# Practical Avian Haematology

**A Hume***

## Introduction

The aim of this paper is to discuss clinically relevant avian haematology techniques. By "clinically relevant" I mean quick, simple, economical and effective methods and materials that can be used "in house". To some this may seem a retrograde step in relation to the North American and European trend towards using increasingly sophisticated outside laboratory services. At this stage Australian avian medicine has a relative lack of demand for and supply of these services for many reasons. It is hoped this paper will contribute to the growing interest in this area.

## Blood Collection [1,3,6,12,20]

a. **Blood volume**

The blood volume of birds is 6-12% of body weight. From a normal healthy bird 10% of blood volume can be safely removed, i.e. 1% of body weight.

| | | |
|---|---|---|
| SCC | 800gm | 8 mL |
| galah | 350gm | 3.5 mL |
| rosella | 200gm | 2 mL |
| budgie | 40gm | 0.4 mL |

b. **Collection equipment**

A fine needle (25 gauge or smaller) and tuberculin syringe are ideal. In smaller species, to overcome venous collapse from syringe back pressure, capillary tubes, microtainers, etc, can be filled directly from the needle hub.

c. **Sample Sites**

i. **Jugular vein**

In most birds the right jugular is larger than the left and is the vein of choice for obtaining large blood volumes. It is generally located under a featherless tract of skin between the cervical vertebrae and the trachea. With care haematoma formation is not a major problem and it can often be performed unaided.

ii. **Cutaneous ulnar (wing) vein**

This runs across the ventral aspect of the elbow joint. It is easily seen after moistening with alcohol. This vein is very prone to severe haematoma formation and usually requires assistance in obtaining samples.

iii. **Medial metatarsal (leg) vein**

Most useful in larger species, e.g. waterfowl, gallinaceous birds, raptors and pigeons. The vein is best visualised and bled on the medial side of the hock joint. Two people are needed

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but haematoma formation is minimal making it ideal for multiple sampling or drug administrations.

iv. **Toenail**

About **a** of a cleaned toenail is clipped using sharp clippers. Do not pinch or milk the toe extend the leg from the thigh and blood will flow freely. Discard the first drop if possible, make a smear from the second and collect further samples directly into capillary tubes. Haemostasis is achieved by ferric sulphate, silver nitrate potassium permanganate or cautery and observe for 10 minutes in case of haemorrhage. This method preserves other blood vessels and avoids haematomas but is not ideal for obtaining large samples. More detailed descriptions can be obtained from Vogelnest[20] or McCracken[12].

d. **Sample Containers** (3,9,12,13,19,20) (see Appendix A)

There are three main determinants; sample size available, haematology and biochemistry requirements.

i. **Microhematocrit tubes**

With or without anticoagulant. Standard 0.07mL (70uL), Caraway tubes (0.25mL), Natelson tubes (0.37mL).

ii. **Micro collection tubes** (0.6mL)

Available with EDTA or heparin and mixing beads and plasma separators if required. Both Microtainers (Becton-Dickinson) and Capiject tubes (Terumo) are useful for collecting blood directly from needle hubs.

iii. **Standard collection tubes** (1mL+)

These should be filled to at least half their capacity to minimise the effects of excess anticoagulant.

## Anticoagulants [3,4,6,7,8,9,12,13,16,17,19,20]

It is widely felt that EDTA and heparin affect cell morphology, stain quality, accelerate haemolysis, and/or change biochemical parameters to some degree. Anticoagulant choice is based on what tests are to be performed. I strongly agree with the view that blood smears be made at time of blood collection before the addition of anticoagulant.

**EDTA**

The recommended concentration for EDTA in avian blood is 1.5mg mL. Excess EDTA can cause shrinkage, degeneration and lysis of erythrocytes and affect leucocyte staining quality. In cases where less than 50% of a sample containers' volume is being used shake out excess EDTA until a few drops remain.

**Advantages**

a. Best anticoagulant for preserving cell morphology and stain quality of leucocytes (WBC) especially.
b. Clotting is prevented for up to 12 hours and WBC and haemoglobin estimates are still accurate for up to 48 hours.

**Disadvantages**

a. Blood from some species shows an unusual reaction becoming dark, viscous and haemolysed after about 15 minutes. Documented so far are *Corvidae* (crows, ravens, magpies); *megapodiae* (brush turkey, curassow, ostrich), kookaburras, crowned cranes and some penguin species.
b. It is not recommended for blood/serum/plasma to be used for clinical chemistry as it interferes with analysis of electrolytes, calcium, LDH, TPP, and lead.

