

BLOOD SMEARS

- From the Haematology teams in Hamilton & Palmerston North

Blood smears created as soon as possible after collection preserve blood cell morphology for smear evaluation, and are a very useful part of any haematology submission. Blood films submitted along with an EDTA sample is always desirable, but become particularly valuable in the following scenarios:

If there is going to be any significant delay between collection and submission of the sample, e.g. long weekend.

For anaemic cats, as *Mycoplasma haemofelis* tends to fall off in EDTA.

For known or suspected leukaemia patients.

Where the EDTA sample is small.

Potential problems when fresh films are not submitted include:

A quick tip on how to make a blood smear:

1. Ensure blood is well mixed.
2. Use a clean dry microscope slide, preferably with a frosted end for ease of labelling.
3. Fill a capillary tube with blood and use this to place a drop of blood at the end of the slide.

Not too small as the smear will be too thin and there will be no white cells to count.

Not too big or you will either go all the way off the slide or end up with a large blob of blood and a very short smear.



4. Without delay place spreader on the slide at 45° angle and move it back to make contact with the drop. The blood will spread out quickly – as this occurs, but before it fully reaches the edge, spread the smear with a rapid, smooth forward movement. The smear should be 3–4 cm in length with a clear, feathery edge to it.

Using a special spreader slide*, with a flat, cut glass end can make all the difference to smear quality – these can be cleaned with a little saline between smears, and re-used many times (don't use to smear blood on!).

To get a good smear alter the angle of the spreader relative to the thickness of sample - the greater the angle the thicker the smear.

Experiment with the speed - a good smear should look as shown below.

5. Label the slide (in pencil – pen or marker may dissolve in the alcohol fixative, or apply a sticker with the patients details) with the animal's name and let completely dry on a flat surface before placing in a slide container. NB – do not blow on the smear to dry, as this can cause lysis of the red cells.

* NZVP will provide spreader slides at no charge.

“EDTA artefact” from prolonged storage of blood in EDTA, or exposure to excessive EDTA solution (e.g. small sample size) – red cells become shrunken and crenated, making examination for potentially important morphologic changes impossible.

White blood cell degeneration – this is particularly important in blood samples from ruminants and camelids, as their white blood cells degenerate even faster than those of companion animals, so if there is delay in sample processing an accurate differential count may not be possible.

Atypical cells – examination and identification of immature or atypical cells (e.g. suspected leukaemias) is much easier when these are fresh.

A poor quality smear can make interpretation difficult – potential causes include dirty slides, water artefact, exposure to formalin, a smear that is too thick or heavily contaminated with blood, and poor smear preparation resulting in large numbers of smudged cells, bare nuclei or strands of nuclear protein. However, as a general rule, any smear is better than no smear so don't be afraid to send your freshly made smears in!

IMPORTANT - store smears at room temperature, DO NOT PUT IN THE FRIDGE!



SYNAPSE



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.....making connections

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CONGENITAL PROTOPORPHYRIA IN LIMOUSIN CALVES

Three Limousin calves on a central North Island farm were seen to exhibit photosensitivity from birth with attempted shade-seeking and subsequent erythema, scaling, alopecia and excoriation of the ears (Figure 1).

Hepatogenous photosensitivity was ruled out on the basis of normal GGT, GLDH and bilirubin results. Primary photosensitivity as a result of plant ingestion containing photodynamic agents was similarly dismissed - partially on the basis of the age of the calves when the photosensitivity was first observed but also on the absence of plants known to cause primary photosensitivity.

Because congenital protoporphyria has been reported in Limousin and Blonde d'Aquitane cattle overseas this was considered the primary differential on the basis of presentation and breed.

Bovine congenital protoporphyria is recognised as an autosomal recessive disease that results in a defect of the mitochondrial enzyme ferrochelatase. Ferrochelatase normally catalyzes the chelation of ferrous iron by protoporphyrin to form haem. As a consequence of the decrease in ferrochelatase activity protoporphyrin accumulates in the blood and tissues. Protoporphyrin is photodynamic. Subsequent transport to the skin and the absorption of sunlight results in photoreactivity. Fast technology for analysis of nucleic acids (FTA) cards were impregnated with the buffy coat from one unaffected and two affected calves with

genetic testing performed by the Department of Veterinary Pathology, College of Veterinary Medicine, University of Missouri, Columbia, USA.

There was no expression of the defective allele for the protoporphyria gene in the DNA of the unaffected calf. However, homozygous expression of the defective allele was identified in the DNA from both affected calves.

This verified the provisional diagnosis of bovine congenital protoporphyria - the first known confirmation of this disease in New Zealand.

