
sysmex

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

+

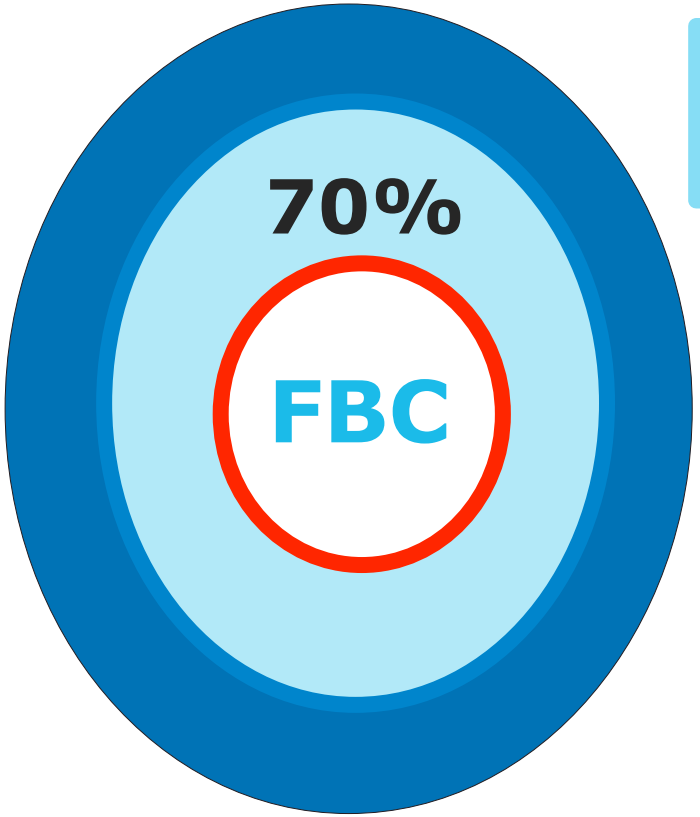
Examination

+

Investigations

=

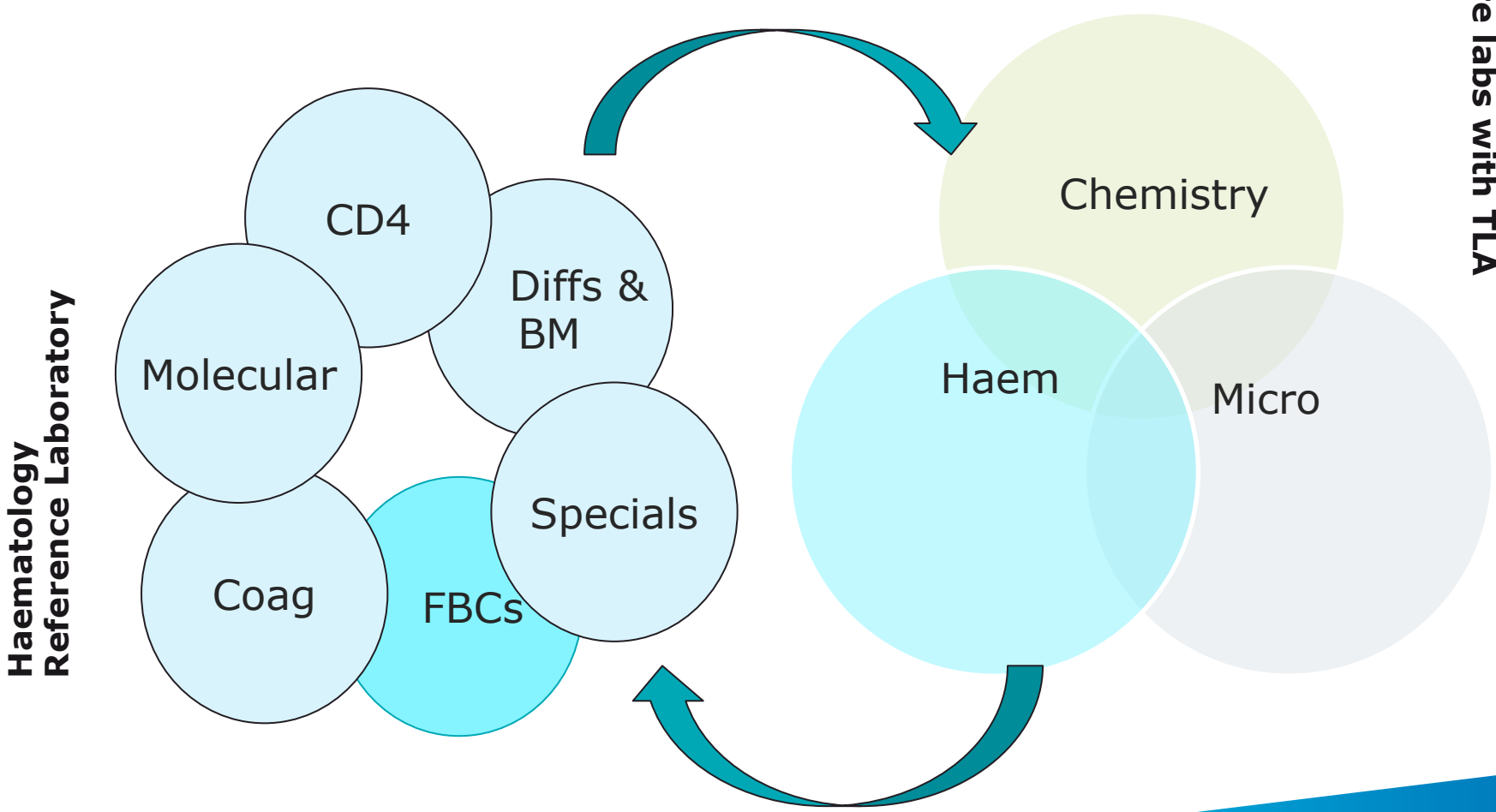
Decision



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

Major technological advancement

2015

Sysmex XN series

State of the Art Haematology Analyser

28 standard + 16 optional parameters

>100 samples /hour

The Traditional Full Blood Count

Haemoglobin

Haematocrit

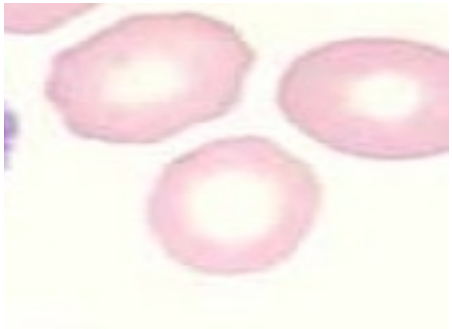
Red Blood Cells

MCV

MCH

MCHC

RDW



White Blood Cells

Platelets

Why do clinicians ask for an FBC?

Anaemia	→	**RBC**
Infection	→	**WBC**
Bleeding	→	**Platelets**

The laboratory Approach to Anaemia

Is anaemia present? **[Hb]**

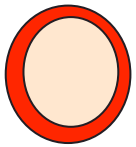
How severe is it?

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

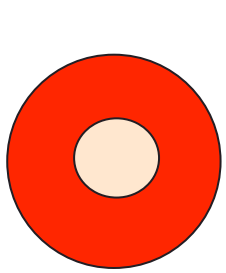
What is the cause of the anaemia? **[Morphology (blood Smear)]**