**Heparin**

The recommended concentration for heparin use is 25U/mL. It is thought best to use anticoagulants in blood taken for clinical chemistry as birds are lacking in the intrinsic clotting pathway. Blood placed in plain tubes may take more than 1 hour to clot affecting biochemical parameters. Lithium heparin is preferred as the sodium or potassium forms will interfere with electrolyte estimation.

**Advantages**

a. Less interference with clinical chemistry parameters than EDTA.
b. Allows plasma to be harvested which provides a larger sample to work with compared to serum. This can be important when dealing with small species.

**Disadvantages**

a. May cause elevations of inorganic phosphate and bile acids
b. May affect cholinesterase assays
c. Prevents clotting for only 8 hours
d. Affects leucocyte staining and morphology.

## To Spin or Not To Spin

An increasingly common recommendation from avian clinical pathologists is that blood collected for clinical chemistry be placed in lithium heparin, spun down as soon as possible, the plasma harvested and placed in a plain tube. This should eliminate artefacts attributed to unseparated blood such as increases in potassium, LDH, and TPP, decreases in glucose and changes to calcium and phosphorus levels. The plasma can be used "in house" on dry chemistry analysers or sent away. Remember that any blood collected in capillary tubes is a valuable source of plasma or serum.

## Suggested Protocol for Blood Collection[12].

a. **Sample size** < 0.6mL

Make smears from fresh blood. Place rest of sample in lithium heparin tube for clinical chemistry. Although not ideal further haematology can be done if needed, before plasma is harvested.

b. **Larger samples**

Make smears from fresh blood, place small amount of blood in EDTA for further haematology and the remainder in lithium heparin for clinical chemistry.

c. **Protocol recommended by Veterinary Pathology Services (VPS) for avian blood biochemistry and haematology**

| | Volume (µL) of plasma/serum |
|---|---|
| Cal | 6 |
| Phos | 5 |
| Alb | 3 |
| Prot* | 5 |
| $HCO_3$ | 3 |
| CPK | 10 |
| Chol | 3 |
| ALP | 5 |
| AST* | 15 |
| Gluc* | 3 |
| GGT | 15 |
| Mg | 4 |
| Uric* | 7 |
| Na | 15 |
| K | 15 |
| Cl | 15 |
| TBIL | 15 |
| TBA* | 40 |

Dead Volume = 25 µL

*Primary tests recommended = 70µL + 25µL = 95µL Serum or Plasma

Full Avian profile = 210 µL sewrum/plasma minimum.

We have fopund that mnicrohaematocrit tubes submitted are suitable for Biochemistry tests, either plain or heparinised tubes. A minimum of 5 full tubes would be needed for a full avian profile. If insufficient sample submitted we would do tests in order of priority requested by the veterinarian. If no preference given then tests would be done according to our pathologists' guidelines * i.e. Primary tests.

Note: Na, K, Cl, BIL, Gluc would be the last tests attempted due to

i. Volume required;
ii. Decrease in values due to delay in analysis after collection time.

## Haematology

Manual white blood cell count : 50 µL whole blood
Haemoglobin, red cell count : 100 µL whole blood
Manual PCV, Plasma protein : 50-100 µL whole blood

Minimal volume : 200 µL whole blood

*Plus : blood smear prepared at time of collection.

## Summary

To achieve the best results in the analysis of avian blood we suggest the follwoing:

1. Clot tube minimum 1 mL blood
2. EDTA tube minimum 200-250 µL blood
   + prepared blood film.

   or

2. Lithium heparin tube min. 1.5 mL whole blood.
   + prepared blood film.
   We will run all haematology first then use plasma to run the biochemistry.

   or

3. If sample collected in microhaematocrit tubes immediately transfer to a lithium heparin tube (use a syringe to blow out the blood into lithium heparin tube). We have found even when samples are submitted in either EDTA or lithium heparin microhaematocrit tubes they still tend to clot making haematology impossible.

Option 1. Best used when blood volume is plentiful.

Option 2 or 3. Are the preferred options when small amounts of blood are collected. Haematology will be performed first and then biochemistry (as per previous notes).

