Awanui Veterinary

Pathology in focus

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Top tips for lymph node cytology

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Lymph nodes are commonly sampled for cytologic evaluation. Cytology can provide a rapid, non-invasive and cost-effective diagnosis in many disease processes including lymphoma, metastatic disease, lymphadenitis and reactive lymphoid hyperplasia. Although lymph nodes can be easy to sample, it is often a challenge to generate diagnostic quality cytology smears. These require a thin, highly cellular preparation of intact cells, from representative locations.

The following are some tips and techniques that can help increase the diagnostic power of lymph node aspirates for cytology, helping avoid a non-diagnostic result. Many of these tips can also be applied to cytology in general.

Sample technique

- **Non-aspiration method**

  Intact cells are critical to be able to evaluate both cell size and morphology. This can be especially challenging when sampling lymph nodes, due to the inherent fragility of lymphocytes. A non-aspiration technique (woodpecker or needle-only) can help maintain cell preservation, and lymphoid tissue generally exfoliates well without the need for the increased pressure of aspiration. Note that ruptured cells should not be evaluated, as they often appear bigger, and can show apparent prominent nucleoli, which may lead to a potential misdiagnosis.

  For the non-aspiration method a 21-23 gauge needle is recommended. In addition, attaching a pre-filled 3-5 mL syringe for this method allows for additional stabilisation, as well as immediate expulsion of the material onto a glass slide. [Note: if using an aspirational technique, pressure must be released from the syringe prior to removal from the tissue, and then the syringe must be detached and re-filled with air to expulse the material]. The non-aspiration method uses capillary action and needle re-direction to collect tissue and usually requires only 3-5 redirections (without removal from the lymph node) to gather sufficient material.

- **Blood**

  A second tip is that blood contamination can be useful. The plasma present in blood can help preserve cellular morphology (Figure 1B). However, very thick blood on cytology tends to obscure cell detail and should be avoided. Reducing redirection attempts and sampling different areas of the node or mass is recommended to help avoid this.

- **Location**

  It is important to generate representative slides for cytologic evaluation, as this increases the diagnostic power of cytology. In cases of generalised lymphadenopathy sample several lymph nodes, ideally two smears each from 3-4 enlarged lymph nodes. When only a single lymph node appears enlarged, sample 3-4 different areas within the lymph node. This is especially true with large or rapidly growing lesions, as the centre may contain non-diagnostic necrotic or haemorrhagic material.

  Note that submitting cytology from multiple lymph node locations generates a single cytology fee when submitting to Awanui Veterinary (see our current price book for full details).

  Sampling too few locations can increase the potential for diagnostic errors. Examples include a misdiagnosis of lymphoma when lymphocytes are

Figure 1. Ideal sample preparation

A: Glass-on-glass smear technique, which generates a thin monolayer of intact cells.

B: Canine large cell lymphoma (100x oil). Note the high proportion of intact large lymphocytes (black arrow), with fewer small mature lymphocytes (blue arrow) and erythrocytes (red arrow). Small lymphocytes and erythrocytes can be used to gauge the size of the lymphocyte population.

