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The practice laboratory – Part 1: a student's guide

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ABSTRACT: The aim of this, and subsequent, articles is to provide student veterinary nurses with a guide to the practice laboratory. It will cover the basic equipment used in most in-house practice laboratories, as well as some of the common tests that as students you will be learning about and performing. It has been my experience that nurses either love or hate laboratory work – and it's my intention that this guide will appeal to both groups.

Equipment

One of the most important considerations to mention about any laboratory equipment is its upkeep and maintenance. Much of the equipment is easily damaged (and very expensive) so careful maintenance is essential to keep the machines working in order to provide accurate results.

A few simple steps performed every day could save much time and effort in the future.

- Keep all the equipment clean and free of spills following your in-house practice cleaning guidelines.
- Switch off the microscope between sessions and store it covered and away from vibrating machines, such as centrifuges, as well as any liquids. The eyepieces and lens should be cleaned regularly and after each session using special lens tissue which is designed so that doesn't scratch the delicate lens.
- After each use the oil immersion lens should be thoroughly cleaned to prevent the build-up of oil, and any oil spilled on the stage should also be cleaned immediately. Most importantly do not use any other lens for oil immersion other than one designed for oil use (usually x50 or x100) as it will damage the lens and a replacement could prove very costly for the practice.
- Electronic analysers should be switched off, where possible, and covered when not in use. Regular servicing is also necessary and should be carried out by an approved engineer to ensure the analyser works correctly and provides accurate results.
- The centrifuge should be cleaned at regular intervals and the safety plate should always be used if the centrifuge has one. In my experience, some vets feel this annoying piece of metal has no use and is better used as a coaster for their coffee!
- Undertaking quality control of biochemistry and haematology analysers is very important and I will cover this later in the article.

These are just a few simple steps and I am sure you can think of many more. It is a good idea to set up a Standard Operating Procedure (SOP) for the upkeep and cleaning of the laboratory, so whoever uses it knows exactly how to maintain it ready for action.

The centrifuge

Probably the most common piece of equipment found in even the most basic of laboratories is the centrifuge (Figure 1). These come in a variety of shapes and sizes and are normally very robust instruments that can be set at varying speeds depending on the type of sample to be processed.

Two types of centrifuge are commonly found within the practice lab:

- The electric centrifuge is used to separate a supernatant from the sediment – most commonly serum or plasma from red blood cells. These can also be used to concentrate sediment, for example, when looking for crystals in a urine sample.
- The microhaematocrit centrifuge is used to separate blood samples that are in special capillary tubes – usually to ascertain a packed cell volume (PCV). Some haematology analysers use a microhaematocrit centrifuge to separate the white blood cells, so an accurate count can be made.

Figure 1: A centrifuge

Most centrifuges are straightforward to use and should always be balanced; in other words, a sample tube of equal size should always be placed opposite the sample. The safety plate should then be fastened down and the lid locked shut. Most centrifuges allow you to set the speed in revolutions per minute (rpm) as well as the length of time that you want to spin the sample.

Table 1 gives you a rough guide as to how long, and at what speed, to spin samples.

The microscope

The microscope is one of the most important instruments in the laboratory. It can be used to examine a wide range of samples, such as blood films to aid the diagnosis of anaemia, or urine samples to look for crystals. It can also be used to look at cytology samples, such as fine needle aspirates (FNAs).

Microscopes vary a great deal in appearance between different manufacturers, but they all have the same basic elements. An in-depth guide to the use of a microscope would take a whole article and such a guide will follow at a later date. However, the following is a brief guide to the correct use of the microscope.

First, the microscope should be placed on a solid, flat surface and at a comfortable height for you to sit and look down the eyepieces. For looking at samples that do not require oil immersion, such as urine and hair:

- Check that the lowest objective lens is in place and that the stage is all the way down. Also check that the rheostat is at its lowest setting.
- Switch on the microscope and adjust the rheostat to roughly half way.
- Place the slide on to the stage and hold it in place using the stage clips.
- Adjust the substage condenser so it is 2-3 mm below the stage.
- Adjust the iris diaphragm so it is half-way open.
- While watching the stage, rack it up until the slide is just below the objective lens.
- Look down the eyepieces and focus on the specimen slide using the course and fine focus. You may also need to move the slide around using directional knobs until the specimen appears in view.

Oil immersion is used to look at specimens, such as blood or cytology samples. The procedure for using oil immersion is very similar to that described above, but a drop of immersion oil is placed on the cover slip or directly on to the fixed sample slide.

The end of the oil immersion lens is lowered into the oil using the fine focus knob and care must be taken not to touch the slide with the lens as this will damage the sample and the lens.

It is also best to open the iris diaphragm completely and have the substage condenser all the way up, as this will allow the most light through and give you a clearer, finer-detailed picture.

Laboratory analysers

The most common types of laboratory analyser are used for clinical biochemistry, haematology and to measure electrolyte levels.

