**Author's response to reviews**

**Title:** Using molecular similarity to highlight the challenges of routine immunoassay-based drug of abuse/toxicology screening in emergency medicine

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**Author's response to reviews:** see over
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To Editor, BMC Emergency Medicine

Re: MS: 1521732942255134

We submit a revised manuscript entitled “Using molecular similarity to highlight the challenges of routine immunoassay-based drug of abuse/toxicology screening in emergency medicine” for consideration as a research article. The original title of the manuscript was “Challenges and limitations of routine immunoassay-based drug of abuse/toxicology screening used in emergency medicine”. We revised the title per the suggestion of reviewer 1, who recommended that the title be more specific to reflect the molecular similarity analysis. We address the other specific points of the two peer reviewers below.

There are a total of five figures, three tables, and two additional files (one Excel and one pdf).

We thank you and the two peer reviewers for the helpful comments and suggestions and hope the manuscript is now acceptable for publication.

Sincerely,

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Response to reviewer 1 (Dr. Alain Verstraete)

Major compulsory revisions

1. “The title should be more specific, and mention e.g. molecular similarity analysis. In its present form, one expects a more clinical article, with an evaluation of immunoassays and GC-MS, and recommendations for clinical and laboratory practice.”

The title of the manuscript has been amended to “Using molecular similarity to highlight the challenges of routine immunoassay-based drug of abuse/toxicology screening in emergency medicine”.

2. P 11&20. “I am surprised that the authors don’t mention venlafaxine (55th most prescribed drug in the US) as a possible cause of PCP false positives, as it was
mentioned in at least 3 publications.”

We mention the documented cross-reactivity of venlafaxine with PCP immunoassays in the Introduction (p. 8, line 2 of the revised manuscript) and in the Results (pp. 20-21), citing the three publications that mention this interaction.

Minor Essential Revisions

3. P17: “the text suggests that figure 2B compares the detection of lorazepam and lorazepam glucuronide, but lorazepam is not shown in the figure.”

We have deleted the reference to Figure 2B in the section referred to. The data allowing for a comparison of lorazepam and lorazepam glucuronide are in the Additional File 1.

4. P17: “the cleavage of glucuronide bonds is included in the reaction of some assays (EMIT & KIMS), so it doesn’t increase analysis time.”

We have amended this section to: “For a drug such as lorazepam, where the glucuronide metabolite is the predominant form in the urine, cleaving the glucuronide bonds would be predicted to enhance the detection rate. Some marketed assays (e.g., Syva EMIT-H® and Roche Online KIMS®) incorporate a glucuronide cleavage step in the reaction to improve benzodiazepine detection, while still maintaining rapid analysis times (Borrey et al., 200; Klette et al., 2005)”.

5. P 24: “please correct the sentence: Of the remaining positive screens … were included patients, e.g. the remaining positive screens … included patients.”

This correction has been made as suggested.

Discretionary Revisions

6. “I wonder whether the explanations on the studies chosen to place the brackets in the figures 2-4 should be mentioned in the text or in the figure legends.”

We considered this option but were not able to integrate the details into the figure legends without exceeding the 350 word limit for figure legends.

7. “Maybe the issue of the cut-off could be discussed as well. Most cut-offs are placed relatively high, to avoid false positives in workplace testing. Lowering them could lower the number of false negatives e.g. for benzodiazepines.”

We have added a paragraph in the Discussion (3rd paragraph of the revised Discussion) discussing the issue of cut-off, including in reference to workplace drug of abuse testing.

8. “Maybe the message in figure 5 B would be clearer in a pie chart.”
Figure 5B has been converted to a pie chart.

Response to reviewer 2 (Dr. Jeffrey Brent)

“Given that these immunoassays have been around for many years, it is clear from the data that you present that many of them are out of date and do not reflect current patterns of either illicit or therapeutic drug usage.”

To better stress the point above, we have edited the last sentence of the third to last paragraph of the Discussion to “It also suggests that development of DOA/Tox immunoassays has not kept pace with the development of new drugs relevant to the ED community or with changes in patterns of abuse of illicit and prescription drugs.”

“Please note that reference 3 on page 5 only deals with drug-drug interactions and not the other issues which are described in that sentence.”

We have moved the first three citations of the paper to be after the second sentence to address this issue and be sure that all issues raised in that sentence are covered by appropriate references.