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Our Packaging and How to use it

Histopathology

Each box that you receive contains four or five separate packages together with loose submission forms bearing your practice address label. The packaging is compliant with the definition given to the BVA by the Royal Mail *(Veterinary Record, March 13 1999,* ***144****, 274).*

Ideal sample size is approximately 2.5cm x 1.5cm and less than 1.0cm thick. Volume of fixative should be at least 10x that of the tissue. If larger samples are to be submitted whole, they should be fixed in an appropriate volume of formalin in a large jar or bucket prior to submission. They can then be sent in a normal pot or wrapped in formalin-soaked tissue and sealed in polythene

Please follow the following procedure outlined below:

Rest Associates

1. Write the **owner and animal names** on the inner container label.
2. Place sample in formalin container and tighten lid securely
3. If more than one container is being submitted per case, please write site/s on the label.
4. Place the specimen container inside the large outer mailer (Cotton wool has been added in case of leakage) Screw the lid on tightly.
5. Complete the submission form with the relevant history.
6. Please write or stamp the practice name and address on the reverse of the postage-paid envelope.
7. Place the specimen and submission form in envelope and seal.

Finn Pathologists

1. Dilute the formalin with tap water to the 'thread line' of the pot.
2. Tighten the lid securely after adding the sample.
3. Please write the **owner and animal names** on the pot label.
4. If more than one pot is being submitted per case, ensure that the site of the sample is also marked on the pot.
5. Place the pot in the plastic bag with the vermiculite (absorbent granules).
6. Place the sealed bag and a completed submission form in the cardboard box and tuck in the box lid.
7. Write or stamp the **practice name and address** on the reverse of the padded envelope.
8. Seal the box inside the padded postage-paid envelope.

Blood Samples

Always remove the needle from the syringe prior to filling blood tubes.

Serum Gel Tubes

1. Slowly fill the gel tube (after removing the needle).
2. Allow the sample to clot by letting it stand for 30 minutes.
3. Centrifuge at high speed for 10 minutes. This results in the gel forming a barrier between the cells and the serum.
4. Check the position of the gel plug and the serum colour visually. If it appears haemolysed, indicating damage to red blood cells, then a repeat sample should be taken before submitting it to the laboratory.

Plain Tubes can be used when the practice does not have a centrifuge. Allow the sample to clot for 30 minutes. Separate the serum by decanting into a fresh plain tube. Label with patient/client details. As before, if serum appears haemolysed, please repeat the sample.

EDTA Tubes are required for haematology. Fill the tubes to the mark (under filling results in artefact and over filling predisposes to clotting). Remember to mix the blood by slowly inverting the sealed tube several times or by gently rolling between the hands. Please also submit 2 fresh, unstained, air-dried smears to preserve cell morphology.

Fluoride Tubes when measurement of glucose is required.

Sample Submission

1. Please write the **owner and animal names** on all of the blood tubes.
2. Complete the submission form with the relevant history and tests required.
3. Place all blood tubes inside the large polypropylene transport tube provided. Cotton wool can also be placed inside the tube to absorb any leakage.
4. Screw the lid on tightly and place in the postage-paid padded envelope, with the submission form, making sure that the **practice name and address** are on the reverse side.

Swabs and Urine tubes

Sample Submission

1. Please write the **owner and animal names** on all swabs and tubes.
2. Complete the submission form with the relevant history and tests required
3. Secure the lids tightly.
4. Place the tubes in absorbent material and then place in a sealed biohazard plastic bag.
5. Place in the postage-paid padded envelope making sure that the **practice name and address** are on the reverse side.

Clinical Pathology Protocols

Chemistry tests

Bile acid stimulation test

The bile acid stimulation test is used to evaluate hepatic function, and is used in the diagnosis of portosystemic shunts. Bile acids are synthesised in the liver, excreted in the bile, reabsorbed in the distal small intestine, and removed from the portal circulation by the liver. Basal bile acid levels in the peripheral circulation therefore reflect the overspill from this enterohepatic circulation. Ingestion of a fatty meal, together with the action of swallowing, stimulate gall bladder contraction and a surge of bile acids reaching the intestine and returning to the liver, and spilling over into the peripheral circulation. Thus, post-prandial levels are slightly higher than basal levels in normal animals. Post-prandial bile acids are elevated beyond normal where liver function is reduced or where there is portosystemic shunting (this can reflect alterations in enterohepatic function secondary to chronic disease sufficient to be detected histologically).

Limitations:

- The bile acid stimulation test cannot distinguish between pathological processes in the liver, and cannot provide assessment as to their reversibility.
- Failure to achieve elevations in the post-prandial sample can be seen where there has been insufficient food intake, malabsorption, premature contraction of the gall bladder prior to starting the test, failure to stimulate gall bladder emptying (individual variation), or bacterial intestinal metabolism.

Protocol:

1. Starve the patient for 12 hours.
2. Collect 1-2 ml blood into a serum gel or plain tube. Label the tube with patient details and time of sampling.
3. Feed a high fat meal – suggested meals include puppy/kitten food, Hill's a/d, n/d or p/d. The addition of oil is not usually necessary and reduces palatability.
4. Collect a second sample 2 hours post feeding, into a serum gel or plain tube. Label the tube with patient details and time of sampling.

Diabetes mellitus diagnosis and monitoring

A marked **hyperglycaemia** at a single point in time can be sufficient evidence of diabetes mellitus, if there are other compatible clinical signs (such as polyuria and polydipsia), and there is no evidence of other disease. However, severe acute stressful illness can cause a marked hyperglycaemia to levels that overlap with diabetic patients. **Glucosuria** is also most often associated with diabetes mellitus. But it also can be seen with other conditions causing hyperglycaemia, and with renal tubular disorders such as the Fanconi syndrome. Consequently, hyperglycaemia and glucosuria alone do not definitively diagnose diabetes mellitus.

Fructosamine represents serum proteins (particularly albumin) strongly bound to glucose because of persistent exposure over the lifetime of the proteins. A high fructosamine reflects persistent hyperglycaemia over a period of 1 to 3 weeks. This is much better evidence of diabetes mellitus. However, it will not detect very recent onset of diabetes mellitus, and can be low in hypoproteinaemic patients.

Fructosamine is also very useful for **monitoring** diabetes mellitus, as it avoids the problems of stress induced transient hyperglycaemia at the time of sampling that could give a false impression of poor control of the condition. It also gets around the problems of collecting and testing urine samples.

Protocol:

Collect 1-2 ml blood into a serum gel tube or plain tube. Label the tube with the patient details. The timing of the sample is not critical for this assay as it is assessing average glucose levels over a period of time. However, a fasted sample is preferable, and the sample should be separated (or a gel tube spun) as lipaemia predisposes samples to haemolysis that can have a significant effect on the result.

Therapeutic Drug monitoring

Phenobarbitone

Phenobarbitone levels stabilise in the serum within 7 to 14 days of starting or changing therapy. The timing of the sample will depend on the aim of sampling. Peak levels (4-6 hours post pill) are used for general monitoring and for checking that toxic levels are not being reached. Average levels occur approximately 8 hours post pill. Trough levels are measured (just before a pill is due) when peak levels appear adequate but clinical signs do not appear to be controlled. This can occur if the drug is being metabolised rapidly and the plasma levels are varying too widely. Phenobarbitone can induce the production of liver enzymes in dogs but not in cats. It can also cause hepatic damage. Monitoring hepatic function during therapy is recommended (ideally with a bile acid stimulation test). Serum gel tubes should not be used for the phenobarbitone assay.

Potassium bromide

KBr levels do not stabilise until the patient has been on therapy for at least 2 to 3 months, by which time plasma levels do not vary much during the day. Most patients are also receiving phenobarbitone. The sample taken for monitoring phenobarbitone levels is acceptable for monitoring KBr levels.

Digoxin

The therapeutic range for digoxin is narrow, and toxicity occurs at levels barely above the therapeutic range. Serum

levels tend to stabilise 5 to 7 days after starting or changing therapy, and close monitoring is recommended. Peak levels occur at 4-6 hours post pill, with trough levels just before a pill is due.

Endocrinology

Hypothyroidism in dogs

Canine hypothyroidism is notoriously difficult to diagnose definitively. Total T4 within the normal range almost excludes hypothyroidism. However, it can be depressed by many non-thyroidal illnesses, drug therapies, and anaesthesia, as well as hypothyroidism. Because of the negative feedback loop, low T4 should trigger an elevation in cTSH production from the pituitary gland. This can also be elevated by some non-thyroidal illnesses (in particular Cushing's syndrome), and is not always elevated in hypothyroid dogs. Free T4 is the active fraction of T4, and is less affected by non-thyroidal illness. Testing for free T4 should be by equilibrium dialysis as other methods are not sufficiently accurate for diagnosis. Approximately 50% of hypothyroid dogs are immune mediated, and these patients may have detectable levels of anti-thyroglobulin antibodies. Anti-T4 antibodies interfere with the T4 assay causing results to be higher than the true value. Dynamic testing with TRH or TSH (when available) theoretically improves the accuracy of diagnosis, but many patients fall within the equivocal bracket.

Protocol:

Basic screening: Total **T4 and cTSH together** on a serum sample.

Further testing where results are equivocal: Free T4 by equilibrium dialysis, anti-thyroglobulin antibodies, and anti-T4 antibodies.

Dynamic testing: TRH and TSH stimulation tests – protocols available on request.

Monitoring treatment for hypothyroidism

The recommended initial treatment for hypothyroidism is the administration of levothyroxine (Soloxine, Arnolds) at a dose of 11-22 mg/kg twice daily. Treatment may be reduced to once daily following resolution of the clinical signs. Some patients appear to require continued twice daily therapy to control clinical signs.

The total T4 concentration should be monitored regularly during treatment, particularly during the initial stages. Measurement of cTSH may also be helpful when monitoring thyroid therapy in dogs as it may reflect the adequacy of the therapy in the preceding days. The total T4 concentration only provides information pertaining to the day of sampling. However, normal/low cTSH levels do not indicate that therapy is adequate, as patients do not necessarily have an elevated cTSH level when T4 levels are inadequate.

Protocol:

Samples for monitoring treatment should be taken **4 to 6 hours post pill**, at which point the concentration should be in the upper half of the reference interval or slightly higher. Where peak levels are adequate, a trough level is also recommended (just before a pill is due), and this should be at similar levels to peak samples. Serum samples are preferred, although heparinised plasma may also be used.