Clinical biochemistry

Biochemistry analysers are used to measure the levels of certain substances in a patient's serum or plasma (Figure 2). They are used to help diagnose certain conditions, monitor therapy or used to provide an 'old age' profile check. Commonly tested 'analytes' include:

- urea
- creatinine
- ALT (alanine aminotransferase)
- ALP (alkaline phosphatase)
- TP (total protein)
- albumin
- glucose
- calcium
- amylase
- lipase.

TABLE 1 A guide to centrifuge speed settings (in rpm)

Type of sample	Speed in rpm	Time
Blood for serum plasma samples	10,000	3-5 minutes
Urine sediment	3,000	3 minutes
PCV	10,000	5 minutes
Fluid cytology samples	3,000	3 minutes

Figure 2: A biochemistry analyser

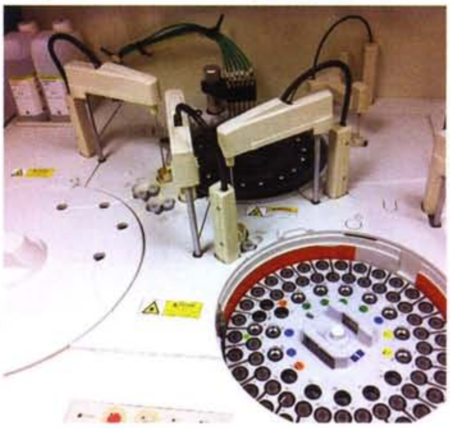

Figure 3: Wet chemistry reagents

There are two types of biochemistry analyser – dry and wet chemistry.

The most commonly used in practice laboratories are dry chemistry systems. These analysers use a small amount of the patient's plasma or serum, which is placed on to a series of slides. A colour change occurs on each slide, which

Figure 4: A haematology analyser

reflects the amount of that substance found in the blood. Most machines then read the colour change and interpret it automatically in order to provide a print-out of the result.

Wet chemistry systems work in a very similar way; but instead of using slides, they use liquid reagents that are placed into cuvettes or wells and mixed with the patient's serum or plasma (Figure 3). The mixture is then incubated at 37°C for varying amounts of time, depending on which reagent is used. For example, in some analysers, the albumin reagent incubates for 30 seconds, whereas lipase incubates for seven minutes.

Once the colour change has taken place, an infrared beam shines through the mixture and this gives the level of the substance being tested.

Whichever system is being used, the results obtained are read against a set of reference ranges for that analyser. It is important to remember that not all analysers are the same and reference ranges will vary.

Part Two of this series will contain a list of the most common biochemical tests and the type of sample required for each one.

I would like to take time here, however, to mention the importance of regular quality-control testing for biochemistry analysers. The most important part of my day in the laboratory is calibrating and quality controlling the analysers. This is done every day – and sometimes twice a day – to ensure the results are as accurate as they can be.

Whilst this would be almost impossible and very expensive for most practices, setting up a practice quality-control procedure – alongside that recommended by the manufacturer – is a good idea.

Sending a sample to an independent laboratory – after you have processed it on your machine, to see if your results match – is an excellent idea. This is called an external quality control and is offered by most laboratories.

Haematology

There are various types of automatic haematology analyser available; but in essence they all do the same job (Figure 4). They are used to measure the numbers of cells which make up whole blood. These cells include:

- white blood cells (WBC) – neutrophils, lymphocytes, monocytes, basophils and eosinophils
- red blood cells (RBC or erythrocytes)
- platelets.

The proportion of red blood cells in whole blood is expressed as the haematocrit or PCV and it is this level that indicates anaemia.

Haematology analysers are also used to monitor the white blood cell numbers, as this can indicate an inflammatory or neoplastic change within the body.

Finally, the platelet count is monitored as this can indicate certain clotting conditions or immune-mediated haemolytic anaemia.

It is also very important that, where possible, a blood film is examined under the microscope alongside the 'automatic' results. Analysers do not check changes within the cell morphology and this can be just as important as the cell count in diagnosing a condition.

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It is also vital to remember that automatic machines can get the platelet count wildly wrong if platelet clumps or giant platelets are present and will, therefore, give a low count. These anomalies can be checked for on a blood film and a manual count can be performed.

Electrolytes

The last type of analyser found in the practice laboratory is the electrolyte machine. Some practices may have a combined electrolyte and biochemistry analyser. Electrolyte analysers measure the patient's serum or plasma levels of:

- sodium (Na)
- potassium (K)
- chloride (Cl).

Analysers work by running the patient sample over the three electrodes sensitive for each electrolyte and this measures their concentration within the blood. Some electrolyte machines will also measure levels of ionised calcium and bicarbonate, but these are not so commonly assessed.

Part Two of this series will explore in greater detail why we test patients' serum, plasma and whole blood using the equipment described in this article.