SEED Haematology

Sysmex Educational Enhancement and Development
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Overview of the benefits of switching from a 3-part differential to a 5-part differential haematology analyser

Why do we need a white blood cell differential count?

The white blood cells are comprised of a number of sub-populations with diverse biological function. The white blood cell count (WBC) on its own is not very informative in evaluating the state of health of an individual. The presence of a normal WBC does not mean that all is well. In view of this, it is common practice to provide a so-called WBC differential count.

The standard WBC differential divides the white blood cells into the 5 major sub-populations which are

- Lymphocytes
- Monocytes
- Neutrophils
- Eosinophils
- Basophils

The traditional method for differentiating the white blood cells is manual microscopic review. This requires the preparation of a thin wedge smear which is then air dried and stained with some form of Romanowsky stain. The first reference method for the WBC differential count, published by the Clinical Laboratory Standards Institute (CLSI) in 1984, was for four operators to each perform a 200 cell differential count on two different smears made from the same specimen tube and for the average of the four counts to be reported. In recognition that this is completely impractical, the current CLSI recommendations have reduced the number of operators to two. However, most laboratories are too busy to follow this time-consuming procedure, consequently in practice manual differential counts are generally derived from a single operator performing a 100 cell count on a single smear. The differential count was originally represented in relative terms only, namely percentage count, but it is now widely accepted that absolute counts are more informative. The white cell populations are therefore routinely reported in absolute counts ($x10^9$/L) as well as percentage count (%) of the total white blood cell count.

With advances in technology, white blood cell differentiation became possible on automated analysers. The major advantage that this development brought was speed and enhanced accuracy. In contrast to the 100 cell manual count, automated analysers counted on average about 15,000 cells per sample.

Automated differential white blood cell counts

Automated haematology analysers with the ability to differentiate white blood cells are now widely available in routine haematology laboratories. These analysers are broadly classified as either 3-part or 5-part differential analysers.

a) 3-part differential analysers

The automated haematology analysers with 3-part differentiation functionality rely on impedance technology to count and separate white blood cells on the basis of size. The red blood cells are lysed using chemical reagents whilst the white blood cells remain intact. Impedance technology involved a stream of cells in suspension passing through a small aperture across which an electrical current is applied. Each cell that passes alters the electrical impedance and can thus be counted. The degree of change is in direct proportion to the size of the cell. The principle of hydrodynamic focusing further enhances the accuracy by ensuring that cells pass through the aperture in single file and eliminate false size estimates if, for example, 2 cells pass through together.

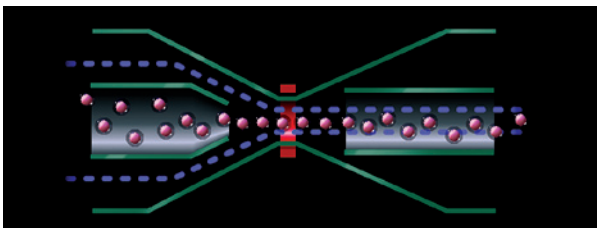

Fig. 1 *Impedance principle with hydrodynamic focusing.*

Three distinct groups based on cell size are identified

- Large cells or granulocytes
- Small cells or lymphocytes
- Medium cells or monocytes or 'middle' cells.

The Sysmex 3-part differential analysers, namely the pocH-100i and KX-21N, are more advanced than other systems as they are able to identify neutrophils as a distinct group on the basis of the chemical make up of the reagents.

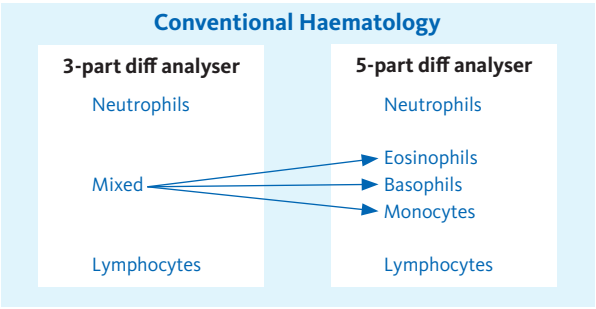

Fig. 2 *Sysmex 3-part differential count. Neutrophils and lymphocytes are indentified as distinct populations with monocytes, eosinophils and basophils being counted together as mixed cells.*

Studies comparing the accuracy of automated 3-part differential counts with the earliest reference method of the 800 manual cell count have revealed that the although precision is excellent, accuracy is less good, especially in abnormal samples where relative cell counts and morphological appearance of cells may become altered. Whilst automated analysis has been a significant advancement, it is generally accepted that the 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).
In disease however

- Ratios become distorted and therefore percentage counts are meaningless in the absence of absolute values
- Sub-populations can increase, for example eosinophils may be increased in response to an allergic reaction
- Sub-populations can decrease, for example 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, for example immature granulocytes in patients with severe infection
- Immature cells that are abnormal can appear, for example blasts in patients with acute leukaemia

A 5-part differential is much more informative than a 3-part differential in identifying the cause of possible illness in sick people.

b) 5-part differential analysers

Automated 5-part differential analysers utilise various combinations of volumetric impedance, high frequency electromagnetic energy, optical and cytochemical staining techniques for WBC differentiation. The principle difference to 3-part differential technology is that cell identification relies on a two dimensional analysis rather than just on cell size.