Feline thyroid disease

Total T4 is the screening test of choice for assessing thyroid function in cats. T4 fluctuates during the day, and some hyperthyroid cats have only marginally elevated T4 at the time of sampling. As in dogs, T4 can be depressed by concurrent non-thyroidal illness (chronic renal insufficiency is common, but almost any disease is a possibility). Hyperthyroid cats with concurrent disease may have T4 levels in the upper half of the reference interval. Measurement of free T4 (by equilibrium dialysis) can be of use where total T4 is within the reference range but clinical signs are strongly suggestive of hyperthyroidism. Care should be taken in its interpretation, as it can be elevated by non-thyroidal illness. Dynamic testing can also be useful in cats, with the T3 suppression test or a TRH stimulation test. The latter frequently causes nausea and vomiting and is not recommended.

T3 suppression test

In healthy individuals, T3 has a suppressive effect on pituitary TSH secretion and subsequently on T4 production by the thyroid gland. This suppressive effect is lost in hyperthyroidism due to the autonomous production of thyroid hormones and chronic suppression of TSH. The serum concentrations of total T4 therefore show minimal decrease in hyperthyroid cats following T3 administration. However, concurrent measurement of T3 is advised to ensure adequate administration and absorption of the T3, avoiding false positive results.

Protocol:

1. Collect a baseline serum sample for total T4 and T3. Label the sample with patient details and time and date of sampling. Store the serum frozen until the protocol has been completed and submit together with the post-Tertroxin sample.
2. Administer Tertroxin orally every 8 hours for 7 doses.

   Cats <5kg: 20 µg of Tertroxin
   Cats >5kg: 30 µg of Tertroxin
3. Collect a serum sample 2-6 hours after the last dose. Label with patient details and time and date of sampling.
4. Submit the samples for measurement of total T4 and T3.

Monitoring treatment for hyperthyroidism in cats

T4 should be measured, with the aim of obtaining levels in the lower half of the reference interval. With regular tablet giving, the serum levels should not vary much, so the timing of the sample is not critical. However, if therapy is interrupted for 24-48 hours, T4 levels will rise significantly.

Canine hyperadrenocorticism

Spontaneous hyperadrenocorticism can develop as a result of a pituitary tumour (usually an adenoma) producing excessive ACTH that stimulates excessive production of glucocorticoids from the adrenal medulla, or a productive adrenal tumour. Approximately 80% of canine Cushing's cases are pituitary dependent. A single cortisol level is not adequate for diagnosis as it is often within normal limits in cushingoid dogs and can be elevated significantly during stress. Iatrogenic hyperadrenocorticism can also be seen, even when patients are on apparently low levels of glucocorticoid therapy. Glucocorticoid therapy (by any route including topical preparations) in the month prior to testing can interfere with adrenal function. Four tests are commonly used:

Urine Cortisol:Creatinine ratio

This test is carried out on a plain urine sample, preferably collected at home to avoid stress during sampling. Cortisol is excreted by the kidneys as well as being removed by hepatocytes. Urine creatinine is used as a comparator as renal excretion is assumed to be constant. A normal result excludes hyperadrenocorticism. A positive result can reflect hyperadrenocorticism, but can also be seen in many stressful illnesses. A positive result should also be confirmed with a dynamic test (LDDS or ACTH stimulation tests).

LDDS test

This test is more sensitive than the ACTH stimulation test, and can in many cases distinguish between adrenal dependent and pituitary dependent disease, but it has some **limitations**:

- The LDDST is not suitable for the detection of iatrogenic hyperadrenocorticism.
- The LDDST is not suitable for monitoring animals on treatment for hyperadrenocorticism.
- False positive results may occur, especially in animals with significant non-adrenal illness, such as diabetes mellitus or chronic renal insufficiency.
- It is very important to avoid any stress to the animal during the test period as far as possible as this may interfere with the results. Stress may cause animals without hyperadrenocorticism to break the suppressive effect of the dexamethasone.

Protocol:

1. Collect a baseline serum sample (1-2ml). Label all tubes with patient details and the times of the samples.
2. Inject 0.01mg/kg dexamethasone intravenously.
3. Collect a second serum sample 3 hours post-dexamethasone.
4. Collect a third serum sample 8 hours post-dexamethasone.

ACTH stimulation test

This test is less sensitive than the LDDST, but less prone to false positive results. It is used in the following situations:

- To detect hyperadrenocorticism in cases where there is stress or concurrent non-adrenal illness.
- To distinguish spontaneous hyperadrenocorticism from iatrogenic disease.
- To monitor patients on therapy for hyperadrenocorticism.

It also has **limitations**:

- It does not allow differentiation between adrenal dependent and pituitary dependent disease. This can be done with a high dose dexamethasone suppression test. This test should only be carried out once a diagnosis of hyperadrenocorticism has been reached. The protocol is as for the LDDST, but using 0.1mg/kg dexamethasone.
- It may not be positive in cases of adrenal dependent hyperadrenocorticism.
- False positives may occur with chronic stressful illness such as diabetes mellitus. However, they occur less commonly than with the LDDST, and the ACTH stimulation test is the test of choice in these cases, preferably once the patient is stabilised.
- False positive results can be seen in some cases of severe gastrointestinal disease where the test may be used to investigate possible Addison's.

Protocol:

1. Collect a baseline serum sample (1-2ml). Label the sample with patient details and the time of sampling.
2. Inject 0.25mg synthetic ACTH (Synacthen) intravenously.
3. Collect a second sample 60 to 120 minutes later. Label the sample with patient details and the time of sampling.
4. Submit samples together for cortisol assays.

SHAP (sex hormone alopecia profile)

Some patients that are clinically cushingoid fail to provide positive results with a LDDST or an ACTH stimulation test. If other differentials are excluded, the SHAP test can be useful. This is based on an ACTH stimulation test, with samples taken at 0 and 60 minutes, and tested for **17-hydroxyprogesterone** as well as cortisol.

Monitoring of therapy for canine hyperadrenocorticism

The test of choice is the ACTH stimulation test (see protocol above). For patients on trilostane (Vetoryl), the timing of the test relative to the most recent tablet is critical for interpretation of the results. The test should be started 4-6 hours post-pill. If the results at this time suggest adequate control, but the patient appears out of control, a test carried out just before the next dose is due is indicated. Some patients require twice daily therapy. Patients on mitotane (Lysodren) can be tested at any time of day.

Addison's disease

In hypoadrenocorticism, the adrenal glands are incapable of responding to ACTH, and cortisol levels remain low in the basal and post-ACTH sample. The **ACTH stimulation test** is therefore the test of choice for diagnosis. The LDDST is not appropriate in these cases, as adrenal cortisol production cannot be suppressed any further than the basal level. The **limitations** include:

- Patients receiving glucocorticoid therapy will have results resembling Addison's cases. Glucocorticoid therapy can suppress adrenal function for up to a month after administration.
- Patients receiving Tardak (delmadinone acetate) can be suppressed for at least 3 months after administration.

Monitoring Addison's patients on therapy

Once a diagnosis has been obtained and therapy is in progress, adrenal function will be further suppressed by the therapy. An ACTH stimulation test will not provide useful information. These cases should be monitored on the basis of electrolyte levels and clinical signs.

Feline hyperadrenocorticism

This is a generally considered a rare condition, with testing most often carried out in diabetic patients not responding to therapy. Interpretation of test results in these cases is complicated by the concurrent chronic stress of the diabetes. Both an ACTH stimulation test and a dexamethasone suppression test can be carried out, but the protocols are different from those in the dog. Both tests have high false positive rates, and should be interpreted carefully.

ACTH stimulation test protocol:

1. Obtain a basal serum sample, label with patient details and time of sampling.
2. Administer 0.125mg synthetic ACTH (Synacthen) intravenously. Intramuscular injections can be used, but injection between muscle bellies in the fascial planes prevents absorption.
3. Collect second and third serum samples at 60 and 120 minutes after the Synacthen injection. Label the samples with patient details and the times of sampling.
4. Submit the three samples for cortisol assays.

Dexamethasone suppression test protocol:

1. Obtain a basal serum sample, label with patient details and time of sampling.
2. Administer dexamethasone intravenously at a dose rate of **0.1mg/kg.**
3. Collect second, third and fourth serum samples at 4, 6 and 8 hours after the dexamethasone injection. Label the samples with patient details and the times of sampling.
4. Submit the four samples for cortisol assays.

Cryptorchid/testicular function

Basal Testosterone:

Testosterone fluctuates during the day. A basal sample can demonstrate the presence of testicular tissue. In most cases where there is no testicular tissue present, testosterone levels are undetectable. For cases where an equivocal result is obtained, or where an absolute diagnosis is essential, the hCG stimulation test is recommended. Basal testosterone should be measured in a serum sample.

HCG stimulation test:

1. Collect a baseline blood sample (serum). Label with patient details and time of sampling.
2. Inject hCG intravenously (Chorulon, Intervet) according to species – see the data sheet.
3. Collect a second serum sample 30-120 minutes later, and label with patient details and time of sampling.
4. Submit samples for testosterone assays.

Detection of ovarian tissue

This test may be carried out in bitches and queens that have been spayed but still display oestrus behaviour. The aim is to detect ovarian tissue remaining after surgery.

Protocol:

1. The test should be started during behavioural oestrus (preferably within the first 3 days).
2. Collect a basal serum sample. Label it with patient details and date of sampling, and store refrigerated or frozen.
3. Inject hCG (Chorulon, Intervet) intramuscularly at a dose rate of 44IU/kg.
4. Collect a second sample 7 to 14 days later, labelled with patient details and date of sampling.
5. Submit the samples together for progesterone assay.

Sample(s) for microbiology:

The following samples should be submitted for microbiology. If in doubt please feel free to contact our microbiology laboratory who will be happy to assist you:

- **Bacterial culture**
  Aimes charcoal transport media swabs.

  Fresh tissue in a sterile container with no more than 1-2 drops of sterile saline solution.

  Any non-blood fluids in a plain sterile container (not EDTA). Please do not add formalin to samples submitted for bacteriology.
- **Fungal culture**
  Scrapings of skin plus hair in a sterile container.

  Fresh tissue in a sterile container with a maximum of 1-2 drops of sterile saline solution.
- **Faecal analysis**
  2-5 grams of fresh faeces in a sterile container.
- **Urine analysis**
  2-5ml of urine in a sterile container.
- **Parasitology**
  Scrapings of skin plus hair.
- **Blood culture**
  Specific blood culture bottles are available from the laboratory, free of charge, on request.

