December 04, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 1 22164-Revised manuscript.doc)

Title: Standardization in Laboratory Medicine - Adoption of Common Reference Intervals to the Croatian Population

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Name of Journal: World Journal of Methodology

ESPS Manuscript NO: 22164

1 The manuscript has been improved according to the suggestions of reviewer (Reviewer's code**:** 03354106):

A - Title:

The first part of the title fits to the content of the review, the second part of the title is less clear for me. Reading this second part l expected to find in the manuscript an overview concerning several procedures/tests in Laboratory Medicine for the definition of common reference intervals and if and how they fits to Croatian population (not present in the manuscript, in which tests were analyzed focused on a single methods and performed by a single laboratory (no evaluation of influence of interlaboratory bias on the definition of reference intervals); on the other hands, the authors want to related this second part of the title to the experimental section (creatinine and AST/ALT), in this case l expected that in the title was underlined that the evaluation was performed only on 3 analytes and by a reference centre.

- The title remains the same according to the following explanation:

  Through the second part of the manuscript title the authors wanted to outline the ongoing harmonization process in medical biochemistry laboratories in Croatia which has started in 2004 as it is presented in chapter: "Harmonization of laboratory test results in Croatia". Verification of "common" reference intervals for creatinine, AST and ALT which is presented in details has to demonstrate the

implementation of CLSI recommended verification protocol for adoption of common reference intervals to our population.

B - Main manuscript

1- Chapter "the role of long term evaluation of national EQA in harmonization of reference intervals in Croatia".
The last sentence could be implemented by a description of the main EQA performed by medical biochemistry laboratories.

- Description of the main EQA performed by medical biochemistry laboratories is implemented in the text:

  Medical biochemistry laboratories in Croatia also participate in the different EQA schemes in the field of clinical chemistry, laboratory haematology and coagulation organized by international EQA providers: Labquality (WHO Collaborating Centre for Education and Training in Laboratory Quality Assurance) Helsinki, Finland; UK NEQAS (United Kingdom National External Quality Assessment Scheme for Haematology and Blood Coagulation); Sheffield, United Kingdom; RFB (Reference Institute for Bioanalytics) Bonn, Germany; ECAT Foundation (External Quality control of diagnostic assays and tests with a focus on Thrombosis and Haemostasis) Amsterdam, The Netherlands; INSTAND e.V. (Society for Promoting Quality Assurance in Medical Laboratories e.V.), Düsseldorf, Germany in order to promote global standardization/ harmonization of the whole laboratory process and achieve a high degree of international interlaboratory comparability.

2-Chapter "Global standardization/harmonization in laboratory medicine". The last sentence is not too much clear.

- improved:
  This ensures a global infrastructure with the aim of defining a systematic approach to determining the list of the complex analytes ~~laboratory test results~~ for which there are no higher-order reference measurement procedures and for which it was unlikely that such procedures could be developed **(28)** in order to increase patient safety through the **best achievable** quality and comparability of all laboratory test results .

3- Chapters "applicability of common reference intervals for serum creatinine concentrations to the Croatian population" and "transferability of aspartate and alanine aminotransferase common reference intervals to the Croatian adult and pediatric population". There is not discussion about if the methodologies suggested are used only in the reference laboratory (Department of Medical Biochemistry and Laboratory Medicine University Hospital Merkur – reference centre of the ministry of health for the production of reference values in the field of general medical biochemistry, Zagreb, Croatia) or in other laboratories. If the intent of the authors is to suggest that these methodologies had to be used in the other Croatian laboratories

or the use of these methodologies are "only" suggested. How the authors intent to export the results to the other Croatian laboratories? The authors suggest to perform EQA trough Croatian Laboratories to monitor these methodologies/reference intervals and extent the evaluation as interlaboratories approach? No discussion about this point, please add some suggestions.

