

Assistant Editor's comments	
<p>This document provides a valuable effort in harmonization of preanalytical procedures in hematology, especially among Croatian laboratories. However, there are two major issues regarding this recommendation:</p> <p>1) It is not clear why authors chose to provide a recommendation only for the EDTA-induced PTCP when they mention in the Introduction section that PTCP might be caused by several other causes. It would increase a value of this document if authors could propose a systematic approach to management of samples with suspected PTCP.</p>	<p>We would sincerely like to thank the assistant editor and reviewers for their valuable and helpful suggestions, which we tried to follow as much as possible while revising the manuscript. We hope that the undertaken changes are acceptable. All changes within the manuscript are highlighted in yellow.</p> <p>Although PTCP might be caused by several other causes, we decided to approach this subject by specifically addressing EDTA-induced PTCP – the most common form of PTCP. We wanted to create a short and easily applicable national recommendation. This document is to be followed by other similar documents addressing specific preanalytical procedures in hematology. The Working group intends to publish a series of short recommendations rather than give one more comprehensive. We believe shorter documents are more easily applied in everyday routine laboratory practice.</p>
<p>2) A clear flowchart described in the manuscript and provided graphically seems to be missing. Please list all steps that need to be done when someone suspects that PTCP is present.</p>	<p>The flowchart was introduced, as suggested. See Figure 2.</p>
<p>Title Please revise the title: National recommendations of the.....: Management of samples with suspected PTCP</p>	<p>The title has been revised as suggested. the revised title is: National recommendations of the Croatian Chamber of Medical Biochemists and Working group for Laboratory hematology of the Croatian Society of Medical Biochemistry and Laboratory Medicine: Management of samples with suspected EDTA-induced pseudothrombocytopenia.</p>
<p>Graphical abstract There is too much text present; please provide most important data in a bulleted form (after revision of the manuscript).</p>	<p>The Graphical abstract was revised, as suggested.</p>

<p>Highlights Highlights are too general, there is no data specific for this manuscript. Please revise.</p>	<p>The Highlights section was revised, as suggested.</p>
<p>Introduction - Introduction is too long - please use this section only to describe background, purpose and aim of this document. Information about PTCP can be provided in the next separate paragraph.</p>	<p>The Introduction section has been substantially shortened. PTCP is described under a separate paragraph (Recommended criteria for raising suspicion on PTCP).</p>
<p>- Please add reference after the sentence: "This benign and rare phenomenon, presents in 0.03-0.27% of the general population and in up to 15.3% of patients with thrombocytopenia, and is not associated with any specific disorder or therapy".</p>	<p>The appropriate reference has been added.</p>
<p>- Authors should consider adding a table with an overview of instrument flags indicating PTCP that appear on most common hematology analyzers.</p>	<p>Table 1 was added with an overview of instrument flags indicating PTCP that appear on most common hematology analyzers.</p>
<p>- Figure 1: Please add the source for this figure. Also please add more detailed description (analyzer, method).</p>	<p>The analyzer, the method, and the source were added in the description of Figure 1.</p>
<p>- Recommended criteria for raising suspicion on EDTA-induced PTCP - why are these criteria specific to EDTA induced PTCP? These are criteria for suspicion to PTCP in general.</p>	<p>We agree with this comment and have removed EDTA-induced from the text in this section.</p>
<p>- After exclusion of preanalytical errors, as a first step in management of PTCP authors propose morphological assessment. However, since this method is rather time consuming, shouldn't the first step be repeating a measurement using a different analytical method, where available (as described in the Introduction section)? It is clear that this approach can not be obtained in all laboratories since many of the smaller laboratories do not have multiple analytical systems for hematology measurement, nevertheless this is a common approach in many laboratories and should be mentioned.</p>	<p>Although reflex testing using an alternative method (i.e. optical or fluorescence-based) has been described in the literature an "effective means of correction" of platelet counts, not enough evidence is available to recommend reporting platelet counts without microscopic confirmation of PTCP and repeated sample collection using a 3.2% sodium citrate tube. The results obtained using reflex testing at this point might be regarded as "informative" but not definitive. This was addressed with the addition of a paragraph after the exclusion of preanalytical errors, and before morphological assessment of the blood smear. The relevant references were</p>