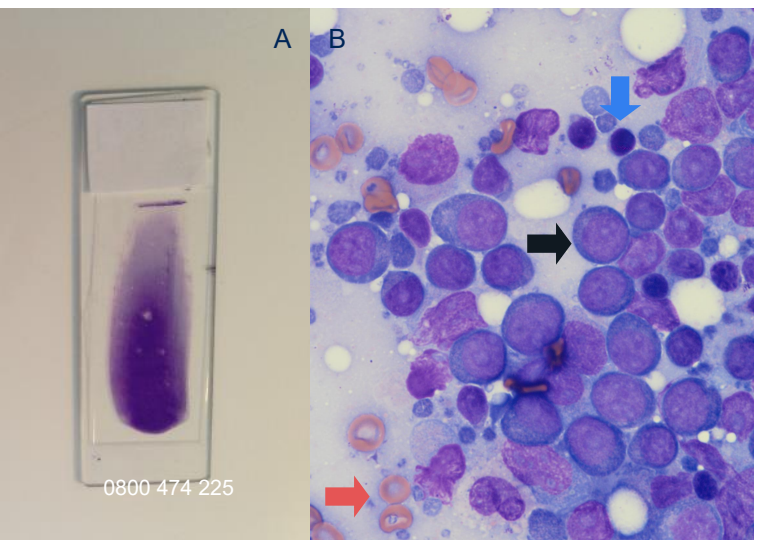
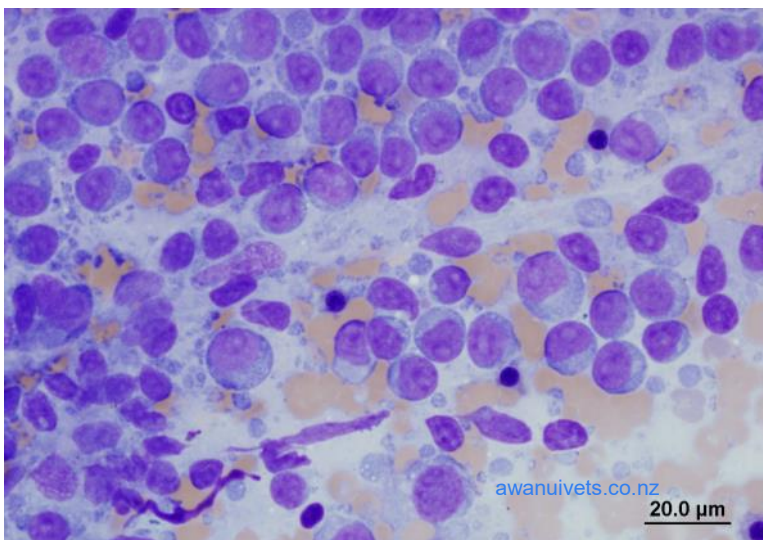


Figure 2. Potential for diagnostic error

This cytology image shows a high proportion of monotypic appearing large lymphocytes, with large central nucleoli, raising concern for lymphoma. In this case, the needle has sampled the germinal centre of a reactive lymphoid follicle, and histopathology confirmed lymphoid hyperplasia. Sampling several areas within enlarged lymph nodes can help avoid this potential diagnostic pitfall.



aspirated from germinal centres in reactive lymph nodes (Figure 2), or missing a diagnosis of metastatic disease when a lymph node is not yet fully effaced by the tumour.

- **Special considerations for the submandibular area**

  Enlargement of submandibular lymph nodes is easily detected and sampled. However, submandibular lymph nodes are constantly exposed to antigens and reactive hyperplasia can complicate cytologic interpretation at this location. In addition, salivary glands also reside in close proximity to the submandibular lymph nodes, and may be inadvertently sampled. For these reasons in cases of generalised lymphadenopathy, it is important to include sampling the popliteal and prescapular lymph nodes alongside the submandibular lymph nodes.

Cytology preparation techniques

1. **Gentle glass-on-glass smear technique**

   Place the needle bevel side down (close to the glass surface) and gently expel material on to the top third of the glass slide. A second glass slide is placed on top of the sample, perpendicular to the bottom slide, and (using the weight of the top slide only), the top slide is glided across the bottom slide, smearing the material in the central area of the bottom slide.

2. **Roll technique**

   This method is similar to preparing an ear or vaginal swab cytology. Aspirated material present in the hub of the needle is gathered onto the blunt end of a clean stick and gently rolled several times across a glass slide (like tire tracks) until all material is dispersed.

3. **Impression smear from an excisional biopsy**

   If submitting an excised lymph node for histopathology, a cut surface can be gently touched a few times onto a glass slide. The transfer of cells is aided if the cut-surface is gently blotted dry first using a paper towel. Submitting cytology alongside histopathology samples can aid pathologists in the evaluation of lymphocyte cell morphology (e.g. granular cell lymphoma).

   ** Note that cytology samples should be submitted separately from formalin fixed samples to prevent premature fixation and poor staining of cytology samples (Figure 4B).*

Smear preparation

Expulsing the tissue on to a glass slide and smearing the material must be performed immediately after sampling, to avoid the tissue drying out.

There are several reported techniques for creating a cytology smear. For lymph node aspirates the gentle glass-on-glass smear technique is considered the most effective, both to maximise cell integrity and generate a thin monolayer of tissue for evaluation (Figure 1A). However, you may want to utilise different techniques for the same case (such as the roll technique, or impression smears described under Cytology preparation techniques), to help increase the chances of obtaining a diagnostic sample.