Red Cell Size

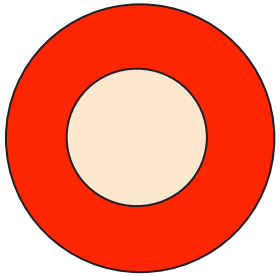
Microcytic

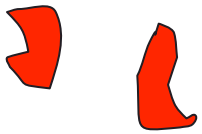


Normal RBC



Macrocytic



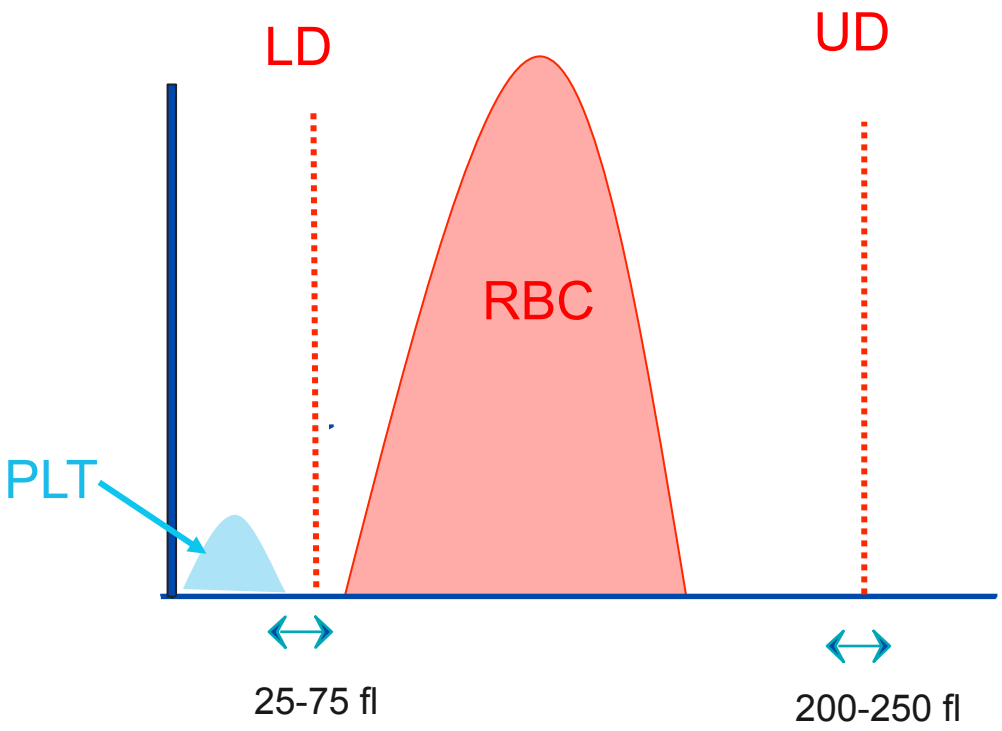


Fragments

Anisocytosis - Variation in cell size

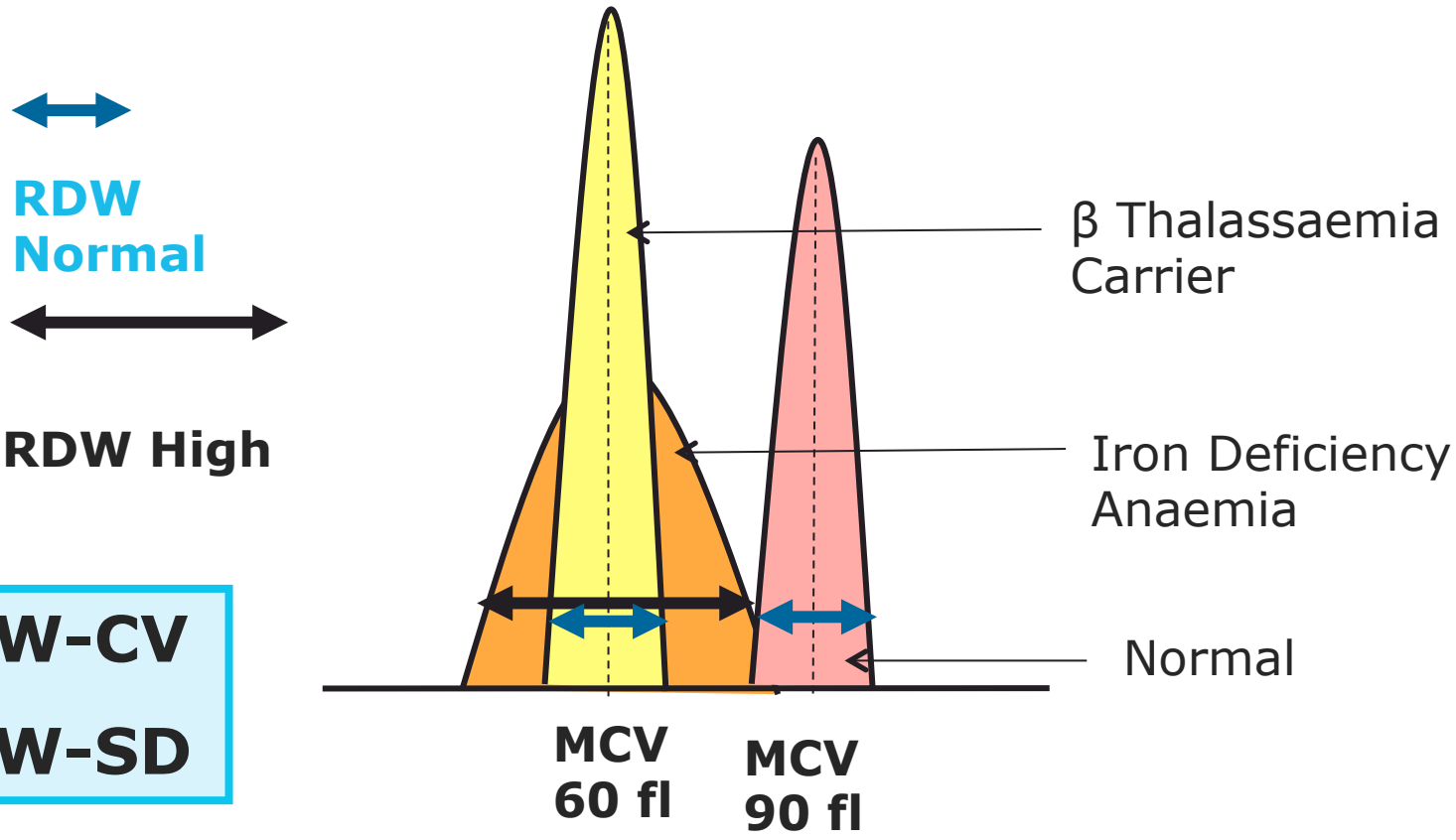
Red Cell Distribution Width (RDW)

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-CV

RDW-SD

Reticulocytes

Very young red blood cells with remnants of RNA

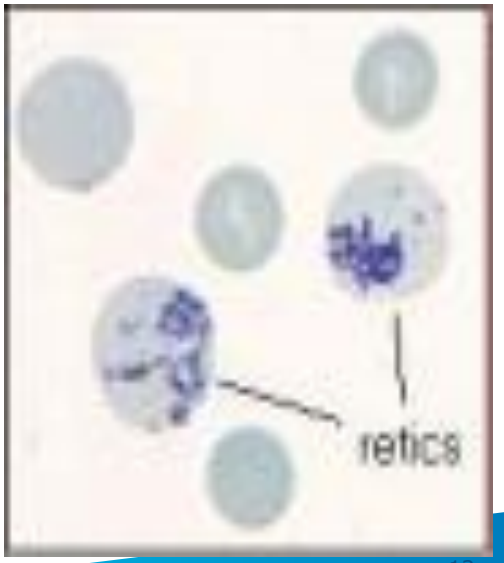
Normally lifespan -2 days in bone marrow, 1 day in peripheral blood

