

INTRODUCTION TO CLINICAL PATHOLOGY - CYTOLOGY

Subjects involved in clinical pathology

Clinical Pathology (Clin Path) involves a study of laboratory techniques and their interpretation as ancillary aids to diagnosis. The main subjects covered by Clin Path are:

- Cytology
- Haematology
- Clinical biochemistry
- Urine analysis
- Serology

The lectures which will concentrate on basics of cytology, haematology and biochemistry with emphasis on interpretation, and will give case examples. Histopathology and post mortem examination (known as Anatomic Pathology) and parasitology are separate but closely related subjects to Clin Path.

Why study Clinical Pathology?

Clearly, it is not always possible to make a definitive diagnosis on history and clinical examination alone, and in many cases it is necessary to carry out further tests to make a definitive diagnosis, identify complicating factors, and monitor progress including response to treatment. **Clin Path is carried out by almost every practitioner on a daily basis, even if it is only interpreting lab results.** It is therefore very important to understand the principles of test selection, sample collection, laboratory techniques and result interpretation.

Introduction to cytology

Cytology is the examination and assessment of cells in fluids and smears, and is a very valuable diagnostic procedure provided care is made in smear preparation, artefacts or non-representative material are not misinterpreted and the limitations of a small number of cells and relative loss of architecture compared with biopsies, are appreciated. It is not always possible to make a definitive diagnosis on cytology and sometimes collection of biopsies for histopathology or other tests are indicated for confirmation or assessment of margins of excision.

Fluid samples for cytology (and biochemistry)

Fluids are assessed for gross appearance and cytological examination. Except for wash preparations (e.g. bronchoalveolar lavage, prostatic wash) where dilution occurs, total cell counts and total protein should also be carried out (e.g. for cerebrospinal fluid (CSF), peritoneal fluid).

Bronchoalveolar lavage (BAL)/transtracheal wash (TTW), pleural, peritoneal, synovial fluid, CSF

- BAL/TTW, pleural, peritoneal and synovial fluid and CSF are submitted in an EDTA tube (for cytology and, if relevant, for total cell counts) plus a plain sterile tube (for biochemistry and microbiology).
- If the samples must be posted, make smears as well (use centrifuge deposit for smears if dilute). For preservation, special solutions can be used e.g. Cytospin collection fluid® which is added 50:50 to fluid samples. Pre-made smears and use of preservative is essential when posting CSF which degrades very quickly.

Urine samples

- Submit in a sterile universal container for routine analysis but place a sample in EDTA if cytology is required.
- Dipstick tests should be carried out on whole, uncentrifuged urine.
- Wet preparations will detect RBCs, WBCs, crystals, casts, bacteria, fat droplets.
- Dried smears from the EDTA sample are made for cytology if inflammation or abnormal cells are suspected as the wet preps are not suitable for this.

Making smears for cytology

Touch imprints (aka “impression smears”)

- Suitable for rapid diagnosis of some external lesions and excised tissues which may subsequently be examined by histopathology.
- Yield fewer cells than scrapings.
- There is a risk of only detecting surface inflammation/blood or contaminants.
- For excised tissues, make a fresh cut surface, blot on hand towel to remove excess blood and then **gently** make multiple impressions on a slide (straight down onto slide and straight up again). The remaining tissue should be undamaged and can then be placed in buffered formalin for histological sections to be made.

Scrapings

- Suitable for the same samples as impression smears but collect more cells.
- Also used for parasitology on skin.
- Use a scalpel blade perpendicular to the surface being scraped or for delicate tissues e.g. conjunctiva, a blunt spatula can be used.
- Smear the sample on slides.

Fine needle aspirates (FNAs)

- Suitable for cutaneous masses, internal masses and organs, lymph nodes etc.
- Avoids superficial contamination.
- Collects samples from deep within the lesion/tissue.
- Risk of coming out of small lesions or missing them altogether.
- Smear after placing on slide.