2. Avoid the following:

- Whilst boric acid is useful for preserving samples for bacteriology, it is not suitable for many of the tests carried out in the urinalysis. Where samples are for urinalysis only, please send them in plain sterile containers. Urine samples for culture should be sent in Boric Acid.
- If submitting fresh tissue for culture please ensure that only 1-2 drops of sterile saline solution is added to keep the sample moist during transit. Please DO NOT send the sample 'swimming' in saline.
- Tissue samples from multiple areas should be sent in separately labelled containers to avoid cross contamination.
- When submitting blood culture bottles, the patient should have had no antibiotics for at least 48 hours (preferably 72 hours) before sampling.

3. Completing the submission form

A succinct and relevant clinical history should be provided for each case being submitted for microbiology. Please include the following information when completing the submission form:

- Client and animal names
- Species, breed, sex, neutered or not, age
- Date the sample was taken
- Site/area sampled
- Brief description of the affected area
- Details of underlying systemic disease
- Recent medication
- Any previous tests carried out and the previous laboratory code if appropriate

Σ

Cytology Sampling Techniques

Bone Marrow

Aims, advantages, and limitations

The main aim of bone marrow aspiration is to assess the cell populations in the bone marrow, and particularly the haematopoietic system. Good smears of bone marrow particles are required to obtain maximum information.

Cytology of bone marrow aspirates allows a more detailed evaluation of bone marrow function than core biopsy, and an approximate assessment of cellularity. However, biopsy of the bone marrow provides a more accurate assessment of cellularity and better information as to other pathological processes occurring in the bone marrow, such as myelofibrosis or myelophthisis. Where a full blood count suggests bone marrow evaluation is indicated, an aspirate should be the first technique used. If the cellularity is particularly low, a biopsy can then provide additional information.

Conditions where a bone marrow aspirate could be useful include peripheral blood abnormalities not explained by peripheral processes, staging of neoplastic disease, some infectious diseases (e.g. Lieshmania), persistent hypercalcaemia, or pyrexia of unknown origin.

The procedure rarely poses a risk to the patient, even where there is severe thrombocytopenia. However, the risk of sedation or anaesthesia should be considered.

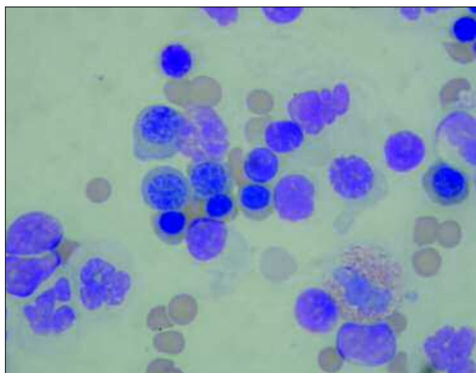


Figure 1.

Myelodysplasia in feline peripheral blood

Preparation

Bone marrow clots very rapidly after sampling. Consequently, it is essential that all equipment is prepared prior to sampling. Several clean glass slides are prepared, placed at an angle of approximately 45° draining onto an absorbent surface. EDTA tubes should also be available. A small surgical kit is required, along with a bone marrow needle; suitable needles include the Rosenthal, Illinois sternal, Salah, or Jamshidi.

Aspiration technique

The ideal sites for bone marrow specimens include the iliac crest, trochanteric fossa and proximal humerus (anterior side) in small animals, and the sternum or rib (dorsal end) in large animals. The patient is gently restrained with light sedation if possible (although cats may require general anaesthesia). The area over the sample site is clipped and prepared for a sterile procedure. Local anaesthesia is infiltrated into the skin and periosteum. A stab incision is made into the skin. The biopsy needle is inserted and, with firm pressure and a clockwise-counterclockwise motion, inserted through the cortical bone. The stylet is removed and a syringe applied (2-20ml depending on personal preference). Negative pressure is applied to the syringe until blood appears in the tip of the syringe. Up to 0.5ml fluid is collected.

For bone marrow biopsy, once the needle is through the cortical bone, the stylet is removed, and the needle advanced by twisting in one direction only.

For both biopsies and aspirates, the needle and syringe are withdrawn as a unit and a skin suture can be placed to close the stab incision if required. Both samples can be taken at the same time if wanted.

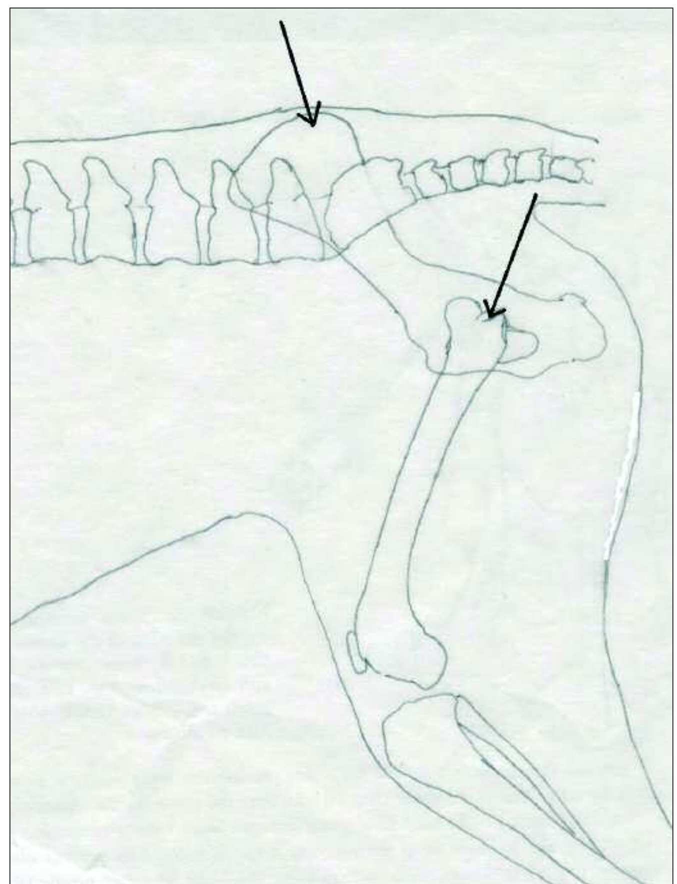


Figure 2.

Sites for bone marrow aspiration

Smear and sample preparation

Several air-dried smears are prepared within seconds of collection. Larger volumes of fluid can be preserved in EDTA, but cell preservation is not as good as with fresh air-dried smears. Even with EDTA, smears should still be prepared within 1-2 hours.

A drop of the aspirated marrow is expelled gently onto the angled slide, where the fluid is allowed to drain off, leaving the marrow particles adhering to the slide. The particles are then smeared by placing a second slide over the sample, clinging by capillary action only, with no pressure, or only slight finger pressure if particles are very thick, and pulling the slides apart gently.

If the sample has been placed into EDTA, the fluid can be emptied into a Petri dish. The particles should be visible as grey flecks that can be picked up with a capillary tube or pipette and transferred to a slide and smeared as before.

The smears should be rapidly air-dried, using a low setting on a hair dryer if necessary.

Bone marrow core biopsy samples are pushed from the needle in a retrograde direction (i.e. from the point of the needle towards the hub) with the stylet or a needle and placed directly into 10% buffered formalin.

Please keep fluids and air-dried smears well away from formalin and its fumes as this significantly affects stain uptake, leading to unreadable slides. It can be helpful to submit them in separate packages to avoid fumes affecting the smears in transit to the laboratory.

Sample preservation and submission to the laboratory

For submission to the laboratory, the practitioner should include a complete history with previous test results, details of any therapy, details of the technique used, and a peripheral blood sample (EDTA and air-dried smear) for haematology to be run at the time of the sample. This is essential for interpretation of the bone marrow smear examination. The smears should be labelled with the patient name and date.

Fine Needle Aspiration (FNA) and Impression Smears

Aims, advantages, and limitations

Cytology of solid lesions is recommended where differentiation between inflammation and neoplasia is wanted. Further classification into type of inflammation, tissue of origin and degree of malignancy of tumours, and involvement of infectious agents is also made where possible.

Cytological sampling is also recommended where a surgical biopsy may pose a significant risk to the patient, or where information from cytology will help to guide the surgeon with respect to the surgical procedure required (e.g. wide margins in the case of mast cell tumours). FNA and impression smears have the benefit of being easy and less invasive than surgical interventions, rarely requiring sedation. Impression smears of incised lesions can add to histological information.

Poor cell exfoliation can limit cytology, particularly in mesenchymal tumours, and where loss of tissue architecture can affect the results (particularly important in feline lymphoma). Impression smears may only allow evaluation of the superficial cell types, often inflammatory in ulcerated lesions.

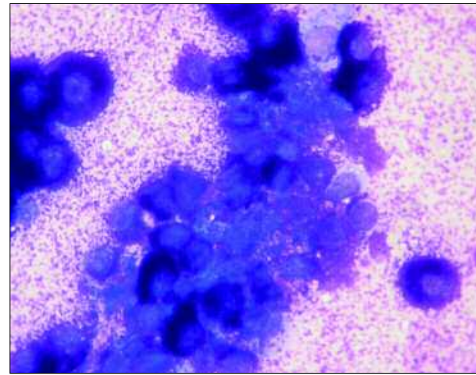


Figure 3.

Mast cell tumour fine needle aspirate

Preparation

Equipment requirements include several needles and syringes, and clean dry microscope slides. For FNA, the skin overlying the lesion can be clipped and prepared for a sterile procedure, although this is not always necessary. For impression smears of superficial lesions, it is useful to remove scabs and scrape away the surface cells to reach more representative cells. If there is oozing of blood from the lesion, it can be gently dried with a gauze swab

Aspiration technique

i) Aspiration

A fine needle (22-25 gauge) is attached to a new, clean, dry syringe (3-20ml, most commonly 10ml). The length of the chosen needle should be suitable for the size of the mass to be aspirated. The mass is then held immobilised with one hand, preferably holding it away from other tissues and blood vessels. The needle is inserted gently into the mass and the plunger of the syringe drawn back to create negative pressure. Ideally, this negative pressure should be held while the needle is redirected within the mass to different directions and depths. The negative pressure is completely released before withdrawal of the needle from the mass.

If blood is obtained, the needle should be withdrawn and a repeat sample taken with a fresh needle and syringe.

ii) Non-aspiration needle sampling

Some cell types are fragile and tend to rupture when they are aspirated with a syringe. Other lesions tend to be highly vascular and aspirated samples can be excessively haemodiluted. In these cases, a non-aspiration technique can help to retain cell morphology. Such lesions include lipomas, lymphoma, liver and spleen samples. In these cases, a needle is used without an attached syringe, inserted into the mass and redirected several times, with some twisting of the needle to use its cutting edge within the lesion.

