Author’s response to reviews

Title: Cross-reactivity of Steroid Hormone Immunoassays: Clinical Significance and Two-Dimensional Molecular Similarity Prediction

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Editor  
*BMC Clinical Pathology*

To the editor:

We are submitting a revised manuscript “Cross-reactivity of Steroid Hormone Immunoassays: Clinical Significance and Two-Dimensional Molecular Similarity Prediction” for consideration as a research article for *BMC Clinical Pathology*. The manuscript provides an analysis of cross-reactivity of immunoassays used in steroid hormone measurement for clinical purposes.

We have carefully considered the comments of the two peer reviewers and include a summary of our responses below. We thank the peer reviewers for helpful critiques and suggestions.

There are a total of six figures, five tables, and one additional file (Excel spreadsheet). All authors declare no competing interests.

Thank you for your consideration,

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**Reviewer #1**

This is a very interesting paper and provides a method to assist in the assessment of the reliability of immunoassays and provides a way to try and predict likely false positive immunoassay results.

My only issue is in understanding the reliability of the similarity scores. For example, the "2D similarity to cortisol" data shows overlap between the "strong" and "none" categories
(Fig 2). Same is true for progesterone (Fig 5). In the Discussion on page 18 you say that compounds with strong and most with weak cross reactivity had scores above 0.8. Are you implying a cut-off to prioritize compounds, i.e., any compound with a score of less than 0.8 need not be tested as it is unlikely to cross-react? My concern centers on the overlap between the "none" and the other categories, in particular strong and weak.

A sentence of two in the Discussion could further clarify this aspect of your data.

**We have added the following two sentences to the last paragraph of the Discussion:**

“Although there is some overlap in 2D similarity scores between compounds with strong or weak cross-reactivity and those with no cross-reactivity, use of a 2D similarity cutoff such as 0.8 would help identify compounds with high likelihood of showing strong cross-reactivity. Conversely, compounds with low 2D similarity (e.g., less than 0.6) are unlikely to show strong or even weak cross-reactivity.”

**Reviewer #2**

The purpose of this study was to determine cross-reactivity of a variety of steroid compounds on the Roche Diagnostics Elecsys immunoassay for cortisol, DHEA sulfate, estradiol, progesterone, and testosterone. In addition the authors utilize computational methodology to attempt to predict cross-reactivity of compounds for steroid hormone immunoassays, which makes this manuscript unique. The two-dimensional similarity analysis seems to correlate well with the cross-reactivity study in the package inserts of the steroid immunoassays.

1. In the results section on page 9, the last paragraph states that “All compounds with strong cross-reactivity for the Roche Elecsys Cortisol assay had 2D-similarities to cortisol of 0.867 or high.” Since the next sentence states that tetrahydrocortisone had a 2D-similarity greater than 0.867 but was not cross-reactive, the sentence should be corrected to state “The majority of the compounds with strong cross-reactivity for the Roche Elecsys Cortisol assay had 2D-similarities to cortisol of 0.867 or high.”

**The suggested edit has been made.**

2. The figure legends for figures 2B – 6B should include a sentence to describe which analytes are represented by the circles, squares, triangles and diamonds.

**As suggested, a sentence has been added to each of these figures legends describing the symbols used.**