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CLSI-based transference of CALIPER pediatric reference intervals to Beckman Coulter AU biochemical assays

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ABSTRACT

Objective: The CALIPER program has established a comprehensive database of pediatric reference intervals using largely the Abbott ARCHITECT biochemical assays. To expand clinical application of CALIPER reference standards, the present study is aimed at transferring CALIPER reference intervals from the Abbott ARCHITECT to Beckman Coulter AU assays.

Design and methods: Transference of CALIPER reference intervals was performed based on the CLSI guidelines C28-A3 and EP9-A2. The new reference intervals were directly verified using 100 reference samples from the healthy CALIPER cohort.

Results: We found a strong correlation between Abbott ARCHITECT and Beckman Coulter AU biochemical assays, allowing the transference of the vast majority (94%; 30 out of 32 assays) of CALIPER reference intervals previously established using Abbott assays. Transferred reference intervals were, in general, similar to previously published CALIPER reference intervals, with some exceptions. Most of the transferred reference intervals were sex-specific and were verified using healthy reference samples from the CALIPER biobank based on CLSI criteria. It is important to note that the comparisons performed between the Abbott and Beckman Coulter assays make no assumptions as to assay accuracy or which system is more correct/accurate.

Conclusion: The majority of CALIPER reference intervals were transferrable to Beckman Coulter AU assays, allowing the establishment of a new database of pediatric reference intervals. This further expands the utility of the CALIPER database to clinical laboratories using the AU assays; however, each laboratory should validate these intervals for their analytical platform and local population as recommended by the CLSI.

Introduction

Reference intervals serve as health-associated benchmarks with which a patient's individual test results are compared and are essential for accurate interpretation of laboratory test results. The levels of many biomarkers may vary with age, sex, and ethnicity; thus, age- and sex-specific reference values are critical for the appropriate interpretation of test results. This is particularly important for the pediatric population, where the high rates of growth and development can alter the otherwise predicted levels of many analytes [1–5]. The use of adult reference intervals for the interpretation of pediatric test results, a common practice in many healthcare centers worldwide, is inappropriate, and represents a major source of post-analytical error that can lead to patient misdiagnoses and inappropriate treatment decisions [6–9].

The lack of pediatric-specific reference intervals has remained a major problem in the pediatric setting due to the difficulty in collecting samples from healthy community children, particularly from young children and infants, where only a small blood volume can be obtained. In an effort to address this important evidence gap, the CALIPER (**Ca**nadian **L**aboratory **I**nitiative in **Pe**diatric **R**eference Intervals) project was established as a collaborative initiative between several pediatric centers across Canada [10,11]. In 2006, the CALIPER project released the first pilot data describing pediatric reference intervals for several basic chemistry analytes [12]. Since then, CALIPER has produced a comprehensive database of pediatric reference intervals for many specialty, immunoassay, and chemistry analytes [1,11,13–21], and has effectively filled many of the previous gaps in pediatric reference intervals [10,11]. A major caveat was that CALIPER reference intervals were established using the Abbott ARCHITECT assays, which limits the application of these reference intervals to pediatric centers that use Abbott analytical platforms.

Abbreviations: CALIPER, Canadian Laboratory Initiative in Pediatric Reference Intervals; CLSI, Clinical Laboratory Standards Institute; Q–Q, quantile–quantile; CAP, College of American Pathologists.

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Establishing new reference intervals is a complex, costly, and daunting task, involving recruitment and sample collection from a large number of healthy individuals. As a result, the Clinical and Laboratory Standards Institute (CLSI) has issued guidelines on transferring reference intervals established in one laboratory (the "donor" laboratory) to other ("receiving") laboratories [22]. This process involves transference and verification steps to ensure validity of the transferred reference intervals. First, transference of reference intervals can only occur if (1) there is a good correlation between methods used in "donor" and "receiving" laboratories and (2) the results produced by both methods are normally distributed [23]. If these criteria are met, a mathematical equation that governs the relationship between the results produced by both platforms is determined, and this equation is used to convert the original reference intervals into transferred reference intervals that can be applied to the platform in the receiving laboratory [22]. Next, the receiving laboratory must verify the transferred reference intervals using specimens from a small number of reference individuals recruited from a healthy population.

We have previously adopted this approach to transfer reference intervals from the Abbott ARCHITECT analyzer to a multitude of assays on four other commonly used clinical chemistry analyzers [24]. There is, however, still a need to transfer reference intervals to additional systems that are commonly used in major pediatric hospitals across Canada, and worldwide. Here, in order to broaden the utility of the CALIPER reference interval database [1], we report transference of pediatric reference intervals to the widely used Beckman Coulter AU assays, which will further enhance the global utility of the CALIPER database.

