Peer Review File
Article information: https://dx.doi.org/10.21037/jlpm-22-65

Reviewer Comments

Reviewer A
Comment 1: This paper is a review article that summarises the reasons for and an approach to unexpectedly elevated troponin tests.

The manuscript sates that it is a "review" and that the methods are not required necessarily for this type of manuscript – however, there are no discussions on the strengths or limitations of the review, which is a major omission.

Overall the manuscript is tricky to read in places and focuses more on laboratory issues rather than clinical issues – this limits the value of the manuscript for the clinician who is on the floor. A useful structure might be a short discussion around myocardial injury -> type 1 and type 2 AMI - then proceed to outline the laboratory analytical issues.

There are several sentences/ideas that are repeated and could be condensed.
Reply 1: The manuscript sates that it is a "review" and that the methods are not required necessarily for this type of manuscript – however, there are no discussions on the strengths or limitations of the review, which is a major omission.
You are correct, this manuscript is an invited review for a focus issue on cardiac troponin. As requested a paragraph on strengths or limitations of this review has been added.

Overall the manuscript is tricky to read in places and focuses more on laboratory issues rather than clinical issues – this limits the value of the manuscript for the clinician who is on the floor. A useful structure might be a short discussion around myocardial injury -> type 1 and type 2 AMI - then proceed to outline the laboratory analytical issues.
There are several sentences/ideas that are repeated and could be condensed.
The topic given was to focus on analytical issues leading to false cTn assay results. The title was given by the focus issue editors, but we modified it to make this clearer. We re-worked the text according to your suggestions, in particular the introduction. Table 1 had already been included as a summary of different important diseases leading to myocardial injury.

Comment 2:
Specific comments

Title	Should be clearer about the intent, purpose and

	content of the manuscript
Reply	Title: modified, the original title was a proposal of the focus issue editors
48	Need to be clearer that current cardiac tropoinin routine assays refer to high sensitivity laboratory based troponin assays. There are many contemporary or point-of-care troponin in use internationally.
Reply	Line 48: modified as suggested
50	Use of phrase daily routine - ?daily clinical practice
Reply	Line 50: modified as suggested
63-66	I am not sure that false low – or negative tests are less problematic as is implied by this statement. A false low test may be falsely reassuring and result in the patient being inappropriately discharged or not referred for appropriate investigations or treatment. Is there a reference that supports the authors statement/conclusion about this?
Reply	Lines 63-66: your point has been included
69	"Clinicians to the fact" – rather than "of the fact"
Reply	Line 69: modified as suggested
91	Repeated sentence from above (69)
Reply	None
100	Thiss sentence isn't clear. Are there words missing?
Reply	Line 100: re-worked
332	Hs-cTn appears more sensitive than imaging … What is it more sentivie for?
Reply	Line 332: detection of myocardial injury (added in the text)
337	This statement, while true needs a reference
Reply	Line 337: We have now added an appropriate reference
324-369	This is a very long paragraph. Consider breaking up.
Reply	Line 324-369: done
Figure 1	This may be related to the format in which it is provided to the reviewers but this flow chart is difficult to read as it is very 'squashed'. Considering increasing the height. There are also inconsistencies in the use of question marks.
Reply	Figure 1: reworked as suggested; now it is split into two parts, figure 1a and 1b

Reviewer B

Comment 1: General comments

This review on troponin interferences is relevant and potentially very useful in routine

practice. Its greatest interest is to concern both lab practitioners and clinicians, and to emphasize the need for both to communicate and collaborate to detect these rare interferences. Overall, it is well written, easy to read and understand. The figures used to illustrate the different types of interference are simple, appropriate, and they greatly facilitate the understanding (except the first one, see the comment below).
Reply 1: Figure 1: reworked as suggested; now it is split into two parts, figure 1a and 1b

Comment 2: P6, L120, "interferences severe hemolysis, hyperlipidemia or hyperbilirubinemia": the title does not specify the type of interferences; therefore, the reader could believe/hope that the impact of HIL interferences on the troponin I/T are discussed, but they are just cited. A brief paragraph should be developed, based on well-chosen references.
Reply 2: re-worked as suggested

Comment 3: P6, L124: ref 5 does not deals with hsTnT, but rather with TnI
Reply 3: corrected

Comment 4: P6, L128-134: the paragraph about biotin interference is interesting but a little short, leaving us a bit unsatisfied with only 2 refs (biotin[ti] AND troponin[ti]: n=17 refs on Pubmed); it should be developed a little.
Reply 4: re-worked as suggested

Comment 5: P8, L170-172: one or two references supporting the usefulness of heterophilic binding tubes (HBT) in case(s) of antibodies interferences in troponin assays would be welcome or, if there is none, at least in other assays (e.g., like TSH).
Reply 5: This paragraph was modified according to your suggestions. There are only published case reports on their use. Therefore, no solid data on the efficacy is available, which also depends on the blocking agents used and the antibodies in a patient sample. Macro-troponin is frequently missed. References had already been included in the original submission including a case report with review of the literature on this topic (29-32).

Comment 6: P8, L177-182: in practice, the logical order should rather be 1) re-centrifugation of the first sample, 2) simple dilution test using the manufacturer's diluent (as in Fig4) and 3) the PEG 6000 protocol (more complicated since it requires to have and/or prepare this solution).
Reply 6: This paragraph and figure 1 were modified accordingly, but dilution testing is the least effective method and always requires additional follow-up testing. PEG 6000 pre-treatment is not more complicated and time-consuming than dilution testing after this method has been established in the laboratory.

Comment 7: P8, L179 refs (28,29) then P9, L196 refs (31,32): but what about the ref 30? (first citation P9, L214); the order of references should be checked.

P10, L228: looking for the word “macrotroponin” in the refs 5 (pmid 10686273) and 39 (pmid 25977070), it does not appear anywhere; is it due to an increment error? or are these references inappropriate?
- Some additional references (pmid below) could be of interest:
Centrifugation/preanalytic: 16776644; 24972004; 25617392; 27346968; 31639761; 36027942…
Macrotroponin: 35929566
Multiple myeloma: 23242077
Reply 7: Thank you very much for recognizing these mistakes, which probably occurred during the frequent revisions before submission. References were added where necessary.

Comment 8: Figure 1 (P26):
- At first glance, the layout seems a bit complex and could put off the reader; a more orderly arrangement of items, connected by vertical/horizontal arrows (not oblique) would make it easier to see and facilitate understanding.
- First step, “Re-draw blood sample…”: what about re-centrifugation of the first sample, as proposed P5, L102?
- L575 (legend), “* A time period of >6 h for re-sampling is recommended to rule out…”: at a time where 2h-delta and even 1h-delta changes in hsTn are beginning to be considered in recommendations, wouldn't it be more appropriate to propose a 3h-delta change rather than a 6h-delta change?
- PEG 600 (Fig1) or PEG 6000 (Table 2)?
Figure 4 (P29):
- “hs-cTnI” instead of “cTnI” on the y-axis
Reply 8: Figure 1: Your suggestions have been followed and the figure has been split into two parts, figure 1a and 1b. We also modified the figure legend to a 3h-delta change sampling protocol, which may also be appropriate. The typo was corrected (PEG 6000 is correct).

Figure 4: This figure has been modified as suggested.