RBC lifespan ~ 120 days

Therefore 1/120th 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 ⇨ Red Blood Cell production hyperactive

Low Hb but NO INCREASE in RETICS ⇨ BM production problem

Normal Hb **High Retics**	Loss/destruction of RBCs in periphery BM still able to compensate
Low Hb **High Retics**	Rate of loss/destruction > BM capacity to produce

Report as RPI

RETIC count allows distinction between peripheral cause and BM cause of anemia

Traditional White Blood Cell Count – Clinical value

Does this patient have an infection?

High WBC? – probably

Low WBC? - maybe

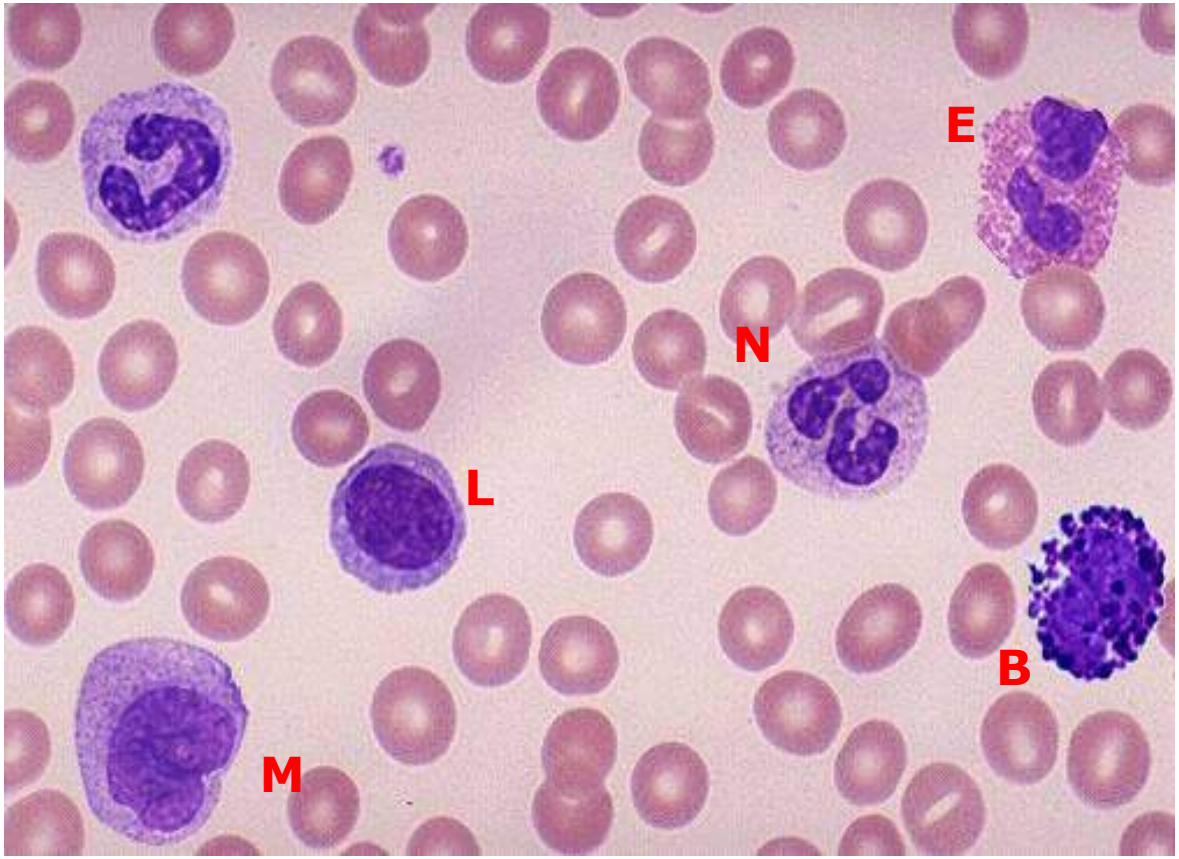
Can we predict what kind of infection?