Smear and sample preparation

i) FNA samples

The syringe is disconnected from the needle (if used) and filled with air, then reconnected to the needle. The sample is pushed onto the slide for smear preparation. Several smears should be made from each mass. Different smearing techniques are appropriate for different sample types:

- **Squash technique:**
  a second slide is placed flat on top of the sample allowing the sample to spread between the slides by capillary action, the slides are slid apart (not lifted off), either perpendicular or parallel – the most likely to be used in most cases. See figures 4 and 5.

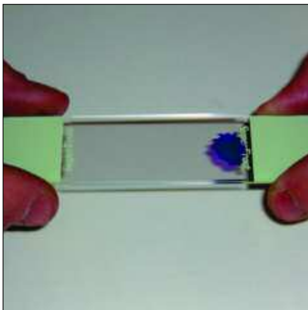


Figure 4.

Allow slides to cling by capillary action.

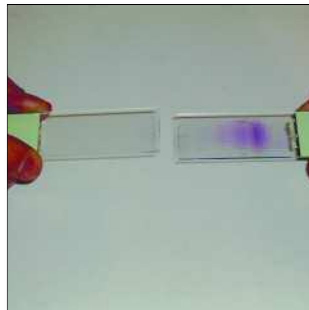


Figure 5.

Pull slides apart gently to spread the cells.

- **Blood smear technique:**
  a drop of sample is placed on one end of a slide, a second slide is placed at an angle into the drop drawing fluid into a line, then pushed away leaving a feathered edge – useful for more liquid samples. Cell morphology is better preserved. See figures 6 and 7.

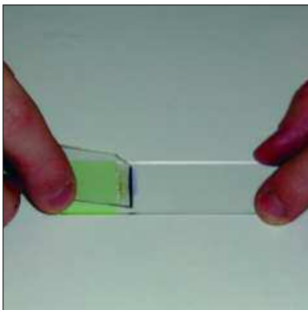


Figure 6.

Allow capillary action to spread the droplet.

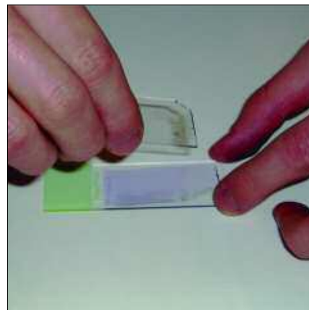


Figure 7.

Spread gently to give a feathered edge.

- **Starfish smear:**
  the sample is placed in the middle of a slide and spread with the tip of a needle into a starfish pattern – useful for small dry samples but more likely to cause cell damage.

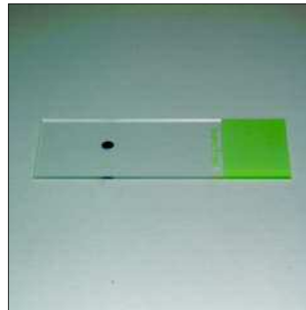


Figure 8.

A sample is placed on the slide.

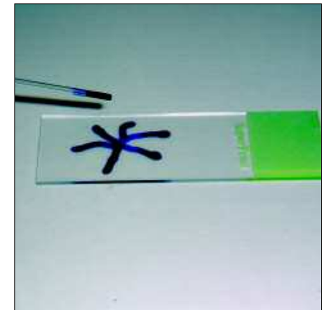


Figure 9.

The droplet is spread with a capillary tube, an orange stick or a needle.

- **Line smear:**
  as for blood smear, but the spreader slide is lifted up instead of continuing on out to form a feathered edge. This tends to concentrate the cells along the line where the slide was lifted up, since cells are carried along the edge of the spreader slide. See figures 10 and 11.

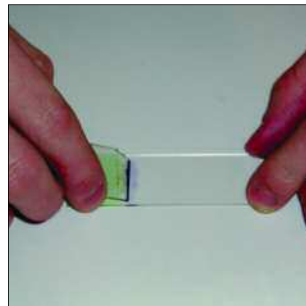


Figure 10.

Start as for a blood smear.

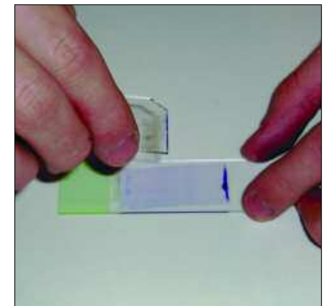


Figure 11.

Lift the spreader before a feathered edge is created.

ii) Impression smears

The lesion or cut surface of the excised mass should be dried with a gauze swab, and then touched onto a glass slide. Several imprints can be made on one slide; the later ones are less likely to be blood contaminated.

Smears should be air-dried rapidly to improve cell morphology preservation. This may mean wafting the slides in air, or using a cool setting on a hairdryer (direct the airflow at the back of the glass slide and hold it at least 15cm from the heat).

Sample preservation and submission to the laboratory

Air-dried smears preserve cell morphology for some time (at least several weeks), although high protein samples may succumb to bacterial contamination. They should be clearly labelled with the patient name, date, and sample site. They should then be submitted to the laboratory, unstained, in slide containers, accompanied by a description of the lesion, the patient history, and the results of any other tests carried out.

Please keep fluids and air-dried smears well away from formalin and its fumes as this significantly affects stain uptake, leading to unreadable slides. It can be helpful to submit them in separate packages to avoid fumes affecting the smears in transit to the laboratory.

Special sites

Lymph nodes: lymphoma cells are fragile and require gentle handling. Smearing techniques should be as gentle as possible. The submandibular lymph nodes should be avoided if possible, as these are often reactive secondary to oral disease, complicating interpretation of the findings. Where there is suspicion of feline lymphoma, biopsy is preferred. This is because the cell morphology is not necessarily conclusive and tissue architecture is more useful.

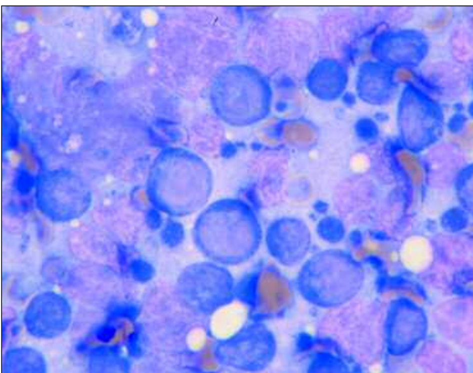


Figure 12.

Lymph node aspirate

Ultrasound-guided FNA: ultrasound gel on smears can confuse interpretation. Please note the use of ultrasound on the submission form.

Liver, spleen, and kidney: sampling can be frustrating because of low cell yield. Several smears should be prepared. Blood contamination is expected at these sites. It s recommended that the platelet count and clotting times are checked before sampling the liver and kidney, whereas the spleen is less likely to haemorrhage because of its capsule. For the liver, sample from the left as the left lobe is larger and this avoids the gall bladder.

Intra-thoracic masses: there is a risk of pneumothorax with fine needle aspiration, particularly with wide bore needles. Close monitoring after the procedure is recommended.

Tumour seeding: there is a theoretical risk of seeding tumour cells along the line of entry of a sampling needle through tissues overlying the sampling site. This risk appears relatively slight in practice, but should still be borne in mind.

Fluids

Aims, advantages, and limitations

Cytology of fluid samples is simple and can be highly informative in a variety of conditions:

Pleural and abdominal fluids – to differentiate between transudates, modified transudates, and exudates, allowing assessment of the cause of the effusion

- Joint fluid – to differentiate inflammatory from degenerative arthropathies
- Urine – to assess cases of cystitis or suspected urinary tract neoplasia
- Prostatic washes – to differentiate neoplasia from inflammation or hyperplasia
- Tracheal washes and bronchoalveolar lavage – to assess the causes of respiratory signs
- Cerebrospinal fluid – as part of the workup for neurological cases
- Pericardial fluid – a diagnosis of haemangiosarcoma can sometimes be made
- Fluid filled masses – to assess the cause of the mass. However, this is the least helpful fluid to sample unless there is inflammation. FNA of the wall of the mass is often more productive.

These samples are relatively easy to take, with little risk to the patient. One major problem is that cells in fluid degenerate rapidly. Consequently, it is very helpful if air-dried smears are made at the time of sampling and submitted with the fluid.

Preparation

For pleural and abdominal fluids, joint fluid, and fluid filled masses, sampling is relatively simple, and requires only suitable skin preparation, syringes and needles, EDTA containers, and several clean microscope slides. For CSF samples, the patient is generally anaesthetised, and spinal needles are recommended. For tracheal washes, bronchoalveolar lavage, and prostatic washes, further equipment would include a sterile catheter and sterile saline. Pericardial fluid sampling would require local anaesthetic, and suitable sterile catheter and three-way tap with a large syringe (30ml approximately).

Aspiration technique

Pleural and abdominal fluids

Sampling is simple, with a needle and syringe. The site of sampling can be empirical or ultrasound guided. If empirical, the more common sites are the 7th to 8th intercostal spaces at the level of the costochondral junction for pleural fluid, or just lateral to the midline midway between the bladder and umbilicus for abdominal fluid. The skin area should be clipped and surgically prepared.

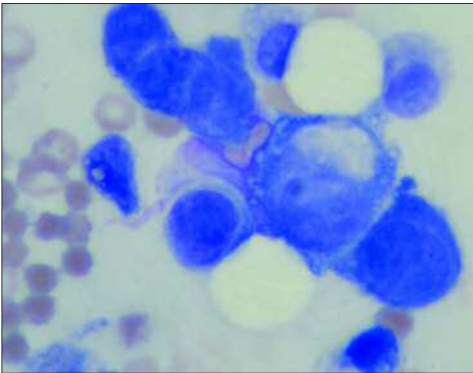


Figure 13.

Pleural fluid smear

Synovial fluid

Sampling from joints is most usually carried out when there are palpable effusions.

Urine

Urine cytology provides additional information when there are cells seen on sediment examination, and further differentiation of cell types is required. It can help to diagnose bladder tumours, and to assess the significance of bacteria culture results. Urine is ideally collected by cystocentesis to avoid contamination from the urethra and external genitalia. The bladder is immobilised with one hand while a sample is aspirated with the second. The bladder should not be left over-full, as this could lead to urine leakage into the abdomen. Care must be taken not to puncture other abdominal organs. Free catch samples can also be used, but this should be noted on the submission forms.

Prostatic wash

Prostatic wash samples should ideally be submitted along with a cystocentesis urine sample. This is because transitional cell carcinoma can occur both in the prostate and the bladder, with similar looking tumour cells. It is the site of sampling that confirms the diagnosis.

Prostatic FNA can also be very helpful. Enlarged prostate glands are often palpable, cranial to the pelvis. If this can be brought against the lateral body wall, FNA can proceed. With prostatic abscesses, this procedure runs the risk of spreading infection within the abdomen.