## Haematology Techniques [3,4,5,9,10,11,12,13,15,18,20]

1 **Making Smears**

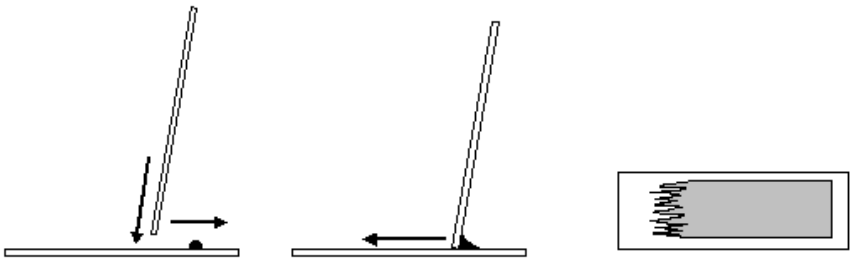
Whilst the technology capable of performing leucocyte counts (WCC), and differentials remains unavailable blood smears will be an integral part of avian haematology. The ideal smear should:

a. be simple to make;
b. consistently provide a monolayer of evenly distributed cells; and
c. cause minimal cell damage (smudge cells). These amorphous blobs are the remains of nuclei of smashed RBC's and WBC's.

**Smearing Methods.**

i. **2 Microscope Slide Wedge Technique**

This traditional method for mammal blood is regarded as the least acceptable technique for avian blood. It is associated with an unacceptable amount of smudge cells and leucocyte (WBC) margination.



A drop of blood is placed near the end of a slide. The spreader slide is placed in front of the blood at a 30 degree angle and drawn back until it touches the drop. After the blood moves across the base of the spreader it is pushed with a steady, even motion.

ii. **Bevel-Edged Microscope Slide**

This technique using a microscope slide with a shortened smearing edge has been found to decrease smudge cells and leucocyte margination[20]. The method is as above.

iii. **Perspex Spreaders**

Instead of glass slides I have used perspex spreaders extensively and found them to produce good quality push smears. Margination of WBC is decreased if minimal downward force is applied to the spreader.

**Advantages of push smears**

They are simple to perform and most people are familiar with this technique which makes for consistent smears.

**Disadvantages of push smears**

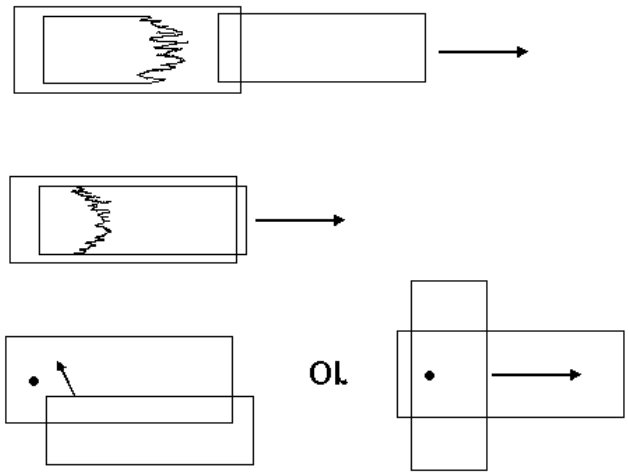
Increased smudge cells and WBC margination.

**Coverslip Techniques** [3,4,5,9,10,11,12,13,15,20]

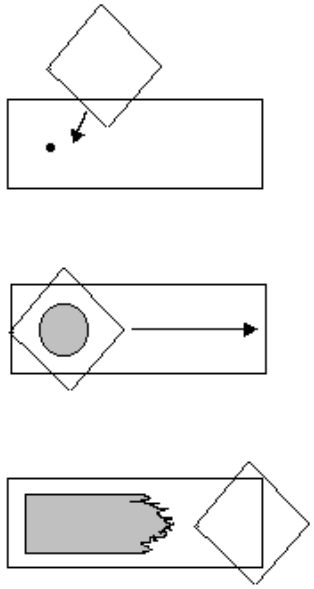
The basic method is the same. A small drop of blood is placed near the end of a slide, a cover slip is placed on it and the blood allowed to fan out. Before the blood reaches the edge of the slide pull the two apart without lifting the coverslip. A normal (but shortened) smear should be formed.