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**

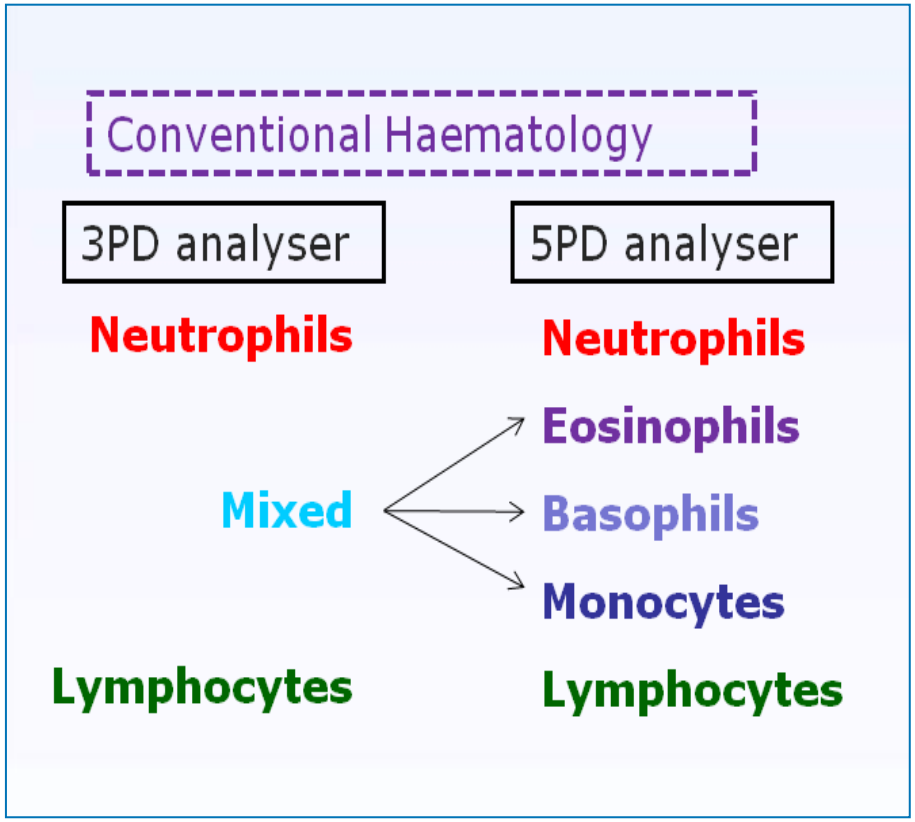
3 Part Differential Count

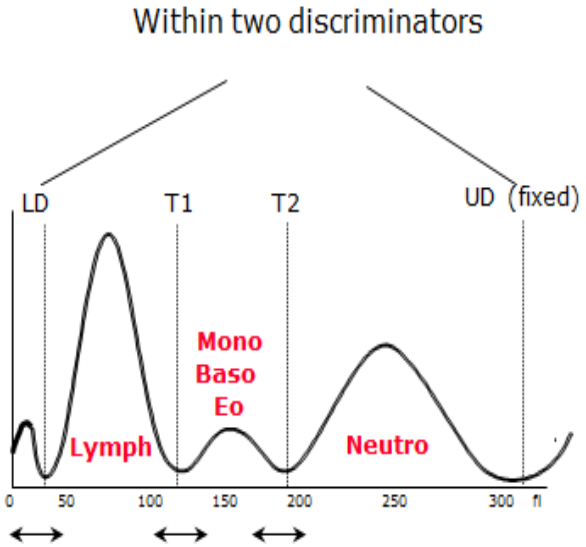
- Less complex haematology analysers use impedance technology to separate cells based on size
- Red cells are lysed
- Three distinct groups based on cell size are identified:
  - Large cells or granulocytes
  - Small cells or lymphocytes
  - Medium cells or monocytes or “middle” cells.

Size here refers to the size of cells after exposure to reagents within haematology analyser – not natural size