Tracheal wash/bronchoalveolar lavage

This is generally carried out under general anaesthesia. A sterile catheter is introduced into the airway, either through the endo-tracheal tube, or as an over-the-needle catheter placed directly into the trachea. The tube is advanced to the level of the carina for a TW, and 0.1 to 0.2ml/kg sterile saline injected and retrieved. For a BAL, an endoscopic examination with visualisation of the target lung lobe is preferred. Alternatively, the affected lobe can be placed downwards, with the catheter passed to the level of the main stem bronchi. The volume of saline used should not exceed 5ml/kg, and is injected in three aliquots, with retrieval of as much as possible between each sample. In large animals, there is a significant difference between the cell populations obtained by these two methods, but in small animals the differences are minimal. Side effects can include oedema, congestion and alveolar collapse.

Nasal flush

The aim with nasal flushing is to obtain cells from the nasal turbinate epithelium. Samples of nasal discharge or swabs are often representative only of the external nares. The patient generally requires anaesthesia, with the endotracheal tube inflated to avoid unnecessary introduction of fluid into the lungs. Sterile saline solution is introduced into the nasal cavity by syringe, possibly with a catheter attached to bypass the nares, sometimes under pressure to increase cell yield. The fluid can be collected through the same catheter, with a modified syringe case in the oropharynx, or with a Foley catheter retroflexed from the oral cavity around the soft palate into the nasal cavity. The catheter can be moved around to dislodge tissue cells and increase yield.

CSF

Patients require general anaesthesia for this procedure. A spinal needle is generally recommended although hypodermic needles can be used in small dogs and cats. Techniques are described more fully in texts. The patient should be in lateral recumbency with the head flexed at 90°, parallel to the tabletop, and held stable by an assistant. The atlanto-occipital region is clipped and prepared aseptically. The needle is inserted on the midline approximately half way between the external occipital protuberance and the cranio-dorsal tip of the axis, just rostral to the anterior borders of the wings of the atlas, with the bevel facing cranially. The stylet is removed frequently to check for the presence of CSF. CSF is collected by gravity into EDTA vials. If the sample appears blood tinged, it is useful to note on the submission form whether this appeared as contamination or as an even colour to the CSF.

Cells in the CSF tend to degenerate rapidly because of the low protein and lipid content of the fluid, and the preparation of smears should ideally be carried out within 30 minutes of sampling, with an appropriate concentration technique. This is considered impractical for many practices. Quoted techniques for improving cell preservation include the addition of fresh, frozen or thawed serum or plasma, or 20% albumin. Sedimentation chambers can be made to allow cell concentration on the slides. A tube is fixed to the slide with Vaseline, 0.5ml of CSF is added, the supernatant is removed in 30 minutes, the tube is removed, and the slide is rapidly air-dried. Direct air-dried smears are useful if there is a high cell content.

Pericardial fluid

The patient is likely to require sedation. An area over the lower thorax at the level of the 4th intercostal space is surgically prepared, and the area at the level of the costochondral junction is infused with local anaesthetic. The needle is introduced through the body wall with negative pressure applied. A “pop” is often felt as the needle penetrates the pericardial sac. The catheter is inserted through the needle and the fluid is withdrawn.

Fluid filled masses

Examination of the fluid within masses often only produces evidence of cystic fluid without suggesting a cause for its presence. FNA of the wall of the lesion can be more helpful. Examination of the fluid can be helpful if inflammation or infection is considered a possibility. Sampling is by simple aspiration.

Smear and sample preparation

Fluid samples for cytology should be placed into EDTA (a normal haematology tube is ideal). All samples should also have fresh air-dried smears prepared as well. This should be carried out with both the blood smear technique and the line smear technique (see above). The latter technique will tend to concentrate cells in fluids of low cellularity. It will also tend to concentrate cancer cells, as the larger cells will be drawn further with the spreading slide. If a blood smear technique goes beyond the end of the slide, these cells will be lost.

Some fluids are of very low cellularity, and concentration before slide preparation is preferred. The line technique is very useful for many fluids. For urine and prostatic wash samples, an aliquot of the sample can be centrifuged and re-suspended before smear preparation. CSF samples have a very low cellularity. Various concentration techniques have been documented, many impractical in practice.

Sample preservation and submission to the laboratory

Please keep fluids and air-dried smears well away from formalin and its fumes as this significantly affects stain uptake, leading to unreadable slides. It can be helpful to submit them in separate packages to avoid fumes affecting the smears in transit to the laboratory.

Blood Smears

Aims, advantages, and limitations

The aims of examining blood smears are to supplement the cell counts and differential white cell count by examining morphological features of the circulating cells.

Sampling technique

The best samples are generally obtained from the largest available vessel, i.e. the jugular vein. If the aim is to search for blood parasites, these tend to localise in capillary beds, and a drop of blood from an ear tip is the ideal sample.

Smear and sample preparation

A drop of blood is placed on one end of a clean microscope slide. The clean dry edge of a second sample is advanced into the drop until it is drawn by capillary action into a line. Pushing the second slide smoothly along the first then makes the smear. This should give a single cell layer and a neat feathered edge, while allowing an even distribution of nucleated cells in the smear.

Sample preservation and submission to the laboratory

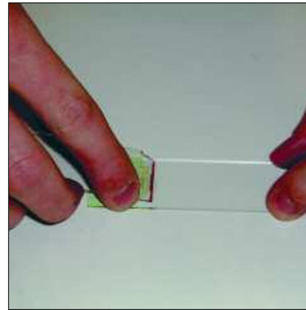


Figure 14.

Spread a droplet by capillary action.

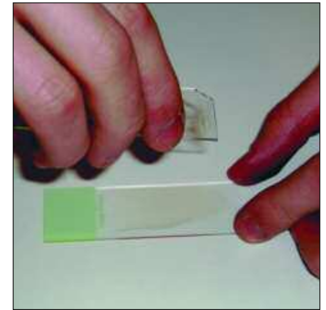


Figure 15.

Spread gently, leaving a feathered edge.

Air-dried smears preserve cell morphology for at least a few weeks, whereas cells in EDTA tend to degenerate over a matter of hours (some species are worse than others). Consequently, it is important to submit air-dried smears as well as an EDTA sample for all haematology submissions.

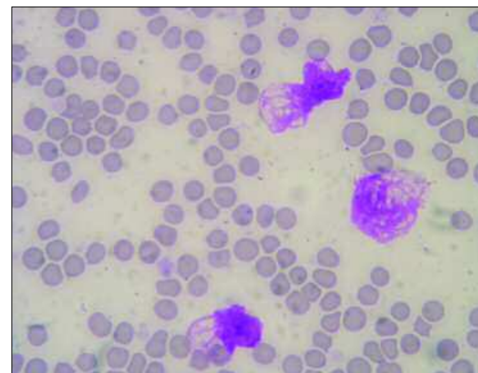


Figure 16.

Smear from blood left in EDTA for several days.

Submitting a biopsy for histopathology

1 When collecting a biopsy sample for histopathology, submission of the best sample available can give valuable information regarding the following:

- **Nature:** Degenerate, inflammatory, hyperplastic or neoplastic
- **Extent:** Localized or diffuse
- **Growth pattern:** Circumscribed or invasive
- **Bone involvement** (where applicable, e.g. digits, oral cavity): Present or absent
- **Infectious agents:** Present, nature and significance
- **Margins of excision**

2 When collecting samples for histopathology:

Σ

- Collect as large and representative a sample as possible
- Use a sharp scalpel blade or biopsy punch
- Avoid very inflamed and/or necrotic tissue (usually present at the centre of the lesion)
- Sample multiple areas where necessary (e.g. skin cases)
- For smaller masses, a total excisional biopsy of the entire mass is advisable
- For lymph nodes, it is always recommended to submit an intact affected lymph node. For cases of generalized lymph node enlargement avoid the submandibular lymph nodes
- Include adjacent normal tissue for orientation of the lesion (especially splenic or liver masses and cutaneous/subcutaneous masses)
- If there are specific areas that you wish the pathologist to evaluate (e.g. very narrow margins) mark those areas with a suture and specify the reason on the submission form

3 Avoid the following:

Σ

- Excessive and rough handling (e.g. crushing) - especially small or fragile samples
- Desiccation - place the sample in formalin immediately after collection
- Cauterization - causes extensive damage of the tissue, which frustrates accurate interpretation
- Excessive cleaning of the area to be sampled

4 Submission of samples:

Σ

- Samples from multiple areas should be labeled as such (sutures or separate pots)
- Samples should be submitted in 10% neutral buffered formalin at a ratio of 1:10 tissue to formalin
- Be sure to dilute the formalin as per the instructions on the sample pot
- Larger samples can be submitted in 2 halves
- Very large samples, e.g. splenic masses or large tumours should be fixed in the practice in a large bucket of formalin for a week and then wrapped in formalin-soaked cotton wool, placed in a plastic bag, carefully sealed and submitted

5 A concise and pertinent clinical history should be provided with all submissions. Be sure to include the following:

- Species, Breed, Sex, Neutered or not, Age
- Site sampled
- Brief description of the lesion
- Radiographic evidence of bone involvement (where applicable)
- Details of underlying systemic disease
- Recent medication
- Any previous tests done on the lesion and previous laboratory code if applicable
- Surgical margins - included or not

6 What information can I expect in my histopathology report?

Σ

- Animal details
- Summarized history (as provided by you)
- Diagnosis (more than one if multiple samples)
- Prognosis
- Brief histological description
- Discussion, giving relevant information with regards the type of lesion and suggestions for further investigations, e.g. immunohistochemistry, serum chemistry, etc.

7 Talk to your pathologist:

Σ

- If there is anything in your report that you do not understand
- If the diagnosis does not match the clinical presentation
- If you wish to discuss any further tests on the tissue

Submission of whole globes for ocular pathology

Detailed information regarding the species, breed, age and sex, along with relevant clinical history and recent treatment is very important. Please indicate the eye affected, and whether the condition is uni- or bi-lateral. Adding your clinical differential diagnoses is also very helpful. Please use the diagrams supplied to mark the position of any particular lesions or areas of interest.

Unless the eyelids or extraocular muscles are suspected to be involved in the disease process, trimming away of excess tissues, including the eyelids and muscle, results in optimal fixation of whole globes; the third eyelid can be left in-situ and will not hamper fixation. The extraocular tissues can be submitted separately within the same specimen container if examination is required. Orientation of the globe should still be possible without lids, however if you wish to indicate the intraocular position of specific lesions of interest (ie masses in the iris or elsewhere) which may not be visible through the cornea after fixation, placing a suture in the residual conjunctival tissue over the site is helpful to allow optimal placement of the primary cut through the eye at trimming.

