Full Blood Count analysis…
Is a 3 part-diff good enough?

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The Role of the FBC in clinical decision making

History

Examination

Investigations

Decision

70%

FBC

Laboratory tests

FBC – most frequently requested laboratory test
Changing focus of Hematology in laboratory practice.....
Evolution of analytical capabilities of automated Hematology Analysers

1953

Coulter Counter Model A
First automated cell counter
WBC & RBC only
10 minutes per sample

2015

Sysmex XN series
State of the Art Haematology Analyser
28 standard + 16 optional parameters
>100 samples /hour
<table>
<thead>
<tr>
<th>The Traditional Full Blood Count</th>
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<tbody>
<tr>
<td><strong>Haemoglobin</strong></td>
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<tr>
<td><strong>Haematocrit</strong></td>
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<tr>
<td><strong>Red Blood Cells</strong></td>
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<tr>
<td><strong>MCV</strong></td>
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<tr>
<td><strong>MCH</strong></td>
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<tr>
<td><strong>MCHC</strong></td>
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<tr>
<td><strong>RDW</strong></td>
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<tr>
<td><strong>White Blood Cells</strong></td>
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<td><strong>Platelets</strong></td>
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Why do clinicians ask for an FBC?

Anaemia  →  RBC

Infection  →  WBC

Bleeding  →  Platelets
The laboratory Approach to Anaemia

Is anaemia present? [Hb]

How severe is it?

What type of anaemia is it? [Red Cell Indices]

What is the cause of the anaemia? [Morphology (blood Smear)]
Red Cell Size

- Microcytic
- Normal RBC
- Macrocytic
- Fragments

Anisocytosis - Variation in cell size

Red Cell Distribution Width (RDW)
RBC and Platelet Histograms

- Distribution curves are separated by flexible discriminators.
- The histogram curve should start and end at the base line within the discriminators.
Red Cell Distribution Width

- **RDW Normal**
- **RDW High**

**RDW-CV**

**RDW-SD**

- MCV 60 fl
- MCV 90 fl

- $\beta$ Thalassaemia Carrier
- Iron Deficiency Anaemia
- Normal
Reticulocytes

Very young red blood cells with remnants of RNA

Normally lifespan -2 days in bone marrow, 1 day in peripheral blood

RBC lifespan ~ 120 days

Therefore $\frac{1}{120}$ of red cell mass has to be replaced each day.

Normal range Reticulocytes 0.2 – 2%

RETICULOCYTE count not part of “traditional FBC”
What’s the value of the Reticulocyte Count?

Presence of reticulocytes is an indication that bone marrow is producing RBCs.

Increased RETICS $\Rightarrow$ Red Blood Cell production hyperactive.

Low Hb but **NO INCREASE** in RETICS $\Rightarrow$ BM production problem.

<table>
<thead>
<tr>
<th>Normal Hb</th>
<th>High Retics</th>
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<td>Loss/destruction of RBCs in periphery</td>
<td>BM still able to compensate</td>
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<table>
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<th>Low Hb</th>
<th>High Retics</th>
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<tr>
<td>Rate of loss/destruction $&gt;$ BM capacity to produce</td>
<td></td>
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RETIC count allows distinction between peripheral cause and BM cause of anemia.

Report as RPI.
Does this patient have an infection?
High WBC? – probably
Low WBC? - maybe

Can we predict what kind of infection?

P.S. reactive and malignant causes of WBC changes
Why do we need a differential count?

- Traditional FBC – Quantitative White Cell Count
- A normal WBC does not mean that all is well
- WBCs are comprised of a number of sub-populations with diverse biological function
- WBC not very informative in absence of Differential Count
Normal WBC sub-populations

- Lymphocytes
- Monocytes
- Neutrophils
- Eosinophils
- Basophils
Manual Differential count

- Reference method
- Manual microscopy
- Thin smear with Romanowsky stain
- CLSI guidelines - 2 smears from same specimen tube
- 4 operators to perform 200 cell count each
- Report AVERAGE of all counts!
- Extremely laborious
- Common practice – 1 operator counts 100 cells on 1 smear

Best Practice 800 cell count  
Actual Practice 100 cell count
Automated Differential Count

- Advances in technology
- Automated differential count
- Major benefit of speed & accuracy
- Most analysers use impedance technology
- In contrast to manual cell count \(~15,000\) cell count
- Automated analysers 3PD or 5PD
3 Part Differential Count

- Less complex haematology analysers use impedance technology to separate cells based on size
- Red cells are lysed
- Three distinct groups based on cell size are identified:
  - Large cells or granulocytes
  - Small cells or lymphocytes
  - Medium cells or monocytes or “middle” cells.

Size here refers to the size of cells after exposure to reagents within haematology analyser – not natural size
SYSMEX 3PD – (POCH100i, KX21N & XP-300)

 Identifies NEUTROPHILS as a separate population
Relative Cell Sizes

- Platelet
- RBC
- Giant Plt
- RBC fragment
- Lymphocyte
- Monocyte
- Neutrophil
- Eosinophil
- Basophil
- NRBC
How good is the 3 part differential?