With thanks to Dr Anyika Thomsen, VetEnt King Country.

Angus Black & Michael Englander

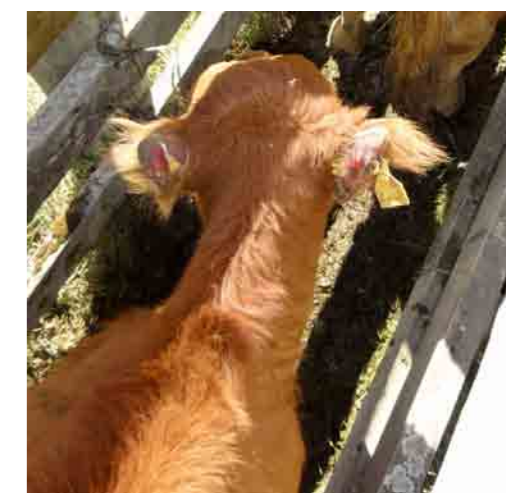


Figure 1: An affected calf several months after initial presentation.

Photo: Anyika Thomsen

NZVP LAUNCHES QUALITY ASSURANCE PROGRAMME FOR IN-CLINIC ANALYSERS

In what may at first seem an unlikely initiative NZVP has launched a Quality Assurance (QA) programme for in-clinic chemistry analysers.

However, I clearly see a linkage. We have consistently said we do not view in-clinic and point of care diagnostics as a threat to our business. All of us involved in the veterinary sector can see the trend is for increasing usage of these technologies and laboratories like ours have to be positive and proactive about where we stand alongside them.

With the interests of the New Zealand veterinary profession at heart NZVP strongly believes that rather than being marginalised it can play a valuable role in ensuring the technologies are used as well as they possibly can be to the benefit of all.

This is a view shared by others as evidenced by the following quote;

"In-house quality assurance programs are essential for monitoring test precision, but it is participation in an external quality assurance program that confirms accuracy."

Dr. James Matthews
VLA website

All referral laboratories such as NZVP are involved in external inter-laboratory comparison programmes for each of the disciplines. Such programmes are fundamental to laboratories

involved in a wide range of analysis and not just those in the health sector. Many readers who have spent holiday job time in dairy factory labs will recall the tin foil packets associated with their inter-lab testing.

The NZVP programme involves the monthly despatch of samples. Veterinary practices run these through their analysers and return their results for a dozen key analytes. An example from a report appears below and this information is replicated for each analyte.

It is important to note that absolute correlation to the NZVP result isn't necessarily the optimum result. Knowing where the in-clinic result sits in relation to that used in the laboratory is sufficient for practitioners to develop their own modified reference ranges and make clinical decisions. The key personnel for this initiative are Rina McCarthy, Cameron Walker and myself.

NZVP has been encouraged by the support for the initiative which has come from The Vet Service Group, the suppliers of the Spot Chem range. It is great to see these people share the same understanding of the role of the programme but there are no barriers to veterinarians with any make of analyser whatsoever participating and all are welcome to be involved.

Further details will be available as we roll this programme out. If you have any queries please call me on 0800 838 522.

Richard Campbell

SUSPECTED MALLOW (MARVA PARVIFLORA) TOXICITY IN A HORSE

"Mid April I was called to examine a 10 year old TB mare that had been showing tremors of the muscles of the neck, had difficulty holding it's head up and was seen to be laying down more than usual for the last few days.

Examination:

37.5, heart and pulse rate 50, no irregularity of pulse, no heart murmur. slightly cyanotic membranes, capillary refill 1 to 2 seconds. A jugular pulse was obvious when the head was down. Had sweated under a light rug overnight. Ambient temperature was "comfortable".

It was at the end of a long drought, feed was short, the horse was in a fenced off section of paddock with one other – little pasture – much mallow in paddock, hay was being fed.

I took some bloods, said goodbye to the owner, and sat watching the horse. The mare started eating mallow. Hang on! Horses don't eat mallow.

I found the owner, advised taking the horse out of the paddock and into a stall, feeding only hay for now. Connor's "Poisonous Plants in New Zealand" described profuse sweating, hurried respiration, plus stiff gait, arched back, and head stretched forward, trembling or shivering.

