





Specialist Diagnostic Services ABN 84 007 190 043 trading as Vetnostics

## Welcome...

By this stage, we are well into 2019 already! I hope the year has started well for everyone - Summer has been a busy time.

This edition of the newsletter includes an update on our website, general information relating to cytology and histopathology sampling as well as focussing on our microbiology service.

As always, please contact Vetnostics Client Liaison Officer Matt Hishon (Tel: 0481 035 612 and email matthew.hishon@ laverty.com.au) or Dr Doug Hayward (02 9005 7272 or email doug.hayward@vetnostics.com.au) if you have any requests for future newsletters, questions or any other queries.

Vetnostics Client Liaison Officer

## **Matt Hishon**

We are pleased to introduce our new Client Liaison Officer, Matt Hishon, who replaces Anna Rys after her retirement.



With more than 20 years of experience in customer service and management roles across several industries, Matt brings his highly developed skills to Vetnostics to ensure a quality client experience.

Please feel free to make contact with Matt to book a clinic visit or to discuss any aspect of Vetnostics' service.

#### Matt's contact details are:

Ph: 0481 035 612 or email: matthew.hishon@laverty.com.au

# Maximising Cytological diagnostic outcomes

## **Dr Sue Jaensch – Veterinary Clinical Pathologist**

Cytology is a very useful tool in evaluation of masses and diseases of solid organs. In comparison to histology, cytology is an inexpensive test, minimally invasive, provides rapid results, and with good site selection provides good correlation with histology.

However, more than any other testing modality, cytology relies very heavily on the clinician to provide a history, to select appropriate sites for collection and to make slides of adequate quality.

## **Setting expectations**

When choosing cytology rather than histology for investigation of a mass or an organ, it is important to set realistic expectations both for yourself and the owner. Cytology is excellent at defining the problem, but may not provide the precision that the evaluation of intact tissue structure in histology can achieve. For example, cytology provides good accuracy for differentiating neoplastic and inflammatory conditions, and in many cases benign or malignant neoplasia. However, grading and more specific classification of a malignancy will usually require histology +/-immunohistochemistry.

Some tissues are more amenable to cytological examination than others. This reflects the likely aetiologies, vascularity of the tissue, and the freedom with which the tissues will exfoliate. Many papers have been published on correlation between veterinary cytology and histology, and the table below provides a guide. Note that this data is usually sourced from secondary and tertiary referral clinics.

Tissue	Correlation between cytology
Skin masses	>80%
Mammary masses	70%
Spleen	60%
Generalised hepatomegaly	50%
Bone	<50%
Discrete hepatic mass	30%

This does not mean that tissues with lower correlations should not be evaluated by cytology, but rather that cases should be individually evaluated based on the major differentials to determine if cytology is likely to be a suitable diagnostic step.

Choice of sampling method can also dramatically affect the diagnostic outcome. For example, prostatic cytology can be highly rewarding when direct FNAs are collected. Prostatic washes and massages are usually non diagnostic regardless of the aetiology. Similarly, targeted aggressive sampling (using a cytology brush or urinary catheters) of identified lesions in the nose can be cytologically rewarding, while nasal swabs or flushes are commonly non diagnostic.

## **Importance of history**

History is vitally important. For every request, please include:

- A brief description of the location of the lesion.
- · the duration of the lesion,
- · growth rate,
- gross appearance,
- · presence or absence of ulceration,
- · medications already administered
- · results from blood testing.

The absence of an adequate history may prevent the pathologist from providing an accurate interpretation or comments regarding further testing, even when an adequate sample is provided.

## **Selection of collection site**

Where to collect the sample from seems a straight forward idea, but can dramatically alter the diagnostic outcome. When there is a choice of lesions, it is preferable to collect from mature lesions, whilst avoiding lesions with evidence of necrosis or ulceration. If only one mass is present and it is ulcerated, please include this information in the history to assist interpretation of the cytological findings.

If fluid is encountered when sampling, make slides from the fluid, but also collect aspirates from the surrounding tissue. Cytology of fluid collected from cysts is rarely diagnostic, but aspiration of the surrounding tissues may provide a cause for the cyst formation.

## **Medway**

MedWay is a web-based application that provides you with access to your patients results as soon as they are available (in real-time) and can be accessed 'on the go' through your regular web browser.