Where possible, please leave enucleated globes intact, as formalin penetrates the eye readily once extraocular tissues are removed, and incision causes collapse and distortion of the globe, hampering gross evaluation.

Whole globes require detailed gross and histological evaluation- if the case is urgent, please mark it as such, and we will do our best to return the results to you as soon as possible. Conjunctival and other biopsies are reported routinely, however whole globes may take up to one week for results.

Front and caudal views of eye correctly submitted. Trimming away excess tissue allows optimal fixation.

Diagnostic Immunohistochemistry

Over the past 2 years our immunohistochemistry service has been expanding and evolving in order to give our clients as comprehensive a service as possible. Here we try to answer some of the commonly asked questions and give you relevant information regarding this very useful diagnostic aid.

My pathologist has advised immunohistochemistry - what is it?

Immunohistochemistry enables us to demonstrate intermediate filaments and other antigens inside or on the surfaces of cells, which may help in identifying the cell type. This is done using antibodies to specific cell markers. For example, the CD3 antibody identifies a T cell lymphoma, as this molecule is only present in the membrane of lymphocytes of the T cell lineage. The marking antibodies are then visualized using a special staining technique. The same principle can be applied for infectious agents.

What tissue samples are required for immunohistochemistry?

Immunohistochemistry can be done on the same formalin-fixed tissue submitted for routine histopathology and in most cases we use the same paraffin wax-embedded sample that was used for the original histopathology.

How will immunohistochemistry help me?

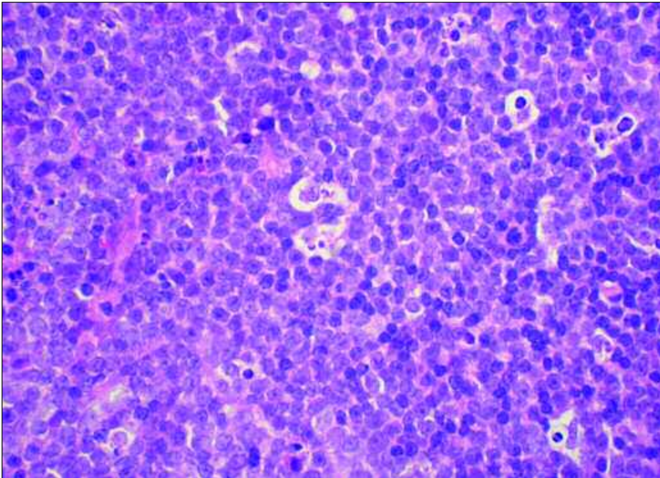
- This technique is useful to phenotype lymphomas - B or T cell origin as demonstrated in the pictures on the right
- It is useful in determining the histogenesis of poorly differentiated tumours
- It can assist in making more accurate decisions with regards to treatment of tumours
- It can aid in giving a more accurate prognosis
- In cases where infectious disease is suspected, it can help to confirm the presence of that infectious agent

How long will it take to get an answer?

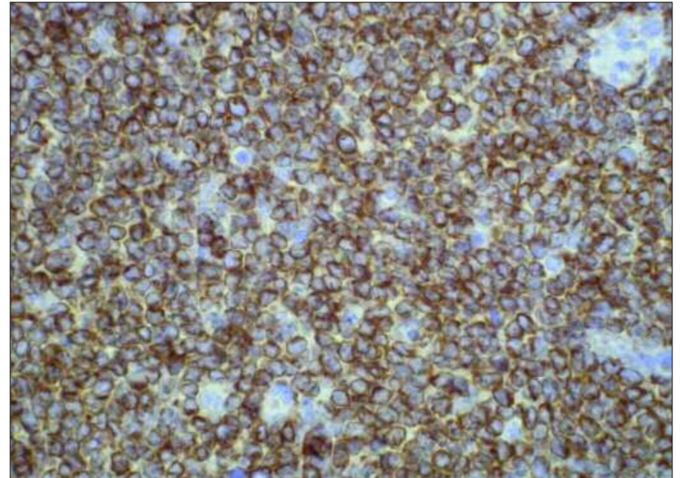
Cases where immunohistochemistry has been requested are batched and currently we stain all of these on a once a week basis. They are then evaluated and reported the same day or early the following day. The waiting period can thus be anything from 2 to 10 days depending on when the staining is requested by the veterinary surgeon. Although most of the stains are done in our laboratory, there are occasional cases where the samples have to be forwarded to another laboratory (the infectious agents mostly) for staining. In such cases you will be informed and it may take slightly longer to get the results.

How will I know if immunohistochemistry is appropriate in a certain case and how do I request the staining?

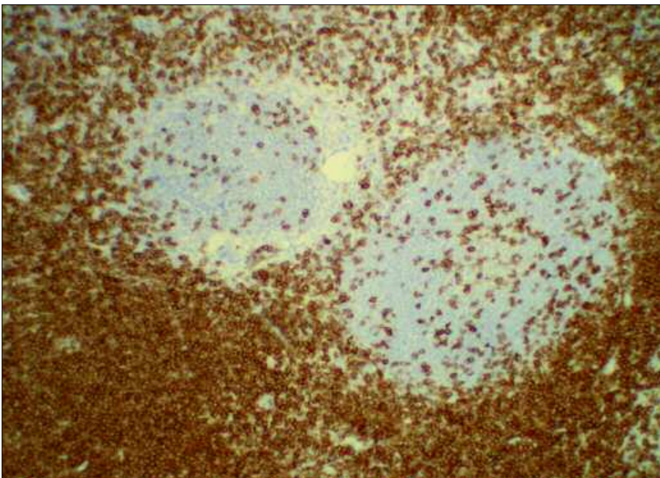
The pathologist reporting on the sample will suggest immunohistochemistry if he/she thinks it is appropriate. They will also include the cost of any immunohistochemistry suggested. If you wish to proceed with the staining, you only need to contact our secretaries who will inform the pathologist who dealt with your case.



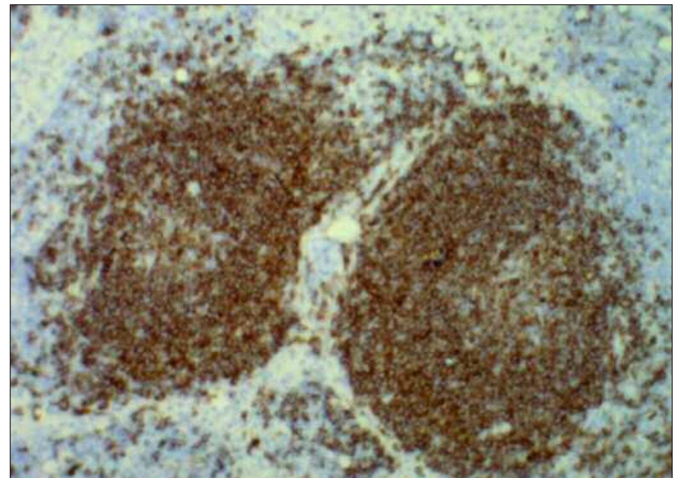
*Routine H&E stain of a lymphoma.
Lymph node*



Immunohistochemical staining with the T cell marker CD3 - T cell lymphoma. Lymph node



Immunohistochemical staining with the T cell marker CD3 - staining of the paracortex of a reactive lymph node



Immunohistochemical staining with the B cell marker CD79a – staining of the germinal centres of a reactive lymph node

Necropsy procedure for dogs and cats

General recommendations:

A necropsy should be performed as soon after death as possible. If this is cannot be, then the body should be kept in refrigeration until necropsy can be performed, preferably less than 24 hours after death. In cases when euthanasia can be scheduled freely, i.e. when there is no immediate need due to suffering or pain in the animal, it should be planned in a way that necropsy can immediately follow.

Following a systematic necropsy procedure ensures that all organs are examined and improves the probabilities of reaching a final diagnosis. The protocol detailed below is meant to serve as a guideline for clinicians who perform in house necropsies and submit samples to a diagnostic laboratory. Remember that, although your examination will concentrate more on the organs or systems known to be affected from your clinical work up, all systems should be examined in case they contain significant lesions that may influence or change the original clinical diagnosis.

Whenever possible, avoid freezing carcasses.

Instruments that you will need:

Basic necropsy instruments include a knife or scalpel, scissors, forceps and bone cutters or a handsaw.

Basic containers that are ALWAYS needed to transport and/or store samples include plastic bottles with 10% buffered formalin for histological samples, and sterile tubes, swabs or bags for microbiology samples.

Containers needed for specific cases include freezer-proof bags to store virology and toxicology samples, syringes and needles to obtain urine, other fluids and aspirates, and glass slides and EDTA tubes to submit cytological smears and fluid samples such as synovial or cerebrospinal fluid when the case merits their collection.

Containers can be requested from Finn Pathologists at 01379 854180.

Remember that the quality of the samples submitted will often reflect on the quality and usefulness of the laboratory results.

Step 1. External examination:

1 Place the animal on its side, head to your left if you are right-handed

2 Are there any…?

- External wounds, bone fractures, tears
- Discharge from eyes, nose, mouth, urethra, anus
- Abnormal mucosal colour (yellow-icteric, pale or white-anaemic, dark blue-cyanotic)
- Abnormal masses

Step 2. Internal examination:

The aim of the internal examination is to visually evaluate all systems in the body and collect the appropriate samples. As you examine each system, keep in mind what samples you want to collect. A list of the samples that are routinely collected at a diagnostic laboratory includes:

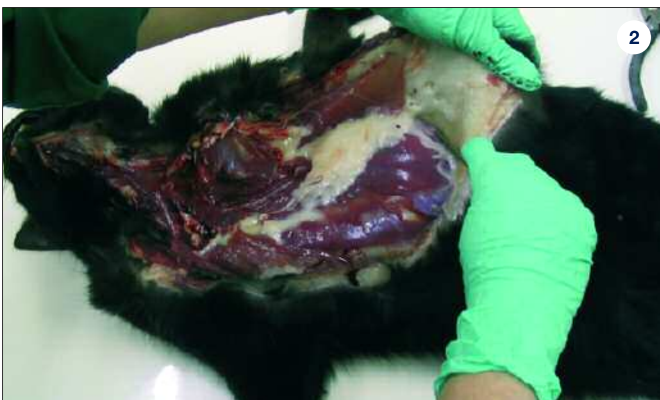
Samples for histology:	Brain Trachea and Lung Heart Oesophagus Stomach Duodenum+Pancreas Ileocaecal junction Liver Spleen Adrenal gland Kidney Bladder Lymph nodes (especially if they are enlarged)
Samples for bacteriology:	Fresh tissue or swab from lung and/or liver and/or kidney. Intestinal samples only useful if the necropsy is performed very soon after death (within a couple of hours).
Samples for virology:	A 1cm^3 piece of brain, lung, liver, kidney, and intestine – freeze as soon as possible
Samples for toxicology:	Gastric contents (in case of acute intoxication) and a 1cm3 piece of liver, kidney and fat (mesenteric or subcutaneous) – keep frozen

Of course, depending on the individual case, more or less samples will be collected. For example, in a case where the signs of the presenting animal are non-specific and there is no presumptive diagnosis, all of the samples listed should be collected to avoid missing something.

