

Reviewer Comments

**Reviewer A**

**Comment 1:** Laboratory investigations play a pivotal role in diagnosing, managing, and following up on diseases. CBC is a frequently requested laboratory test. While the demand for laboratory tests continues to increase technological developments have been introduced in the hematology analyzers.

Decisive technical and methodological advances have accumulated over the years, so the systems have become more sophisticated, and enhanced instruments and techniques not only contribute to greater throughput and efficiencies but also increase accuracy and allow a better characterization of the blood cells.

This is an interesting and well-presented overview of the principal milestones in the development of technologies applied to hematology counters over decades

**Reply 1:** *We are really thankful to the referee*

**Comment 2:** I only raise minor comments and suggest some references, interesting and relevant in this field

Hematological analyzers: history of an evolving technology

Page 4 lines 106-108 The red blood cell volume measurement using the optical method was influenced both by the erythrocyte biconcave discoidal shape and the different refraction index of each cell (depending on hemoglobin concentration)

2 articles illustrate this point

YR Kim & L Ornstein. Isovolumetric sphering of erythrocytes for more accurate and precise measurement by flow cytometry. *Cytometry* 1983; 3(6):419-427.

N Mohandas et al. Accurate and independent measurement of volume and hemoglobin concentration of individual red cells by laser light scattering. *Blood* 1986; 68: 506-513  
Red cell diseases and diagnosis.

**Reply 2:** We added the two articles suggested in the references (References 17 and 18)

**Comment 3:** Page 6 lines 156-159 a Few years before, the same author, reported on 156 the possibility of using an index obtained with the Advia-2120© (Siemens) (% 157 microcytic/% hypochromic ratio index) to differentiate between microcytic anemia and 158 beta-thalassemia with an excellent rate of success (32). Ref 32 was an update of the results reported by G d'Onofrio et al. Automated measurement of red blood cell microcytosis and hypochromia in iron deficiency and  $\beta$  thalassemia trait. *Arch Pathol Lab Med* 1992; 116: 84-89 In the nineties with the Technicon H\*3 devices, using the most modern Advia 2120 The good performance of this formula was proven in a metanalysis JJML Hoffmann, E Urrechaga, U Aguirre. Discriminant indices for distinguishing thalassemia and iron deficiency in patients with microcytic anemia: a meta-analysis. *Clin Chem Lab Med* 2015; 53(12): 1883-94

**Reply 3:** We added the metanalysis as suggested (References 33)

**Comment 4:** Research and modern analyzers

J. R. Furundarena et al. The utility of the Sysmex XE-2100 analyzer's NEUT-X and NEUT-Y parameters for detecting neutrophil dysplasia in myelodysplastic syndromes. *Int. Jnl. Lab. Hem.* 2010, 32, 360–366.

**Reply 4:** Added as suggested (see reference 51)

**Comment 5:** Sang Mee Hwang & Youngwon Nam. Complete blood count and cell population data parameters from the Abbott Alinity hq analyzer are useful in differentiating myelodysplastic syndromes from other forms of cytopenia. *Int J Lab Hematol.* 2022;44:468–476.

**Reply 5:** Added as suggested. (see reference 50)

We are really thankful to the referee for this very valuable input.

**Reviewer B**

**Comment 1:** Title. There should be more focus on the data described because in the article only red blood cell analysis is covered more in deep than white blood cells for example I recommend changing the title.

**Reply 1:** We have changed this as requested **MODERN HEMATOLOGY ANALYZERS:**

**BEYOND THE SIMPLE BLOOD CELLS COUNT (with focus on the red blood cells**

**Comment 2:** In the part of Hematological analyzers: history of an evolving Technology □ there are needed some figures illustrating the evolution of the methods for a better illustration of the Technology evolution. Also, some histograms and cytograms showing the different systems are needed.

Table 1 can be changed to a chronologic line dating the most relevant year and discovery.

**Reply 2:** Table 1 has been deleted and replaced by Figure 1

**Comment 3:** If the article has to focus on general hematology not only on red blood cells, then further information on red blood cell lysing agents and days evolution for leukocyte differentiation, reticulocytes count and immature platelets detection needs and important extension, as well as the part of leukocytes changes, are needed.

**Reply 3:** As requested also by other reviewers we have changed the title in line with the fact that in the text red blood cell analysis is covered more in detail compared to other cells.

**Comment 4:** Line 125 a case example of the histogram is needed.

**Reply 4:** We think it may be of greater interest to add the figure related to a more recent work on the same topic, as requested by the reviewer in the next point (see below)

**Comment 5:** Line 127 examples of RBC cytogram pattern are needed to better illustrate of the article.

**Reply 5:** Figure added as requested (see Figure 2)

**Comment 6:** Line 132, 141, 157, and 170 improved or summarized decision trees can be added considering this is a review article.

**Reply 6:** We do appreciate the point made by this reviewer, however, it is very difficult to report an improved decision tree without being able to demonstrate it. Furthermore, the reference work (Nivaggioni et al. Int J Lab Hematol 2020;42:697-704) is very exhaustive on this topic.

**Comment 7:** Also, a line time with the decision trees will be interesting to the reader. Line 216 if the focus of the article is red blood cells this part is not needed. If the willingness of the authors is to be included then a significant extension to detect leukemia and other leukocytes diseases is needed.

**Reply 7:** We agree with the reviewer, a further review would be needed to describe this part in detail. As suggested by reviewer #1, we added some references. We think that this paragraph, although probably not exhaustive, can certainly be interesting for readers. The fact that hat some studies have identified in simple parameters a valid aid for the diagnosis of myelodysplastic syndrome can be an interesting topic.

