Peer Review File
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Reviewer Comments

Reviewer A
Comment 1: General comments:
The aim of this review is not defined very well. If not sufficient data on a particular topic is available for a specific assay, this must be mentioned as well.
Reply 1: We have re-written our manuscript to better address this.

Abstract:
Comment 2: Line 59: "speed" – what do you mean?
Reply 2: We have re-written our manuscript and abstract and this is no longer part of the manuscript.

Comment 3: Lines 68-71: What is the aim of this review?
Reply 3: We aim to assess whether there are population differences in the 99th percentile URLs of high-sensitivity cardiac troponin I (hs-cTnI) and high-sensitivity cardiac troponin T (hs-cTnT) derived from healthy reference populations in different populations across different geographic locations.

Introduction:
Comment 4: The introduction is too long and not straight to the point.
Reply 4: We agree and have shortened and re-written the introduction.

Comment 5: Page 5, line 84: should be "contractile" apparatus
Reply 5: Corrected.

Comment 6: Line 88: … rise or fall, delta … - what do you mean?
Reply 6: We have re-written the manuscript and this is no longer included.

Comment 7: Line 92: defined – what do you mean? labelled?
Reply 7: Correction made to labelled on line 75

Comment 8: Page 6, line 106: What do you mean?
Reply 8: We have re-written the manuscript and this is no longer included.

Comment 9: Results, Conclusions:
Must be totally reworked according to general comments
Reply 9: Done.

Comment 10: Tables 1-4:
Does this indicate "data not available"? If so, N/A is more appropriate.

Reply 10: Not reported (NR) used as suggested.

Comment 11: Table 4: Split according to manufacturers into “Beckman” and Ortho and Singulex into 2 separate tables
Reply 11: Tables reworked. As we only included studies with at least 800 subjects (400M, 400F) there was only 1 study for each of these manufacturers. Hence they have been kept in the same table rather than as separate tables and provided as supplementary data as there are no other comparators.

Reviewer B
Comment 1: It would be helpful to state that this is a review in the title.
Including setting or population would help as well.
Consider: “A Review of Population Differences in High-Sensitivity Troponin Levels”
Reply 1: Title has been modified as suggested

Comment 2: The manuscript covers a lot of ground and includes summarized data from prior publications but lacks a clear focus and purpose. A clearer aim for this review and more definitive conclusions would help this paper's contribution to the literature. Even if this meant covering less topics, but clarifying and addressing a clinical challenge or unmet need. For example, combining these many studies to answer a question that no single study can confidently answer on its own.
Reply 2: We have re-written our manuscript to better address this.

Comment 3: Since one of the conclusions is that the current studies are underpowered, this seems to be an ideal opportunity for a meta-analysis with these data.
Reply 3: As the study designs vary and would make it difficult to conduct a meta-analysis, we have written a narrative review.

Comment 4: The Intro is quite long and gets into detail that goes beyond a typical background section. It may be worth keeping the Intro to <=3 paragraphs that highlight the purpose of the review, and then create a separate heading for the IFCC requirements that are discussed later in the Intro.
Reply 4: We have re-written our manuscript to better address this.

Comment 5: Line 148-149 is repetitive from the first line of the paragraph (line 139). Would delete.
Reply 5: We have re-written our manuscript and this is no longer included.

Comment 6: Some of the paragraphs are long and could benefit from breaking them up. For example, the first paragraph in the co-morbidities section is 46 lines long.
Reply 6: We have re-written our manuscript to better address this.

Comment 7: The manuscript would benefit from the addition of a Figure or visual aid to go along with the 4 tables. The 4 tables are very data heavy which is fine, but it would

be useful to have at least 1 table (or figure) that synthesizes all these data into the clear summary/take-home points that the authors want to convey in this review.
Reply 7: We have since only included studies that have 800 subjects (400M, 400F) – one for Roche, one for Abbott, and one for the rest; those with only 1 study are relegated to a single Table as supplementary information. We hope that this new arrangement will be clearer and easier to digest.

Reviewer C
Comment 1: The introduction should be more to the point. The main body is very descriptive (can probably be condensed) and needs more focus on the interpretation of the data. Can the authors identify clearer concepts? A clear summary (conclusion) and take-home messages are needed.
Reply 1: We have re-written our manuscript to better address this.

Comment 2: While we understand the authors' point that it is difficult to come to a conclusion around whether differences in population exist due to confounding effects of study differences in age and co-morbidities, additional focus on the section of specific populations is needed (this should help the aim and focus of the review as well, although pretty well-defined in lines 68-71). The authors should separate race and ethnicity. The authors should suggest how more definitive studies might be accomplished.

Reply 2: We have re-written our manuscript to better address this and have focused on ethnicity. We have made some suggestions regarding cohort composition and statistical treatment of studies.

Comment 3: Discussing all potential influences separately by assay would significantly increase the word count and worsen the focus of the review. It is the principles that are important. The assay focus is already too long.
Reply 3: We have re-written our manuscript to better address this.

Comment 4: We would not suggest doing a meta-analysis because of the variability intrinsic to the studies.
Reply 4: We concur.

Comment 5: L156 "this may be due to" please check considering the previous sentence
Reply 5: We have re-written our manuscript and this is no longer included in the manuscript.

