**Supplemental material**

**Supplemental methods: Detailed description of the RMP measurements**

A reference laboratory with accreditation according to ISO 17025 and ISO 15195, performed the RMP measurements on a Cary 300 analyzer (ThermoFisher Scientific). The RMP was an automated version of the manual PRMP developed by the Committee on Reference Systems for Enzymes (C-RSE) of the IFCC, applying the same measurement principle. ALP catalyzes the hydrolysis of NPP, forming phosphate and free 4-nitrophenol; under alkaline conditions, 4-nitrophenol is converted to the 4-nitrophenoxide ion. AMP and H2O used as phosphate-acceptors. The concentration of 4-nitrophenol is determined by measuring absorbance at 405 nm.

**Calculations for dCRM and U(dCanRM)**

BCS is the mean ofthe differences Bi.

**List of supplemental Figures, Tables and An ethical approval for using patient serum samples**

**Table S1**: The basic information of ALP Kits for commutability evaluation.

**Table S2**: Imprecision of routine methods for serum ALP measurements. The concentrations of QC materials were 96.9U/L, 245.7U/L and 499.2U/L.

Verification of the imprecision of the routine methods was carried out in accordance with CLSI guideline EP15-A3 [10]. Two levels of GCs were tested in triplicate over 5 consecutive days.

In the imprecision study, the calculated within-run imprecision values for ALP using the three control materials for the five assays were 0.63%-2.98%, 0.45%-1.71%, and 0.51-2.11%, while the corresponding within-laboratory imprecision values were 0.89%-1.86%, 0.77%-1.44% and 0.70%-2.26%, respectively. The imprecision of routine methods are listed in Table.S3.

**Table S3** Regression information and Fig.S1

The results from the reference methods were designated as the x-axis, and the results of each of the routine methods were designated as the y-axis. The results from the CSs were used to generate a scatter plot, and the slope, intercept and correlation coefficient of each regression line were calculated.

1. Clinical and Laboratory Standards Institute. User verification of precision and estimation of bias; approved guideline, 3rd ed.; ISBN 1-56238-965-3; CLSI document EP 15-A3, 2014.

**Table S1**: The basic information of TG Kits for commutability evaluation.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Manufacturers  | orgin | Calibrator lots | ALP Reagent lots  | Catalogue number  |
| Beckman | America | 1121B | AUZ6780 | OSR6004 |
| Biosino | China | 170122 | 180951 | 100020020 |
| DiaSys | Germany | 50239185 | 00004625 | 14104171701 |
| KH | China | 877UE | 20181012 | 30812010205 |
| Roche | Switzerland | 10759350 | 381222 | 05166888190 |
| Wako | Japan | AJ730 | AH993 | 996-63891/99663991 |

**Table S2:** Imprecision of routine methods for serum ALP measurements.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Instrument | Method | Level 1 |  | Level 2 |  | Level 3 |
| CVr% | CV1% |  | CVr% | CV1% |  | CVr% | CV1% |
| Beckman AU5400 | Beckman  | 2.58  | 1.86  |  | 1.71  | 1.44  |  | 2.11  | 2.26  |
| Hitachi 7180  | Biosino | 1.07  | 1.13  |  | 1.14  | 1.01  |  | 0.51  | 0.71  |
| Hitachi 7180  | Diasys | 1.01  | 1.17  |  | 1.11  | 0.93  |  | 0.91  | 1.04  |
| Hitachi 7180  | KH | 0.63  | 0.98  |  | 0.45  | 0.95  |  | 0.73  | 1.26  |
| Roche C701 | Roche  | 0.69 | 0.73 |  | 0.70 | 0.72 |  | 0.62 | 0.71 |
| Hitachi 7180  | Wako | 0.74  | 0.89  |  | 0.60  | 0.77  |  | 0.49  | 0.70  |

**Table S3**: Regression information

|  |  |  |  |
| --- | --- | --- | --- |
| Analytical system | Intercept | Slope | R2 |
| Beckman | 1.3107 | 1.0343 | 0.9998 |
| Biosino | 2.0152 | 0.9336 | 0.9998 |
| Diasys | 2.7166 | 1.0193 | 0.9998 |
| KH | 2.3922 | 0.8296 | 0.9992 |
| Roche | 3.443 | 0.9096 | 0.9995 |
| Wako | -2.2656 | 1.0058 | 0.9996 |

**Supplemental Figure 1**



**Supplemental Figure 2**

Scatter plots of CRMs ALP concentrations measured by reference method and routine methods.

Scatter plots of 9 CRMs concentrations measured with the comparative method (x-axis) and the routine methods (y-axis). The black solid lines are the regression lines, and the black dotted lines are the two-tailed 95% prediction lines. The black circles, red squares, yellow triangles and blue crosses represent results of CSs, ETVs, GCs and SRMs measured with the reference method and the routine methods.

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**An ethical approval for using patient serum samples**

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