**Author's response to reviews**

**Title:** Rapid and reliable detection of alpha-globin copy number variations by quantitative real-time PCR

**Authors:**

Runa M Grimholt (runamg@medisin.uio.no)
Petter Urdal (Petter.Urdal@medisin.uio.no)
Olav Klingenberg (Olav.Klingenberg@ous-hf.no)
Armin P Piehler (apiehler@furst.no)

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**Date:** 17 January 2014

**Author's response to reviews:** see over
Dear Dr Peter O'Donovan

Please find enclosed a revised version of our manuscript “Rapid and reliable detection of α-globin copy number variations by quantitative real-time PCR”. We have addressed all issues raised by the Journal Editorial Office, and we have carefully proofread the manuscript and improved language.

The study received approval from the institutional research ethics committee of Oslo University Hospital as a quality assurance study.

Yours sincerely,

Runa Marie Grimholt
Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author

Hemoglobin MS #: LHEM-2013-0062

The paper describes the discovery and characterization of a new hemoglobin beta-chain amino acid point mutation. The mutation was pinpointed at position 78 by DNA analyses which are well described, and the data appear convincing.

SPECIFIC COMMENTS

1- The chromatograms of figures 1 are of a non-acceptable quality. Axes and peaks must be properly labelled.

The quality of figure 1 has been improved, and axes and peaks have been correctly labelled.

2- The electrophoretical information is missing. This is suited for the readership of this journal.

We thank Referee 1 for the suggestion and agree that electrophoretical information should be presented. We have analyzed the sample on a capillary electrophoresis (CE) instrument, Capillarys II from Sebia (Figure 1). The novel hemoglobin variant is not detectable using this method. The extra peak in zone 11 occurs due to high
sample age probably representing denatured hemoglobin. The CE result is mentioned in the revised manuscript. Unfortunately, we do not have access to a fresh sample and have therefore decided not to include the CE-plot in the revised manuscript.

Figure 1. Capillary electrophoresis

<table>
<thead>
<tr>
<th>Fractions</th>
<th>%</th>
<th>Normal Values %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z11 zone</td>
<td>9,2</td>
<td></td>
</tr>
<tr>
<td>Hb A</td>
<td>89,1</td>
<td></td>
</tr>
<tr>
<td>Hb A2</td>
<td>1,7</td>
<td></td>
</tr>
</tbody>
</table>
3- One may recommend to leave out the molecular modelling (Fig.3), since it is somewhat redundant rather speculative, and besides the analytical focus of the paper. Also the figure 2 B does not improve the quality of the paper. The interesting information in this paper is the interference of the new variant with HbA1c measurement on Tosoh G7- and BioRad Variant-Analyzers.

Following the suggestions of the reviewer, we have omitted Figure 2B and 3 in the revised manuscript.

Referee: 2

Comments to the Author

This is a well written short communication describing a new Hb variant which interferes with HbA1c measurement. The authors should add that the presence of a Hb variant may not only alter the region where HbA1c elutes causing an erroneous value of HbA1c but in a more general way decrease the HbA1c level if there is some, even mild, decreased RBC life.

Figure 3 is not necessary.

We thank Referee 2 for bringing up the issue of falsely low HbA1c value due to shortened lifespan of erythrocytes. This point is now addressed in the revised manuscript. Figure 3 has been omitted.
We would like to thank the reviewers for reading our manuscript and for their expert comments and criticisms. They have helped to improve the quality of our paper. Thank you indeed for your interest in our work.