TECHNICAL REPORT

— SERIES 10 —

The Thing about Fluorescence Technology

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As a direct consequence of nucleic acid staining with fluorescent dyes, Sysmex Hematology is in a unique position. The recent FDA approval of the IPF (Immature Platelet Fraction) a Reticulated Platelet equivalent, is further evidence of the Sysmex technological advantage. The IPF parameter was made possible by the fact that all of the cells sampled on the Sysmex instruments are exposed to fluorescent dyes with affinity for nucleic acids.

Since the late 1990's, cell counter manufacturers have been looking at flow cytometry techniques as a way of increasing the capability of their already burdened cell counters. Traditional flow cytometry instrumentation requires the use of highly specific antigen-antibody interactions. The adoption of these complex approaches often would require delays due to pre-incubation requirements, and the reagents are expensive. In addition, the gating of cell scattergrams on traditional flow cytometers is complex, and is often performed manually by a skilled operator. The complexity of the reagents used, requires manual pipetting, and not all traditional flow cytometry applications are fully automated.

Sysmex corporation uses Polymethine dye (***Fig. 1***) staining of all sampled cells, which increases specificity and allows extension of clinical applications beyond the realm of traditional cell counting, without the complexity and cost of antigen / antibody reactions. The principle function of a cell counter is to act as a screening tool, and as such, the reagent systems used should not compromise throughput or reliability.

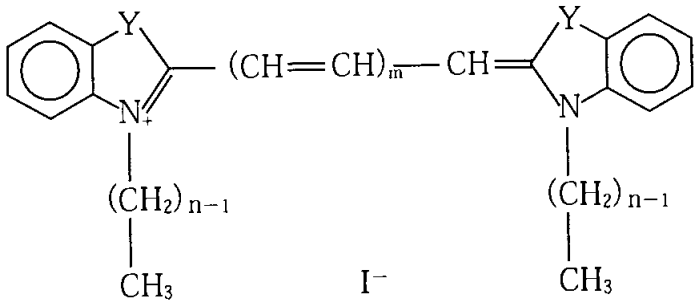

Fig.1 *Polymethine dye*

2 endocyclic nitrogen atoms are responsible for emission of intense light signals in this group of molecules specifically designed to fluorescently label nucleic acids. Fluorescence amplification upon nucleic acid binding is due to steric hindrance of the dye molecule. Customized excitation and emission wavelengths for fluorescence of the polymethine dyes, allow the use of laser diodes. Laser diodes are significantly cheaper and more reliable than Argon ion lasers. They do not require delay for temperature equilibration prior to use, and last longer.

By staining all components of peripheral blood with Polymethine dyes, the analytical sensitivity of cell counters is enhanced and signal - to - noise ratios are high. By extending the use of flow cell technology, and replacing other older technologies, reportable ranges are extended, there is enhanced separation of cell types, and interference is managed better than before.

The impact of the enhanced capability at a reagent level is evident when assessing microscopic review rates. Auto-validation and "Reflex" testing are rightly the focus of any laboratory while assessing Hematology cell counters. "Reflex" testing implies testing by an alternate method, if the initial result is inaccurate due to interference. The "Reflexed" test should increase specificity of analysis by eliminating the interference.

If an instrument does not use an alternate methodology for reflex testing, "Reflex testing capability" can be translated as, "repeated inaccurate result". The increased specificity available on Sysmex instrumentation, provides users with uncomplicated solutions that very efficiently reduce microscopy requirement.

During 2002, 13,000 samples were analyzed in an effort to make recommendations for the establishment of consensus rules1). Contributions were gathered from multiple analyzers, and the results generated were a reflection of the existing status within a broad cross-section of laboratories.

The truth table summary for a broad range of analyzers and laboratories revealed in ***Table 1***.

	%
True Positive	11.2
True Negative	67.3
False Positive	18.6
False Negative using consensus rules	2.9
False Negative using own lab rules	3.8

Table1 *Truth Table Summary*

Contributions to the false positivity rate of 18.6% are due to interference and limitations of the existing methodologies:

Interference and Limitations flagging

- *PLT counting (microcytosis, large PLTs)*
- *WBC Differential (RBC Lysis resistance, Linearity limitations NRBC's, IG's, false monocytosis, lack of specificity)*
- *CBC (Turbidity, Leukocytosis)*

Sysmex analyzers provide users with a fluorescent optical PLT count. This functionality has been shown to provide significant reduction in the interference with PLT counting when large PLTs, microcytic RBCs, and RBC fragments are present.
Alternate lysing reagents and the differentiating superiority of the fluorescent labels reduce interference and enhance specificity of cell counting across all cell lineages.

False negative contributions were due to the presence of IGs in 52% of cases. The IG master software package can therefore effectively halve the incidence of false negative results, and reduce false positivity due to inaccurate classification of IGs.

Since the consensus rules were developed for application on a broad range of instrumentation, the criteria considered for the development of the consensus rules are limited to traditional hematology parameters. Sysmex instrumentation has capabilities that go beyond the criteria considered for rules development, and allow non-traditional applications to be done cheaply and efficiently. (IG, IPF, RET- He, Body Fluids[2)], HPC).
Fewer vote-outs and a reduced need for dilution / manual counts due to linearity restraints are features of Sysmex cell counting.

By gating the least mature fluorescent platelets, a parameter synonymous with the Reticulated PLT count is now available on a routine cell counter. The IPF (Immature Platelet Fraction) parameter is routinely available on the XE-2100 instrument equipped with the IPF master software package.

Clinical applications include detection of increased thrombopoiesis in active ITP (Idiopathic Thrombocytopenic Purpura), and other disease states with increased peripheral destruction / consumption. Early evidence suggests that the IPF parameter may be a useful tool in distinguishing between bone marrow suppression of thrombopoiesis, and peripheral destruction / consumption of platelets.

References

1) The International Consensus Group for Hematology Review: Suggested Criteria for Action Following Automated CBC and WBC Differential Analysis. Laboratory Hematology, 11:83-90, 2005 .

2) Lesley K, et al.: Performance Evaluation of the Application of Body Fluids on the Sysmex XE-2100 Series Automated Hematology Analyzer. Laboratory Hematology, 11: 24-30, 2005.