Peer Review File

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<mark>Reviewer A</mark>

Comment: I am confused, were the patients informed that the remaining clinical samples would be saved in a biobank for future research? So, they could opt out? please explain. Reply: We feel great thanks for your professional review work on our article. We apologize for the poor language of our manuscript. Regarding ethical issues, there may be ambiguity in the expressions of our manuscript. We have modified our text as advised (see Page 4, line 14) Changes in the text: The samples studied were the remaining clinical specimens with EDTA-K2 (dipotassium ethylenediaminetetraacetic acid) anticoagulant for which test reports had been sent and did not involve the privacy and interests of patients. The study was approved by Medical Ethics Committee of West China Second University Hospital, Sichuan University (No. 2021S160).

Reviewer B

Comment 1: Abstract: "anthropic factors" - recommend "human factors", high hct or high triglyceride samples

Reply 1: Thanks for your suggestion. We have modified our text as advised (see Page 2, line 8 and 21)

Changes in the text: "anthropic factors" has been changed to "human factors" and "in samples with high HCT or triglycerides" has been changed to "high HCT or high triglyceride samples"

Comment 2: Introduction: It would be important to discuss recent CBC devices that specifically handle capillary blood. This includes: Sight Diagnostics OLO, Pixcell Hemoscreen, Boule Medonic devices.

Reply 2: We feel great thanks for your professional review work on our article. Although these CBC devices use imaging technology instead of the impedance and light scattering technologies to make CBC efficient and convenient. And even just two drops of blood can complete the test avoiding the hassle of mixing samples. However, these devices just used for point-of-care test (POCT) in a limited clinical setting like small laboratories, outpatient clinics and emergency departments(1,2). And Sight Diagnostics OLO is not suitable to use on samples from patients less than 3 months of age and patients with extremely high WBC counts(1). But newborns or hemodialysis patients are the main population for capillary blood used in CBC. We need the hematology analyzers that are suitable for most laboratories and meet the needs of as many population as possible. The sufficient blood volume of samples is a prerequisite for accurate results, therefore, samples need to be sufficiently mixed, which is the focus of our article. Therefore, we think that we can dispense with the discussion of these types of devices in the introduction.

1. Leite R, Woodcock S, Brady S, et al. Performance analysis of the compact haematology

analyser Sight OLO. Int J Lab Hematol 2022;44:1078-87.

2. Ben-Yosef Y, Marom B, Hirshberg G, et al. The HemoScreen, a novel haematology analyser for the point of care. J Clin Pathol 2016;69:720-5.

Comment 3: Methods: This a very large amount of blood and would generally result in squeezing a finger to obtain this volume, allowing introduction of tissue factor and clotting. Also, word choice, should be "histogram" or "scattergraph".

Reply 3: Thank you so much Reviewer for your kind feedback. In this part we used venous blood to investigate whether the blood volume affects the effect of automatic mixing. We have modified our text as advised (see Page 4, line 30 and Page 6, line 3)

Changes in the text: We have added "In this part we used venous blood" in our manuscripts, and "histogrammic or scatter graphic abnormalities" has been changed to "those with abnormal histogram or scattergraph."

Comment 4: Results: Be good to see what the normal results look like – Table 2 states they are in range between the manual and automatic, but correlation and Bland-Altman graphs would be a must to establish the method. There is data for the four confounding groups (thalassemia, high fibrinogen, high TG group, and high HCT group. Table 2 is presented before Table 1. Also, for each, there should be a correlation graphs of the manual versus the automatic.

Reply 4: Thanks for your suggestion. Table 1 is the industry standard Analytical Quality Specifications for Routine Tests in Clinical Hematology (WS/T 406-2012), therefore we put it at first in the part of methods. According to WS/T 406-2012, we could evaluate the comparability between the two modes by the relative deviations. Due to the limitation of article capacity, only some of the graphs with bad correlation are shown in the article.

Comment 5: Discussion: Please state more clearly what samples were used (venous or capillary) and for which parts of the experiments. It would be good to actually see correlation plots to determine the ICC. The method was validated on normal healthy; it would be good to see the correlation graphs and the Bland-altman without the four confounding sets.

Reply 5: We set four groups of specimens with interference factors in the mixing process, of which the blood volume group used venous blood and the remaining groups used capillary blood. However, the purpose of our experiment was to evaluate the consistency between the two modes of automatic and manual mixing of the samples, so the use of venous blood did not affect our comparison between the two modes. As regards the graphs, the capacity of the article is limited and we can provide it separately if needed.