Swabs

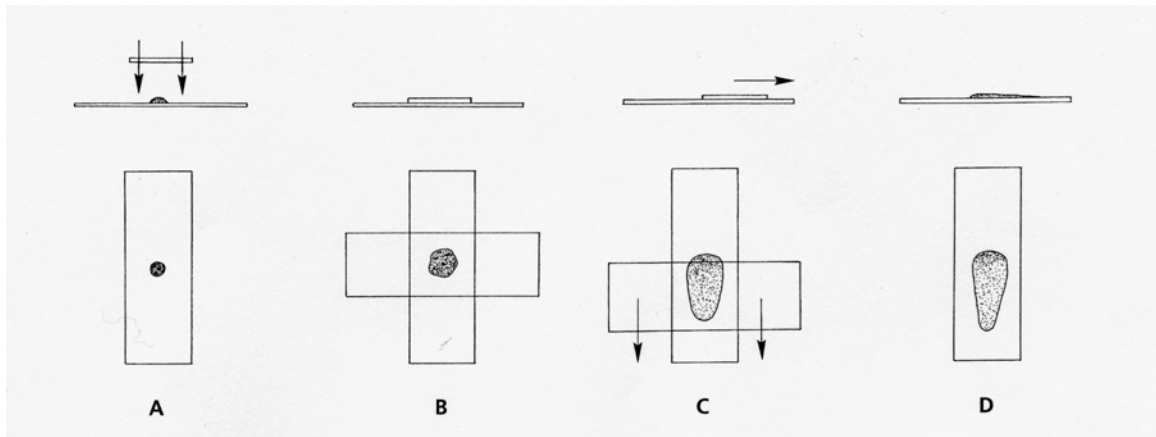
- Suitable for tissues which are not readily reached e.g. vagina, fistulous tracts.
- Use cotton swab moistened in 0.9% NaCl.
- **Roll** swab down middle of the microscope slide – do not slide across.

Techniques for smear preparation

The technique used depends on the cellularity and viscosity of the sample and presence of particulate material. If in doubt, contact the lab before you make the smears.

Squash preparation

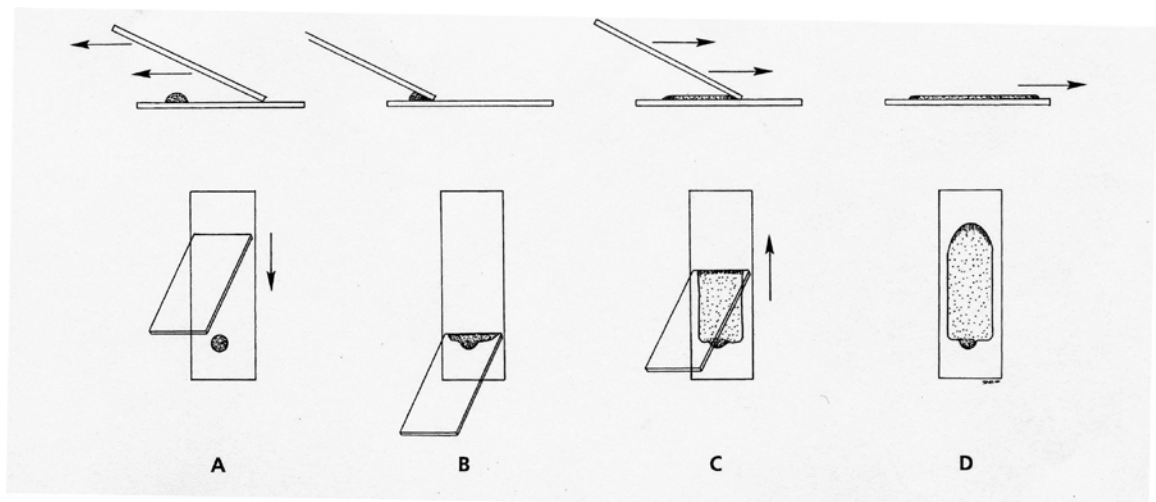
Suitable for FNAs, scrapings, viscous fluid samples, samples concentrated by centrifugation, samples with flecks of cellular material and bone marrow aspirates.



The sample is placed in the middle of the slide and the spreading slide placed on top then the two slides are moved apart by gently sliding the spreading slide towards one end of the sample slide. The spreader can be at right angles or parallel to the sample slide.

Blood smear technique

Used for blood smears, fluids of medium to high cellularity, samples concentrated by centrifugation.



The sample is placed near one end of the slide. The spreader slide is **just** brought into contact with the drop and the spreader slide is then moved smoothly towards the opposite end of the slide. This should produce a feathered edge. Ensure that the sample does not spread right to the long edges of the slide or off the far end.

Slide labelling

Lack of, or incomplete labelling of slides causes confusion and potential misdiagnosis with serious consequences. Glass slides with a frosted end must be used and the patient identity and source of the material written clearly in pencil because pen marks come off during staining. The details of what has been sampled and clinical details should also be written on the submission form.

