic laboratory has been using TEGO devices to measure BVDV antibodies. The TEGO device was designed by an Australian, Craig Burke, and has been marketed globally by Innovating Technologies for Life (ITL). Designed to collect blood for genetic testing, the device clips to the ear using an All Flex tagging gun. Sharp guarded blades cause the ear to bleed onto a specialized absorbent card which can be easily freighted for analysis. We had previously validated and implemented the TEGO's for the detection of antibodies to BVDV using an ELISA developed by IDEXX. I was keen to see if we could use TEGO's to measure PAG's.



Marianne Gould, working for ITL, introduced me to Professor Kyoung-Jin Yoon, a veterinary virologist working and teaching at Iowa State University. He had successfully used the TEGO devices to run the IDEXX PAG ELISA and was amenable to assisting me to attempt the same.

Last year, working with Michael Lawrence from Murdoch University we ran a preg testing elective prac for eight final year veterinary students. One morning, we collected blood samples, TEGO samples, and pregnancy data from a mob of cows. Later with assistance from other Murdoch students I collected additional blood samples and TEGO samples to augment the sample size. Pregnancies ranged from 2 months to 5 months of gestation. It is



unlikely that any animals were pregnant below physical detection limits on the well managed herds we screened.

Both the serum samples and TEGO samples were analysed in our in house laboratory. Our technicians were blinded to the ultrasound preg testing results. Following the IDEXX PAG ELISA kit instructions, our findings from the serum samples were very similar to the IDEXX validation data.

	Ultrasound Positive	Ultrasound Negative
Serum Test Positive	118	1
Serum Test Negative	2(2.5 and 3.5 m)	52

Sensitivity = 98.3% (95% CI: 94.1%-99.8%)

Specificity = 98.1% (95% CI: 89.9%-100.0%)

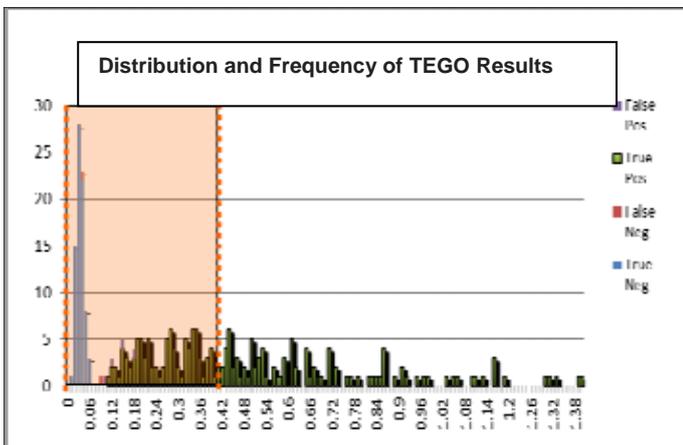
Following Dr. Yoon's protocol, we then analysed the TEGO samples. The TEGO validation data, derived from analysing the whole blood elution from the TEGO cards resulted in data almost identical to the IDEXX plasma validation data described by Dell'Osa.

	Ultrasound Positive	Ultrasound Negative
TEGO Test Positive	203	4
TEGO Test Negative	2(4 m)	79

Sensitivity = 99.0% (95% CI: 96.5%-99.9%)

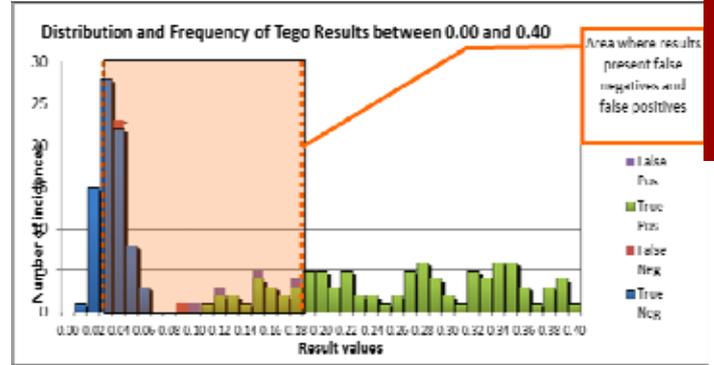
Specificity = 95.2% (95% CI: 88.1%-98.7%)

Dr. Yoon had suggested a cut off point of 0.1 to discern negative and positive results. All of the inconsistent results were located near this diagnostic cut off. The distribution of the results from analysing the TEGO cards is summated below.



It would appear that perhaps a better cut off value could be 0.08. It would be reasonable to retest animals between 0.06 and 0.20 from this data. We will report actual results with a positive cut off value of 0.08 in our laboratory.

ry. Presumably, some of the false positive results may have been associated with the recent loss of a conceptus and an associated diminishing serum PAG level.