Materials and methods

Method comparison

The present study was approved by the Institutional Review Board (IRB) at the Hospital for Sick Children (Toronto, Canada) and the review boards of collaborating hospitals. Approximately 200 pediatric pooled patient serum specimens (The Hospital for Sick Children, Toronto, Ontario) were analyzed on the Abbott ARCHITECT c8000 (at Eastern Health Authority, St. John's, Newfoundland) and the Beckman Coulter AU Systems (at Beckman Coulter, Brea, California). Samples represented a wide range of analyte concentration or activity. A full list of analyzed markers, assay specifications and analytical parameters are provided in Supplemental Tables 1 and 2.

Transference protocols and statistical analysis

Data analysis and reference interval transference were done in accordance with CLSI C28-A3 and EP9-A2 guidelines [22,23]. Statistical analysis was performed using Excel (Microsoft) and R statistical computing program [25]. For each assay, the Abbott ARCHITECT results were plotted as a function of the corresponding results obtained with Beckman Coulter AU. Graphs were visually examined for outliers, and gross outliers, which occurred in rare cases, were removed. Results below the lower end of the reportable range were excluded.

A simple linear regression assumes that the X variable is known (without error). If the $R^2 \geq 0.95$, any error in X is adequately compensated by the range of data, and simple linear regression using the least squares approach can be used to estimate the slope and intercept [23]. Therefore, this regression method was used to determine the line of best fit and its corresponding equation in cases where $R^2 \geq 0.95$. However, if $0.70 < R^2 < 0.95$, linear regression does not accurately estimate the slope or the Y intercept (where the error in X is not adequately compensated by the range of the data). In this case, Deming regression was used, which allows both methods to have measurement error. In cases of poor correlation ($R^2 < 0.70$), the corresponding reference intervals were deemed non-transferable.

In order to assess the appropriateness of a linear model with normally distributed data points, we generated standardized residual, Bland–Altman, and quantile–quantile (Q–Q) plots. The standardized residual and Bland–Altman plots were visually examined to confirm that the data points did not cluster into distinct patterns. Q–Q plots served to assess whether the residuals followed a normal distribution. A Q-Q plot shows the distance between a point and the regression line (i.e. the standardized residual) on the y-axis as a function of what that distance would be if the residuals were normally distributed (i.e. the theoretical quantile) on the x-axis. We visually examined Q–Q plots to verify that the data followed a straight line of the equation $y = x$, indicative of normally distributed residuals. In cases where all these criteria were met, the equation of the line of best fit was used to transfer the CALIPER reference intervals established using the Abbott ARCHITECT platform [1] to the corresponding Beckman Coulter AU assay. In cases where the criteria were not met, the corresponding reference intervals were deemed non-transferable.

The root of the mean-squared error (RMSE) was used to determine 95% confidence intervals around each lower and upper reference limit, calculated as the reference limit $\pm$ 1.96 $*$ RMSE. The 95% confidence intervals were used as secondary limits with which to verify the transferred reference intervals.

Verification of transferred reference intervals using samples from the CALIPER biorepository

Next, the transferred reference intervals were verified in accordance with the CLSI C28-A3 guidelines [22]. For this purpose, approximately 100 CALIPER reference specimens were analyzed on the Beckman Coulter AU platform. Selected samples spanned as many pediatric age range and gender partitions as possible. A conservative approach was adopted to define and remove outliers. In brief, the results were visually inspected for gross outliers, which were defined as being several-fold higher/lower than the next highest/lowest data point. If these points were considered outliers by the Tukey test (a method for finding outliers using the interquartile range to filter out very large or very small numbers), they were then excluded. The total percentage of samples that fell within the appropriate reference limits was then calculated (total verification across all pediatric age and gender partitions). This process was repeated for the lower and upper reference limits inclusive of the 95% confidence intervals. Reference intervals were considered verified when >90% of reference samples fell within the confidence intervals of the transferred reference intervals.

Results

First, correlation between assays on the two platforms was carefully assessed using 200 pediatric serum samples. The results from 32 Abbott ARCHITECT assays were correlated with 60 corresponding assays on the Beckman Coulter AU system, where Beckman Coulter offered more than one assay for the majority of the tested analytes, with the exception of apolipoprotein B (APOB), anti-streptolysin O (ASO), complement C3 (C3), complement C4 (C4), C-reactive protein-high sensitivity (CRPHS), haptoglobin (HAPT), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), prealbumin (PAB), and transferrin (TRN), for which only one assay was tested on the Beckman Coulter AU. Results from the vast majority of Beckman Coulter AU assays (51/60) strongly correlated ($R^2 > 0.7$) with those from Abbott (Fig. 1A, Supplementary Fig. 1), except for 9 assays that did not sufficiently correlate between the two platforms (Supplementary Fig. 2), including: carbon dioxide (2/2 assays; R^2 ranged from 0.15 to 0.2), calcium (2/2 assays; R^2 from 0.32 to 0.52), albumin (1/2 assays, $R^2 = 0.69$), alanine aminotransferase (1/3 assays; $R^2 = 0.52$), aspartate aminotransferase (1/3 assays; $R^2 = 0.4$), magnesium (1/2 assays; $R^2 = 0.61$), and total protein (1/2 assays; $R^2 = 0.66$). As a result, the reference intervals

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