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SYSMEX EDUCATIONAL ENHANCEMENT AND DEVELOPMENT | June 2024

# SEED Haematology

## Benefits of transitioning 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 (WBC) are comprised of several subpopulations with diverse biological functions. While an abnormal white blood cell count on its own can already indicate a pathological condition, a normal WBC count does not guarantee that the patient is healthy. For example, in patients with lymphoma a basic WBC count might still appear normal [1]. 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 five major subpopulations (see Fig. 1) which are:

- lymphocytes (A)
- monocytes (B)
- neutrophils (C)
- eosinophils (D)
- basophils (E)

Modern haematology analysers go even further than these five subpopulations and offer automated counts for immature granulocytes (IG) as well. The IG count on Sysmex analysers includes promyelocytes, myelocytes and metamyelocytes but not band cells.

The traditional method for differentiating the white blood cells is a 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

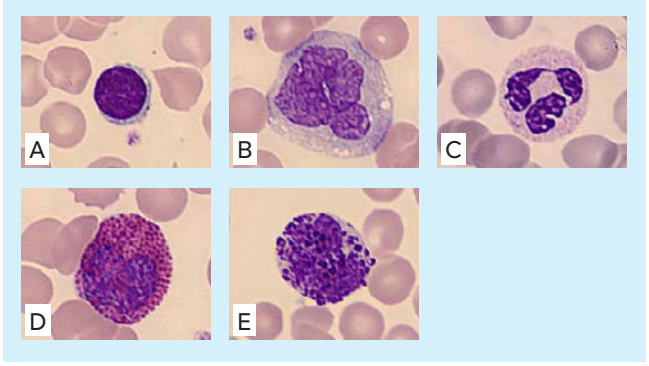


**Fig. 1** Microscopic view of five white blood cell subpopulations.

1992, was for five operators to each perform a 200-cell differential count on five different smears made from the same specimen tube and for the average of the five counts to be reported [2]. However, it is common practice that manual differential counts are derived from a single operator performing a 100-cell count in 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 as absolute counts (e.g. × $10^9$/L) as well as a 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 manual count, automated haematology analysers count several thousand WBC [3].

## Automated differential white blood cell counts

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

**3-part differential analysers**

The automated haematology analysers with 3-part differentiation functionality rely on impedance technology to count and separate white blood cells based on their volume. The red blood cells are lysed using chemical reagents whilst the white blood cells are shrunk to a certain extent but remain intact. The DC detection method involves a stream of cells in suspension passing through a small aperture across which an electrical current is applied. Each cell that passes through alters the electrical impedance and can thus be counted. The degree of change is in direct proportion to the volume of the cell (see Fig. 2).

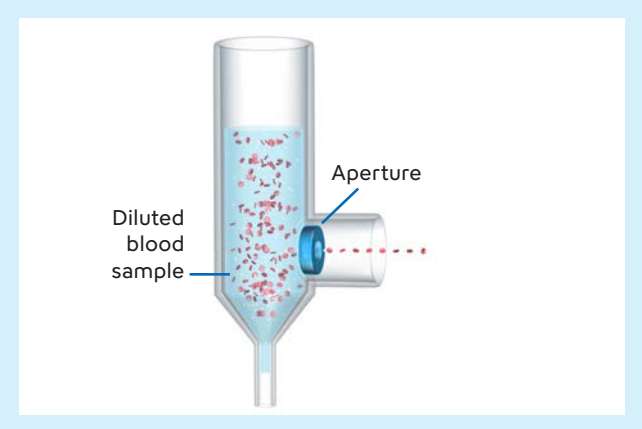


**Fig. 2** DC detection method.

Three distinct groups of WBC based on cell volume are identified:

- small cells or lymphocytes
- medium cells or monocytes, basophils and eosinophils or 'mixed' cells
- large cells or neutrophils (see Fig.3)

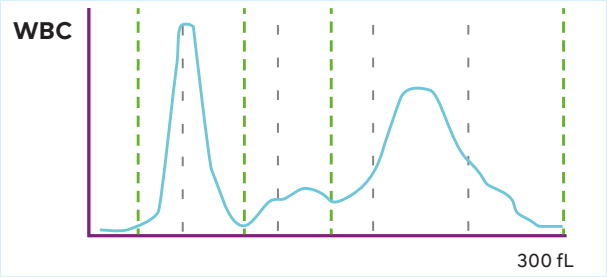


**Fig. 3** Histogram of a 3-part differential analyser showing three white blood cell populations.

The Sysmex 3-part differential analysers can identify neutrophils as a distinct group based on the chemical composition of the reagents (see Fig.4).

