

# continuing education

## Making the most of laboratory samples

In the second part of our series on effective clinical pathology, PETER WEBB, Managing Director of Axiom Veterinary Laboratories, looks at how to get the best results when sampling a clinical case.

Whether you have an in-practice laboratory or use a referral laboratory, taking samples is unavoidable. And, taking these samples accurately is essential - there is nothing worse than taking samples from a case only to find you have taken the wrong ones. This can lead to an embarrassing situation with the client and a loss of confidence on their part.

There are a number of samples that can routinely be taken to help with diagnosing a condition and, among others, this article will look at blood, urine, and faeces samples, as well as biopsies and fine needle aspirates.

Serum	Derived from the separation of blood cells and fluid after clotting.
Plasma	Derived from the separation of blood cells and fluid from unclotted blood through centrifugation.

Table 1: The main types of blood tubes available

tube	colour	function
serum	red	biochemistry, serology, endocrinology
heparin	green or orange	whole blood: selenium, lead, genetic tests, virus PCR, BVD virus, reptilian/ bird haematology serum: biochemistry, serology, endocrinology
separated serum	brown or speckled yellow	biochemistry, serology
EDTA	purple or pink	mammalian haematology, fluid cytology, fibrinogen
oxalate/fluoride	grey or yellow	glucose only
citrate	pale blue or green	clotting times



Figure 1: Pipette and serum tube.



Figure 2: Two types of heparin tubes

### Choosing the correct blood sample tube

The main tubes that are available for blood sampling are serum tubes, heparin plasma tubes, EDTA tubes, oxalate fluoride tubes and citrate tubes (Table 1).

#### Serum tubes

Classically, serum tubes have a red top but the exact colouration depends upon the manufacturer. These types of tubes contain no anticoagulant thus the blood coagulates within the tube and eventually separates to form serum and a cellular clot. The clot is not required for any analysis and if you are considering posting sera to a referral facility the removal of the serum from the clot using a small pipette is recommended (Figure 1). If the serum and the clot are not separated, the clot will break up during transit and the serum will become haemolysed. This haemolysis can affect certain biochemical measurements and severe haemolysis makes the sample useless. It is always best to send separated serum but it should be noted that there is a time lag between taking a sample and the formation of a decent clot, usually of three to four hours.

#### Heparin tubes

Heparin tubes (Figure 2) traditionally have green or orange tops and contain small amounts of heparin to prevent clotting. The contents of heparinised tubes can be separated through the use of a bench top centrifuge into plasma and red cells. Between the plasma and the



Figure 3a (above) sodium citrate and 3b (below) separated serum tube.



Figure 5: Oxalate/fluoride tubes.



Figure 4: EDTA tubes.

red cells is a small area termed the Buffy coat which is rich in white cells. Heparinised plasma can be used for serology, biochemistry and endocrinology in a similar way to serum. For some parameters, however, there will be minor differences in the values obtained from serum and plasma. Unseparated heparin blood is the sample of choice for red cell enzymes such as glutathione peroxidase (selenium), lead, virus PCR and genetic analysis. Whole blood heparin samples are also the sample of choice where analysis is performed on the Buffy coat, e.g. identification of BVD antigen, and can be used for haematology to give a reasonably accurate total red and white cell count. In haematology, however, the blood cells stain poorly and performing a differential on a heparinised sample can be problematic and the results obtained inaccurate. Heparin blood samples are not recommended for routine mammalian haematology, but they can be used in an emergency. In some non-mammalian species whole heparin samples are the sample of choice. If in doubt, call your referral laboratory to clarify.

#### Sodium citrate tubes

Sodium citrate tubes (Figure 3a) have light blue or green tops and are used exclusively for coagulation studies (clotting times, clotting factors etc.). They have a small mark on their side and it is important that tubes are filled to this mark so that the correct citrate/blood ratio is calculated. Incorrect ratios can lead to incorrect clotting times.

#### Separated serum tubes

Separated serum tubes (Figure 3b) are used exclusively in human medicine but less so in veterinary medicine. They contain no anticoagulant but have a small gel that, on centrifugation, forms a barrier between the red cells and the plasma. The advantage of this over normal serum tube sampling is that the gel prevents haemolysis of the serum and the serum does not have to be pipetted into a separate tube. However, the tubes are ineffective unless they are centrifuged in-practice. Serum obtained from serum separated gel tubes has similar properties to that of serum obtained from non-gel separated tubes, but there are concerns about the measurement of some endocrine parameters and drug concentrations on serum separated tubes. For example, they are not recommended for measuring barbiturates levels.

#### EDTA tubes

EDTA tubes (Figure 4), commonly with purple or light pink tops, contain the anticoagulant EDTA. They are only suitable for routine mammalian haematology and cytology and fibrinogen. EDTA plasma can be used for some emergency biochemistry and serology analyses but are unsuitable for many others. There is a small mark on the side of the tube and it is important that the tube is filled to this mark. When taking a sample for haematology, it is recommended to send an unstained blood smear on a slide as well. Blood cells are still living at the time of sampling but gradually die and, over time, produce degenerate changes. A smear made at the time of sampling helps overcome these problems and allows the pathologists to study cell morphology in greater detail.