Blood test results of significance showed:

CK	29424	(63 – 469)
AST	5533	(0 – 700)
GGT	14	(7 – 45)
GDH	21	(1-8)

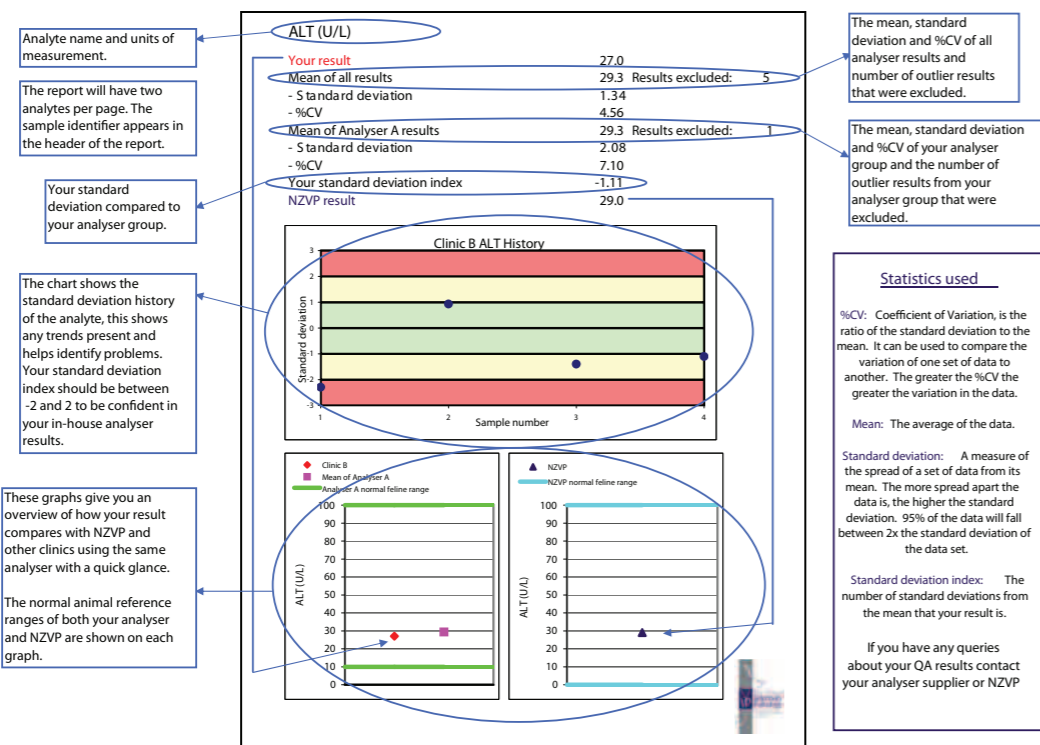
By 4 days off the pasture the shivering was reduced, and by 7 days had stopped completely. 6 weeks later the horse was doing well.



Many thanks to Dr John Mace, Veterinary Centre, Richmond, for writing up this case. There is an unidentified toxin in mallow which causes skeletal muscle necrosis and the plants may also contain toxic quantities of nitrates which cause the methaemoglobinaemia. Sheep are reported to be more susceptible than cattle or horses. Mallow is a weed of stock yards, crops, pastures and waterways which is difficult to control with herbicides.

Jenni Donald

NZVP QA Programme Report Breakdown



HISTO – CYTO PACKAGE

Cytology and histology are complimentary diagnostic techniques, each with advantages and disadvantages. As a general rule, cytology is less invasive, less labour intensive and can give a quicker result. In contrast, surgical biopsies often require anaesthesia and the histology takes longer to process, but this technique does allow important evaluation of tissue architecture. There are a few different options with the pricing of cytology and histology submissions to the lab, and we also offer a histo/cyto package at a significant discount to take advantage of the two techniques.

Cytology pricing:

Examination of multiple smears from a single site are charged a single cytology fee - keep this within reason, there is seldom any advantage to examining 16 smears from one lesion, as opposed to three or four!

Where multiple sites or lesions are sampled, charging becomes more discretionary. For example, if two lipomas are aspirated then we tend to charge only one fee but if samples of liver, spleen and abdominal fluid from the same animal are submitted then there will be one or more additional site fees.

Histology pricing:

Multiple biopsies from a single lesion are charged as a single site.

Two or more samples from multiple lesions or organs, and also multiple biopsies from generalized skin disease, will be charged a multiple histology fee.

Histo/Cyto packages:

Submission of both cytology and histology samples from the **same lesion** are eligible for a fee discount.

Both samples can be submitted at the same time, and the cytology will usually be examined first. If this is diagnostic, the histology is placed on hold. If non-diagnostic or equivocal, the histology is processed, and the discounted fee applied to the histology sample. You can also request the histology to be processed first.

Histology submitted after the cytology has been reported is also eligible for discount. Please let us know the previous case number so the discounted histology fee is applied. If two masses are removed and only one had cytology the discount does not apply.

If you have any queries about histology and cytology pricing, please feel free to give us a call to discuss the case or to receive an estimate for your client.