**Introduction and background**

Full blood counting is a relatively easy process, usually simply involving the mixing of blood in an EDTA collection tube, mode selection, introduction of sample for sampling and start of analysis. A full profile of patient results follows within two minutes offering an invaluable tool for in vitro diagnosis of a myriad of possible pathological conditions. This allows for prompt and accurate medical intervention by clinicians that can be lifesaving. Therefore, with today’s heavy burden of disease and without the currently highly automated and sophisticated blood analysis systems, patient testing would be very difficult to manage.

As pointed out in part 1 of this topic, despite the rapid advances made in automated laboratory testing systems, challenges in assuring the quality of results still exist. This includes factors related to sample collection and handling prior to analysis, the testing process itself (system related) and the so called post-analytical influences. (For the full detail on the pre- and post-analytical influences please see SEED 4 2013). Interferences that take place during analysis need to be understood and eliminated or reduced where possible and taken into account when interpreting results in order to ensure that the accuracy of the results is acceptable.

**What is interference?**

Aberrant, unreliable patient results may occur due to the presence of substances other than the parameter of interest interfering with the measurement principle and thus affecting the accuracy of the final result. This may include external or non-haematological substances such as lipids but may also occur as a result of one blood cell type interfering with the measurement of another cell type. It is important that the measurement system should at least detect these occurrences and alert the operator so that appropriate action can be taken. These occurrences are known as interferences and the accompanying alert messages are termed flags. This discussion will concentrate on those interferences affecting the white cells and the related flags.
What type of flagging messages can we anticipate?
Some messages are common and their sources are readily identifiable while others may have more complicated origins depending on the analytical technology used.

1. Abnormal or reference range messages
These are messages that emanate from what the laboratory has set as a reference or normal range for each parameter and should reflect the profile of the population being served. When properly set they allow the analyser to judge such results as either normal or abnormal and indicate whether they are below or above the defined range. Therefore the message “thrombocytopenia” identifies the presence of a low platelet count whilst “thrombocytosis” is indicative of an elevated platelet count.

2. Suspect messages
Flagging messages alert the operator about possible disturbances in the measurement chamber which may affect the accuracy of the results as well as the possible existence of abnormal cells which may assist in reaching timeous diagnosis of an underlying pathology. Examples here may range from detection of presence of platelet clumps which may have an effect on the platelet count to detection of presence of leukaemic cells, e.g. blasts.

White cell interferences and flagging on Sysmex 3 part diff analysers
In 3 part diff analysers initial information about the possible cause of an abnormal white blood cell count (high or low) is provided by the classification of white blood cells into three separate subpopulations. This gives rise to the so-called 3 part differential count which broadly separates cells according lineage. Sysmex 3 part differential analysers separate normal white blood cells into lymphocytes, neutrophils and a mixed group comprised of monocytes, basophils and eosinophils.

Reagent effect on cells
During the analytical process, the white blood cells are treated with specific lyse reagents. The lyse reagent effect on white blood cells causes the cells to shrink to a defined volume according to their cell type whereby they are identified as a separate population in the histogram. After this special lysis treatment, cells will be shown in a histogram according to their size (see figure 1).

LD = lower discriminator, UD = upper discriminator, T1 = trough between lymphocytes and mixed cells, T2 = trough between mixed cells and neutrophils.

Based on the analysis of many thousands of normal samples during the development phase of the analyser software, the expected range of cell size has been determined. This information is used to set the so-called discriminators of the WBC histogram. Some of these are flexible (i.e. the boundary of population can be located between a set range) whereas others are fixed on an absolute value. The WBC histogram has to fulfil certain criteria in order for the resultant cell counts to be considered reliable:

- The distribution curve should fall within the discriminators.
- The curve should start and end at the base line.
- The LD, T1 & T2 are flexible and can be adjusted for the latest sample just processed by performing the manual discrimination procedure but LD cannot be set lower than 30 fl. The results are then recalculated and displayed.

The measurement sample in the WBC channel includes white blood cells and platelets but not red blood cells as these have been lysed.
The volume of platelets is usually between 8 - 12 fl, therefore the LD of the WBC histogram separates the white blood cells from the platelets and thus they do not interfere with the WBC result. However, please note that large PLT clumps can be located immediately to the left of the 30 fl lower WBC discriminator.

In 3 part diff measurement only volume of cells is used to differentiate the white cell subpopulations. As stated above the distribution curve must be within the discriminators and each curve must start and end at the base.

Any particles in the measurement distorting this pattern may result in flagging and possible incorrect results.

The following flag messages may appear:
- **WL** - abnormal height at lower discriminator.
- **WU** - abnormal height at upper discriminator.
- **AG** - high number of cells to the left of the lower discriminator.
- **T1** - no differentiation between lymphocytes and mixed cells.
- **T2** - no differentiation between mixed cells and neutrophils.
- **F1** - relative height of T1 or T2 exceeds the limit.
- **F2** - relative height of T1 or T2 exceeds the limit.
- **F3** - relative height of T1 or T2 exceeds the limit.

Possible causes of white cell interferences and flags in 3 part diff analysers

The various interferences and flags are described and presented in tables 1 and 2 below. Possible causes and solutions are also suggested (see also figures 2 and 3 for illustration of related histograms).