Other methods (including splatter, squash, scribble or starfish techniques, see Figure 3.) often produce suboptimal quality samples, generating both thick areas of tissue (which causes cell contraction and under-staining), and excessive rupture of cells (Figure 4C-D). Assessing lymphocyte size is a crucial part of interpretation, and contracted cells in thick areas can appear small, and ruptured cells can appear large which may result in inaccurate interpretation.

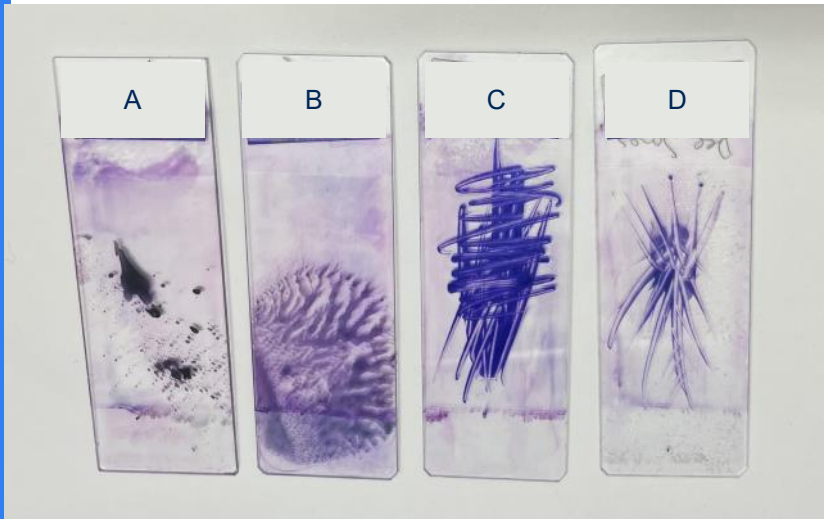
Note that when ultrasound guidance is used, the gel must be wiped from the skin surface prior to sampling for cytology, as even small amounts can reduce stain quality and obstruct viewing of tissue cells (Figure 4A). Clearly label slides in pencil (as pen or markers can dissolve during staining).

Regardless of the technique used, grossly a successful lymph node cytology sample appears fluidic and creamy white, indicating the presence of many tissue cells. Allow to air dry completely before staining. In colder climates, cytology samples can be dried rapidly with a hair dryer (on low setting) to avoid slow drying artifacts which reduce cell preservation. This also works well for joint cytology samples.

Sample staining and submission

Whether cytology slides are to be evaluated in-house or

Figure 3. Examples of suboptimal sample preparation techniques
These techniques should be avoided as they create areas of thick and ruptured tissue, which limit cytologic evaluation.
A: Splatter B: Squash C: Scribble D: Starfish



sent to a reference lab for pathologist review, ideally at least one representative slide should be stained and evaluated in-clinic, to assess the diagnostic quality of the sample (i.e. check if there is adequate cellularity and preservation to enable an interpretation). This increases sensitivity and maximises the diagnostic power of cytology. Doing this before the patient goes home allows time for a repeat sample if required.

Follow your routine staining procedure for in-house evaluation. Performing a quick scan on low power after initial staining can identify whether the sample needs more time in the stains (which can be required with thicker lymph node preparations). Poorly stained smears often look pale and pink (rather than the purple and blues of nuclei and cytoplasm respectively).

Note also that quick stains (such as Diff-Quik) can make lymphocyte chromatin appear coarse and nucleoli more prominent, compared with alcohol-based stains (such as modified Wright stains). The latter are used in both the reference laboratory and are also commonly seen in the images used in many reference materials.

Remember to use a cover slip when using the 40x (dry) objective, otherwise the cells will appear fuzzy. A drop of oil can then be placed on top of the coverslip when rotating around to the 100x (oil) objective.