Results viewed on MedWay can be saved as a colour PDF or printed. Please register online at www.medway.com.au

## **Green Specimen Submission Bags**

To facilitate rapid turnaround times and the priority which we place on the processing of veterinary specimens within our laboratory, Vetnostics recommend the use of green specimen submission bags only. Please use these bags for ALL your submissions to Vetnostics.

# The Vetnostics Microbiology Service

Vetnostics is proud to offer the premier veterinary microbiology (bacterial and fungal) service in Australia.

The vast majority of bacterial culture submissions are reported out within 48 hours, with longer times required for specific specimens (eg. those requiring extended culture, etc.). Vetnostics has on-site access to the most advanced instrumentation to assist with identification of bacterial and fungal organisms allowing fast and accurate reporting of culture and sensitivity results.

## Aspiration of the sample

When collecting the sample, secure the lesion with the fingers of your non dominant hand. Introduce the needle into the tissue. If using a syringe, apply mild negative pressure. Redirect the needle in the tissue several times using a sewing machine needle like motion, without withdrawing the needle from the tissue. Release the negative pressure (if using) before withdrawing the needle. You should see a small amount of material in the needle hub. Splattering of material into the syringe usually reflects excessive negative pressure was used.

## **Spreading the sample**

Transferring the material to the slide seems very simple, but this step is often the cause of major artefacts.

- 1. Remove the needle from the syringe.
- 2. Draw up a small amount of air into the syringe.
- 3. Place the tip of the needle in contact with the slide.
- **4.** Very gently express a small amount of material onto the slide you should have a small round droplet. Do not spray the sample onto the slide.
- 5. Repeat on additional slides if there is more material.

To spread the slide, use a second slide held at a shallow angle, just as you would make a blood film. Place the slide in front of the droplet and draw it back to make contact with the droplet. Let the material spread along the bottom of the slide. Now slowly slide the spreader slide along the sample slide. No downward pressure is necessary. You should now have a smear that looks like a blood smear.

Dry the slides rapidly by waving them in the air, placing in front of a fan, or using a hair drier on a warm setting. There is no need to fix cytology slides prior to submission.

Spray slides are commonly submitted to Vetnostics. These are slides where the material has been sprayed onto the slide, often from a distance and with some force. The material is then usually not spread. Slides made in this manner are rarely diagnostic as the cells are commonly damaged by the spraying process.

Please label slides with the animals name and the site of collection. Please use pencil to label all slides – the ink from most pens including permanent markers is removed during staining. Pencil writes well on all frosted slides. If you use non frosted slides, a diamond pencil can be used to etch details on the slide.

For supporting images of sample collection and smear preparation, please visit our updated website.

## Staining slides in house

It is often an advantage to fix and stain a slide in house and evaluate it for the adequacy of sample collection and cellular morphology. This can prevent submission of non diagnostic samples and can allow immediate recollection if required, without having to have the animal revisit the clinic. We are happy to receive slides that you have stained in house. Where possible, please submit at least one unstained slide as well.

# Submission of cytology samples with histology samples

We commonly receive cytology samples submitted at the same time as histology samples. If the cytology samples are exposed to formalin vapours from the fixed samples, this causes marked artefact in the samples and usually renders them non diagnostic. To avoid this, please submit cytology and histology samples in separate bags and with separate request forms.

If you have any questions about cytology sample collection, staining or submission, please feel free to call Vetnostics and discuss this with one of the pathologists.

### **Further Reading**

The Accuracy of Fine-Needle Aspiration Cytology in the Diagnosis of Canine Skin and Subcutaneous Masses, Radostin Simeonov, Comparative Clinical Pathology 06/2010; 21(2).

Historical Overview of Evidence-Based Diagnostic Cytology Including Bone Marrow in Veterinary Medicine, Rose E. Raskin, ACVP conference, 2013

Accuracy of US-guided FNA of focal liver lesions in dogs: 140 cases (2005-2008). Bahr KL1, Sharkey LC, Murakami T, Feeney DA., J Am Anim Hosp Assoc. 2013 May-Jun;49(3):190-6.





## Histopathology Sample Considerations and Expectations

## **Dr David Taylor – Veterinary Pathologist**

The final part of the JAVMA commentary (Vol. 244, No. 4, February 2014) begun in newsletter Autumn 2015, covers issues to be considered when taking samples and how those samples can influence pathologist interpretation and the diagnosis you receive.

Diagnostic interpretation by pathologists isn't an exact science. It is often helpful for vets to understand how sample features can influence the diagnostic process. Pathologists do the best they can at interpretation, but the limitations of damaged/small/insufficient samples should be acknowledged. These limitations make clinical context vastly more important.

**Small samples.** Aspirates, endoscopic and needle biopsies often present significant challenges for pathologists in interpretation and diagnosis. Three problem areas are key: poor sample quality, sample fragmentation, and limited or no architecture. Small samples collected with laser or electrocautery can be severely altered resulting in significant artefact. Excessive or aggressive use of forceps can be just as damaging. Some tissues such as lymph nodes and certain tumours need architecture for lesion interpretation. As an example, small lymphocytes constitute much of the cortex of a normal lymph node while follicles are few. Needle biopsies may fragment and/or not contain any follicles confounding diagnosis of small cell lymphoma and reactive hyperplasia. Similar issues are encountered with endoscopic GI biopsies, especially when only the superficial mucosa is sampled. Because the laboratory cannot always orient these samples optimally, taking multiple sections often yields enough tissue in the correct plane allowing interpretation.

**Skin samples.** Nowhere else is the collaboration between clinician and pathologist as important than when making a dermatopathologic diagnosis. Apart from a few instances, a diagnosis is often highly dependant on clinical context. Minimum objective data provided to the pathologist should include: age at the onset of clinical signs, duration of clinical signs, presence or absence and intensity of pruritus, characteristics of the lesions (eg, papular, macular, crusting, or ulcerative), distribution of the lesions, and key laboratory findings such as results of FBC, serum biochemical analyses, measurement of serum hormone concentrations, Wood's lamp examinations, and skin scrapings. Pruritus while perhaps the most frequent reason for examination for skin biopsies, is often forgotten when submission forms are completed. This becomes important because pruritus definitely impacts microscopic findings. Other information that helps formulate the clinical context includes seasonality, home environment, food sources, exposure and travel history, drug history, and response to attempted treatments.

The list of differential diagnoses is a critical component of the diagnostic process for skin samples, given that the pathologist's primary role is indicating whether one or more of the listed differential diagnoses is supported by or ruled out by the histologic results. It is not the pathologist's role to advance a list of differential diagnoses, and this certainly cannot be done in the absence of a good history, even by those highly experienced in dermatopathology. Challenging cases may benefit from consultation with a dermatologist prior to sampling who can advise on differential diagnoses and sample selection. Finally, skin biopsy samples should (in most but not all cases) reflect early and developed lesions and should avoid clearly ulcerated skin. The histologic appearance of ulcerated skin is dominated by the reaction to the ulcer and rarely indicates syndrome-defining microscopic changes.

**Liver samples.** Needle or wedge biopsies are often accompanied by a history of 'high' enzyme levels, but this doesn't provide the pathologist with sufficient context. It's more helpful to know if the animal is sick and the actual enzyme values. Elevations over an extended period of time (30 days) are more meaningful than a one-off measurement. The half life of ALT is such that levels may remain elevated while 'one hit' liver damage has mostly been repaired. When portocaval shunting is suspected, it is fundamentally important to know whether the liver is of normal size or small, the neurologic status of the animal and bile acids concentration. Finally, consider collecting additional samples in anticipation of other tests (ie, fresh samples for copper, bile for culture).

**Kidney samples.** Results of histologic examination of kidney biopsy samples should always be interpreted in the context of renal function, including BUN and serum creatinine and phosphate concentrations, urine protein concentration and specific gravity, degree of proteinuria, and estimated glomerular filtration rate. Evaluation of H&E-stained sections of kidney biopsy specimens provides only limited information. That said, histologic examination of needle biopsy specimens can identify advanced glomerular disease, interstitial disease, tubular nephrosis, and amyloidosis if we understand the clinical context well. Again, sample quality is paramount, and clinical context provides some indication of whether the histologic changes are clinically important and whether they correspond to the severity of alterations in renal function.

### **Conclusions**

Complicated and sometimes expensive diagnostic testing is best approached with an organized and logical plan to define the problem, outline the objective findings, hypothesize what may be happening (ie, develop a differential diagnoses list), and perform appropriate testing to rule differential diagnoses in or out. Providing an organized, complete history on submission forms will facilitate case analysis.