There are 3 basic variations:

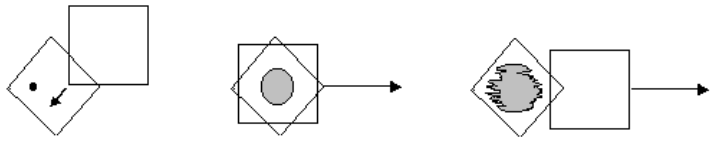
1. **Microscope slide and large coverslip (50x22mm)**




2. **Microscope slide and small coverslip (22x22mm)**



3. **2 small coverslips at right-angles**



**Advantages**

WBC margination and smudge cells are greatly reduced.

**Disadvantages**

a. The coverslip/coverslip method is highly recommended, especially by American workers[4,9,10]. However these workers have access to dip-type quick Wright's stains and coverslip staining jars that are not currently available here.
b. During transport, coverslips are more prone to damage than slides.

## To Fix or Not To Fix [3,12,15,19,20]

It has long been recommended to fix blood smears in absolute methanol if staining is delayed more than 48 hours. It has recently been shown that unfixed smears can be stored for a month without loss of staining intensity[15]. I found this to be correct and found no staining deficiencies in unfixed smears over 3 months old that had been kept clean and dry.

Perhaps more importantly I concurred with the same findings[15] that if there was a delay between fixing and staining the quality of staining of leucocyte granules especially is markedly reduced. Don't fix smears until the actual time of staining.

## Common Avian Haematology Stains [1,2,3,4,8,10,11,12,13,14,15,19,20]

**Introduction**

In this study three Romanowsky stains were evaluated. Romanowsky stains are generally defined as combinations of eosin and methylene blue in methanolic solutions. The three stains evaluated were Diff Quick, Wright's and May-Grunwald-Giemsa

**General Considerations**

Romanowsky stains do two things; fixation followed by staining.

1. **Fixation**

It has been shown that the use of strong fixatives such as methanol on air dried smears greatly facilitates stain penetration and subsequent colouring of cells[11]. This effect increases with time but it seems that little benefit is gained after 4 to 5 minutes of fixation. It is also reported that fixation times of greater than 1 minute can disrupt WBC granules[4]. So far I have not found this to be significant. What is important is that fixing solutions remain water free as water causes many serious artefacts in unfixed smears. Poorly fixed stains will be under stained or show patchy staining especially of nuclei.

2. **Staining**

The time and method vary greatly. Interestingly (confusingly) the instructions accompanying many commercial stains and stain recipes can be quite different for ostensibly the same stain. Initially I attempted to make various Wright's, Wright-Giemsa and May-Grunwald-Giemsa (MGG) stains following a number of recipes[1,2,10,12,20]. This proved to be time consuming, laborious, required expensive ingredients and laboratory equipment and failed to produce satisfactory stains. Another problem is that in Australia powdered stains are not required to be certified as they are in the USA so quality and results are variable.

In contrast I was able to purchase good quality economical and reliable stains from two sources; Bacto Laboratories and Fronine P/L. Their specific instructions are supplied if requested.

It is a good idea to mount dry, stained smears with coverslips and mounting media. This preserves them indefinitely and creates a useful reference library. Stains may be rapidly dried using a hair drier.

3. **Buffers**

Many stain recipes require the use of buffers. These can be bought ready made or made up from tablets. In general acid pH will favour reds and alkaline pH, blues. However in most cases I found the same or better results came from using good quality distilled or deionised water.

**Diff Quick** [1,3,10,12,13,20]

A three-part stain consisting of a methanol-based fixative, eosin and methylene blue. Usually longer staining times are required for avian blood. Adjust staining times to effect and slides can be stained, dried, examined and restained if desired. Diff Quick works best if the reagents are well protected from moisture and evaporation. It deteriorates rapidly, especially the fixer, and this will cause a marked decline in stain quality.

**Advantages**

a. Rapid, versatile and ubiquitous
b. It is a good stain for examining RBC parasites and mononuclear cells.
c. With experience it is very effective in clinical situations if immediate basic information is required.

**Disadvantages**

a. Granulocytes can be difficult to differentiate especially heterophils and eosinophils due to coalescence or disruption of granules
b. RBC polychromasia may not be evident
c. Subtle WBC changes indicating problems may not be as obvious as with other stains.

**Instructions**

**Step 1 Fixation**: Recommended fixation times vary from 5 seconds to 3 minutes. At least 30 seconds is recommended.

**Step 2 Eosin**: 10 to 20, 1 second dips, drain off excess. Extra dips will increase cytoplasmic and granule eosinophilia.