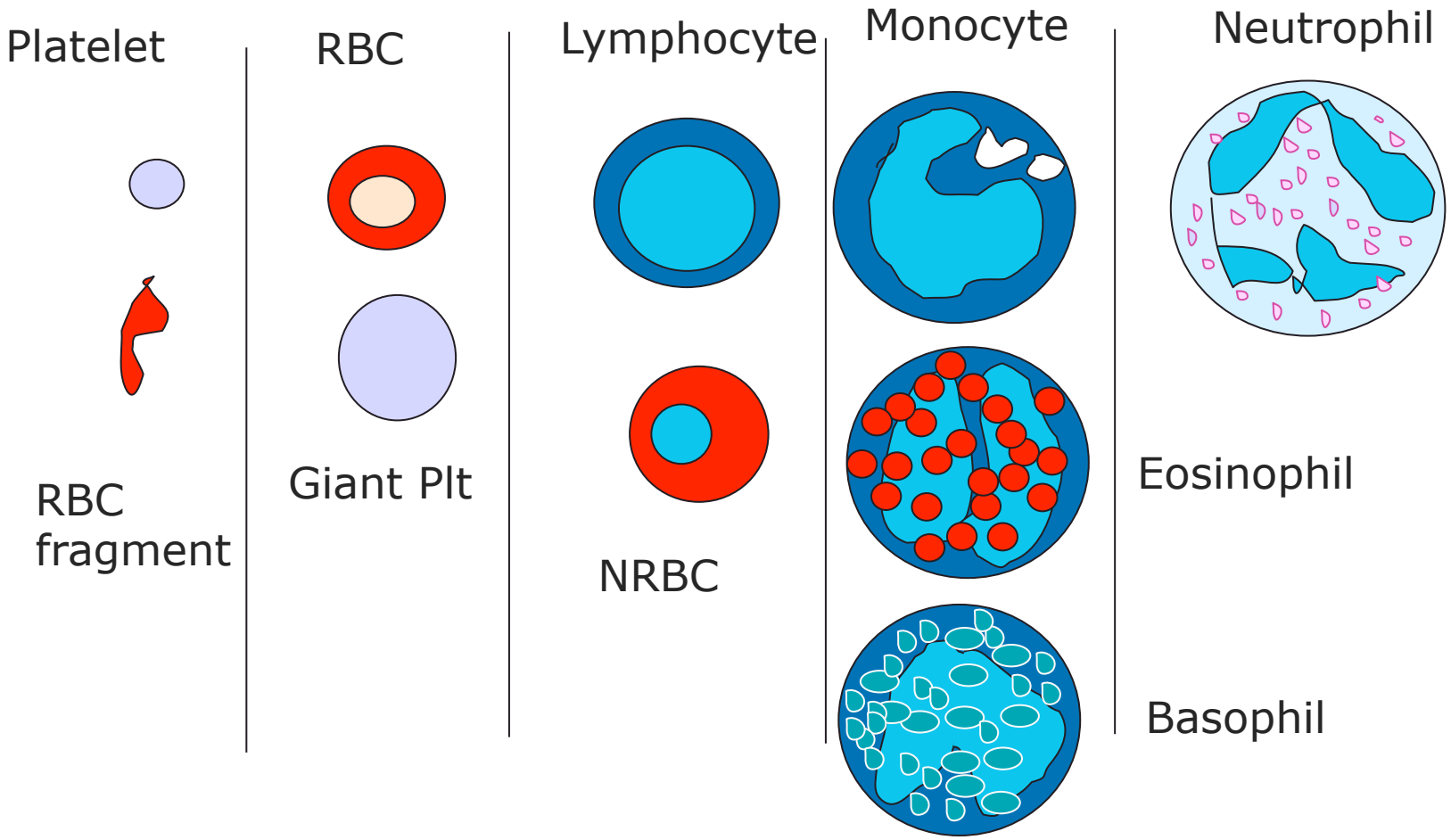
SYSMEX 3PD – (POCH100i,KX21N & XP-300)

Identifies NEUTROPHILS as a separate population





Relative Cell Sizes



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

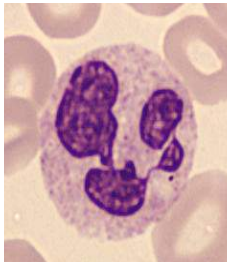
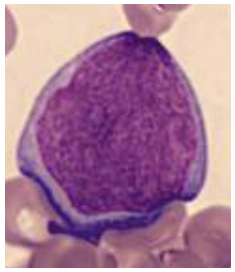
Differential Count in Disease

- 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.

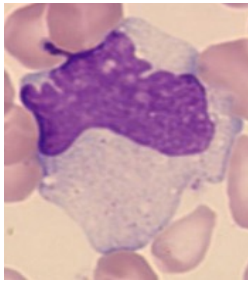
Why should one consider a 5PD rather than a 3PD?

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.

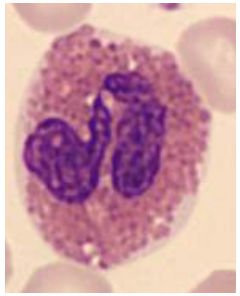
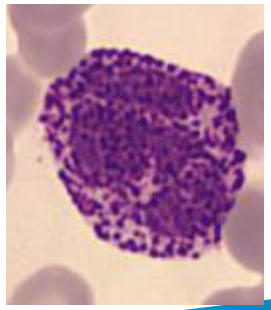
5 Part-Differential Count

5 Part Diff



- Neutrophils — ↑ Bacterial Infection
- Lymphocytes — ↑ Viral Infection
- Monocytes — ↑ Any Chronic Infection (eg TB)
- Eosinophils — ↑ Parasitic infection, allergies
- Basophils — **REACTIVE CAUSES**

Percentage Count (%)

Absolute Count (#)