	added.
- Also, authors should specify do they recommend morphological assessment by manual staining and microscope or automatic staining and digital microscopy or is it not relevant.	We added that the smears can be stained either manually or using an automated slide maker and stainer, while inspection of the smear for the purpose of detecting platelet clumps and confirming PTCP should be done exclusively manually using light microscopy, since this is the method of choice recommended by ICSH. The appropriate document has been cited and it becomes reference 12 in the revised manuscript. The reference list has been revised accordingly.
- Platelet aggregates and platelet satellitism are not specific to EDTA induced PTCP. How do you know at this point that PTCP is caused by EDTA? How can you confirm that PTCP is caused by EDTA prior to obtaining citrate sample?	We completely agree with this comment. Therefore, the section was rephrased.
- Authors should present clearly that the difference in number of platelets should be observed in the citrate sample in order to confirm EDTA induced PTCP. Also, it would be usefully to present approximate percentage difference that would confirm EDTA induced PTCP.	We completely understand the rationale behind this comment, however, it is not possible to determine an exact rise in platelet count which should be obtained in the citrate sample to confirm that in the EDTA sample PTCP was EDTA-induced. The percent difference in platelet count is difficult to estimate because it depends on multiple factors. Instead, in the revised manuscript, we added a sentence that the platelet count and platelet-related indices are reported only after microscopic exclusion of the presence of platelet aggregates in the peripheral blood smear prepared from the 3.2% sodium citrate tube. The absence of platelet aggregates in the citrate tube confirms that the observed PTCP was EDTA-induced.
- "Alternatively, platelet count might be determined after repeated collection in an EDTA tube (lavender cap) if sample analysis can be performed without delay and immediately after venipuncture." - Why is sampling in EDTA tube proposed here? Without delay and immediately are	We understand that this part is not clear, as also indicated by reviewer 1. Therefore, we completely removed this part, as suggested.

subjective terms and have different meaning in different hospital settings. Either give exact recommendation in minutes or remove this part.	
- Please describe procedure in the second EDTA tube, how would you assess results and confirm/exclude EDTA induced PTCP.	The procedure related to the second EDTA tube has been removed, as previously suggested.
- Instead of: "should be corrected with a 1.1 correction factor" please write "should be multiplied by 1.1"	This has been corrected as indicated by the editor.
- It is not clear what is the difference between the first and the third paragraph of the section: 4. Reporting platelet count from samples with confirmed PTCP. - recommendations are practically the same.	We agree that these two paragraphs refer to the same issue; therefore, the third paragraph was removed.
The second paragraph of this section is not clear: "Alternatively, if repeated collection was performed using an EDTA tube immediately transported to the laboratory and platelets counts were determined without delay, the platelet count should be reported from the EDTA-sample". What should happen in the second EDTA tube to allow reporting of the platelets? Increased number of platelets in regards to the original sample? This part is not described well.	We understand that this part is not clear, as also indicated by reviewer 1. Therefore, we completely removed this part in the revised version of the manuscript.
Authors should provide a flowchart of concrete steps for exclusion/confirmation of PTCP.	The flowchart was added, as suggested.
Authors are also encouraged to provide an example of laboratory report with confirmed and excluded PTCP.	Examples of laboratory reports for each described situation have been added in the revised manuscript as Figure 4.
Reviewer 1	
The authors have submitted a short review of EDTA induced pseudothrombocytopenia and recommendations for management and action in the laboratory. The information given is not new but the specific recommendations are intended to provide a national framework for harmonisation of practice.	We sincerely thank the reviewer for appreciation of our work, valuable and helpful suggestions that we tried to follow as much as possible while revising the manuscript.
I would like to make the following observations: 1. Please check the text for occasional	Thorough English proofreading has been done and all spelling errors have been corrected.

