Author's response to reviews

Title: Paired measurement of urinary creatinine in neonates based on a Jaffe and an enzymatic IDMS-traceable assay

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Version: 3
Date: 6 March 2014

Author's response to reviews: see over
We thank the reviewers for their suggestions and time invested to evaluate the paper on urinary creatinine measurements in neonates. We have carefully considered their comments, and tried to adapt the revised version upon their requests. To further facilitate subsequent re-evaluation by the editorial board and reviewers, we provided a point-to-point answer. We obviously remain available for any further discussion, if this is felt to be appropriate.

Answers to Reviewers

Reviewer 1

Reviewer’s report:

The authors describe the results of paired urine creatinine test results in neonates based on a compensated Jaffe and an enzymatic creatinine method. The authors focus on the method differences, rather than on the traceable reference values for urine creatinine in neonates. Although traceable reference values for creatinine in urine from neonates are welcomed very much, the manuscript suffers from methodological flaws which should be solved.

Firstly, the authors should be aware of the fact that we currently live in an era where metrological traceability of test results from type A analytes is a necessity. For a metrologically sound study, direct comparison to a gold standard method (or to value assigned reference materials) is needed. I doubt the interpretation of their findings without having insight into these data.

Response to Reviewer: We agree direct comparison to a gold standard method is missing in our paper. This is not always possible due to sample requirements (e.g. sample volume needed) and financial issues. The inserts of the Jaffe as well as the enzymatic assay state as “Traceability: This method has been standardized against ID/MS”, without further detail. We hereby assume that this is a comparison to value assigned reference materials, and we have also added this limitation in our discussion part.

Secondly, although the authors recognize the importance of IDMS-traceable creatinine methods, they do not give any insight into the analytical performances (IQC/EQA) of the test assays and applications ran in their lab. The authors focus on interpreting tiny mean differences between methods without considering the random analytical variation, the calibration bias, total (allowable) error, lot-to-lot ,differences of reagent and/or calibrator lots used etc. They should have certained themselves of exchangeability of the applications in e.g. adult and children's urines upfront. The observed slope (= bias of about 5%) can e.g. be the resultant of pre-existing calibration differences; how do the authors exclude that?

Response to Reviewer: We added the assay specifications in Material and Methods section.

As suggested by the reviewer, the upfront exchangeability study (using random urine samples) results are discussed in the revised paper. This method comparison also showed a negative slope using Passing Bablok regression of enzymatic versus Jaffe values. Checked against biological total allowable error
criteria (desirable) for random urine samples as described by C Ricos et al., the
difference was acceptable. We are also aware that such method comparisons are “snapshots” using certain lots of calibrators, reagents,... A new comparison later on might yield (slightly) different results. In this context, we agree that we focused too much on interpreting minor differences and philosophizing on a origin. In our revised paper, we put more emphasis on the effect of calibration, reagent, lot-to-lot-variability in the discussion part.

The philosophizing exercise is, in our opinion, nevertheless interesting. For example the independent significant impact of PNA on the assay difference is clinically not relevant, but a scientific paper should at least try to give an explanation. We obviously agree that the broader context should not be lost and the core message that indeed the tested IDMS-traceable assays produce clinically commensurate results is more emphasized in our revised paper. As discussed in the paper the observed minor difference was remarkably good, given previous reports on differences up to 80% in children using Jaffe and enzymatic assays.

Thirdly, a compensated Jaffe method was used. No insight is given in the M&M section regarding the absolute value of the compensation factor used in the analyzer application. May be the fixed intercept that the authors describe in their regression equation when comparing both creatinine methods is created by this compensation factor?

**Response to Reviewer:** For the Roche urine Jaffe method application no compensation factor is used. This is scientifically sound as these factors are used predominantly to compensate for the Jaffe reaction’s cross reactivity with pseudocreatinines and proteins, normally present in much lower concentrations in urine. We adapted the description of the Jaffe assay.

Fourthly, the authors did not predefine the quality level of performance at which they aimed (what is a clinical acceptable difference between methods? e.g. based on clinical outcome or biological variation), and did not reflect in their discussion on the difference between clinically usable and statistically different. Expertise and involvement from clinical chemists is needed to answer the above questions.

**Response to Reviewer:** As described above, we added biological criteria to evaluate the clinical acceptability of the difference of paired creatinine results (desirable Tea, Ricos). We further clarified the difference between the statistically significant slope and the minor clinical impact in the discussion part.