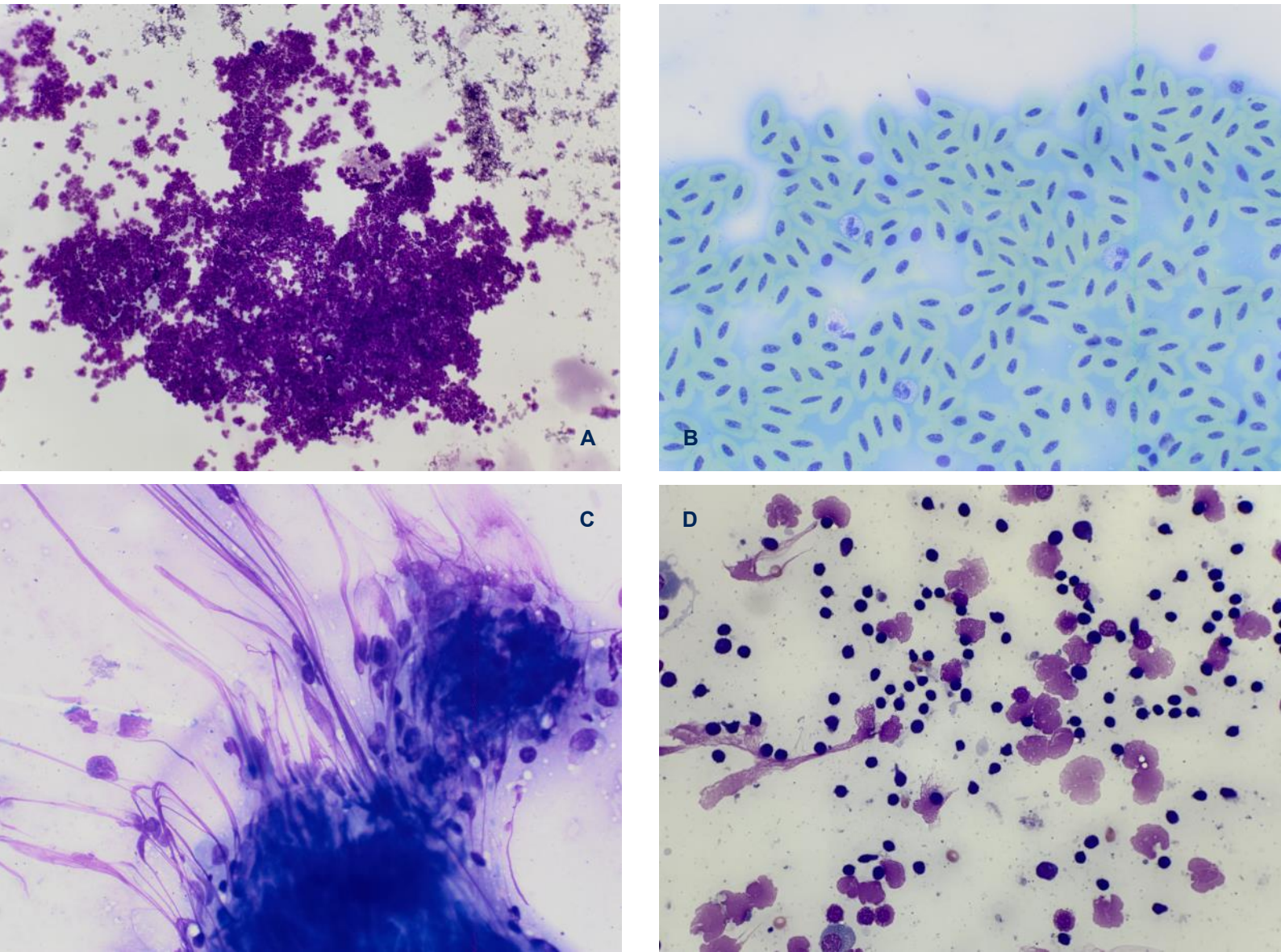
If sending the samples to a reference lab be sure to also include the pre-stained slides (as these may be the most diagnostic samples) and remove any coverslips (as these can stick to the glass as the oil dries out). Also include the signalment, relevant history and clinical description, as these form a critical component of the interpretation.

Useful references:

- Meichner K. Cytology of Lymph Nodes. *Today's Veterinary Practice*. Issue: May/June 2023.
- Raskin R. and Meyer D. *Canine and Feline Cytology*. Third Edition. Elsevier. 2016.
- Schlemmer S. Obtaining a Sample for Cytology Using Fine Needle Biopsy. *Today's Veterinary Practice.* Issue: January/February 2023.
- Sharkey L. C. *Veterinary Cytology*. John Wiley & Sons, Inc. 2021.

