

Peer Review File

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Reviewer A

The paper describes the Detection of an unlabel Hb variant by a Mindray BC6800 plus counter and the data appear convincing.

The principle for the Detection of WBC is inadequately described. I believe all the X-axis reflects the leukocytes. The Y-axis reflects the amount of Nucleic acids within the cell.

Reply: Absolutely right This section has been modified accordingly

The hematological and biochemical results should be presented in a table together with the reference intervals for each parameter

A table has been added to the letter , thank you for the suggestion

Figure 2 is not necessary. Figure 3 and 4 can be put together in one figure.

Furthermore, the axes and peaks must be properly labelled in figure 3.

Reply: The figures have been changed

I advise you to have a professional editing service correct your language or seek assistance of a fluent English-speaking colleague.

Reply: The manuscript has been edited to improve language

Reviewer B

- 1) I would advise you to organise the laboratory results in a table. If you are using too many figures then you could omit the sequencing figure.

Reply: A table has been added to the letter , thank you for the suggestion , the figures have been also organized

- 2) I think you need to add a sentence to make it a little bit clearer, which error we are talking about. I think adding an arrow to indicate the deviation of the plots in figure 1 would be helpful

Reply: We have tried to clarify the point

- 3) I think you need to discuss in more detail how target cells affect the Mindray and therefore and compare your error to liver disease

Reply: Thank you for the suggestion we have added information

- 4) HPLC - P stands for High-performance not High pressure

Reply: Absolutely right

Reviewer C

Here, the authors present an interesting case report describing the detection of a hemoglobinopathy via its interference in the WBC differential channel on the Mindray BC 6800 Plus analyzer. This appears to be a novel finding as the authors note there is no other published incidence of this phenomenon except with Hb Johnstown.

I have a couple of suggestions that I think will make the paper stronger:

1. The lab test data presented in the first paragraph of the case presentation needs reference intervals for all of the tests. Furthermore, please check the accuracy of the units for ferritin and haptoglobin.

Reply: Laboratory results are presented in a table , and units corrected

2. The English in the manuscript is sometimes awkward. Please have it reviewed and edited for clarity.

Reply: The manuscript has been edited to improve language

Reviewer D

The authors report a case of an asymptomatic patient carrying a rare unstable variant (Hb Shelby). The variant has been suspected because of an abnormal pattern of a low fluorescence signal for WBC.

Although the Hb Shelby causes mild phenotype even when associated with other variants. This could be an interesting and accessible way to detect carriers of unstable variants that may cause mild to severe phenotypes.

The main idea of the manuscript is clear. However, some revisions are needed.

1- Some sentences need to be rephrased because of Grammar mistakes and unclarity. Example: line 30-31, line 34-35 (variants are reported to be...), line 38-39 ...leads or is leading, Line 68-69

2- Line 68-69 Sequencing "og the gen"?!

3- Line 68-69 it's better to write the correct nomenclature of the variant c.position C>A and p. ...

4- Line 70-79 : This part doesn't fit with the case presentation. It involves literature results and discussion so it's preferable to put it in discussion.

Reply: The manuscript has been edited, so all the mistakes have been corrected

5- Another point to clarify: Did authors make HLPC because of the WBC pattern or because of the smear results? Did they suspect the unstable variant?

Reply: Some previous articles on Sysmex analyzers described the characteristic low signals along Y axis when unstable Hb is present; we found the same effect for Hb Johnstown in the Mindray counter; so we run the chromatogram to verify that the patient was a carrier.

6- It's important to emphasize the importance of the detection of carriers of unstable variants especially as this technic is accessible in all the labs.

Reply: Absolutely agree