**Step 3 Methylene Blue**: 10 to 20, 1 second dips, drain off excess. Extra dips in this solution will increase nuclear and granule basophilia.

**Step 4**: Rinse slide with firm stream of water to remove excess sediment. Tap water is OK. Air or blow dry.

## Wright's Stains [1,3,10,11,12,13,20]

These are the most commonly used avian haematology stains.

**Advantages**

a. Provide excellent structural detail enabling cell differentiation and accurate assessment of subtle pathological changes .
b. They are readily available, economical and easy to use.

**Disadvantages**

a. Is slower than Diff Quick
b. Inconsistent staining of nuclei. This is mostly seen in heterophils, blast cells and spleen and bone marrow smears. Nuclear staining can be enhanced by following up with a Giemsa stain.

**Wright's Staining Procedures**

Good, consistent results were obtained with three commercially prepared Wright's stains. Two standard stains coming from Bacto Laboratories and Fronine NSW and a recently arrived "fast" Wright's stain supplied by Lyppard NSW.

Wright's stains have 2 parts. Firstly fixation of the smear usually by the methanol in the undiluted stock solution with little or no staining occurring at this stage. Secondly the addition of buffered or distilled water that causes staining.

**Method For Bacto Laboratories and Fronine NSW Wright's stain.**

Note: The instructions received from each of these companies I found to be unnecessarily complex and time consuming. The directions below are my recommendations and not endorsed by either company.

**Step 1**: Place air dried, unfixed smear on staining rack
**Step 2**: Fixation. Flood smear with stock solution for 2-4 minutes.
**Step 3**: Staining. Add an equal volume of distilled or buffered (pH 6.4 - 6.8) water. Gently blow on slide to facilitate mixing. A greenish, metallic sheen should appear. Leave for 3-6 minutes
**Step 4**: Rinse quickly with distilled or buffered water and shake off excess.
**Step 5**: Clean back of slide with alcohol moistened swab and air or blow dry.

**Remember**

a. Increased fixation time will increase stain depth and intensity especially of nuclei.
b. Increased staining time will increase stain depth and intensity. Excessive basophilia indicates overstaining.
c. Increasing the pH of the distilled water or the buffer will generally cause increased basophilia and decreasing it will enhance eosinophilia.
d. As stains age staining times will increase.

**"Finger Tip" (R) Wright's Fast stain**

The apparent advantage of this stain is a short stain time and done in a single step. Unfortunately following the instructions did not produce good results despite repeated efforts. The instructions require a pre fixed smear be used but no method or time is indicated. Other instructions are somewhat unclear.

However the following method produced excellent results:

**Step 1**: Place air dried unfixed smear on rack
**Step 2**: Flood with stain for 3 minutes. Increase or decrease time to suit.
**Step 3**: Rinse quickly with distilled water.
**Step 4**: Shake off excess water and air/blow dry.

**May-Grunwald-Giemsa or Pappenheim Stain** [1,11,13,14,15,20]

May-Grunwald-Giemsa (MGG) is commonly used in Europe and Canada and readily obtained from Fronine NSW.

**Advantages**

a. It is a relatively intense stain and is excellent for staining blast cells, haemopoetic tissues.
b. It gives good nuclear detail making it very useful in differentiating mononuclear WBC's

**Disadvantages**

It is much longer and more complex than Diff Quick or Wright's stain taking 15-20 minutes.

**Methods for MGG**

a. **Adapted from Miller**[13]

**Step 1**: Place air dried, unfixed smear on rack
**Step 2**: Dilute May-Grunwald stain 1:1 with distilled water and flood slide for 2.5 minutes.
**Step 3**: Add equal amount of buffer (pH 6.8) for 1 minute then rinse briefly.
**Step 4**: Dilute stock Giemsa 40 drops in 40mL of buffer (pH6.8) and flood slide for 11 minutes.
**Step 5**: Rinse in buffer and air/blow dry.

b. **As described by Lucas and Jamrosz**[11]

**Step 1**: Flood slide with May-Grunwald stock solution for 6 minutes.
**Step 2**: Add equal amount of distilled water for 1.5 minutes. Then shake off excess, don't rinse.
**Step 3**: Dilute stock Giemsa 1:10 with distilled water and flood slide for 15 minutes.
**Step 4**: Rinse in distilled water and air/blow dry.