The Sysmex X-Class analysers utilise fluorescence flow cytometry to perform 5-part differentiation. The sub-populations are separated on the basis of cell complexity or side scatter and fluorescence signal. The sample is exposed to a fluorescent dye which binds to intracellular RNA and DNA. The fluorescent signal strength is proportional to individual cell RNA/DNA content. Furthermore, the differentiation is achieved with a high level of accuracy because of the adaptive cluster analysis system (ACAS) software. This ensures that there each cell population forms a clear cluster before all events are counted as belonging to that cell subtype. In contrast some other systems utilise fixed gating which sometimes causes cells to be counted as part of an incorrect group, especially in pathological specimens.
The ability of 5-part differential analysers to enumerate the less abundant cell types, namely monocytes, eosinophils and basophils, separately rather than as a mixed cell population is a significant enhancement.

As already alluded to, individual white blood cell types are diverse in their function and hence alterations of the absolute quantity of the individual population provide very valuable information to enable a doctor to hone in on the most likely clinical diagnosis as well as to monitor response to treatment.

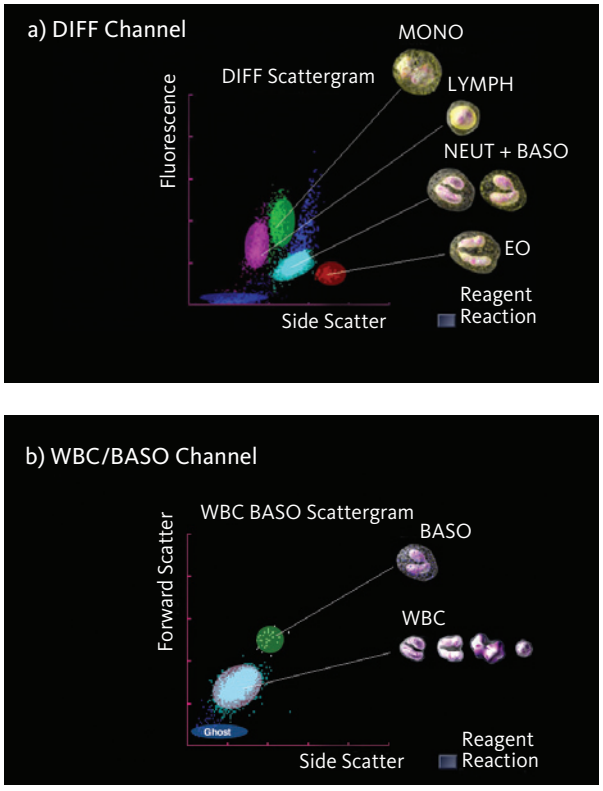

Fig. 3 *Sysmex X-Class analyser scattergrams. a) Differential scattergram: fluorescence which is a measure of RNA/DNA content of cells is represented on the Y axis and cell complexity or side scatter is represented on the X axis. b) WBC/BASO channel – this channel, present on the XT and XE analyser, utilises a chemical reaction which keeps basophils intact to separate them from the remaining white blood cells on the basis of forward scatter (Y axis) and side scatter (X axis).*

So why is fluorescence flow cytometry based white blood cell differential counting superior to 3-part differential and to competitor 5-part differential technologies?

a) Assessment is independent of cell size

Fluorescence flow cytometry differentiates white blood cells on the basis of nucleic acid content and internal structure or complexity. The major advantage of this approach is that, unlike 3-part differential and 5-part differential analysers from other manufacturers, the analysis does not rely on cell size. The fact that WBC differentiation is independent of cell size is a significant benefit because

- Cell size changes occur quite rapidly once blood is collected into EDTA as this is a non-physiological environment for blood cells placing them under metabolic stress which lead to glucose depletion. This in turn leads to an inability to maintain the balance of movement across the cell membrane leading to cell swelling and ultimately disintegration.
- 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.

b) Identification of immature cells

Another equally important benefit is that fluorescence flow cytometry enables the identification of immature cells on the basis that they have higher nucleic acid content in comparison with their mature counterparts. This has enabled the generation of a 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 with the exception of the immediate post-partum period and a neonate less than 3 days old. The precision of the automated IG count is much better than manual microscopy making it ideal for serial monitoring of patients thereby eliminating labour intensive manual counting.

c) Extensive flagging system for identification of abnormal cells

Another major advantage an X-Class 5-part differential analyser over a 3-part differential analyser is a sophisticated flagging system which enables the qualitative identification of immature and abnormal cells. 3-part differential analysers also have a flagging system but it is less informative as it is based entirely on cell size aberrations.