- Precision is very good compared to “proper” manual cell count
- Accuracy less good – especially if person is unhealthy
- Relative cell counts and morphology become altered
- Automated analysis is a significant advancement, BUT generation of only a 3 part differential for pathological samples is not ideal.
- In health, the 5 major sub-populations are within so-called normal limits and ratios (% counts).
- BUT in DISEASE, the differential count is often abnormal
Differential Count in Disease

- Ratios become distorted → percentage counts are meaningless in the absence of absolute values.
- Sub-populations can increase, e.g. eosinophils may be increased in response to an allergic reaction.
- Sub-populations can decrease, e.g. lymphocytes typical become progressively reduced in untreated HIV infection.
- Immature cells that are normally only found in the bone marrow can appear in the peripheral blood, e.g. immature granulocytes in patients with severe infection.
- Immature cells that are abnormal can appear, e.g. blasts in patients with acute leukaemia.
Why should one consider a 5PD rather than a 3PD?

A 5 part differential cell count provides much more information than a 3 part differential cell count in identifying the cause of possible illness in sick people.
5 Part-Differential Count

**Percentage Count (%)**

**Absolute Count (#)**
Automated 5PD analysis

- Rely on a combination of two types of measurements for WBC differentiation:
  - volumetric impedance
  - high frequency electromagnetic energy
  - optical light scatter
  - cytochemical staining techniques for WBC differentiation.
- The principle difference to 3 part differential technology
  - Cell identification relies on a two dimensional analysis rather than just on cell size.
SYSMEX 5PD automated analysis

- fluorescence flow cytometry
- Sub-populations separated on basis of
  - cell complexity or side scatter
  - fluorescence signal – measure of DNA/RNA content.
- Adaptive cluster analysis system (ACAS) software
  - Ensures that each cell population forms a clear cluster before all events are counted as belonging to that cell subtype.
  - In contrast NON Sysmex systems use fixed gating which sometimes causes cells to be counted as part of an incorrect group, especially in pathological specimens.
Gating strategies for WBC differentiation

Non SYSMEX Flexible ACAS gating

SYSMEX Fixed gating
Sysmex Fluorescence Flow Cytometry
So what are the benefits of 5PD over 3PD?

- Benefits are greater than just the expansion from a 3PD to 5PD count.
- The answer lies in the underlying technology of fluorescence flow cytometry.

So why is fluorescence flow cytometry based white blood cell differential counting superior to 3 part differential and competitor 5 part differential technologies?
Advantages of Fluorescence Flow Cytometry

- Assessment is independent of cell size
- Identification of immature cells
- Extensive flagging system for identification of abnormal cells
Differentiation independent of Cell Size

• 3PD and all other non-SYSMEX 5PD systems rely on size – limitations

• Cell size changes occur quite rapidly once blood is collected into EDTA
• Progressive cell swelling and ultimately disintegration over time.
• Differential counts relying on cell size therefore become unreliable within 24 hours.
• In contrast Sysmex X-class analysers produce a reliable differential count in specimens up to 48 hours post collection.
Ability to identify immature cells

- Immature cells have higher nucleic acid content in comparison with their mature counterparts.
- 6 part differential count by addition of immature granulocytes (IG).
- The IG count includes promyelocytes, myelocytes and metamyelocytes but not band cells.

The presence of immature granulocytes is always pathological except:
- immediate post-partum period and a neonate less than 3 days old.

Precision of automated IG count is much better than manual microscopy making it ideal for serial monitoring of patients thereby eliminating labour intensive manual counting.
Extensive flagging system for identification of abnormal cells

Major advantage of 5 PD analyser over a 3 PD analyser is the sophisticated flagging system

Enables qualitative identification of immature and abnormal cells

3PD analysers also have a flagging system but it is less informative as it is based entirely on cell size aberrations.
Flags and an messages

- Almost all analysers provide some kind of flagging system for abnormalities detected
- DO NOT IGNORE them – they provide very important information which will guide further review on the same sample in the lab OR aid diagnosis
- Histograms and scatterplots
Summary of FBC

Traditional FBC

- HB
- RBC
- HCT
- MCV
- MCH
- MCHC
- RDW
- WBC
- PLT

EXTRA Parameters – routinely available

- Reticulocytes
- Differential Count
  - Neutrophils
  - Lymphocytes
  - Monocytes
  - Eosinophils
  - Basophils
Peripheral Blood Microscopy

Traditional FBC – **quantitative** information

Often need manual review of peripheral smear to assess **qualitative** features

**BUT**

- Time consuming
- Skilled staff required
- Extra materials required
Is there still a need for manual differential count?

YES, BUT not to count cells.

Automated count >>>>> more precise than manual count

Only for review of specimens that analyser flags as suspect for abnormal cells - to confirm morphological abnormality

NOT routine manual differential counting

But rather **selective** manual smear review
Consequence of poor quality smears

Erroneous microscopic interpretation
Incorrect differential count
Pathology can be missed or over-diagnosed

**May have serious consequences for patient care!**

**SOLUTION** – standardisation

Best form of standardisation is automation
In most labs limited to staining – but mostly “open” systems
Any stain can be used
Only timing is controlled

Potential for stain overuse, “home-made solutions” not eliminated

Automated slide-makers usually only available in large labs
To ensure that microscopic review will provide a report that can be trusted for clinical judgement........

**Quality of smear and stain MUST be optimal**

**Best way to achieve this is by means of automation of slide-making and staining**
Sysmex Staining Solutions – RAL Stainbox and RAL Stainer

Semi-Automated Solution
• 5 slides at a time
• Various staining protocols
• Methanol free stain
• 300 slides/kit or 28 days
• Timing, rinsing, drying – controlled.

Fully Automated Solution
• 10 slides at a time
• Various staining protocols
What else does a 5PD analyser offer beyond WBC information?

The advanced technology together with specific reagents allows for qualitative and quantitative detection of other cell populations

e.g. Advanced RBC and PLT information
Thank you very much for your attention!