

Review Form 1.7

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	Ms_IJBCRR_111182
Title of the Manuscript:	A multicentric verification of hereditary and acquired thrombophilia coagulation assays
Type of the Article	Short Research Article

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<p>Compulsory REVISION comments</p> <p>1. Is the manuscript important for scientific community? (Please write few sentences on this manuscript)</p> <p>2. Is the title of the article suitable? (If not please suggest an alternative title)</p> <p>3. Is the abstract of the article comprehensive?</p> <p>4. Are subsections and structure of the manuscript appropriate?</p> <p>5. Do you think the manuscript is scientifically correct?</p> <p>6. Are the references sufficient and recent? If you have suggestion of additional references, please mention in the review form.</p> <p><u>(Apart from above mentioned 6 points, reviewers are free to provide additional suggestions/comments)</u></p>	<p>Yes. Like any other test, the thrombophilia panel test may have limitations. Disorders will affect the result and interpretation of the thrombophilia test. A multicentric verification of hereditary and acquired thrombophilia coagulation assays can be more reliable for the attending physician.</p> <p>Yes.</p> <p>Yes.</p> <p>No, in the abstract, it is better to add the Background section. It is also better to write the results and discussion section separately.</p> <p>Yes.</p> <p>Yes.</p>	<p>Thank you for your comment, we appreciate it.</p> <p>Thank you for your comment. We have completely rewritten the study design paragraph: An extensive and multicentric verification of coagulation assays included in thrombophilia testing was performed on the BCS XP coagulation analyzer (Siemens Healthineers, Marburg, Germany): antithrombin activity (AT) (Innovance Antithrombin), protein C activity (PC) (Berichrom Protein C), protein S activity (PS) (Protein S Ac), free protein S antigen (free PS:Ag) (Innovance Free PS Ag), activated protein C resistance (APCR) (ProC Ac R and ProC Global + Coagulation Factor V Deficient Plasma), lupus anticoagulant (LA) screening (LA1 and activated partial thromboplastin time by using Dade Actin FSL as reagent) and confirmation test (LA2), factor VIII activity (FVIII) (Dade Actin FS and coagulation FVIII Deficient Plasma).</p> <p>Also, some concrete results were added in the Results paragraph: Results: All of the obtained imprecision CVs were within the manufacturer's claims (<5/10/15%). While the observed bias for PC (+1.9%) was within the EFLM performance specifications (6.7%), the average bias for AT was higher than acceptance criteria (+10.8% vs 3.2% allowed). P&B regression revealed a significant positive proportional difference (slope=1.14).</p> <p>The Methodology paragraph was shortened to preserve the allowed number of words.</p> <p>Regarding the results and discussion section, we have used the Journals template where those two sections are combined.</p>
<p>Minor REVISION comments</p> <p>1. Is language/English quality of the article suitable for scholarly communications?</p>	<p>Requires little editing.</p>	<p>Some editing has been done throughout the text, marked in yellow.</p>
<p>Optional/General comments</p>	<p>In the discussion section, the results of the study should be compared with the results of similar studies. The discussion section is poorly written.</p>	<p>Thank you for your comment. We have significantly altered the discussion by introducing six new references that have been commented on in the manuscript:</p> <p>1. Flieder T, Gripp T, Knabbe T, Birschmann I. The Sysmex CS-5100 coagulation analyzer offers comparable analytical performance and excellent throughput capabilities. Practical Laboratory Medicine 2016;6:38 – 47</p>

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		<div>2. Legnani C, Palareti G, Boggian O, Cavallaroni K, Oca G, Lo Manto G, et al. An evaluation of several laboratory tests and test combinations in the detection of lupus anticoagulant. Int J Clin Lab Res 1992;22:106-110</div> <div>3. Favaloro EJ, Mohammed S, Vong R, Chapman K, Swanepoel P, Kershaw G, et al. A multi-laboratory assessment of lupus anticoagulant assays performed on the ACL TOP 50 family for harmonized testing in a large laboratory network. Int J Lab Hemato. 2022;44:654–665</div> <div>4. Hörber S, Lehmann R, Peter A. Evaluation of the Atellica COAG 360 coagulation analyzer in a central laboratory of a maximum care hospital. Int J Lab Hematol 2020;42:28–36</div> <div>5. Scherer-Burić RA, Lesser-Wetzold K, Nagel D, Weigand M, Spannagl M, Teupser D, et al. Performance testing of four automated coagulation analyzers in a university hospital setting with focus on global coagulation assays. Int J Lab Hematol 2022;44:643–653</div> <div>6. Strande BJN, Sridharan M, Leger RR, Stuart MS, Tange JI, Navitska SD, et al. Effect of residual platelets in frozen-thawed plasma on results of dilute Russell's viper venom time assay for lupus anticoagulant testing. Am J Clin Pathol 2023;aqad138. doi: 10.1093/ajcp/aqad138. Epub ahead of print. PMID: 37878771.</div> <div>The following changes have been introduced in the Discussion section:</div> <div>3.1 Imprecision</div> <div>Although similar findings were obtained by Flieder and Hörber [11,12], their verification studies were performed on different coagulation analyzers (Sysmex CS-5100 and CS-2000i vs Siemens Atellica Coag 360), thus disabling the direct comparison with our study performed on Siemens BCS XP analyzer. However, it is worth emphasizing the surprisingly high CVs for Protein C on the Sysmex CS-2000i analyzer in the Flieder study [11]: 7.92% for Control N and 10.02% for Control P, which even exceeds the manufacturer's claim of 10% allowed in the imprecision study. Protein C chromogenic assay was the assay that excelled in its performance during the verification study, thus the observed differences could be attributed to different coagulation platforms used. Surprisingly, the CVs for Protein S observed in the study by the Hörber et al. [12] were significantly lower than numbers obtained in our study. Possibly we can employ the same explanation as for the Protein C assay, incomparability of the coagulation analyzers.</div> <div>3.2 Accuracy (method comparison study)</div> <div>Although, similar results were observed in a comparison study of four automated coagulation analyzers by Scherer-Burić et al. [13], with a significant positive slope for AT (1.05), and in the Hörber study [12], with a positive antithrombin bias of 11.2%, the first received ECAT report revealed unsatisfactory result for AT activity with a positive bias (+9.8%) observed in comparison to other participants that used the same</div>
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		<p>method. Although it is worth emphasizing that the biases of the commercial quality controls during the whole verification period were within the allowed manufacturer's claims ($\pm 20\%$), the received results were in accordance with the positive bias observed in the comparison study (irrespective of the centers involved in the comparison study), and could not be ignored, nor explained by the incomparability of the coagulation analyzers, as the observed bias was consistent in all studied samples.</p> <p>We preferably decided to compare the final classification of all received samples, then to assess the comparability of the individual measurements of the screen-mix-confirm protocol, as it was previously confirmed that some minor differences between reagents and instruments are to be expected and are also observed in EQA data [14].</p> <p>Two of the discrepant lupus anticoagulant results when compared to University Hospital Centre Zagreb and Sestre milosrdnice University Hospital Center were characterized as positive in those two centers with established thrombophilia testing, but they were falsely reported negative in our department. It is well documented that all tests examined could detect patients with strong anticoagulants, none was able to detect all patients, especially those patients with weaker anticoagulants could be missed [15], which could be the possible explanation for the observed misclassification. Additionally, as the verification protocol included frozen remnant plasma samples, perhaps residual platelets in plasma samples could result in shortening of DRVVT assay which may result in a false-negative LAC test result [16].</p>
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PART 2:

	Reviewer's comment	Author's comment <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
Are there ethical issues in this manuscript?	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	