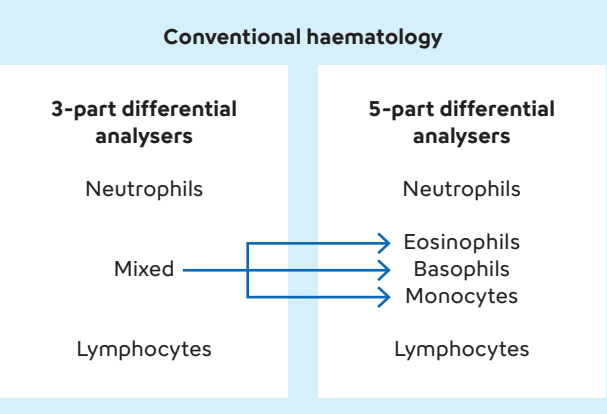


**Fig. 4** Sysmex 3-part differential count. Neutrophils and lymphocytes are identified as distinct populations with monocytes, eosinophils and basophils being counted together as mixed cells.

Studies assessing the accuracy of automated 3-part differential counts and comparing their performance to 5-part differential analysers have revealed that 3-part differential analysers show very good accuracy and precision even at low cell counts [4]. However, in abnormal samples where relative cell counts and the morphological appearance of cells may become altered, more advanced 5-part differential analysers show superior performance [5, 6]. Whilst automated analysis has been a significant advancement, it is generally accepted that only generating a 3-part differential for pathological samples is not ideal.

In healthy humans, the five major subpopulations lie within so-called reference intervals. In disease however, ratios become distorted and therefore percentage counts can become meaningless in the absence of absolute values. In some cases, subpopulations may increase; for example, eosinophils may be increased in response to an allergic reaction [7].

In other cases, subpopulations can decrease; for example:

- lymphocytes typically become progressively reduced in untreated HIV infection [8].
- immature cells that are normally only found in the bone marrow can appear in the peripheral blood, such as immature granulocytes in patients with severe infection [9].
- immature cells that are abnormal can appear, such as blasts in patients with acute leukaemia [10].

While 3-part differential analysers provide reliable results of high quality, 5-part differential analysers offer additional productivity values and clinical insights.

**5-part differential analysers**

While in 3-part differential analysers cell identification relies on cell volume, Sysmex 5-part differential analysers utilise fluorescence flow cytometry to perform WBC differentiation. The subpopulations are separated based on cell size (FSC), cell complexity (SSC) and RNA/DNA content (SFL). The sample is exposed to a fluorescent marker 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). As shown in Fig. 5, this ensures that each cell population forms a clear cluster before all events are counted as belonging to that cell subtype and abnormal cells are flagged [11, 12]. 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 [13].

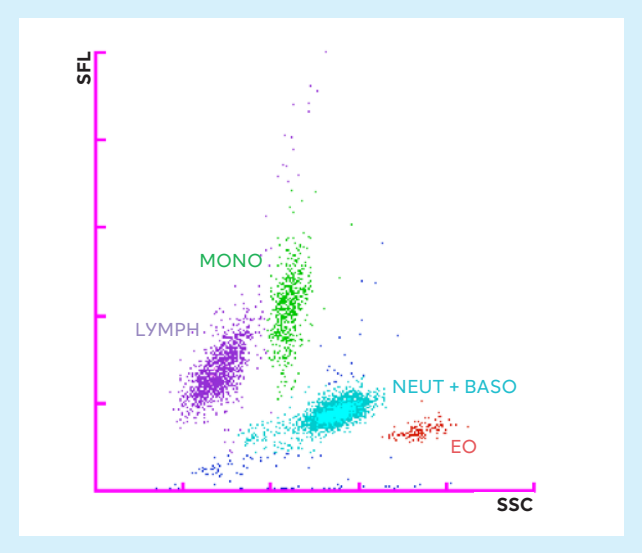


**Fig. 5** Sysmex 5-part differential analyser WDF scattergram: Side fluorescence light (SFL), which is a measure of the nucleic acid content of cells, is represented on the Y-axis, and side scatter (SSC) is represented on the X-axis, providing information about the internal cell structure and its content.

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

## So why is fluorescence flow cytometry-based WBC differential counting superior to 3-part differential technologies?

**Assessment is independent of cell size**

Fluorescence flow cytometry differentiates white blood cells based on nucleic acid content, internal structure or complexity and cell size. The major advantage of this approach is that, unlike 3-part differential analysers, the analysis does not only rely on cell size or cell volume. 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 leads 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 [14].

**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 a higher nucleic acid content in comparison to their mature counterparts. This has enabled the generation of a 6-part differential count with the addition of immature granulocytes (IG). The presence of immature granulocytes is always pathological except for during the immediate post-partum period and in a neonate less than three days old [15, 16]. The precision of the automated IG count is much better than manual microscopy making it ideal for the serial monitoring of patients and thereby eliminating labour-intensive manual counting [17].