Stains for cytology

Many stains for cytology are available but it is best to stick to a simple Romanowski type of stain (e.g. Diff-Quik®) for general work. The main stains are:

- Diff-Quik® - general cytology
- Gram - bacteria
- Ziehl-Neelsen – acid-fast bacteria

Diff-Quik® is ideal for in-house haematology and cytology. Bear in mind that it does not stain some granules in cells well, especially mast cell granules. It is easier to examine slides if you coverslip them by placing a few drops of DPX mounting fluid on the coverslip and inverting the slide on top to pick up the coverslip.

General assessment of cytology slides

1. Using low power (x4 and x10), look all over the smear for large structures such as particulate artefacts, large clusters of cells, parasites etc.
2. Then go to x20 and x40 to look more closely for areas of interest and other structures that might be missed at x100.
3. To look in more detail, place a drop of oil on the smear and move to the x100 oil immersion lens. This is not necessary in every case.
4. Make sure you **look all over** the smear. Sometimes the diagnosis is made on a few cells at the edge!

A logical approach to cytological interpretation

When examining cytological preparations, try to determine:



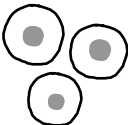
- The type of cell(s) present
- The degree of abnormality
- The nature of the process(es) present

Cell types

Cell types can be divided into **three main categories**, so when evaluating slides, decide which category(ies) is present first then decide what the cells are:

- **Epithelial** - e.g. squamous cells, basal cells, glandular and duct lining cells
- **Mesenchymal** – mainly connective tissue cells e.g. fibroblasts, osteoblasts
- **Round cell** - e.g. lymphocytes, histiocytes, plasma cells, mast cells etc.

Cytological criteria of the 3 basic cell categories

Tumour type	Cell size	Cell shape	Schematic representation	Cellularity (aspirates)	Clumps or clusters
Epithelial	Large	Round to polygonal/caudate		Usually high	+++
Mesenchymal (spindle cell)	Small to medium	Spindle to stellate		Usually low	- to +
Round cell	Small to medium	Round		Usually high	-

Cell arrangements

In cytology preparations, architecture is often lost but residual architecture can often be seen. This helps analyse the smear. Residual cellular arrangements include:

- Clusters – epithelial cells
- Rows, acini and papillary arrangements – epithelial cells
- Storiform (streams and whorls) – mesenchymal cells
- Perivascular (around capillary fragments) – epithelial or mesenchymal cells

Process(es) present

Once you have decided on the types of cells present and whether they look normal or abnormal and (if the latter) in what way they are abnormal, you can decide which process(es) are present:

- **Normal or hyperplastic tissue** – Normal/near normal cells e.g. benign prostatic hyperplasia. There may be mild changes in hyperplasia such as mild anisocytosis (variation in cell size) and slightly variable nucleus to cytoplasm (N:C) ratio.
- **Cyst** – Contains fluid and few cells e.g. sweat gland cyst, prostatic cyst. The fluid is usually low in protein (pale pink background) with a small number of cells, often with reactive macrophages predominating. There may be haemorrhage (RBCs, erythrophagocytosis [engulfed RBCs in the cytoplasm], blue/black haemosiderin granules in macrophage cytoplasm) or secondary inflammation. Some plump mesenchymal cells (reactive fibroblasts) may be seen.
- **Inflammation** – Variable numbers of neutrophils, lymphocytes, plasma cells, macrophages, eosinophils, or mast cells e.g. abscess, peritonitis, eosinophilic bronchitis. Free and intracytoplasmic bacteria (usually in neutrophils) may be present, or other agents e.g. yeasts, fungi or protozoa.

The number of inflammatory cells can be used to classify the inflammation and the categories may overlap:

- Purulent (suppurative) - >85% neutrophils (a subcategory of acute)
- Acute - >70% of inflammatory cells are neutrophils
- Subacute (=chronic-active) – 50-70% neutrophils, 30-50% macrophages
- Chronic - >50% macrophages
- Granulomatous – predominance of macrophages and lymphocytes. Variable numbers of neutrophils. May be giant cells.
- Eosinophilic – predominance of eosinophils (>10% but may be much higher)

N.B. If neutrophil nuclei appear swollen and disrupted, suspect sepsis, even if you cannot see bacteria. However, normal-looking neutrophils do not rule out sepsis.