## Conclusion

In my opinion the stain that best fulfils the criterion of being quick, simple, economical and effective in a clinical setting would be a Wright's stain followed by Diff Quick. There are a number of other Wright's 'quick stains' which are not yet available in this country but hopefully this will soon change.

**Avian Clinical Haematology: "In House" Techniques** [1,3.4,5,6,8,9,10,11,12,13,15,18,19,20]

This discussion will concentrate on parameters readily collected in a clinical setting. The referenced texts provide excellent descriptions of the more involved techniques.

### Evaluating Erythrocytes

Packed cell volume (PCV), total red cell count (RBC), haemoglobin concentration, MCV, MCH, MCHC and reticulocyte count can be calculated for birds using methods developed for mammalian blood but modifications are often required. As avian RBC's are relatively large ( RBC size roughly increases in proportion to species size) and nucleated.

### Packed Cell Volume

A quick, practical method for evaluating the avian erythron. Spin at 12,000g for 5 mins. Although many factors such as age, sex, species, diurnal variation, season, etc can affect PCV the normal range for most birds is 35-55%.

Additional information can be obtained by examining the plasma in the capillary tube:

a. Total plasma protein (TPP) can be done with 2.5 to 5gm/dL being normal for most species.
b. Lipaemia may be dietary (seed diets) or may indicate ovarian activity prior to egg laying.
c. Buffy coat can give a rough estimate of total WCC with less than 1% considered normal.
d. Light yellow plasma is commonly caused by carotenoids in the diet. Darker shades can indicate jaundice.
e. If TPP is not performed plasma can be saved for clinical chemistry.

### Polychromatophilic Index

With Wright's stain immature RBC tend to be more rounded, have a basophilic cytoplasm, and a less densely staining nucleus (polychromasia). Normal birds have 1-5% polychromasia but numbers can rapidly increase with blood loss. Also many normal birds can moderate levels of polychromasia which is thought to be due to the relatively short lifespan of avian RBC's (20 to 45 days).

### Avian Polychromatic Index

| Grade | Comment | % polycromasia |
|---|---|---|
| 1 | almost no polychromasia | 1-5 |
| 2 | A few polychromatic cells | <10 |
| 3 | moderate numbers | 10-20 |

| | | |
|---|---|---|
| 4 | Significant numbers | 20-50 |
| 5 | Highly polychromatic and significant poikilocytosis | >50 |

**A simpler reported method is**:

| | |
|---|---|
| Slight | 5-10% |
| moderate | 10-20% |
| Heavy | 40-50% |

Other parameters possible but not terribly practical in a clinical situation are;

1. Manual reticulocyte counts using vital stains
2. Total RBC count. Due to differences in avian RBC it is felt that this parameter is less useful than a PCV so it is not often performed.
3. Haemoglobin estimates and red cell indices can be done

**Avian Thrombocytes** [1,3,5,8,10,11,12,13,20]

Avian thrombocytes (TBC) are nucleated cells ranging from 20,00-30,00/ul or approximately 1 TBC/75 RBC or 1-2 TBC/oil immersion field (OIF) at 1000x magnification. Total TBC count is difficult to calculate due to TBC tendency to clump. An estimate can be made from a smear using the following formula[20]:

$$\text{Estimated TBC/ul} = \frac{\text{average number of TBC in 5 OIF}}{1000} \times 3{,}500{,}000$$

3,500,000 = average number of RBC in 5 OIF
1000 = average number of RBC/mL of blood

If PCV is outside the normal range a correction can be made:

$$\text{Corrected TBC/uL} = \text{estimated TBC} \times \frac{\text{observed PCV}}{\text{Average normal PCV (47.7\%)}}$$

**Significance of changes in thrombocytes**:

Reactive thrombocytes are characterised by cytoplasmic bleb formation and vacuolation. They are seen in serious viral or bacterial infections, heavy metal toxicities, anaemia's and chronic disease.

Thrombocytopaenias are noted in septicaemia's, DIC and haemopoetic neoplasia.
Thrombocytosis is seen with severe infections and parasitaemias.

**Leucocytes** [1,3,5,8,10,11,12,13,18,19,20]

**Total Leucocyte Count (WCC)**

Due to the presence of nuclei in avian erythrocytes and thrombocytes automated cell counters used for mammalian blood are not useful. Manual methods have to be used which are time consuming and require experience in identifying the different cells. There are two basic approaches:

1. Direct counts using a haemocytometer
2. Estimated counts from a stained blood smear.

**Direct Methods** [1,3,6,8,12,13,19,20]

In this study direct methods were not used for several reasons.

a. They tend to be time consuming, complex and require extra equipment and solutions making them less applicable to a clinical situation.
b. The solutions tend to deteriorate rapidly so cell counts must be performed without delay
c. It is debatable that they are any more accurate than estimated methods due to technique errors and cell damage caused by transportation time and anticoagulants.