Automated 5PD analysis

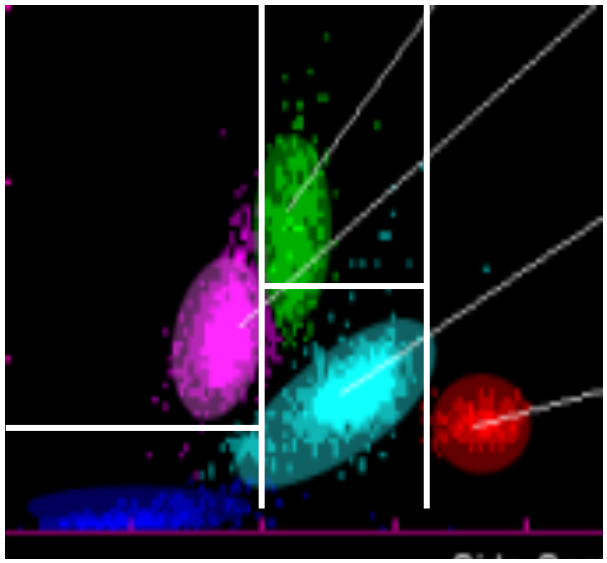
- Rely on a combination of two types of measurements for WBC differentiation:
  - volumetric impedance
  - high frequency electromagnetic energy
  - optical light scatter
  - cytochemical staining techniques for WBC differentiation.
- The principle difference to 3 part differential technology
  - Cell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

SYSMEX 5PD automated analysis

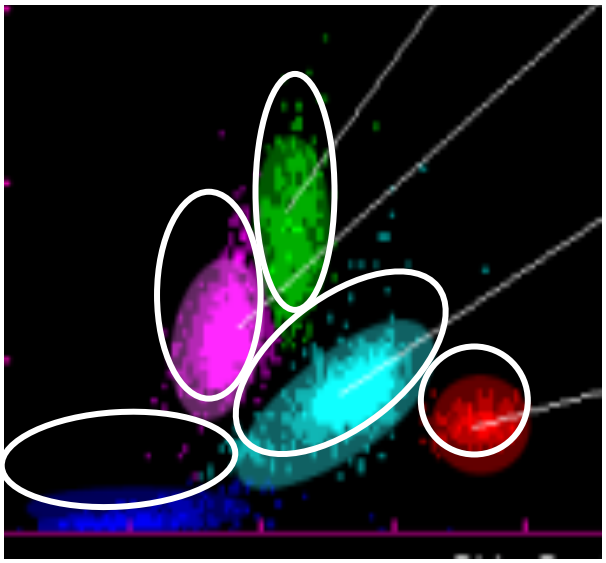
- fluorescence flow cytometry
- Sub-populations separated on basis of
  - cell complexity or side scatter
  - fluorescence signal – measure of DNA/RNA content.
- Adaptive cluster analysis system (ACAS) software
  - Ensures that each cell population forms a clear cluster before all events are counted 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