- **Response to tissue injury** – Haemorrhage, fluid, fibrosis, necrotic material and inflammation e.g. haematoma, seroma. There are likely to be macrophages, possibly with dark haemosiderin granules or erythrophagocytosis. Plump elongated fibroblasts are also expected as they form fibrous scar tissue. Necrotic material creates a “dirty” blue/grey smudged background.
- **Neoplasia** – Benign or malignant neoplastic cells. Benign neoplasia may resemble the normal cells of that type, or appear mildly pleomorphic. For appearance of malignant cells, see below.

N.B. More than one process may be present at the same time e.g. an inflamed tumour or a cystic tumour.

Abnormal cell morphology


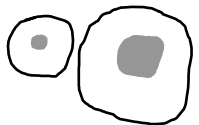
- **Hyperplasia** – May look normal or have a higher N:C ratio and/or increased cytoplasmic basophilia.
- **Dysplasia** – Can mimic neoplasia and results from asynchronous maturation of different parts of the cell e.g. immature nucleus in mature cytoplasm. Can occur with chronic inflammation/irritation. Mild to moderate variation in cell size (anisocytosis), variation in nuclear size (anisokaryosis), increased N:C ratio, occasionally coarse chromatin. **Be careful in diagnosing neoplasia where there is significant inflammation – it may just be dysplastic change.**
- **Neoplasia** – See below.

Neoplasia

Cytological criteria of malignancy


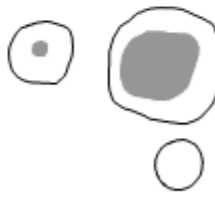

Certain criteria of malignancy are used in cytology to try to decide whether malignant neoplasia is present. If they are absent it is more likely the lesion is benign, but some tumours e.g. endocrine tumours and anal sac gland adenocarcinoma tend to look benign even when they are malignant. Conversely, some e.g. cutaneous histiocytoma sometimes look malignant even although they are benign. The following are some general rules.

Criteria of malignancy (General / whole cell)

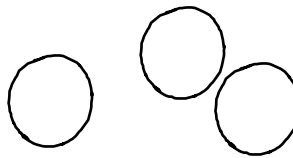


Criteria	Description	Schematic representation
Pleomorphism	Variable size, shape and nucleus:cytoplasm ratio in cells of the same type (exception: lymphoid tissue)	
Anisocytosis Macrocytosis	Marked variation in cell size, some cells > 1.5 times larger than normal	
Hypercellularity	Increased cell exfoliation (decreased cell adherence)	
Disordered alignment of the cells in clusters		

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Criteria of malignancy (Nucleus 1)

Criteria	Description	Schematic representation
Anisokaryosis	Marked variation in nuclear size especially nuclei of multinucleated cells	
Macrokaryosis	Increased nuclear size especially nuclei with diameter > 10 µm	
Increased nucleus: cytoplasm (N:C) ratio	≥ 1:2 (normal, nonlymphoid tissue: 1:3 to 1:8)	
Multinucleation	Multiple nucleation in a cell, especially important if nuclei vary in size (exception: osteoclasts, megakaryocytes)	

Criteria of malignancy (Nucleus 2)

Criteria	Description	Schematic representation
Increased mitotic figures	Mitosis is rare in normal tissue	 normal abnormal
Abnormal mitosis	Atypical mitotic figures, single chromosomes, division in three parts, incorrect alignment of chromosomes	
Atypical chromatin pattern	Cord-like, coarse, clumps, fragments, nuclear wall hyperchromasia, variations in chromatin content	
Nuclear moulding	Deformation of nuclei by other nuclei within the same cell or adjacent cells	

Artefacts

It is important to be able to recognise artefacts in smears so they are not misinterpreted. These include nuclear streaking due to too much pressure when making the smear, haemorrhage during sample collection (the presence of platelets suggests this whereas the presence of erythrophagocytosis suggests the haemorrhage was present before sample collection), and ultrasound and lubricant gel (bright pink granular material).

Limitations of cytology

Cytology can be very valuable but its limitations must be appreciated:

- The anatomic context is at least partially lost – but look for residual architecture
- Is the smear representative of the lesion?
- Beware of artefacts

INTRODUCTION TO CLINICAL PATHOLOGY

IN-HOUSE LABORATORIES

Clinical pathology may be undertaken at an external laboratory or some may be done within the practice using small biochemistry analysers (e.g. VetTest, VetScan, iStat) or haematology analysers (e.g. ProCyte, Hemat5). Cytology and blood smears may be made and stained in-house using rapid methods such as Diff-Quik®. Basic serology can also be done using simple kits e.g. FIV and FeLV, but they are not the tests of choice and their performance varies between manufacturers.