**Indirect Methods** [3,4,5,9,10,12,13,19,20]

**Advantages**

a. With practice they are simple, quick, accurate and consistent.
b. If smears are made at time of blood collection they avoid the effects of anticoagulants and transportation.
c. They can be examined at any time especially if they are mounted with a coverslip.
d. Less equipment and expense are involved.

**Disadvantages**

a. Monolayer smears with evenly distributed cells that are well stained must be made consistently.
b. Experience is needed in recognising cells.

**Method 1**

This is probably the most commonly used and best regarded. Scan the smear on low power to check general cell distribution and find a well stained monolayer. A straight edge is ideal and parasites such as microfilaria should be watched for at this stage. Formula for estimation of total WCC:

1. Count all WBC in 10 fields using high, dry objective (400x)
2. Divide by 10 to get the average.
3. Multiply by 2 which gives the number of WBC x 10(9)/l

The estimate should be given as a range and a common guideline used is;

| **WBC x $10^9$/L** | **Range Spread** |
|---|---|
| up to 25 | 2 |
| 25-40 | 4 |
| 40-65 | 5 |
| 65-140 | 10 |
| 140+ | 20 |

e.g., a total WCC of 26 x $10^9$/L would be given as 24-28 x $10^9$/L

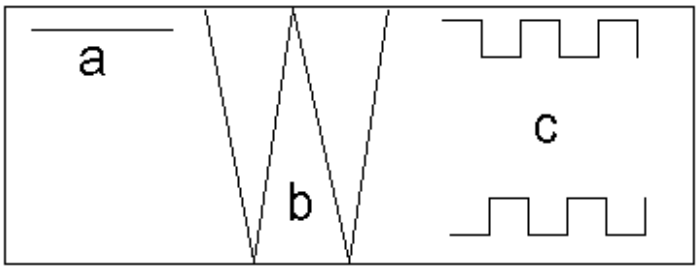
If PCV is abnormal the estimated WCC can be corrected using the following formula;

$$\text{Corrected WBC} = \text{total WCC} \times \frac{\text{observed PCV}}{\text{Normal PCV (47.7\%)}}$$

WCC estimations using buffy coat are quite subjective but normal birds have a buffy coat of < 1%

## The Differential Leucocyte Count [1,3,4,5,9,10,11,12,13,18,19,20]

Accuracy depends on having a well stained monolayer smear, the counting method and experience. Counting is done using oil immersion (1000x) and 100 cells should be counted. More cells can be counted but statistically greater accuracy does not occur until 400 cells are counted.
Consideration should also be given to general cell morphology, polychromasia and blood parasites. Besides random selection of fields, there are 3 basic methods:




a. Straight edge, i.e. counting along the edge of the smear.

b. Cross section. Counting fields as you cross from one side of the smear to the other.

c. Battlement. This is the preferred method and reported to be the most comparable to counting the cells on the whole film[18]. A straight edge is found and three horizontal edge fields are counted followed by two vertical fields then two horizontal fields and so on until 10 fields are counted.

## Basic avian Blood Cell Morphology [1,3,4,6,8,9,10,11,12,13,19,20]

Avian blood cells are more difficult to examine than mammalian ones due to both variations in the same cell types and similarities among different cell lines. Whilst there are many good descriptions of avian blood cells there are few texts with comprehensive illustrations especially of aviary species. Some of the better examples are noted in the references, specifically 1, 8, 11, 12, 19 and *A Colour Atlas of Veterinary Haematology* by Hawkey CM and Dennet TB (1989), Wolfe Medical Publications Ltd, London.

In general the cells should be examined with regard to

a. Size, shape and mature versus immature forms

b. Cytoplasmic colour, granules, parasites and vacuoles.

c. Nuclear shape, staining and chromatin structure.

The following brief descriptions are based on Wright's stained cells.

**Erythrocytes**

a. Mature erythrocytes

Oval cells of uniform size and shape that generally increase in size proportional to the size of the species. The cytoplasm is usually orange pink with no inclusions except for parasites. The nucleus is centrally placed with condensed, darkly staining cytoplasm.

b. Immature erythrocytes.