spelling errors (I noted benign and harmonisation but there may be others)	
2. Please rewrite or remove the phrase in parentheses "(physician or nurse in his absence)". Not all physicians are male and the test may have been requested by a nurse practitioner or other healthcare professional. Also consider the impact on the laboratory and the patient of suggesting contacting the patient directly. Is this advisable unless the platelet count is very low?	The phrase in parenthesis has been removed in the revised manuscript, while regarding direct contacting of the patient, we added a remark that in case when the patient's healthcare provider cannot be reached the patient should be contacted for a repeated blood sampling.
3. The figure at the start of the manuscript is not given a figure number or referenced in the text. A flow chart is very useful in this type of instruction.	Revised as suggested. The flowchart was added, as suggested.
4. I'm not sure that 'recommendations' is specific enough to be used as a keyword but will be guided by the editors.	The keyword 'recommendations' has been replaced by 'procedures', a keyword that is included in the MeSH keywords database.
5. I suggest that the colour of the tube caps is omitted since it may vary in other regions.	The color of the tube caps has been omitted in the revised manuscript, as suggested.
6. The authors note the impact of preanalytical errors and I suggest that the term clot(s) and/microclot(s) is used in place of coagulum. I am unsure that the note about venepuncture site is relevant - hemodilution will cause pancytopenia and is to be avoided for all specimen collection, not just a possible cause of thrombocytopenia.	The term 'coagulum' has been replaced by 'clot'. The part about the venipuncture site has been modified to make it clear that dilution will cause false lowering of all cell counts, including the platelet count.
7. I suggest that recommendations are more direct - there is a recommendation to take a sodium citrate and 'alternatively' a repeat EDTA with immediate analysis. I suggest that it is better to collect one of each anticoagulant and arrange for immediate analysis, to reduce the number of venepunctures required and to obtain the best possible count in the shortest time possible.	We understand that this part is not clear, as also indicated by the associate editor. Therefore, we completely removed this part in the revised version of the manuscript.
8. I suggest that the authors say that the Fonio counting method may be used rather than should be used. The Fonio method requires substantial technical skill and microscopic counting ideally should be	Revised as suggested.

<p>undertaken using a graticule to section the visual field.</p>	
<p>Reviewer 2</p>	
<p>Dear Editor, According to the guidelines for reviewers, I would like to report on my reviewing progress. The manuscript is comprehensive, up to date and includes the relevant and new information. The type of article is well selected, since the reviews are intended to encompass a comprehensive overview of a topic, including clinical and analytical information, current relevance and future directions, not exceeding 5,000 words, 8 tables or figures and 100 references. The title relates to the content of the article. Keywords are appropriate and reflect the content of the article. The Abstract is according to guidelines: "Reviews should include an unstructured abstract." In Introduction section, the authors provided sufficient details to explain the background of the problem. Relevant literature is listed. However, the Figure 1 is not technically clear, so it needs improvement. The references are up-to-date.</p>	<p>The analyzer, the method, and the source were added in the description of Figure 1.</p>
<p>In the revised version of the manuscript you have added a step of inspecting blood smear from the citrate tube as an additional step for confirming PTCP. Although this is the only definite way for confirming PTCP, I would suggest to add an alternative way - PTCP can also be confirmed if the number of Plt is significantly higher in the citrate sample. This should be added in GA, Figure 4 and section 3. Measuring platelet count in samples with suspected EDTA-induced PTCP. After this section you can explain that the exact criteria for the significantly higher</p>	<p>The manuscript was revised as suggested (please refer to 3. Measuring platelet count in samples with suspected EDTA-induced PTCP).</p>

number can't be strictly defined, and it depends on number of clumps, number of Plt and medical history of the patient.	
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