It is rarely possible or even desirable to duplicate the external Clin Path lab in the practice setting. However, provided the limitations are recognised, they can be valuable and most practices have some lab facilities.

There are both **advantages and disadvantages to having a practice laboratory**, some of which are outlined below. The most important ones are underlined.

Advantages and disadvantages of a practice laboratory

Advantages

- Useful for emergency care (rapid results)
- Adds interest to job
- Impressive for clients
- May be lower cost per test for some tests

Disadvantages

- Results may be unreliable if quality control is poor
- Requires some technical skill
- Requires interpretation – no clinical pathologist involved
- Takes time (therefore money) to do assays
- Artefacts may not be recognised
- Can slow down diagnosis due to poor test selection and interpretation
- Equipment set-up costs
- Possible legal implications if errors
- Safety issues must be observed
- No required manufacturing standards for in-house equipment

Quality control

One of the major pitfalls for in-house labs is **quality control (QC)**

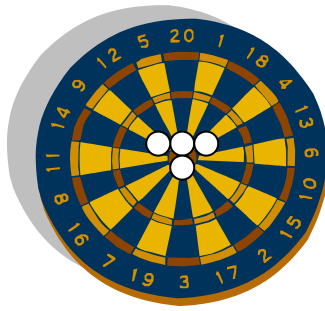
All analysers, regardless of type, require regular assessment of performance (precision and accuracy) but few practices carry this out properly, which can lead to erroneous results. There is no such thing as an analyser that does not require QC.

Precision = how similar results of repeated assays on the same sample are

Accuracy = how close assay results are to the true value



precise but inaccurate



precise and accurate



neither precise nor accurate

Quality assessment can be done by:

1. **Quality assurance (QA):** If you join an **external quality assurance programme**, unknown samples will be sent for you to test. You report the results which are compared with those of other labs (accuracy) and a summary of performance is sent back.
2. **Quality control (QC):** Analysis of a **commercial quality control serum** or a **single or pooled patient serum with known values** alongside patient samples (accuracy).
3. **Repeat analysis of the same sample** multiple times in the same sample run (within-run precision) or multiple times on different days (between-run precision)

The details of QA and QC are beyond the scope of this course but as a **minimum**, items 1. and 2. above should be carried out. External QA is carried out every few weeks/months depending on the individual scheme, whereas QC is a daily process.

BLOOD SAMPLE COLLECTION AND HANDLING

Samples should be collected into appropriate anticoagulant for the tests required.

Some vary with the lab and you should take the advice of the lab you use.

- Routine haematology and platelets – ethylenediamine tetra acetic acid (EDTA) (purple top vacutainer, pink plastic tube)
- Most biochemistry – clotted sample (serum) (red top vacutainer, white plastic tube). Many practices use gel separator tubes for serum but they cannot be used for some analyses e.g. phenobarbitone and progesterone. Their advantage is that they separate the serum from the blood cells quickly so there is less interference from leakage of blood cell/platelet contents into the serum.
- Coagulation tests – trisodium citrate (pale blue vacutainer, purple plastic tube)
- Glucose – fluoride oxalate (grey top vacutainer, yellow plastic tube)
- Hormone tests – most use serum or heparinised plasma (green top vacutainer, orange plastic tube)
- Most serology – serum for antibody, whole blood in heparin for virus isolation

Some interfering factors

- Interference with absorbance of biochemistry assays (haemolysis, lipaemia, icterus).
- Alteration of pH of enzyme reactions (haemolysis).
- Release of red cell contents which are high in some analytes or alter the reaction (haemolysis).

PRINCIPLES OF INTERPRETATION OF HAEMATOLOGY AND BIOCHEMISTRY

Interpretation of lab data requires a great deal of practice, so start now! Look at as many lab reports as you can and try to work out what (if anything) might be going on using your knowledge of the case. Rarely are diagnoses made on the basis of a single test result i.e. we are usually trying to recognise **patterns of abnormalities** in a group of tests. In many cases only part of the pattern is evident and further tests may be required. You will receive further lectures on these tests in other parts of the BVM&S course – the emphasis here is on interpreting sets of results.