Remember that not all samples have to or will be submitted. Some samples, like those for virology and toxicology, are meant to be kept at the practice in case they are needed.

How to proceed with the necropsy:

1. Make an incision through the skin and subcutis along the ventral midline from the mandibular symphysis to the anus. To peel the skin back, cut the nerves of the brachial plexus to free the left scapula and thus lay the left fore leg on the table. Then cut into the left coxofemoral joint, expressing the femoral head and reflect the left hind leg (see pictures below).



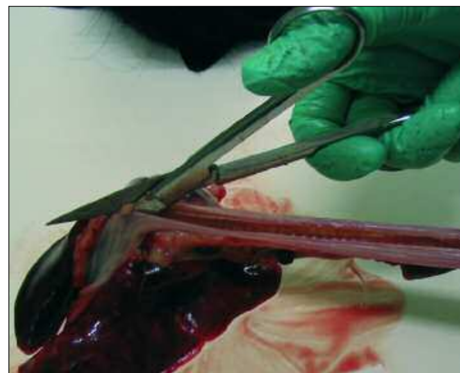
2. Remove abdominal muscles to expose the peritoneal cavity. Use the muscles as a "handle" to remove the ribs cutting at the level of the costochondral joint (the softest portion of the rib) and along the sternum. Young and small animals can be handled with scissors but larger ones require bone cutters.

The Thorax

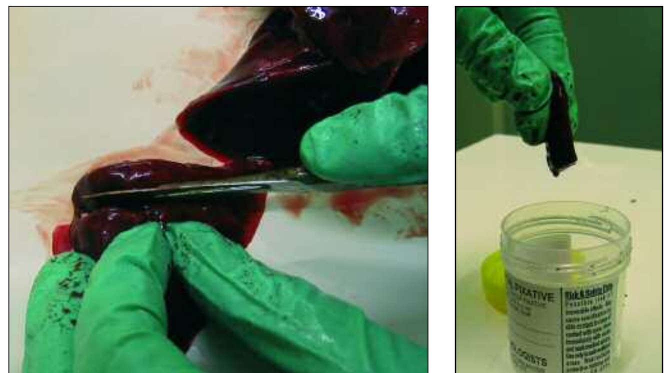
3. Remove the ventral muscle of the neck to expose the trachea and oesophagus. Cut along the medial surface of the mandible to free the tongue. With gentle traction and cutting along the parietal mediastinum, remove together the tongue, the respiratory tract, heart and cranial oesophagus (pluck). Detach the pluck at the level of the diaphragm, cutting the esophagus just cranial to the cardia.

4. Expose the oesophageal surface by cutting along its dorsal axis with the scissors. The mucosa should be pale pink, smooth and shining.

Then, cut the larynx and trachea open, also with the scissors, exposing their mucosa (see picture). Follow the bronchi into the lungs. Note colour changes, presence of foam, parasites, nodules (granulomas or neoplasms). Remember to take a section of oesophagus and trachea for histology.



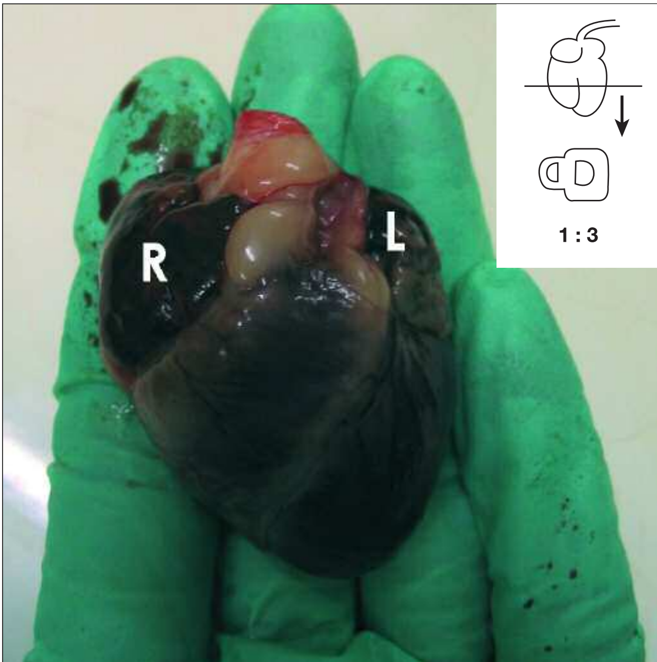
5. Collect a thin sample from the lungs. Use either scissors or scalpel. Be sure not to squeeze the tissue as it will cause compression artifact. Remember to make the sample approximately 5 mm thick so that the fixative has good penetration. Place in formalin and make a note if the sample sinks (lungs with severe pneumonia will sink).



Remember to collect a sample for bacteriology and, maybe, virology.

6 Examine the heart. Cut into the pericardial sac and free the heart. Detach the heart from the lungs. If you suspect a congenital defect, the best thing to do is to fix the heart whole in formalin and submit as such. Otherwise, you will need to make 4-5 cuts into the heart as follows.

First get oriented. It is easy to lose track once the heart is detached from the lungs, particularly once you start cutting into a distorted heart. If you lose orientation, place the heart on your hand so that the free portions of the atria, i.e. the auricles, are facing you. That way you will always have the heart in a "shake hands" position (see picture).



If you are concerned about hypertrophic cardiomyopathy, you will have to examine the thickness of the ventricular walls. Make a cut parallel to the coronary groove at approximately the middle of the ventricles (see picture for normal ratio).

If the cardiac silhouette is normal externally and there is no indication of hypertrophy, it is best not to make this cut, as it does make the rest of the examination slightly more cumbersome.

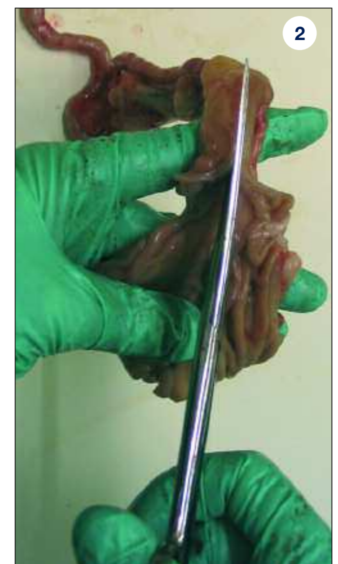
There are many ways to examine a heart. One of the easiest to remember is to make the cuts follow the flow of blood. Thus, start at the right atrium, cut into the right ventricle and thus expose the atrium, ventricular chamber and right AV valve (cut 1). Then follow the blood out through the pulmonary artery and examine its lumen, which should be white and smooth, and the pulmonary valve (cut 2). Cut through the left atrium into the left ventricle; examine the atrium, chamber and left AV valve (cut 3). To follow the aorta, which is the next step, you will have to cut the AV valve (cut 4); expose the aortic valve and lumen.

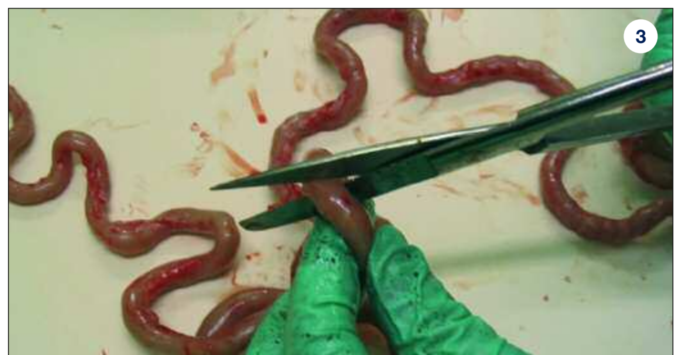
Once you have looked at the heart, collect a sample for histology, preferably one from each ventricle, cutting along the long axis.

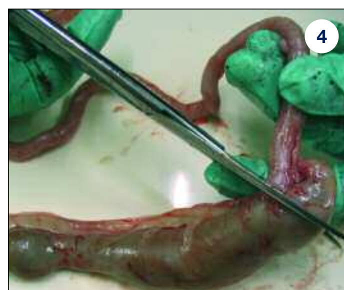
The Abdomen

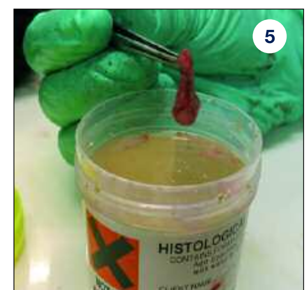
7 Remove the spleen, liver and gastrointestinal tract. Dissect each organ and examine them individually. Make several cuts into the parenchyma of the liver and spleen, even if no lesions are obvious externally. Cut a thin section of liver and spleen for histology and a larger piece of liver for bacteriology. Collect some abdominal fat to be frozen for toxicology.

8 Detach the gastrointestinal tract from the omentum and the mesentery. This can be done by tearing them apart gently. Expose the entire GI tract (1). Open the lumen of the stomach, from the cardia to the pylorus, following the greater curvature (2). Open several sections of the small and large intestine, either randomly or focusing on areas that appear abnormal (3). Collect a section of stomach, duodenum+pancreas, jejunum and ileocaecal junction (4) for histology (5). Remember that intestinal samples for bacteriology are only useful if collected within the first few hours after death.