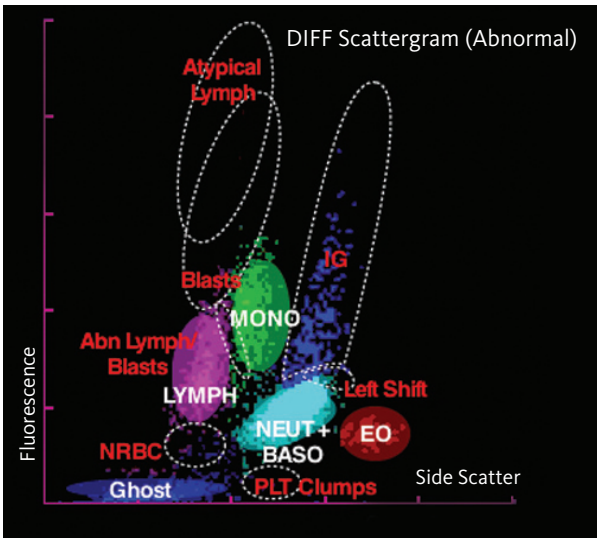

Fig. 4 *Sysmex differential scattergram showing the positions where abnormal cell populations are likely to be found.*

Is there still a requirement for a manual differential count?

Manual microscopy will continue to play a critical role to confirm the presence of abnormal cell populations that the automated analysis has identified as suspect and flagged for the operator's attention. The purpose of the manual review is not to check the differential cell count but rather to confirm the presence of abnormal cell populations and to record any noteworthy morphological features. Manual counting will never be as precise as automated counting by virtue of the fact that the number of cells counted is so much lower. Likewise, the automated analysis will never be able to accurately identify every possible abnormal cell variant. The converse however is that if the analyser has not flagged a specimen as having any suspected abnormality, that it will be extremely unlikely that manual review will add any additional value. In order to obtain true benefit from manual microscopic review, individuals have to be highly skilled and have the necessary experience. In this context, the 5-part differential analyser, by dramatically reducing the need to perform a manual differential count and directing the reviewer to the look for specific pathology (by virtue of the flags generated), contributes to greater lab time and cost efficiency as such individuals are a scarce resource.

So why choose a Sysmex 5-part diff analyser?

If laboratories intend to only reviewing basic parameters such as haemoglobin, platelet count and white blood cell count, which is common practice, there is no benefit to investing in a 5-part differential analyser. 3-part differential analysers are in general more cost effective than 5-part differential analysers but 5-part differential analysers are however undoubtedly superior for the analysis of sick people because of:

- White blood sub-population identification
- The improved ability to detect abnormal cells
- Superior flagging system
- Additional parameters with clinical utility which are beyond just being able to provide a more complete differential count.

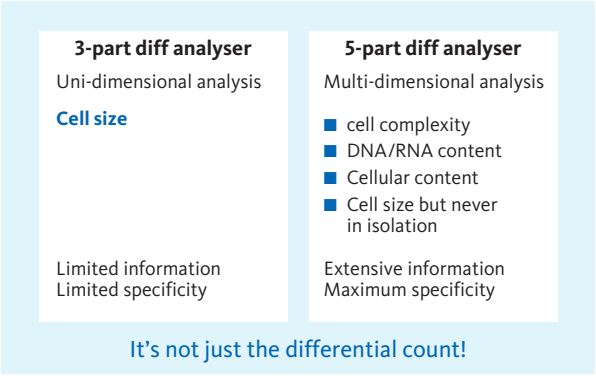

Fig. 5 *Why a Sysmex 5-part differential analyser is superior to 3-part differential analysers.*

Conclusion

Provided that all information generated by a more sophisticated 5-part differential analyser is shared with doctors to inform their patient management decisions and therefore benefit the patient, the 5-part differential is definitely as cost effective if not more so than the more basic 3-part differential analyser. The reduction in time required for manual microscopy of specimens processed on a 5-part differential analyser in comparison to a 3-part differential adds to the overall benefit to be gained by the laboratory. In general, the features and measurement parameters available on different haematology analyser become more advanced in parallel with increasing throughput capacity. This ordinarily puts the more advanced features, including 5-part differential capacity out of reach of smaller laboratories. Sysmex has recognised this limitation and filled the gap by introducing the XS-series analyser. It is designed for the same relatively low throughput laboratories as the KX-21N, but provides superior analysis, especially of pathological specimens as described in this article. 'X-Class analysers are much more than just cell counters – the focus has shifted to the identification of subtle qualitative features in order to distinguish between normal and pathological conditions.'

Compiled by
Dr. Marion Münster

Sysmex Europe GmbH
Bornbarch 1, 22848 Norderstedt, Germany · Phone +49 40 52726-0 · Fax +49 40 52726-100 · info@sysmex-europe.com · **www.sysmex-europe.com**