

## Reviewer's report

**Title:** A high confidence, manually validated human blood plasma protein reference set

**Version:** 3 **Date:** 16 April 2008

**Reviewer:** Tim Griffin

### Reviewer's report:

The authors have done a good job in addressing the main concerns from the first review. The manuscript is generally improved. Before publication I would suggest the following revisions:

Points that should be addressed before publication:

1. Although the manuscript has been shortened, both in terms of text length and number of figures, one final suggestion to further shorten the manuscript would be to consider removing Figure 5. Given that the pie chart shown in this figure is showing a very simple binary comparison of peptides with or without signal sequences, it seems that this can be simply covered in the text by describing the proportion of proteins with a signal sequence (44%) compared to those without (56%). Putting these numbers into a figure does not add to the discussion of this result and adds to the length of the manuscript.
2. On the issue of using MS3 in order to increase confidence in peptide identifications, some confusion still exists. The authors have added in a better explanation of how their MS3 experiment works (loss of phosphate), however this is cause for some questions. For one, there is no reason presented to the reader as to why they decided to include this operating mode in their experiments? One would assume that they are working on the hypothesis that there are a high number of phosphorylated peptides within the plasma which would benefit from an MS3 scan? Some rationale should be given in the Results section to clue the reader into why these experiments were included. Also, if the reason is to analyze phosphopeptides, does this mean that all the peptides that were identified with the aid of MS3 were phosphorylated peptides? Or did the MS3 scan randomly sequence fragments from non-phosphorylated peptides as well which still helped to identify these?

Minor points:

- The inclusion of the discussion on peptide length seems warranted based upon the authors explanation. One suggestion would be to include a brief explanation of why peptide length is an important parameter, perhaps with a citation to the Adamski et al publication where this issue is also discussed.
- Throughout the manuscript "Orbitrap" should be capitalized because it's a product name
- On p. 28 the authors explain that all "identifications based on 1 peptide unless a

given peptide was validated by a third MS spectrum (MS3).” What is meant by “third MS spectrum”? What are the other two spectra?

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests