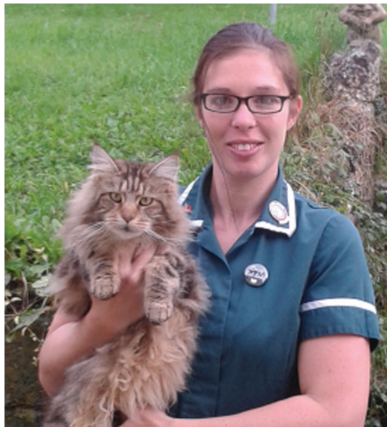

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Anji qualified as an RVN in 2005 and has a keen interest in medical nursing and laboratory diagnostics. She has completed nurse training at Axiom and IDEXX Laboratories and is in the final year of her Dip AVN at Harper Adams University. Anji works in a mixed first opinion practice, is a clinical coach and also lectures and teaches laboratory techniques. In practice she is often found behind a microscope getting overexcited about an unusual blood smear or infecting students with her enthusiasm.

Ten top tips to make the most of your practice laboratory

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ABSTRACT: The majority of first-opinion practices have an in-house laboratory and RVNs are well placed to become adept at maximising the diagnostic information that can be obtained. Refreshing skills learnt during VN training, in addition to learning a few new techniques, can provide greater diagnostic yield from samples being examined externally and more information from samples examined in-house. Laboratory work is interesting and enjoyable and providing our veterinary surgeons with the best information with which to treat patients is the ultimate reward.

Keywords: laboratory techniques; diagnostics; cytology; urinalysis; general practice

Introduction

Using your practice laboratory to its full potential is a useful skill in general practice, as the ability to obtain diagnostic information faster and produce high quality samples undoubtedly improves patient care. These top tips require no specialist or expensive kit other than basic laboratory supplies and equipment and the additional information you can obtain benefits patients with a variety of disease processes across all species.

1. Double up the diagnostic yield of a packed cell volume (PCV) measurement by reading total protein

Once your haematocrit tube is spun and read using your reader, the tube can be snapped at a level just above the buffy coat (an ampoule snapper allows you to do this safely) and the plasma applied to a refractometer. Do not tap the tube on the glass to remove the plasma as you will damage the refractometer; fit a disposable 1 ml pipette over the end and using the pipette, gently blow the sample out, do not attempt to blow the plasma out by mouth! (**Figures 1a** and **1b**). Most refractometers are dual scale, with one scale for urine specific gravity and the other for plasma protein. Once read, the plasma protein value provides the veterinary surgeon with useful information for differentiating between fluid shifts caused by disease processes and anaemia. Remember, however, that haemolysis, lipaemia and icteric samples will falsely elevate plasma protein (Villiers, 2005).

Box 1. The interpretation of PCV and plasma protein values together can allow your vet to narrow down the diagnosis far more than either value on its own (TS = total solids/proteins)

Interpretation of PCV and plasma protein (TS) values

- decreased PCV/normal TS – anaemia, haemolysis
- increased PCV/normal TS – hyperthyroidism, Cushing's syndrome, haemorrhagic gastroenteritis
- normal PCV/decreased TS – protein-losing enteropathy, protein-losing nephropathy
- normal PCV/increased TS – severe dehydration and anaemia, FIP, multiple myeloma
- increased PCV/increased TS – haemoconcentration
- decreased PCV/decreased TS – chronic or subacute blood loss

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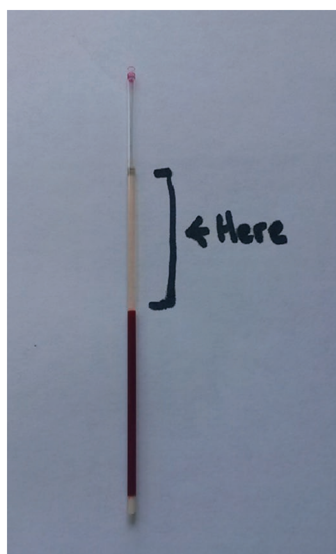

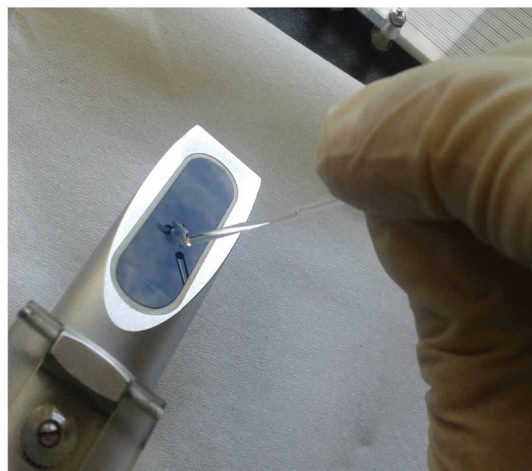

Figures 1a and 1b: Determination of total protein value using a PCV tube: snap the tube above the buffy coat, blow the plasma onto the refractometer prism and read from the total protein scale

2. Prepare a fresh blood smear whenever you are taking blood for haematology

Blood samples stored in anticoagulant deteriorate and begin to develop artefactual changes after around four hours (Valenciano, Cowell, Rizzi & Tyler, 2014). The best way to eliminate these changes from hampering assessment is to prepare a fresh smear *at the time of sampling*. Fresh blood is placed directly onto a slide from the collection needle and a blood smear is made. Fresh blood smears should accompany all external laboratory submissions to assist the pathologist. Alternatively they can be stained and examined in-house to provide extra information alongside your analyser cell counts. Omitting fresh smears can lead to errors such as:

- false negative results for Heinz bodies and blood parasites (such a *Mycoplasma haemofelis*) as these can detach from erythrocyte surfaces
- false decreases in platelet numbers as they degrade with storage
- difficulties with leucocyte counts as their nuclei degrade during storage

(Valenciano et al., 2014).

Storage also increases crenation of erythrocytes, which can hamper morphological evaluation. The best way to become proficient at smear preparation is to practice! Prepare a smear with every sample for a few weeks and your technique will rapidly improve.

The perfect blood smear is around an inch long with a feathered edge and is slightly narrower than the width of the slide (**Figure 2**).

3. Get used to looking at blood smears

If you start following tip number 2, you will soon have plenty of good quality smears in your lab, which you can stain. The main benefit of this tip is to learn to identify things that your in-house analyser cannot. Many analysers cannot recognise platelet clumps, count feline platelets as erythrocytes and fail to accurately identify the giant platelets that regularly occur in samples from Cavalier King Charles Spaniels (Rebar, MacWilliams, Feldman, Pollock & Roche, 2002).

Being able to recognise platelet clumps, examine a slide manually for platelet numbers and identify abnormal platelets are useful skills for determining the origin of an unexpected thrombocytopenia. In the same way, nucleated red blood cells are often counted in error as leucocytes, so learning to spot them allows you to distinguish a regenerative RBC state from a high WBC count. Finally, impedance analysers do not differentiate between mature and band neutrophils (Rebar et al., 2002), so being able to spot band neutrophils on a smear, indicative of a left shift, is a skill for which your veterinary surgeon will be grateful.

4. Try a stained wet preparation for urine sediment analysis

Once you have prepared a slide for a standard, unstained sediment examination use the leftover sediment to prepare a stained wet preparation. Simply add one drop of methylene blue (Solution 3 of your Diff-Quik® if you don't have a stand-alone bottle), mix, wait 30 seconds and then examine the stained urine under a coverslip (**Figure 3**). This technique allows you to see bacteria, white blood cells, red blood cells and epithelial cells in good detail. It is a useful addition to your standard sediment exam when you are short of time or don't wish to carry out a full Diff-Quik® staining.

5. Use good quality, frosted-end slides where possible

Whenever a slide is prepared, immediately label the slide with the patient's details. This is best done in pencil, as

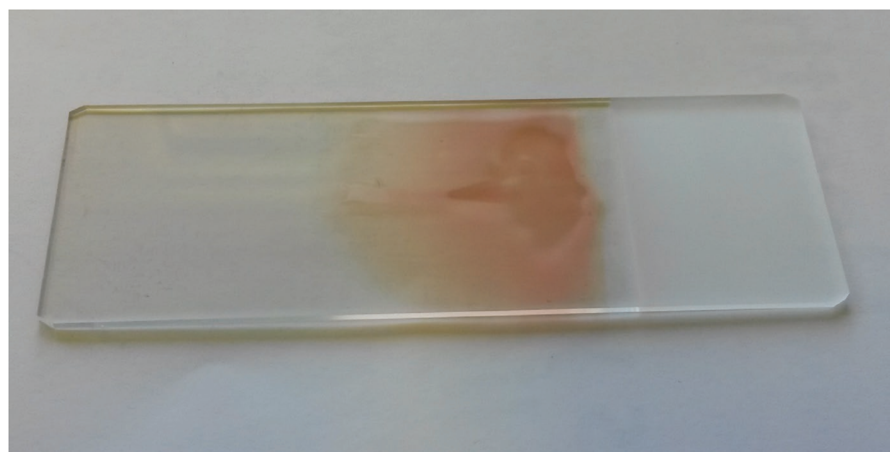

Figure 2: An unstained blood smear: a smear around 4 cm long with a good, feathered edge and no loss of blood from the edges is a great asset to a haematological analysis

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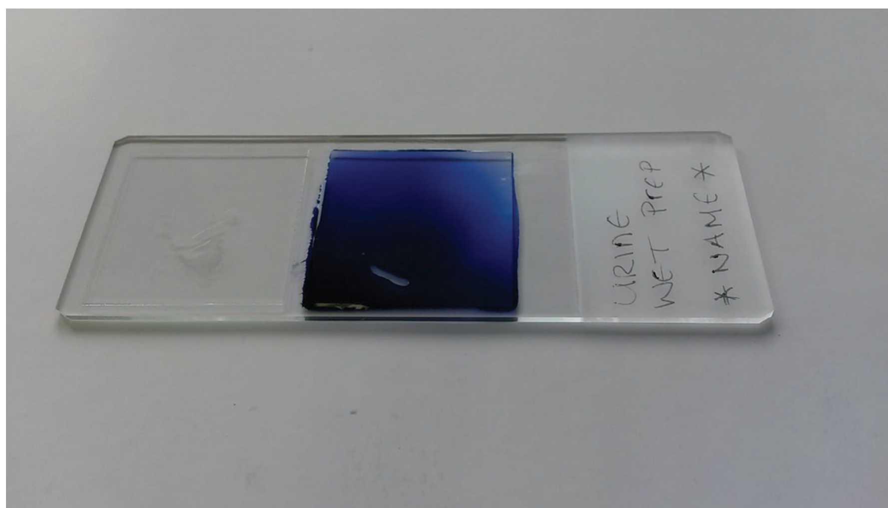

Figure 3: A urine sediment wet prep, both stained and unstained preparations can be examined side by side using a single slide and two coverslips

sometimes pen will run and compromise your staining. Most slides do not require cleaning if they are used directly from the box, but take care when handling to hold them at the edges as it is easy to contaminate them with squames (skin cells) from your own fingers (Stone & Reppas, 2010). Labelling slides not only prevents loss or confusion between slides but it also reminds you which way up the slide is! Indeed, if your veterinary surgeon tells you that the high power microscope objective is broken because they cannot focus on the material, your first check needs to be that the slide is the right way up!

In addition, using slides with cut corners also aids your smear preparation, as the spreader edge is naturally narrower than the bottom slide, so you are less likely to lose material off the slide edges.

6. Ace your fine needle aspirates - learn to squash prep

Spraying the collected material onto a slide through a needle often results in thick blobs of material on the slide. This is not conducive to a good cytological evaluation and increases your chances of a non-diagnostic report being received from your cytologist. To improve these slides, a good squash preparation technique will spread the material more thinly across the slide and greatly improve the chances of a helpful interpretation (Box 2 and **Figure 4**).

Box 2. Squash preparation technique, adapted from Stone and Reppas (2010)

Squash preparation technique

- Expel the aspirated material onto a glass slide.
- Place a second, spreader slide at right angles to and resting on top of the original slide.
- Draw the spreader slide quickly and smoothly once across the bottom slide.
- Do not press down on the top slide – its weight alone is sufficient to spread the material.
- The smear produced on the underside of the spreader slide is then examined.

Figures 4 (a and b). Fine needle aspirates: (a) shows the material sprayed directly onto the slide, the clumps are dense and difficult to evaluate; (b) is a squash preparation and shows the material thinly spread, facilitating good staining and evaluation

7. Evaluate cytology smears before referring them

When sending slides away to an external laboratory for cytological evaluation, examine them in-house first to make sure that there is material for the pathologist to comment on! This helps to reduce non-diagnostic samples and frustrated veterinary surgeons. Once you have prepared the slides, ensure they are fully dry. If you are short of time, a hairdryer set on low can be used to speed the drying process (Stone & Reppas, 2010). Scan the slides on 4x or 10x magnification to check that cellular material is present. Ideally you are looking for a monolayer of well-preserved cells.

8. Look after your stains!

Most general-practice laboratories have a staining set with the most common being a Diff-Quick® or Rapidiff® three-part set. These stains are simple to use and provide useful information across a variety of samples. Maintaining your staining set is very important in a general practice laboratory as, inevitably, they get used for anything and everything!

One good tip is to have two sets of stains, a dirty set and a clean set. The clean set is

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Figure 5: Separate staining sets for 'clean' and 'dirty' slides helps to minimise stain contamination thus improving the accuracy of microscopic analysis

reserved for staining blood smears, fine needle aspirates and other 'clean' samples, whilst the dirty set can be used for ear slides, skin impression smears and all else gunky! This helps to minimise stain contamination and reduces your chance of diagnostic errors due to compromised stains.

It is important that you are confident that the bacterial populations you are examining have come from your sample and not from your stain, so it is good practice to stain a clean glass slide periodically as a quality control to check that your stains are not contaminated.

Stains also need to be changed regularly as they become exhausted (Duncan, 2005). The time interval will vary depending on how many slides you are staining, but in an average general practice laboratory it is good practice to replace stains every 4–6 weeks.

9. Invest in a microscope eyepiece with a measuring gauge

A measuring eyepiece is worth its weight in gold when you are assessing slides at the end of a long working week or in the middle of the night and your eyes are more than a little bleary. It will allow you to determine cell sizes more accurately and will give you a definitive measure of polymorphism. I for one, would far rather measure several cells to be sure, as my visual assessment as a general practice RVN will be less accurate than that of a clinical pathologist. These eyepieces are inexpensive and can be slotted in and out of your microscope in seconds. They are also useful when assessing faecal flotations, as knowing the size of the egg you are examining can narrow down the species a great deal and thus facilitate identification.

10. Practice, practice, practice

Nobody becomes an expert in the laboratory overnight, but if this is an area of diagnostics you are interested in, you can become a huge asset to your veterinary surgeons and your patients.

Invest in some good textbooks and start by looking at as many 'normal' blood smears as you can; learn to identify different leucocytes, assess platelets and look for clumps. Then progress to looking at samples from ill patients, which are being referred to an external pathologist and keep a note of what you thought the slide was showing you. When these results are returned, compare the pathology report with your findings and learn from it; re-examine the slide to look for things you may have missed.

The same can be done with fluid samples and fine needle aspirates, just keep practicing and before long your confidence will increase. RVNs are not expected or allowed to make diagnoses but it is well within our remit to identify and draw our veterinary colleague's attention to abnormal morphologies, to use our knowledge to produce the best samples and to quality check them in order to increase the accuracy of the samples taken from our patients.

In conclusion, laboratory work may seem daunting but by starting small, learning from your mistakes and using your external laboratory reports as a learning tool, your confidence will quickly increase and your skill set will grow. Your colleagues and patients will value your new skills and before long you too will have a set of images detailing the amazing things that you have identified in the practice laboratory.

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