Figure 4. Cytology artifacts.

A: Ultrasound lubricant gel (purple granular material). B: Formalin fixation (green staining erythrocytes). C: Thick smear with cell rupture (nuclear streaming). D: Squash preparation with dark contracted lymphocytes and pale ruptured lymphocytes.



Pathologist spotlight

Cristina Gans is originally from Florida, where she completed a BSc. (Biology and Chemistry with honours) at the University of Miami. She moved to New Zealand and completed a BVSc. (with distinction) in 2008 at Massey University. After university, she enjoyed living in New Zealand so much that she decided to stay permanently and spent the next 9 years in companion animal practice.

Having harboured a love of a pathology since vet school, Cristina returned to Massey University to complete a three-year residency and masters program in anatomic pathology, focusing on the pathology of *Mycoplasma bovis*. She successfully completed the ACVP certifying examination in 2021 to become an ACVP diplomate in Anatomic pathology.

Cristina enjoys all aspect of veterinary pathology, including both companion and production animal medicine, and enjoys working with veterinarians in this context.

When not studying or working, she enjoys hiking, reading fiction and travelling to exotic places.

Aquamation is coming to Auckland!

Awanui Veterinary can now support more clinics by expanding our Fond Farewells end-of-life services with Aquamation, available soon in Auckland.

What is Aquamation?

Aquamation is a water and alkali based alternative to traditional flame cremation. This process uses 90% less energy and produces no harmful emissions*, so is an effective tool for making significant steps in reducing your clinic's carbon footprint.

Are there still ashes?

Yes, as the process leaves bone material, this is ground into 'ashes' in the same way as they are for traditional flame cremation. The pet owner still receives these 'ashes' and has access to a full range of memorial items and urns.

What's the process in clinic?

We have simplified the end-to-end process. Pick up services# are booked through an online portal and a dedicated courier will respectfully transport it to our Aquamation location. The ashes and any memorial items and urns will then be delivered back to the clinic.

How long does it take?

We aim for a 3-5 day turn around.

Why should my clinic offer Aquamation?

An increasing number of pet owners are now actively seeking out sustainable alternatives. Aquamation is a more sustainable choice, aligning with growing consumer demand for environmentally friendly options. A 2024 report highlights that 78% of consumers value sustainability, and 62% actively seek out sustainable products.

Can pet owners access this services directly?

No. We are offering this opportunity purely B2B, so that your staff can support your customers through the difficult decision.

How can I get this service at my clinic?

We are currently registering clinics so that they can start accessing this service as soon as the doors open. If you are interested contact Rachel.howie@awanuigroup.co.nz or 0276 048 690.

If your clinic is located outside of Auckland and you are interested in this service, please contact us as we will be expanding this offering to other interested areas.

** Source: NCCEH. Alternative disposition services: Green burial, alkaline hydrolysis and human composting (2022)*

 # Pick up services currently only available in Auckland metropolitan area for Aquamation and Christchurch city area for flame cremation.

Equine melanoma

Clinical history

A 19-year-old Grey warm-blood horse presented as lethargic and anaemic, with very pale mucous membranes and covered in melanomas. Bloods were submitted for biochemistry and haematology testing.

Haematological findings

Haemoglobin (69g/L) and haematocrit (0.20 L/L) were both below the normal reference interval and red cells displayed anisocytosis, and poikilocytosis. Small numbers of acanthocytes were seen.

A neutrophilia (8.8 x109/L) supported inflammation, despite the fibrinogen being normal. Low numbers of cells containing very dark granules were also noted in the blood smear (see Figures 1 & 2).

Discussion

The granulated cells are melanocytes. Finding them in circulation supports the potential for metastatic disease in this case.

Equine melanoma can be benign or malignant. The skin is the most common site for the tumour in horses, with the typical locations being the ventral tail, perineal region, prepuce and commissure of the lips. It is most prevalent in grey horses and it's estimated that up to 80% of grey horses more than 15 years old have melanomas.

Tumours are usually firm and nodular, may be solitary or multiple, and may be hairless and ulcerated. They are usually black. Melanoma can be suspected based on clinical signs and visual inspection, and confirmed via either FNA cytology or biopsy histopathology. Further diagnostics are recommended especially if the mass is atypical in appearance.