Minor Essential Revisions
The dilution factor used in both urine applications should be mentioned in the ms.

**Response to Reviewer:** We added the dilution factors in the M&M section.

pg 3: 2n paragraph. Despite the calibration of these assays to IDMS,... display interference. Reply of hte reviewer: adequate standardization is one thing; assay specificity is another important but independent issue in order to be able to generate
accurate test results. The independency of both points should be emphasized by rephrasing this sentence.

Response to Reviewer: We rephrased the introduction putting more emphasis on adequate standardization and we make a clear distinction with assay specificity.

pg 3. last sentence: quantification of the differences is not interesting; generating IDMS-traceable urine creatinine results in neonates should be the goal.

Response to Reviewer: Historically, creatinine assays showed great variation depending on reaction mechanism and manufacturer. Consequently, creatinine values in neonates depended on the specific method used. Since IDMS-traceability has been introduced and widely used, this problem should have diminished. It is, however, always desirable to check such assumptions experimentally, especially for neonatal samples (with variable matrix compositions) and using assays with different reaction mechanisms. In children, differences up to 80% have been reported using enzymatic and Jaffe assays.

In this context (and assumed the IDMS traceability worked), we acknowledge the importance of reporting on neonatal urinary creatinine concentrations measured by a IDMS-traceable method. Our paper aids to fill up this gap. In our revised version, we put more emphasis on this part of our study in the introduction and discussion.

M&M: See appeal to medical journal editors sinds 2012: full description of lab methods and specimen handling is now required. CCLM 2012; 50(3): 411-413.

Response to Reviewer: We adapted M&M section incorporating the required information.

pg 4: last sentence: I miss the predefined quality requirements for clinical use.

Response to Reviewer: We used biological criteria for acceptance (Ricos), and have added this in our M&M section.

Results
pg 5: why do the authors use medians for describing the absolute creatinine concentrations in urine versus mean difference for describing the magnitude differences in test results?

Response to Reviewer:

pg 5: linear regression should not be used when comparing two routine methods; P/B of Deming regression are preferred.

Response to Reviewer: We used Passing Bablok instead of simple regression in the revised paper.

Discussion
The constant INTERCEPT (this is not a bias term!) of -0.41 mg/dL is rather large ....

**Response to Reviewer: We adapted the terminology in our revised paper.**

Because of an incomplete M&M section, the discussion should be re-interpreted and rewritten after (a) using Deming or P/B regression, (b) taking into account the compensation factor in the Jaffe application and (c) after verification of preexisting calibration bias between both assays. It is advised to put less emphasis on the (assumed) differences between assays and to put more emphasis on the clinical relevance of the (differences in) creatinine urine data in neonates.

**Response to Reviewer: We re-interpreted and nuanced our results taking into account above-mentioned remarks.**

Reviewer 2

**Reviewer’s report:**
This is an interesting manuscript, aiming at quantifying the differences in urine creatinine values measured in neonates with Jaffe and an enzymatic IDMS traceable assay. This has been performed in adults, but not yet in children.

We thank the reviewer for the overall positive evaluation of the paper.

**Minor essential revisions**

**Methods**
1) I still lack data regarding any medication and bilirubin levels in neonates in whom creatinine was measured in urine samples.

*We agree, and have added the data as available (drugs, but bilirubin only rarely collected, since this was not part of the study). Cephalosporins were not used in these cases (amoxy-amikacin, or amikacin-vancomycin are the ‘routine’ antibiotics used).*

**Results**
2) 84 urine samples, for how many babies? How many urine samples per baby? *This has been added in the results section of the paper.*

3) Please give the range of urine output/kg/h *This has been added in the results section of the paper.*

4) Authors report a significant linear regression between both creatinine measurements (r²=0.9957). As measures were performed on term and preterm babies, did the values have a normal distribution, or were they bimodal? Is the R² the same in preterm and term babies?

*As suggested, we have re-analysed the data for preterm and term cases, but there were no significant differences between both subgroups. This has been added in the results section of the paper. We only provided the figures in this response to the reviewers text to further confirm that we indeed have done the*
analysis, but that – in our opinion - there is no real add on value to add these figures to the revised version of the paper.

5) Please present the beta instead of the R2 in the multiple regression model explaining the difference between assays.

This has been added, while the further adaptations in this part of the paper were also driven by the requests of the first reviewer.

6) Figures lack titles. Please don’t truncate words in the figures or please define them as abbreviations beneath the figures.
Sorry, adapted.