Case examples will be discussed at the end of the lecture and will be on LEARN soon after the lecture.

Interpretation of haematology

It is essential to look at blood smears as well as the numerical data from the analyzer to get a full picture of the haematological findings in each sample.

Red blood cell picture

Numerical data: In order to assess the number of circulating RBCs, their size, haemoglobin and regenerative capacity, five estimations can be performed:

- Total RBC count
- Packed cell volume (PCV)
- Haemoglobin concentration (Hb)
- Mean cell volume (MCV) and mean cell Hb concentration (MCHC)
- Reticulocyte count

The **MCV and MCHC** indicate whether the **size and Hb content**, respectively, of the cells is normal. They are useful in classifying the type of anaemia present.

- A high MCV indicates macrocytosis, a low MCV microcytosis and a normal MCV normocytosis.
- A low MCHC indicates hypochromia and a normal MCHC normochromia.

For example, regenerative anaemias may be macrocytic hypochromic in some species e.g. dogs (because immature RBCs are larger and have less haemoglobin) and non-regenerative are usually normocytic normochromic. Iron deficiency anaemia may be microcytic hypochromic.

Smears: The terms **anisocytosis** (variation in size) and **poikilocytosis** (variation in shape) are useful for describing RBC morphology and can indicate the cause of anaemia e.g. spherocytes are a type of poikilocyte seen in immune-mediated haemolytic anaemia.

Reticulocytes are anucleate, immature RBCs with clumps of cytoplasmic RNA (reticulin) seen when stained with New methylene blue. The ones with a lot of reticulin can also be seen in normal blood films as slightly purple cells called polychromatophils. **Polychromatophils and reticulocytes are essentially the same thing.** In dogs and cats they appear in increased numbers in regenerative anaemia but less so or not at all in large animals. Another indicator of regeneration is normoblasts (nucleated RBCs which are one stage less mature than reticulocytes).

RBC tests

Increased RBCs, PCV and Hb occur in dehydration, fit animals, hypoxia and some tumours. Sight hounds e.g. greyhounds, whippets, borzois have “high” normal values.

Decreased RBCs, PCV and Hb - anaemia

a) *Regenerative*

Reticulocytosis, anisocytosis, may be macrocytic hypochromic

- Haemorrhagic anaemia
- Haemolytic anaemia

b) *Non-regenerative*

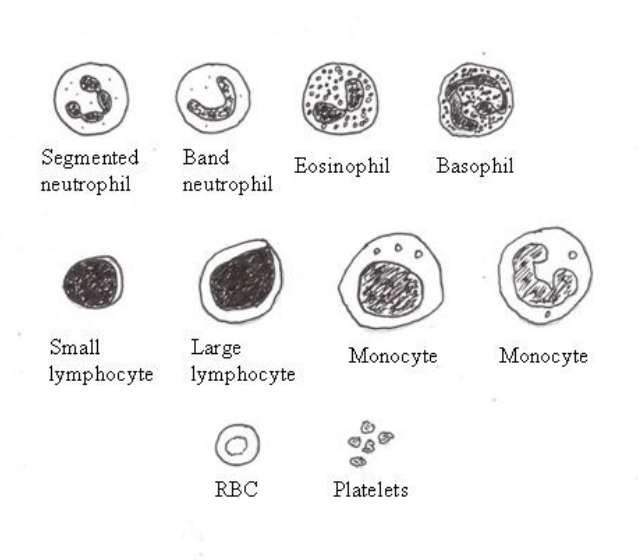
- No evidence of regeneration (usually normocytic normochromic except in iron deficiency). Some examples:
 - Pre-regenerative stage of a regenerative anaemia (it takes 2-4 days for reticulocytes to appear in the circulation)
 - Iron deficiency anaemia (usually from chronic haemorrhage)
 - Bone marrow aplasia/hypoplasia
 - Anaemia of inflammatory disease
 - Neoplastic cells in bone marrow (haematopoietic or other)

WBC tests

WBC examination includes total WBC count and a differential count on a blood smear. **Both are essential.** Some practice lab analysers give a differential count but few are reliable and they can give erroneous results with increased numbers of nucleated RBCs and in leukaemias where WBC morphology is abnormal.

Classify WBCs in smears as:

- Neutrophils (mature - segmented)
- Neutrophils (immature – bands or earlier precursors)
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils
- Other e.g. mast cells, immature blast cells



It is very important to correlate total and differential WBC counts. In dogs and cats, use differentials in terms of absolute figures rather than percentages. In horses, ruminants and rabbits, significant changes in percentage can occur without a significant increase in the total count, so examine percentage and absolute figures.