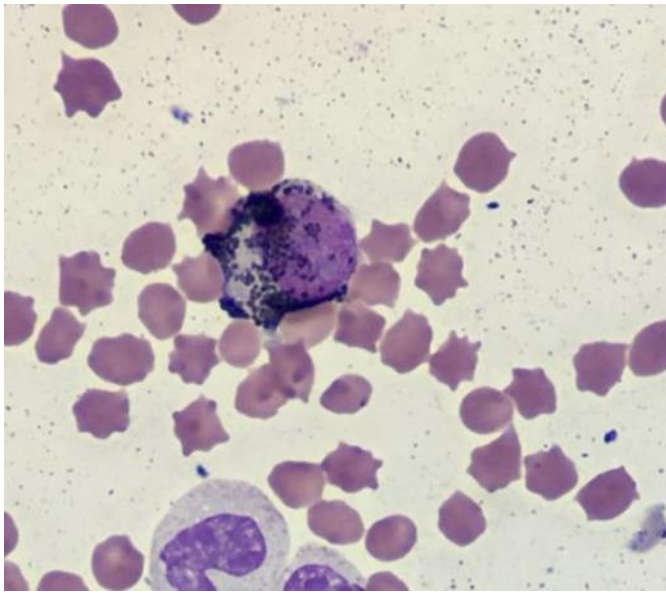
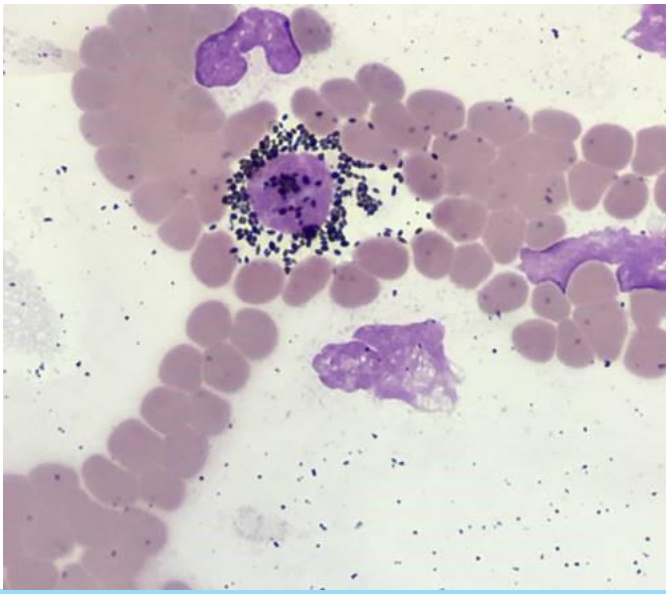


Figure 1. *(above) Intact cell with coarse, dark granules.*

Figure 2. *(below) Disrupted cell showing the granules more clearly.*



ezyVet Standard Diagnostic Integration (SDI)

We're pleased to share that 80% of our clients using ezyVet have successfully transitioned to the full SDI integration for submitting requests and receiving results. This integration offers a more streamlined and reliable experience compared to the legacy email-based system.

ezyVet has advised that the old email integration will be phased out in the near future. As such, we strongly recommend making the switch to the SDI integration as soon as possible to avoid any disruption to your workflow.

Several clients have already taken advantage of the ezyVet support desk to assist with configuration changes, and we encourage you to do the same if needed. For those who have not yet transitioned, we urge you to consider this change proactively, rather than waiting until the old system is retired.

Jo Archibald our IT Solutions and Systems Manager is available and ready to assist you through the process to ensure a smooth and seamless transition. Please don't hesitate to reach out to Jo if you have any questions or need support – jo.archibald@awanuigroup.co.nz or 027 444 7095.

In brief

- **Our newsletter frequency** has now changed to quarterly. Issues will be sent out in June, September, December and March. You can also tune in to our website news section or our Facebook page for updates.
- **Matariki long weekend** - All of our laboratories will be closed the ENTIRE of the Matariki long weekend (20—22 June), reopening on Monday 23 June.
- Thank you for visiting our stand at the NZVA conference last week. If you haven't already, don't forget to complete our **customer satisfaction survey** and go in the draw to win a bottle of Moët & Chandon Champagne Brut. **Access the survey here.**

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- contacting Awanui Veterinary couldn't be easier.

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