![Fig. 2 The WBC histogram showing the a) "WL" b) "AG" and c) "WU" respectively.](image)

It is important to note that in cases where the histogram does not reach the base at the lower or the upper discriminators, this may result in incorrect results. In such cases the described flags will be displayed besides the numerical results.

**Table 1** The “WL”, “AG” and “WU” flags

<table>
<thead>
<tr>
<th>Flag</th>
<th>Possible causes</th>
<th>Possible solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) &quot;WL&quot; -</td>
<td>Lyse resistant RBC.</td>
<td>Retesting a diluted sample (with Cellpack or 0.9% NaCl) will help to obtain correct results.</td>
</tr>
<tr>
<td>abnormal height at lower discriminator.</td>
<td>PLT clumps.</td>
<td>Samples showing platelet clumping should be recollected in trisodium citrate anticoagulant and retested.</td>
</tr>
<tr>
<td></td>
<td>EDTA-incompatibility.</td>
<td>Clotted samples should be recollected and retested.</td>
</tr>
<tr>
<td></td>
<td>Coagulated sample.</td>
<td>Presence of NRBCs poses a challenge because after lysing, their nuclei, which are about the same size as lymphocytes, remain.</td>
</tr>
<tr>
<td></td>
<td>Nucleated red blood cells (NRBC) – seldom.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RBC cold agglutinins.</td>
<td></td>
</tr>
</tbody>
</table>
They will therefore be counted as white cells and if a differential count is also performed, then the lymphocyte count will also be distorted.

b) “WU” flag - abnormal height at upper discriminator.
- Extreme leukocytosis.
- WBC aggregation.
- PLT satellitism – seldom.

In cases of extreme leukocytosis the sample should be diluted at a ratio of 1:5 to obtain a reliable count.

In rare cases of PLT satellitism phenomenon (aggregation of PLTs onto neutrophil membranes, often seen together with EDTA-incompatibility) using trisodium citrate as an alternative anticoagulant for blood collection may resolve the problem.

c) “AG” Flag - High number of cells to the left of the lower discriminator.
- Platelet aggregation due to EDTA incompatibility
- Lyse resistant RBC (see WL)
- NRBC – seldom (see WL)

PLT count should be microscopically checked for platelet aggregation.

In case of EDTA incompatibility sample should be recollected in citrated tubes and reanalysed.

The “T1” and “T2” flags
Although T1 and T2 discriminators are flexible and adjust to the sample, in some extremely pathological samples it may not be possible to differentiate between the lymphocytes and the mixed populations (T1) and between the mixed populations and neutrophils (T2). This is illustrated in figure 3 and summarized in table 2 below. However if either the T1 or T2 appear, the total white cell count is not affected.

<table>
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<th>Possible solution</th>
</tr>
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<tbody>
<tr>
<td>Flags T1 and T2 (Figures 3a and 3b respectively) - No differentiation between lymphocytes and mixed cells or between mixed cells and neutrophils respectively.</td>
<td>Abnormal white blood cells</td>
<td>Check smear for abnormal white blood cells e.g. blasts etc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If only “T1” or “T2” flag appear the total white cell count is not affected.</td>
</tr>
</tbody>
</table>

Fig. 3 The WBC abnormal histograms showing a) combined “T1” and “T2” interference and b) “T2” interference.

Table 1 The “WL”, “AG” and “WU” flags
The “F1”, “F2” and “F3” flags
In some abnormal samples the T1 and T2 do appear on the histogram but there is still no clear separation of white cell types because the histogram does not reach the base at both these discriminators. See figure 4 and table 3 below for possible causes and suggested action.

Fig. 4 The “F1”, “F2” and “F3” flags showing identification of “T1” and “T2” discriminators but no clear separation of cell populations.

Table 1 The “F1”, “F2”, and “F3” flags and interferences

<table>
<thead>
<tr>
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<th>Possible solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1, F2, F3</td>
<td>Abnormal distribution of WBC populations showing morphological features that do not allow clear distinction between them.</td>
<td>Check smear. Please note: In the absence of “WL” or “WU” flags the total WBC count is not affected.</td>
</tr>
<tr>
<td></td>
<td>Abnormal or immature WBC</td>
<td></td>
</tr>
</tbody>
</table>

Summary
The warning messages described above enable the user to detect abnormal samples and to react with follow-up actions based on the warnings. Morphological abnormalities – mostly preliminary development stages of normal cells or abnormalities of the myeloid and lymphatic cell series – require manual differentiation. Pre-differentiation information will reduce the rate of microscopic differentiation by restricting this to clinically relevant cases only. Manual work can thus be minimised without any compromise in quality of results.

Conclusion
Automated full blood count analysers add great value in terms of providing useful information that aids laboratory staff to correctly interpret results and to help pathologists and clinicians reach a correct diagnosis timeously. Efficient or optimal utilization of the analyser capability that helps in this process requires understanding of the basic workings of the analytical systems. The numerical results must be reviewed and interpreted in conjunction with careful inspection of the histograms and any accompanying alert messages or flags.
Take home message

- While numerical values on their own give important information about the patient state of health, all related information generated during analysis must be taken into consideration in order for result interpretation to be meaningful.

- Any suspect or abnormal flags must be noted and their source identified. The potential impact of interference on the accuracy of results must be assessed. Appropriate action must be taken, wherever possible, in order to eliminate or minimize the effect of interferences.

References:
2. Sysmex XP 300 Instructions for Use.

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