Comment 6: L187-188 "females being less likely to be recognized as having a MI when using sex-specific URLs at first blood draw" please double-check, I believe females are more likely to be recognized as having MI when using sex-specific URLs
Reply 6: You are right. We have re-written our manuscript and this is no longer included.

Comment 7: L227 "5mg/L" should be "5 ng/L"
Reply 7: Thanks for spotting the typo. We have re-written our manuscript and this is no longer included.

Comment 8: L423 "shown" should be "are shown"
Reply 8: We have re-written our manuscript and this is no longer included.

Comment 9: Please cite the references in Table 4. I can't find them in the main body of the text either.
Reply 9: We have cited the references for tables in the revised manuscript.

Comment 10: L435-437 and Table 4. Please check the analyzers and assays these studies were performed on. "Access" is not an analyzer, but the name of the assay (i.e. Access hsTnI). For example, the study by Kim et al. was performed on a DxI800 analyzer. "Access 2" on the other hand is an analyzer, not an assay name.
Reply 10: We have revised our tables as suggested.

Reviewer D

Comment 1: The authors have done a good job re-writing and focusing the manuscript according to the reviewers' comments. I have a few additional comments:

Supplemental Table A should be included in the main manuscript as Table 4.

Reply 1: Table 4 has been included as suggested.

Comment 2: The authors still use "racial" and "ethnic" populations interchangeably without properly defining the difference. For example in lines 31 and 87, "racial population" is used, while lines 51, 82, 150, and 302 refer to ethnicity. Race refers to a classification system based on physical characteristics, while ethnicity refers to a shared cultural heritage and identity. In this context, I believe it would be more appropriate to focus on race compared to ethnicity. However, the the distinction is not always clear-cut and these constructs are rather arbitrary. Therefore, my suggestion is to avoid this altogether and just refer to population differences, as the authors have done on many occasions in the manuscript. THERE ARE ISSUES WITH VERBIAGE THAT TODAY ARE SENSITIVE ISSUES. IT SHOULD BE MALES AND FEMALES WHICH IS DONE, SEX RATHER THAN GENDER WHICH HAVE DIFFERENT DEFINITIONS AND RACE RATHER THAN ETHNICITY.

Reply 2: As advised, "ethnicity" and "race" have been changed to "population differences", and "gender" has been changed to "sex".

Comment 3: L120-121: "Studies using hs-cTnT are slightly easier to compare as hs-cTnT assays are from a single vendor, Roche".

Hs-cTnT is now also supplied by ET Healthcare. Suggest to reword this sentence to "Studies using

hs-cTnT included in this review are slightly easier to compare as they are from a single vendor, Roche"

Reply 3: Sentence has been edited as suggested (line 121-122).

Comment 4: L250-252 "A practical solution could be a 3-band age-specific 99th percentile URLs as has been done for NT-proBNP: <50, 50 – 75, >75 years" and L300-301 "Age-specific bands for the 99th percentile troponin URL may also be needed akin to that for NT-proBNP".

While I understand what the author are trying to say, there is a slightly nuanced difference here. The three different age categories for age in case of NT-proBNP are clinical confirmatory ("rule-in") cutpoints based on optimal clinical specificity, and are not based on a reference interval study for these different age groups. Please reword these sentences. The issues with age relate in part to whether or not increases are part of normal aging or commodities. The data should be looked at and commented on. The same is the case for renal failure since we know that CTN is not cleared in any significant amount renally.

Reply 4: Reworded to line 249-250: "A practical solution could be a 3-band age-specific diagnostic threshold as has been done f or NT-proBNP: <50, 50 – 75, >75 years to optimize clinical specificity (58)."

Line 306-307: "Age-specific diagnostic thresholds for troponin may also be needed akin to that for NT-proBNP."

Issues with age and troponin have been addressed from line 236 -240: "The development of a relationship between troponin and age later in life is consistent with age-related accumulation of cardiovascular risks and disease. Elevations in troponin concentration with age may also be attributed to physiological processes such as increased release of troponin due to myocyte turnover, cardiomyocyte proliferation and increased cardiac mass (48)."

New comment on renal dysfunction and troponins added from line 264-267: "Although the clearance of c-Tn is not well understood, a dual clearance model has been proposed where at high concentrations, as seen in MI, c-Tn is predominantly hepatically degraded, while at lower levels, such as in chronic heart failure, renal clearance dominates and can partially explain higher troponin concentrations in individuals with renal dysfunction (60,61)."

Comment 5: L292-293. "However, when using hs-cTnI (Abbott), some differences in the 99th percentile hs-cTnI URLs may be noted."

Can the authors provide an extra sentence or two on what kind of differences can be noted here?

Reply 5: Added line 294-298: "However, when using hs-cTnI (Abbott), some differences in the 99^{th} percentile hs-cTnI URLs may be noted – male hs-cTnI 99^{th} percentile URLs were higher in Australian than Dutch cohorts with different compositions of subclinical disease and statistical handling of outliers. Differences in hs-cTnI (Abbott) were less prominent when comparing cohorts

of more similar composition."

Comment 6: The issues related to comorbidities vs other reasons for increases should be reflected in the conclusions.

Reply 6: Added line 300-302: "Male sex, increasing age, comorbidities such as subclinical heart disease, renal dysfunction and dysglycemia, and including outliers in analysis can raise hs-cTn 99th percentile URLs."