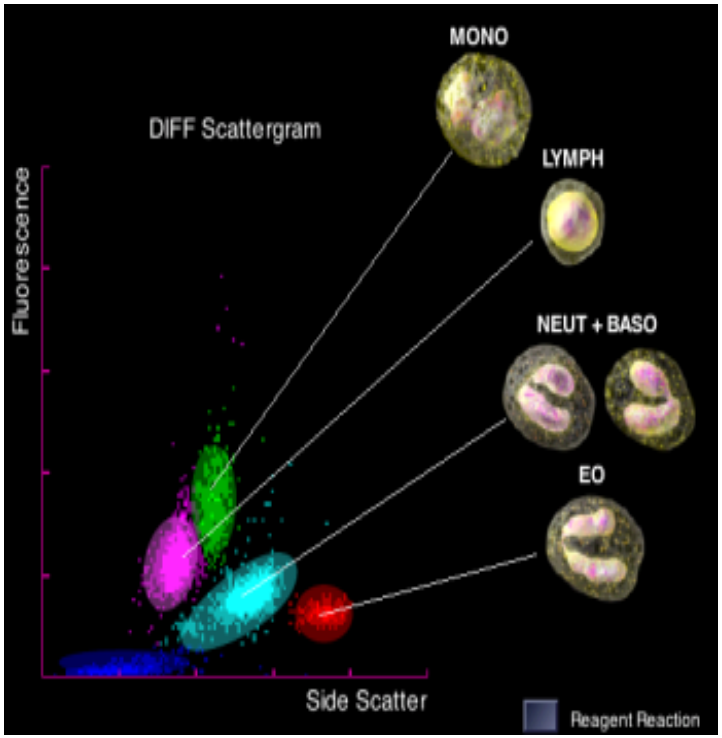
Non SYSMEX Fixed gating

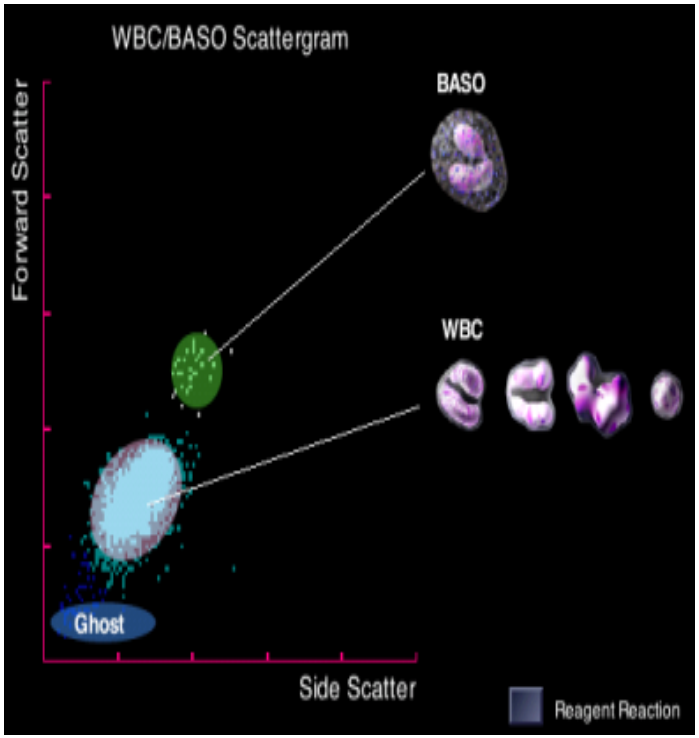


SYSMEX Flexible ACAS gating



Sysmex Fluorescence Flow Cytometry





So what are the benefits of 5PD over 3PD?

- Benefits are greater that 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

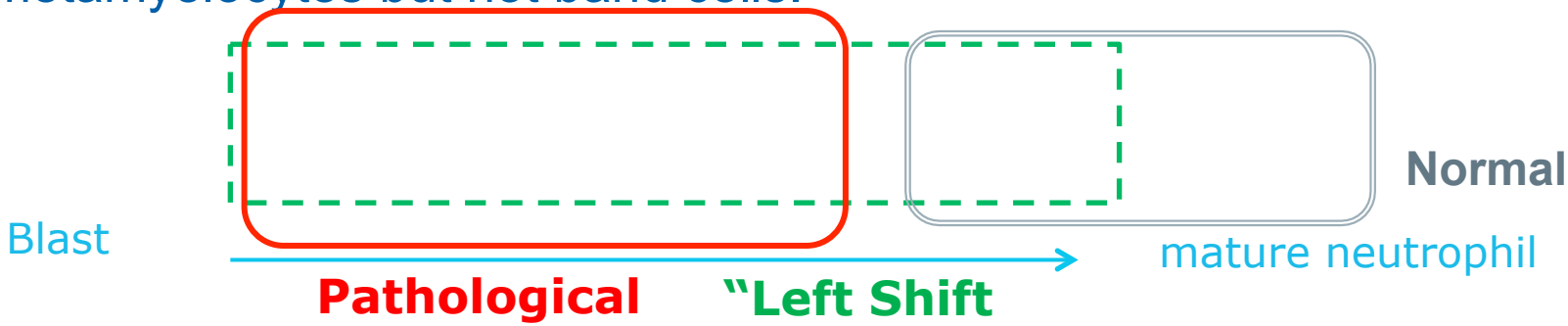
Differentiation independent of Cell Size

- 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.



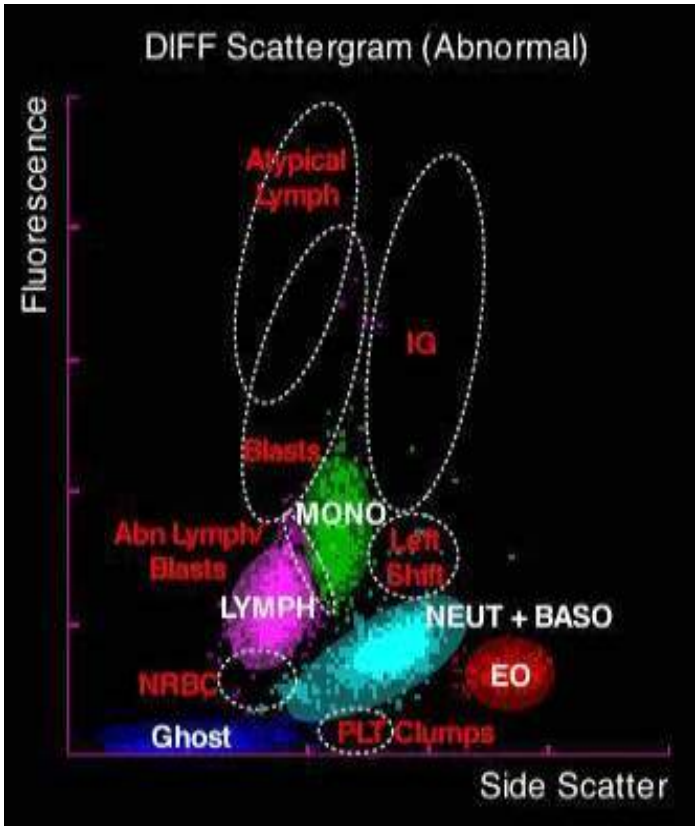
- The presence of immature granulocytes is always pathological except
  - immediate post-partum period and a neonate less than 3 days old.
- Precision of automated IG count is much better than manual microscopy making it ideal for serial monitoring of patients thereby eliminating labour intensive manual 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

Manual smear review caveats

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.

Kit RAL StainBox MCDh

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!