- improved:
  In 2014, as a part of the ongoing harmonisation project the Croatian Chamber of Medical Biochemists has recommended specific enzymatic method to be used as routine analytical method for the measurement of serum creatinine concentrations and IFCC recommended methods for the measurement of AST and ALT activity concentrations. Based on the evaluation of the reference intervals and verification studies application of „ common" reference intervals to the Croatian population was recommended. The results of these long-term evaluation and improvement processes as well as interlaboratory variability of the obtained results are clearly demonstrated through national external quality assessment program which is obligatory for all medical biochemistry laboratories and is one of quality indicators in scope of the external professional audit of medical biochemistry laboratories in Croatia.

4- The data concerning the evaluation of serum creatinine concentration are closed to the IFCC data (more females than males) (table 1). It is not too much clear what the authors want to conclude from this experiment: are the IFCC "common" reference intervals for global application suitable for all age/sex and for Croatian population, too? It means the main results of this analysis is that the use of specific enzymatic method is better, more accurate, and gives the same intervals of IFCC common reference intervals?

- According to the obtained results of the verification studies the authors want to conclude that the IFCC "common" reference intervals for global application are suitable for all age/sex in the Croatian population as it is presented in the chapter: Applicability of common reference intervals for serum creatinine concentrations to the Croatian population: „Creatinine results in the reference population group of 240 healthy adult persons obtained under controlled pre-analytical conditions and with analytical quality within desirable target values using specific enzymatic creatinine method, showed almost identical results as the recently recommended "common" reference intervals for global application by the IFCC Committee on Reference Intervals and Decision Limits Table 1, (32). Due to the fact that the trend of creatinine concentration considering age and sex in our population-based study using uncompensated Jaffe kinetic method (10-14) is quite similar to that presented in recently recommended "common" reference intervals for global application (32), it seems acceptable that "common" reference intervals could be adopted for all age groups. Based on obtained validation results all prerequisites for adopting

established common reference intervals for creatinine measurement were fulfilled (33)".

5 -Table 2 and 3. These tables should be revised regarding the format: same values are not aligned properly so it is difficult to read appropriately the tables. It is not clear if the evaluation concerning ALT and AST in table 2 and 3 in Croatian population is represented by the "1st line" and in the 2nd the data of IFCC.

- The tables are revised and aligned properly.

6 -The data concerning the evaluation of aspartate and alanine aminotransferase activity are closed to the IFCC data (table 2 and 3, respectively) even if two different methods were used for the evaluation. So, the main conclusion of this study is that the "IFCC reference intervals fit to Croatian population" independently of the methods used for the evaluation? Add some considerations.

- improved:
  Verification of reference intervals for aminotransferases for Croatian adult and pediatric patients using IFCC recommended analytical methods in comparison to previously produced reference intervals recommended by Croatian Chamber of Medical Biochemists (CCMB) in 2007, are presented in Table 2 and 3 together with related references (10,14,15,41-45). The obtained results confirmed that the IFCC recommended common reference intervals for aspartate and alanine aminotransferase activity concentrations are appropriate for the adult Croatian population.

7- In table 2 and 3 it is not clear if the "verification%" is related to the overall evaluation of pediatric reference intervals of the three listed studies or only concern the first study (CALIPER), please format appropriately this data. Please verify the discrepancies between the "percentage of verification" in the tables and in the text.

- The tables are revised and aligned properly.

8- Please, add some comments concerning the comparison of the bias among the suggested methodologies (for creatinine) and those used by Croatian laboratories.

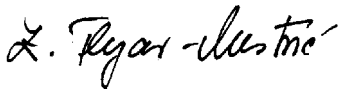
- Added comments:
  Method comparison between Creatinine Enzymatic (X) and uncompensated Jaffe kinetic method (y) gave the following Passing/Bablock equation for the whole group (n=240): y=1.00x +17.00 and a correlation coefficient of 0.968. As expected, using the creatinine uncompensated Jaffe method substantially higher values were found than using the enzymatic method. Those results are in accordance with several comparisons studies between specific enzymatic methods and non-specific conventional Jaffe methods indicating that Jaffe methods based on alkaline picrate reaction overestimate true serum creatinine concentrations mainly due to the nonspecific protein interference.

2 References and typesetting were corrected. References 7, 14,15 are not available on line.

3 Tables were aligned properly

Thank you again for the privilege of publishing our manuscript in the *World Journal of Methodology*

Sincerely yours,



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