Peer Review File
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Reviewer Comments

Comment 1: The authors examined the detection of interference in the FT4 assay on the Beckman DXi analyzer through dilutions, as previously described by Oostendorp and Lentjes. They provide valuable additions by better defining the reference range and studying the effect of dilutions in a small selection of pregnant and critically ill patients. However, there are some comments to make:

Lines 60-63: Which phase of pregnancy is considered? This determines the increase in TBG, and the Beckman DXi FT4 assay is sensitive (responds with a decrease) to TBG elevations. Are the patients admitted to the intensive care unit severely ill with a non-thyroidal illness? The severity of illness can vary significantly among the population in an intensive care unit. Is there any additional information available?
Reply 1: The pregnancy subjects were in the second and third trimesters (already pointed out in the text, lines 62-63) and among the increase of binding capacity there was a significant increase of TBG. Anyway, regardless of the possible decrease in fT4 measurement, the dilutions work well.
The patients were admitted in the intensive care unit for infectious diseases or cardiac failure, and in this case, the effect evaluated was the low recovery due to a probable low binding capacity of the samples (lines 62).

Comment 2: Line 62: It is not investigated whether there is a significant difference in the dilution effects on FT4 between saline and buffer in the population that was later examined: hyperthyroid, hypothyroid, and patients with inconsistent results. Why was a tris buffer chosen instead of a readily available saline solution?
Reply 2: No differences were found in the cases examined in the first part of the study. It was therefore decided to use the saline solution, more readily available, for the subsequent evaluations, as in Oostendorp 2017 (reported at lines 110-112)

Comment 3: Line 88: Does the 1/5 dilution mean 1 part sample + 4 parts buffer or 1 part sample + 5 parts buffer? Please clarify this more explicitly. The same applies to the 1/10 dilution. In my experience, this can sometimes cause confusion.
Reply 3: We modified the text according to the suggestion (lines 99-100).

Comment 4: Lines 91 to 95: The authors demonstrate the effect of multiple dilutions in a small selection of patients and healthy individuals (Figure 1). However, they do not explain why they ultimately chose the 1/5 dilution. Why was this choice made instead of the 1/10 dilution?
Reply 4: We explained the choice at lines 111-112.

Comment 5: Line 105: "In 22 of the 43 suspected cases…". However, in Figure 5, there

are 23 points in the SN group and 21 in the SI group, making the total 44 and not 43 (Line 105). Please correct this in the text or in the figure.
Reply 5: We thank you for the careful evaluation, and we apologize for the mistake. Indeed, while the figures were made using the final cases, some draft numbers were left over in the text (my clerical error). We have made the corrections and now the numbers of cases are correctly reported in the text as well (lines 35-36, 86, 124, 153, 163).

Comment 6: Line 106: This sentence is somewhat unclear. Add "in undiluted sample" in the text and change "confirmed." Suggestion: "...and the values of FT4 in the undiluted samples were also within the reference range of the Liaison XL platform." This makes the sentence clearer.
Reply 6: Actually, our sentence is not clear. We modified the text to clarify the concept and in part according to the suggestions of both reviewers (lines 124-127).

Comment 7: Line 106: What criterion is used to confirm the DXi results with the Liaison method? When is a point in Figure 4 considered abnormal? In other words, what is the acceptable range around the regression line?
Reply 7: As stated also at lines 126-127, we used to evaluate the suspected cases the practical finding of the agreement of the results in relation to the respective reference intervals of the two methods.

Comment 8: Line 106: It is stated that 22 cases are confirmed on the Liaison XL (Figure 4), but Figure 4 shows 19 points (empty circles) falling within the Liaison's reference range and outside that of the DXi, not 22 cases (Line 105) or 21 cases (Figure 5). Please correct this.
Reply 8: In Figure 4 (now Figure 5) there are 23 filled rhombi (the correct number of non-interfered samples) and 19 empty circles, the interfered samples were evaluated with Liaison. In the two cases interfered by biotin supplementation was not necessary to compare the data on a different analytical platform, as stated in lines 133-135. We pointed out this in the figure legend.

Comment 9: Line 110: Which company manufactures the blocking tubes? Blocking tubes often target unspecific antibodies and may miss some interferences, based on my experience. Is there a literature reference available for the effectiveness of these blocking tubes?
Reply 9: The Company was reported at line 97.
We confirm, blocking tubes may miss some interferences, as see in this study (7/22 interferences identified). Considerations about the effectiveness of HBT was outside the aim of this brief report. Anyway, we softened the sentence (line 132) and we added a review article as reference (16).

Comment 10: Lines 130 and 131: Is it 43 cases or 44 cases (Figure 5)? In line 131: 22 cases or 23 cases (Figure 5)?
Reply 10: Please refer to the comment relating to your question “line 105”

Comment 11: Line 140: The text currently mentions 20 samples with abnormal recovery: Should this be 21?
Reply 11: Please refer to the comment relating to your question “line 105”

Comment 12: Line 141: "...showed normalized results...": How is normalization achieved? Does this mean that the values are located around the regression line?
Reply 12: We realize that the sentence was not clear. We better explain at lines 126-127

Comment 13: Figure 4: Do all 19 points (open circles) falling outside the DXi reference range also have abnormal recovery, as described in the legend? However, 21 values with abnormal recovery are shown in Figure 5. This is very confusing and not clearly described. Please clarify.
Reply 13: The two cases with the supplementation with biotin was not verified in Liaison (not necessary, as outlined in lines 133-135). We clarify this in the figure legend.

Comment 14: Line 146: The additional explanation provided is "the presence of non-specific low-affinity antibodies." However, the use of blocking tubes aims to detect this type of interference. Is something else meant here? Please clarify.
Reply 14: Actually, our sentence is not clear. Unfortunately, the issue of the nature of heterophile antibodies and how blocking agents work is complex and largely outside the aim of the study. We have tried to make the concept clearer (lines 168-171) and we have added a bibliographic reference (16).

Comment 15: Line 150: What does "Even though the results are particularly satisfactory" mean in relation to the predictive value of the test? Please clarify or delete this part of the sentence.
Reply 15: The sentence was clarified (lines 172-175)