**White blood cell functionality**
Some Sysmex 5-part differential analysers offer a set of parameters called 'Extended Inflammation Parameters' consisting of NEUT-RI, NEUT-GI, RE-LYMP and AS-LYMP to quantitatively measure neutrophil and lymphocyte activation. The combination of the RE-LYMP and AS-LYMP parameters, which quantify the numbers of all reactive lymphocytes and antibody-synthesizing lymphocytes respectively, provides additional information about the cellular activation of the innate and adaptive immune response. Furthermore, the granularity and reactivity of neutrophils (NEUT-GI and NEUT-RI, respectively) reflect an innate immune response to bacterial infections [18].

The value of this parameter set is described in more detail in the Sysmex SEED article 'Looking deeper into inflammatory conditions from a laboratory and clinical perspective' and in the white paper 'Novel haematological parameters for investigation of the immune system response'.

Most Sysmex 5-part differential analysers can be equipped with a WPC channel. The WPC channel can classify suspect samples into one of three clearly defined categories ('reactive', 'suspected malignant' or 'negative'). Together with the WDF channel, these categories translate into analyser flags with a high sensitivity for blasts and abnormal lymphocytes [19] as well as leucocytosis of neoplastic and reactive origin [20].

More information about the assessment of WBC functionality using the WDF and WPC channels can be found in the white paper 'Going beyond the visible: Reliable characterisation of WBC functionality'.

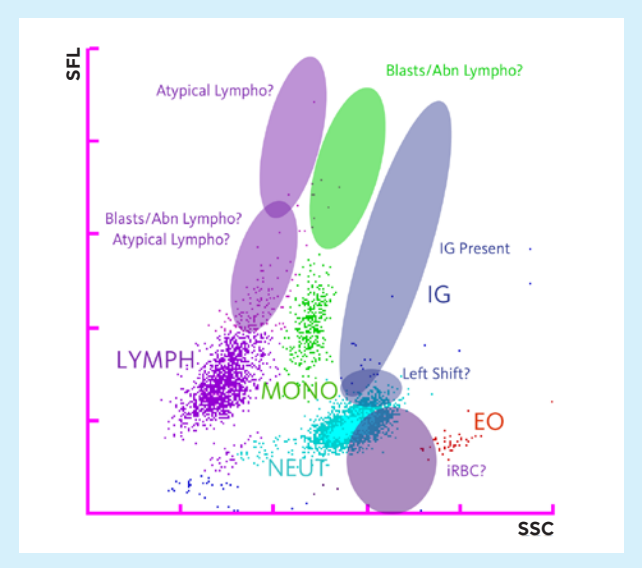


**Fig. 6** Sysmex WDF scattergram showing the positions where abnormal cell populations are likely to be found.

**Extensive flagging system for identification of abnormal cells**
Sysmex 5-part differential analysers offer an adaptive flagging algorithm based on shape recognition (AFLAS). The algorithm analyses the appearance position of individual clusters as well as the shape of the cluster and the position of the centre within the three scattergram dimensions. It helps to detect abnormal patterns with a high sensitivity (see Fig. 6). This flagging algorithm is superior to the 3-part differential flagging system, which is based on cell size aberrations.

## Is there still a requirement for a manual differential count?

Smear reviews will continue to play a role in confirming 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 repeat the differential cell count but rather to confirm the presence of abnormal cell populations and record any noteworthy morphological features [21]. Manual counting will never be as precise as automated counting, as 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, it will be extremely unlikely that manual review will add any additional value. To obtain true benefit from a manual smear review, individuals must 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 look for specific pathologies (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 differential analyser?

If laboratories intend to only review basic parameters such as haemoglobin, platelet count and white blood cell count, 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, with the latter offering additional analytical values for faster and more efficient diagnostic support because of:

- WBC subpopulation identification
- the improved ability to detect abnormal cells
- superior flagging system
- advanced clinical parameters with a value beyond providing a complete differential blood count

**It's not just the differential count**

| 3-part differential analysers | 5-part differential analysers |
|---|---|
| Uni-dimensional analysis | Multi-dimensional analysis |
| Cell volume | Internal cell structure and content |
| Limited information | Nucleic acid content |
| Limited specificity | Cell size |
| | Extensive information |
| | Maximum specificity |

**Fig. 7** Why a Sysmex 5-part differential analyser is superior to 3-part differential analysers.

## Conclusion

Sysmex 5-part differential analysers offer tailor-made solutions for low to medium-throughput laboratories up to large scalable automation lines. High throughput and maximum workload, as well as workflow improvements thanks to reduced smear rates, benefit laboratories of all sizes.

Using the Sysmex applications concept, all 5-part differential analyser series are scalable and configurable for the laboratories' and clinicians' needs.

The Sysmex Advanced Clinical Parameters, such as the Extended Inflammation Parameters, offer clinical insights beyond a complete blood count.

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