Have a more rounded shape. The cytoplasm has a bluish tinge (polychromasia). The nucleus has less condensed nuclear chromatin.

c. Erythroblasts

Round cells with a high nuclear:cytoplasmic ratio and a dark blue cytoplasm. Uniform dark staining nucleus often with nucleoli.

**Thrombocytes**

These have a phagocytic as well as haemostatic function. They are an oval, nucleated cell, smaller and more rounded than erythrocytes. They are often clumped or in twos and threes that can help differentiate them from small lymphocytes. The cytoplasm is colourless to pale blue and may have formed blebs or may not be visible. The nucleus more rounded than an erythrocyte, and the chromatin is deeper and more uniformly stained.

**Granulocytes**

a. **Heterophils**

The most common granulocyte and the most common WBC in the majority of species. They are the functional equivalent of the mammalian neutrophil.

**Mature Heterophil**

Round cells with a uniform size and shape on the same smear. The cytoplasm is colourless or occasionally pink if degranulation has occurred. The characteristic granules are usually rod or spindle shaped. They may be long and thin or short and fat. The nucleus may fail to stain consistently with Wright's stain. It is lobular with 2 or more lobes.

**Immature Heterophils.**

Uncommon in healthy birds. They are often larger and significant numbers suggest a poor prognosis. They have fewer granules which are often spherical or otherwise different from mature forms in the same smear. The nucleus is unlobulated, round or bean shaped.

**Toxic heterophils**

Changes such as basophilic cytoplasmic granules, nuclear and cytoplasmic vacuolation and degranulation are seen.

b. **Eosinophils**

Their function is unclear but they often increase with parasitism. They are uncommon (2-3%) and have variable morphology between species. They are a similar size and shape to heterophils. The cytoplasm stains pale blue. The granules are usually round and stain brick red or they may be absent. Ducks tend to have rod shaped granules and psittacines may show variable basophilic staining. The nucleus is lobed and often stains darker than heterophil nuclei in the same smear.

c. **Basophils**

The most easily recognised granulocyte. It is round and has a similar function to mammalian mast cells. The cytoplasm is clear but mostly obscured. The granules are round, uniform

size and intensely basophilic. The nucleus is usually single lobed, pale staining and often obscured by the granules.

**Mononuclear Leucocytes**

a. **Lymphocytes**

They are the most common mononuclear leucocyte and the most common leucocyte in the so called lymphocytic species; e.g. canaries, finches, chickens and some amazons. The resemble lymphocytes of mammals. Mature lymphocytes are as the small and medium sizes whilst large lymphocytes are the rarer immature form. They are generally round but may be moulded to the shape of adjacent cells. The cytoplasm is basophilic and occasionally contains magenta granules. Reactive lymphocytes show cytoplasmic blebbing. The nucleus is usually central with a high nuclear:cytopasmic ratio. The chromatin stains darkly and is densely clumped. Note; small lymphocytes can be confused with thrombocytes.

b. **Monocytes**

Less common than lymphocytes they are often seen in chronic diseases such as chlamydia and TB. They are relatively big cells but there is a confusing over lap between smaller monocytes and larger lymphocytes. They tend to be round cell with a smooth outline (no blebs). They have much more cytoplasm than lymphocytes that is greyish, reticular, and often vacuolated. The nucleus is often eccentric and paler staining than lymphocytes It is often kidney shaped due to a shallow indentation that faces centrally. In contrast to lymphocytes the chromatin is smooth and reticular.

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# Appendix.

**List of Products and Suppliers**

| Product | Supplier |
|---|---|
| Natelson Tubes | Crown Scientific PMB 8, Moorebank NSW 2170 |
| Wright's stain<br>Giemsa stain<br>Buffer | Bacto Laboratories P/L, PO Box 295, Liverpool NSW 2170 |
| Microtainers | Becton-Dickinson P/L, 80 Rushdale St, Knoxfield VIC 3180 |
| May-Grunwald Stain<br>Wright's stain | Fronine P/L, 33/2 Richard Cl N Rocks NSW 2151 |
| Wright Finger Tip Stain | Lyppards P/L, 3/5 Hudson Ave Castle Hill NSW 2154 |
| Terumo Capijects | Allhank Trading Co, 102 Tope St, S Melbourne, VIC 3205. |