**Comment 8:** Lines 236,246, 251 if possible examples of cytogram of these diseases will be needed.

Line and section 261 -> need examples and a full explanation of the CPD changes. Also, a timeline for leukocytes to discover diseases with automated blood cell analyzers is needed.

**Reply 8:** As requested by other reviewers we have changed the title in line with the fact that in the text red blood cell analysis is more exhaustive

### **Reviewer C**

**Comment 1:** The topic of the manuscript is interesting and has the potential to be published

**Reply 1:** We are really thankful to this reviewer

**Comment 2:** However, the purpose of the manuscript as such remains unclear. Is this a review article? Then it should be far more comprehensive, and cannot be considered for publication as a review article as such. If this is an article describing the practical implementation of novel hematology analyzer parameters in the authors' laboratories or based on their experiences, more hands-on examples, testing, implementation, etc. with statistical analysis are needed.

**Reply 2:** Our intention is to present a short and concise clinical review with practical reflections

**Comment 3:** The manuscript focuses mainly on erythrocytes, with a minor focus on leukocytes. I'd suggest focusing on one topic/blood cell type to avoid confusion especially if the aim is to focus on practical experiences. For example, there is a massive amount of publications on hematology analyzers' ability to detect infections or to aid in infection diagnosis. In this manuscript, the paragraph regarding this is very limited in data and references.

**Reply 3:** We understand the reviewer's considerations, but being able to be fully exhaustive on such a vast topic is very difficult. We have tried to describe the aspects that are commonly seen in clinical practice. Moreover, while responding to a comment from another reviewer we have decided to change the title to better focus our work.

### **MODERN HEMATOLOGY ANALYZERS: BEYOND THE SIMPLE BLOOD CELLS COUNT (with focus on the red blood cells)**

**Comment 4:** In the Introduction, the authors describe that they present some examples of the potential diagnostic information provided by haematology analysers. In the Abstract, it is said that the examples described are significant. Please specify how you define significant? What is the clinical relevance of the parameters?

**Reply 4:** We understand the reviewer's considerations. We wanted to give some examples of how some parameters offered by analyzers can help physicians. The term "*significant*" perhaps seems inappropriate and we have deleted it (see Abstract)

**Comment 5:** Table II: I suggest considering switching the presentation so that the main purpose is the origin of data presentation and then presenting the various studies regarding this purpose, together with their outcomes. Also, now the studies presented here are spurious. There are several studies regarding Ret-He and IDA, for example. Please, define which analyzer manufacturers and which models the data refers to.

**Reply 5:** We have added the type of analyzer used in the different studies (see page 7, last paragraph of the section "**Red cell diseases and diagnosis**")

**Comment 6:** Row 73: I can't see the relevance of describing the history of hematological analyzers here. The history is not comprehensive and thereafter remains vague. Please revise.

**Reply 6:** We understand the point of this reviewer, however our intention was to give a brief touch of key historical points as reported in the new figure (NOTE this was also requested by another review)

**Comment 7:** Table I: See comment 1. Should this manuscript be a review article, a description of history might be relevant. In that case, please consider which data is relevant enough to be presented in a table. Also, in that case, refer to more recent advances after 1977.

**Reply 7:** Table 1 has been deleted and replaced by Figure 1, as requested by reviewer

**Comment 8:** Row 158. “microcitic”, please correct.

**Reply 8:** This has now been amended

**Comment 9:** Row 191 forward: Please specify, which analysers from different companies are referred to.

**Reply 9:** Done as requested

**Comment 10:** Row 216: The title of the section is unclear. What is meant by research? Research parameters? Please clarify. We agree with the reviewer, the title of the section is unclear.

**Reply 10:** We changed the title to: “Modern *Analyzers in the Diagnosis of Myelodysplastic Syndrome*”

**Comment 11:** Row 251 forward: There are several comprehensive publications regarding Sysmex XN and the detection of malaria-infected RBC instead of one case study. Please review the literature comprehensively.

**Reply 11:** References 59, 60, 61, and 62 refer to malaria detection with Sysmex analyzers.

**Comment 12:** Row 261: In this section, the authors describe the usability of CPD. Are these for research use only? If so, why are they not under the title Research and Modern Analyzer?

**Reply 12:** We understand the reviewer's considerations, however, these parameters (CPD) have been described in a dedicated section, since they have been considered an aid for inflammatory and/or infectious diseases.

#### **Reviewer D**

**Comment 1:** Mark of copyright © should be changed to Trademark ™ or registered trademarks.

**Reply 1:** We have changed as suggested

**Comment 2:** The abbreviation (Iron-Def) is defined on line 175, so three copyright symbols on lines 181 and 185 are not needed.

**Reply 2:** We have changed as suggested

**Comment 3:** I couldn't find the citation of document number 35 in the text. Please confirm. A citation 31 on line 173 might be citation number 35.

**Reply 3:** We apologize for the error. With the addition of some articles as requested by

another reviewer, the corrected reference is now number 37

**Comment 4:** Please add some citations for the text on lines 98-100: "The amplitude of scattered light collected at narrow-angle (forward scatter) was found to correlate to the cell volume, while the refraction index at greater angles was associated with the cellular structural complexity".

**Reply 4:** We added the missed reference (reference number 14)

**Comment 5:** There are many useful measurands (parameters) reported, but some experiences that different manufacturers and different models might recognize different results for the same name parameters. From this perspective, if you have any views on measurement items that need international standardization, it would be good if you could add them for readers.

**Reply 5:** We understand the reviewer's intentions, but most of the additional parameters are technology and instrument-dependent. A real standardization is extremely difficult to achieve. As reported in our conclusion is very important that laboratory specialists become aware and familiarise with the potential offered by the current and different instruments and technology available.