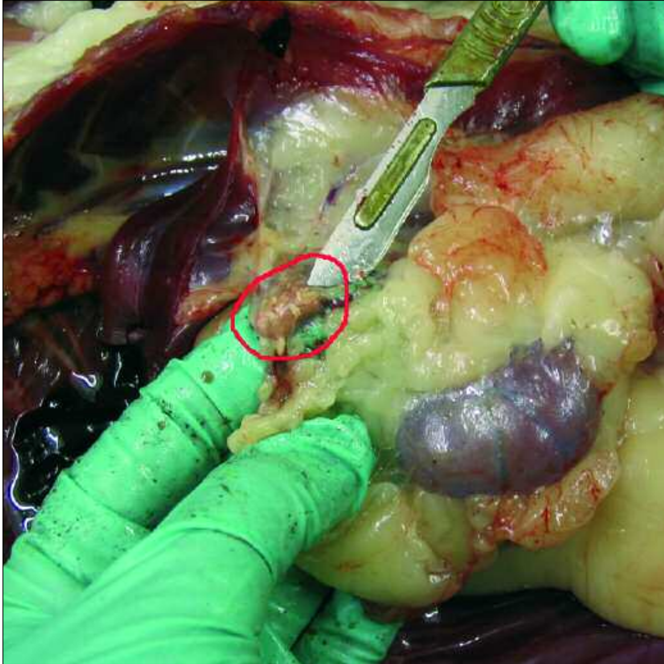




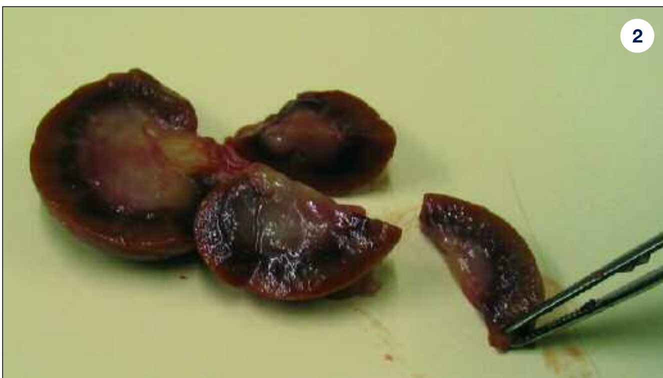


9 Examine the outer and mucosal surface of the bladder. Take a section for histology.

Before removing the kidneys, locate and dissect the adrenal glands. This can be difficult in obese animals, as they are deeply embedded in the perirenal fat. You can see the adrenal in this picture, surrounded by a red circle. Remove and place in formalin.



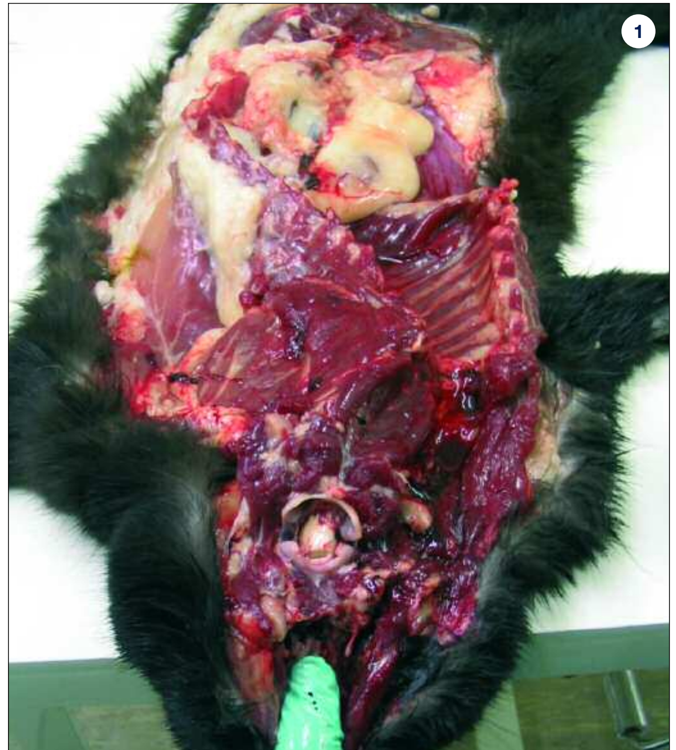
10 Take out the kidneys from the cavity, remove their capsule. Cut into the parenchyma to observe the cortex and medulla. Take a section for histology, a section for bacteriology and freeze some for virology and/or toxicology, if necessary.

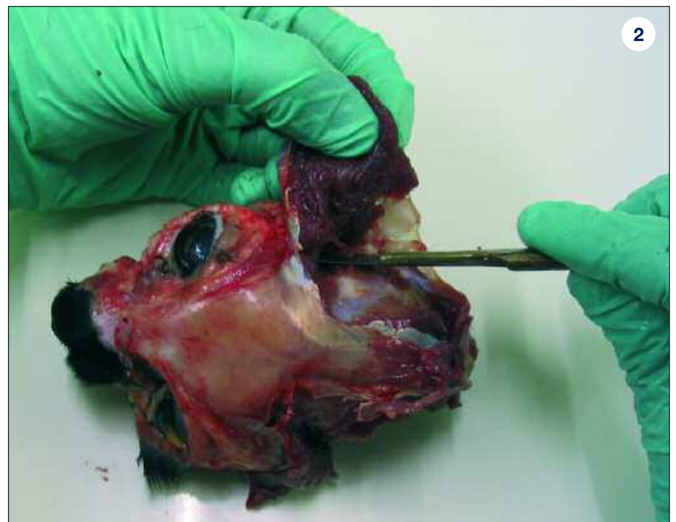


The Cranium

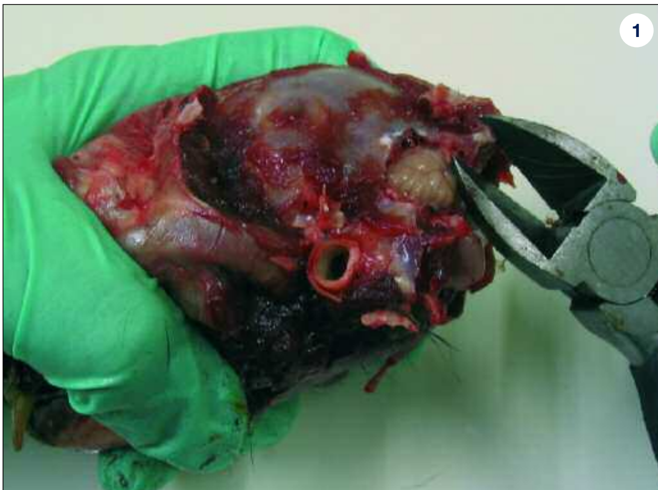
11 Remove the head to obtain the brain. Place the animal so that its head hangs from the edge of the table. Cut through the atlanto-occipital joint to free the head.

Remove the skin and muscles from the calvaria.





12 Chip away the calvarian bones with wire cutters



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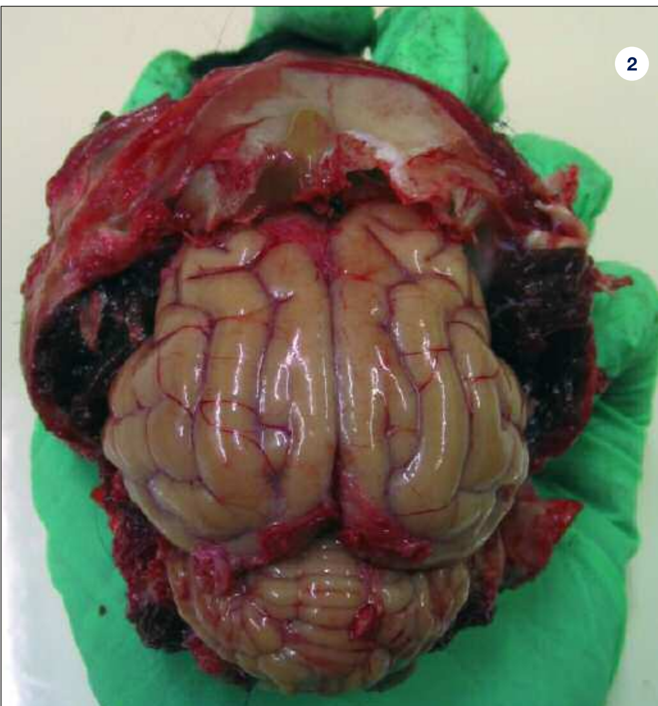

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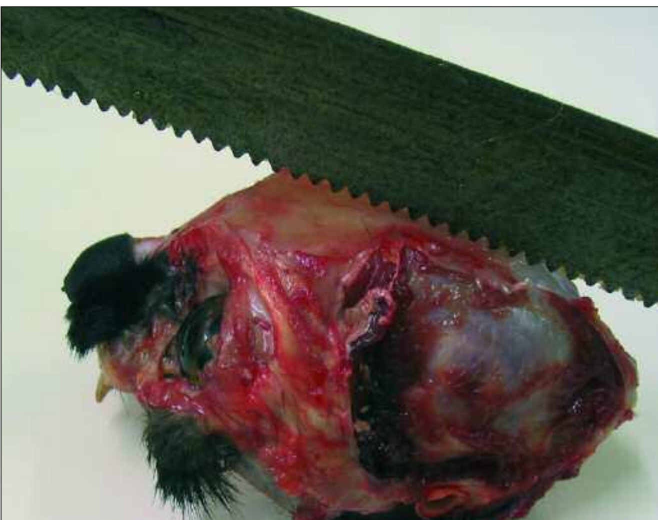
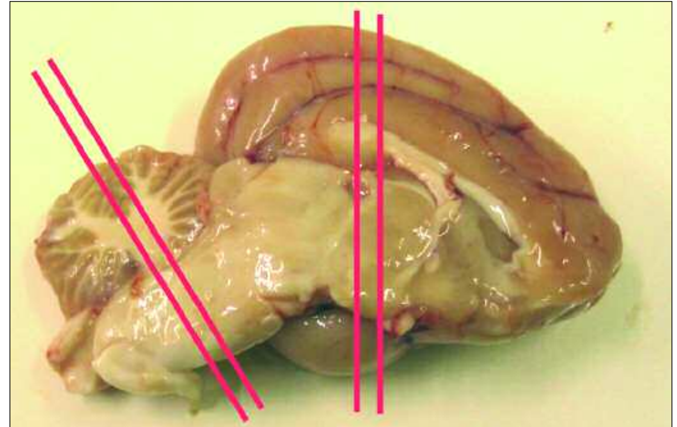
or, alternatively, cut it in half with a saw and thus obtain the brain.



Once the brain is out of the calvaria, examine the outer surface of the cerebrum, cerebellum and brain stem. Then cut the hemispheres apart and examine the lateral ventricles. Obtain a section of cerebellum+brain stem and of cerebrum for histology, as shown in the picture.

You may also want to freeze some brain tissue for virology.



Other Tissues:

In specific cases, examination of the bone marrow may be in order. If so, cut the femur free of the surrounding skin and muscles. Use a hammer to crack the bone towards the proximal end. Expose the marrow. Blot the marrow on paper to remove excess water and proceed to do several impression smears on to a clean glass slide. Bone marrow smears are generally more useful than histological samples.

Questions?

Remember that you can always contact a pathologist before performing the necropsy to discuss the case and agree on the best way to proceed and which samples to obtain.

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