The WBC response varies between species. In the dog and cat, inflammation results in a marked increase in total WBCs, mature neutrophils and immature (mainly band) neutrophils. **An increase in immature neutrophils is called a “shift to the left”.** In cattle and sheep, the WBC changes in inflammation are less marked. Horses are intermediate between small animals and ruminants in their response.

N.B. The terms **–philia** or **–cytosis** indicate an increase and **–penia** indicates a decrease.

Neutrophils

- Neutrophilia in inflammation but also in excitement, stress and hyperadrenocorticism (Cushing’s disease).
- Neutropenia in overwhelming infections and endotoxaemia.

Monocytes

- Monocytosis in chronic (and sometimes acute) inflammation, necrosis and in stress.

Eosinophils

- Eosinophilia may be seen in hypersensitivity, parasitism, some cases of hypoadrenocorticism (Addison’s disease), neoplasia and some specific eosinophilic and mast cell disorders.
- Eosinopenia in stress/hyperadrenocorticism

Basophils

- Rarely seen in peripheral blood but basophilia may be seen in conjunction with eosinophilia and in mast cell diseases.

Lymphocytes

- Lymphocytosis in excitement and chronic infection.
- Lymphopenia in stress, lymphoma (sometimes), early viral diseases and lymph loss

Increases in each cell type may also occur in neoplasia affecting that cell line e.g. myeloid (granulocytic) leukaemia, lymphocytic leukaemia etc.

Platelets (see notes on Disorders of the circulation)

Decreased numbers (e.g. immune-mediated thrombocytopenia, bone marrow hypoplasia) or platelets with abnormal function may result in defects in primary haemostasis. Increased numbers can occur in excitement and inflammation.

Interpretation of clinical biochemistry

This will be considered briefly as more details on use of the tests will be given in separate lectures.

Routine biochemical assays can be divided into:

- Proteins e.g. total protein, albumin, globulin, haptoglobin
- Enzymes e.g. alanine aminotransferase (ALT), alkaline phosphatase (AP)
- Metabolites e.g. urea, creatinine, glucose, bilirubin, bile acids
- Electrolytes and trace elements e.g. calcium, phosphate, potassium
- Hormones e.g. cortisol, T₄, TSH
- Others e.g. therapeutic drugs such as phenobarbitone

Serum proteins: Total protein, albumin and globulin are the standard 3 measurements and **only relatively large changes in these are considered significant**. Increased albumin indicates dehydration and increased globulins suggests inflammation or an immune response. Low proteins suggest loss, or lack of synthesis. Serum protein electrophoresis shows in more detail which broad protein groups are involved. Individual proteins, e.g. the acute phase proteins C-reactive protein (dogs) or serum amyloid A (cats, horses), may

be measured using specific assays. Acute phase proteins are used to detect an inflammatory response.

Enzymes increase in the serum due to escape from damaged cells e.g. ALT from hepatocytes in canine hepatitis, or increased synthesis e.g. gamma-glutamyl transferase from bile duct epithelium in equine ragwort poisoning. Usually **only relatively large changes are considered significant for most enzymes.**

Metabolites indicate how the animal is handling certain products of metabolism e.g. glucose is increased in diabetes mellitus, urea and creatinine are increased in renal failure and bile acids are increased in severe liver disease.

Electrolytes also indicate metabolic changes in disease and loss from the body e.g. high potassium (hyperkalaemia) can occur in metabolic acidosis while hypokalaemia can occur in metabolic alkalosis or diarrhoea. **Small changes in concentration of electrolytes may be significant** as levels are normally maintained within strict limits.

Hormone assays can confirm the presence of endocrine disease e.g. low cortisol in hypoadrenocorticism, high T₄ in hyperthyroidism. However, single values are not always helpful and stimulation or suppression tests (“dynamic tests”) must be used e.g. dexamethasone suppression test for hyperadrenocorticism in dogs.

Remember, some analytes are affected by other, sometimes non-pathogenic, factors:

- Breed e.g. higher PCV and lower T₄ in sight hounds
- Age e.g. AP and phosphate are increased in active bone growth
- Drugs e.g. glucose is increased by some anaesthetics
- Exercise e.g. increased CK release from muscle

Keep practicing interpretation of clinical pathology data!