The ability to guide a producer to collect and ship their own blood sample using a TEGO device for the analysis of pregnancy in animals down to the 28th day of their gestation will provide many Australian veterinarians more flexibility. Assuming a sensitivity of 99.1% and a specificity of 95.2% a few scenarios are worth exploring:

Many dairies may well benefit from this diagnostic tool to identify non pregnant animals between herd health visits, especially in instances where the expected pregnancy rate is quite low. Animals identified as empty could be administered prostaglandin in the process of being re-enrolled in breeding programs and the animals diagnosed as pregnant reaffirmed physically at the next herd health visit. When prostaglandin will be administered, we want all or virtually all of the test-negative animals to be non-pregnant. The negative predictive value of the assay results describes the percentage of the test-negative animals that are, in fact, not pregnant. As an example, if the expected pregnancy rate was 50%, the negative predictive value would be 99%. In other words, we would expect 99% of the negative testing animals to be non pregnant. However, if a higher pregnancy rate was expected, producers would need to be made aware that more of the negative testing animals will be pregnant. For instance, if the expected pregnancy rate was 80%, the negative predictive value would fall to 96%, and perhaps the producer should consider having the animals manually retested.

Beef veterinarians may welcome a test which can allow them to screen small or geographically isolated mobs of animals remotely, accepting a small reduction in accuracy (by using the assay rather than rectal pregnancy testing) to reduce travel costs. In beef herds, the cost of animals misdiagnosed as pregnant can be greater than those misdiagnosed as empty, assuming misdiagnosed animals are retained for an entire production year and optimal stocking densities are being achieved. In this instance we would prefer that virtually all of the test-positive animals are in fact pregnant. The positive predictive value of the assay results describes the percentage of the test-positive animals that are, in fact, pregnant. This would be quite high in circumstances where the expected pregnancy rate is high. For example, if 90% of the animals were expected to be pregnant, the positive predictive value, or the probability that a test-positive animal is in fact pregnant would be 99.5%. However, if the expected pregnancy rate was, say, 65%, the results would need to be interpreted in light of this as the probability of test-positive animals being pregnant, or the posi-



tive predictive value would drop to 97.5%. Essentially, in this situation, we would expect 2.5% of the animals testing positive on the assay to in fact be empty, perhaps warranting wearing the travel to have the animals manually preg tested, with the additional benefit of picking up any false negatives.

Some practices with a very small production animal case load may find the test useful to diagnose pregnancy or to use as a skill assessment tool in the process of developing their (or their new graduate's) manual preg testing skills. Incongruous results could be retested at a later date.

AQIS may find the assay an excellent tool to measure the accuracy of some of the pregnancy testing associated with export work, requiring that incongruous results be investigated. Perhaps in groups of animals deemed too immature for manual palpation, and presumably prepubertal, the assay could further be of use. In this instance, AQIS would be seeking assurances that the animals are truly empty, therefore a high negative predictive value would be the goal. If the expected pregnancy rate was 1% the negative predictive value would be 99.989%; very nearly all test negative animals will in fact, be non-pregnant. At a 5% expected pregnancy rate, it drops to 99.94% and the likelihood of a test-negative animal being pregnant rises to 0.06% or 6 of 10,000 test-negative animals. Whilst manual palpation of empty tracts is the gold standard, this level of negative predictive value may well exceed that of cursory ultrasound examinations for the confirmation of empty status in lines of mostly empty export heifers.

Like rectal palpation, ultrasound, and blood tests before it, the IDEXX PAG ELISA is another tool that in my opinion should be administered with veterinary oversight. By understanding this new technology, we will remain in a position to respond to both the questions and the demands of the industry we service.

Great thanks to Dr. John Morton for calculating sensitivities, specificities, and discussing the merits of both the positive and negative predictive values, to Dr. Kyoung-Jin Yoon for his advice, to Dr. Michael Reichel for his statistical insight, to the then Murdoch veterinary students, now veterinarians Wanlin Lin, Lara Burgstad, Rachel Farquharson, Hennie Swanepoel, Russel Brown, Collette Sims, Jess Longley, and Rebecca Taylor, to Marianne Gould, Craig Burke and ITL for the TEGOs and assistance, to Rosemary Santangelo, Alison Kelleher and IDEXX for the ELISA kits and assistance, to Sharon and Lauren in the BVDV lab for running the samples, and to Aidan Sinnot for summing and graphing the results.



Strep ag could be lurking in your herd!

Do you purchase cows? Have you checked for Strep ag lately?

- > *Strep agalactiae is a highly contagious mastitis causing bacteria which spreads from cow to cow during milking*
- > *Strep ag infections lead to increased somatic cell counts and decreased milk production*
- > *Many Strep ag positive cows show no obvious signs of infection*
- > *With a good management plan, Strep ag eradication is practical and cost effective*

Don't purchase cows without testing for Strep ag! Screen and monitor your herd with bulk tank testing.

*Talk to DTS or your udder health advisor about an **RtMastitis PCR test** - convenient sample submission, same day results.*

clientservices@dtsfoodlabs.com.au

www.dtsfoodlabs.com.au

Dairy Technical Services Ltd
5/352 Macaulay Road, Kensington VIC Australia 3031
Tel: (03) 8371 7600 Fax: (03) 9372 2013

DTS 
FOOD LABORATORIES
YOUR TRUSTED PARTNERS IN QUALITY

facta 
Food Allergies Control Training Analysts