#### Oxalate/fluoride tubes

Oxalate or fluoride (Figure 5) samples have grey or yellow tops and are used exclusively for the measurement of blood glucose. Oxalate and fluorides are biochemical poisons which stop red cells from metabolising glucose after a blood sample is taken. They are of use where the practice does not possess a bench top centrifuge for separation of serum and red cells, and an accurate blood glucose analysis is required. However, if the practice has a bench top centrifuge and the sample can be separated within one hour of sampling, heparinised plasma is perfectly adequate for measuring glucose.

## Sampling for microbiology

### Bacteriology

When it comes to bacteriology, plain dry swabs have limited usefulness and can sometimes yield disappointing results. Thus, transport swabs are the recommended choice for microbiological sampling. Transport swabs are either thick tipped or fine tipped. The fine tipped versions are useful for small sinuses and nasal cavities (particularly for identification of MRSA carriage in man). They are not used routinely but are available from most referral laboratories on request. In addition, they come with two different media: a clear or Stewart's media and a black or charcoal media. The former will support both aerobes and anaerobes whilst the latter only supports aerobes. Generally, the sample of choice for microbiology is a transport swab containing charcoal media (**Figure 6**).



**Figure 6:** Transport swabs.



**Figure 7:** Blood culture bottles.

Some organisms, particularly mycobacteria, certain fungi and other meticulous organisms, are best grown from fresh material. This material should be biopsied, then placed in a sterile universal pot on top of a small piece of gauze dampened with normal saline. In tissues where few bacteria are present (e.g. CSF, joints and suspected bacteraemias), it is recommended that fluids and/or whole blood are inoculated straight into broth-containing blood culture bottles (**Figure 7**) and pre-incubated at 37°C before sending to the referral laboratory. In these cases it is best to contact the referral laboratory prior to sampling.

### Viral culture

Viral swabs have a green tip and contain a specialised transport media for the preservation of viruses. Sometimes they are not very effective in veterinary medicine, and special viral transport media is usually available from the referring laboratory. Bacterial transport swabs are no use for viral cultures.

### Other samples

Hair samples and skin scrapings should be placed in a small universal container with a screw top. Unguarded blades should not be sent through the post. Sometimes it is better to put smears from skin scrapes on to slides and send the slides. Hair for ringworm should not be sent in plastic or standard envelopes as retrieval of the sample at the recipient laboratory can be dangerous to the personnel and, sometimes, a fruitless exercise due to static electricity.

Urine samples should be collected in a 20ml sterile universal container; those taken by cystocentesis or catheter give the best results. Free-flow samples are likely to be contaminated by commensal bacteria of the vulva and lower urogenital tract and must be interpreted accordingly.

Fresh faeces should be collected into a sterile universal 20 ml pot with a screw lid. Aged faeces, those usually brought in by the client, are unlikely to be of use.

### Histology

Samples for histology should be placed immediately into buffered formal saline to preserve cell architecture. This should be available from your referral laboratory. It is important that lumps sent should not be too large - for adequate fixation, the ratio of formalin to the size of lump should be 10:1. Thus a 100g piece of tissue would require 1 litre of formalin! As it is expensive and dangerous to send large volumes of formalin through the post, and it is likely that large lumps will be fixed inadequately, lumps should be trimmed in size. Several smaller pieces of tissue are much better than one large piece. Samples from the periphery of the tissue, especially at the junction of normal to abnormal tissue, offer more information than central core pieces as the latter often show tissue necrosis and/or degenerative changes. Very small pieces of tissue, such as small intestine or skin biopsies, should be enclosed within special cassettes, available from referral laboratories on request. Skin samples are best taken using a 5-10mm punch and, once again, multiple samples will yield more information than a single sample.

**Cytology**

**Fine needle aspirates**

Fine needle aspirates should be sent as smears on slides. Fluid should be split between a sterile universal pot for culture and an EDTA blood tube/formalin tube for cytological examination. Your referral lab will dictate whether it prefers EDTA or formalin as a cell preservative - this will depend on the individual cytologist's preference. Smears from fine needle aspirates and blood smears should be sent to the referral lab within a slide box.

**Bone marrow samples**

Core biopsies should be placed in formalin but fine needle aspirates should be smeared onto a glass slide. It is important to make as many smears as possible, as up to 75% of smears may be inadequate for examination. Slides should be sent unstained and a peripheral blood sample in EDTA should accompany them.

**General tips**

Having taken the precious samples from a case, it is important that they are analysed quickly in-house or sent safely and rapidly to a referral laboratory. Samples are best kept refrigerated until they are sent and some endocrine examinations require the sample to be kept cool/frozen during transmission to the lab. Do not leave samples for hours behind car windscreens, directly in the sun or in metal post boxes. It is also important to package the samples appropriately. This helps prevent damage to the samples and inadvertent exposure of

couriers or postal personnel to biological hazards. Packaging should conform to national standards - 'Jiffy' bags are not suitable (Figure 9).



Figure 9: A wide range of packaging is available from referral labs.

For more information on taking and transporting pathology samples, contact Axiom Veterinary Laboratories. Email: [admin@axiomvetlab.co.uk](mailto:admin@axiomvetlab.co.uk), telephone: 1850 946 912.

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