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CLSI-based transference of the CALIPER database of pediatric reference intervals from Abbott to Beckman, Ortho, Roche and Siemens Clinical Chemistry Assays: Direct validation using reference samples from the CALIPER cohort

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ABSTRACT

Objectives: The CALIPER program recently established a comprehensive database of age- and sex-stratified pediatric reference intervals for 40 biochemical markers. However, this database was only directly applicable for Abbott ARCHITECT assays. We therefore sought to expand the scope of this database to biochemical assays from other major manufacturers, allowing for a much wider application of the CALIPER database.

Design and methods: Based on CLSI C28-A3 and EP9-A2 guidelines, CALIPER reference intervals were transferred (using specific statistical criteria) to assays performed on four other commonly used clinical chemistry platforms including Beckman Coulter DxC800, Ortho Vitros 5600, Roche Cobas 6000, and Siemens Vista 1500. The resulting reference intervals were subjected to a thorough validation using 100 reference specimens (healthy community children and adolescents) from the CALIPER bio-bank, and all testing centers participated in an external quality assessment (EQA) evaluation.

Results: In general, the transferred pediatric reference intervals were similar to those established in our previous study. However, assay-specific differences in reference limits were observed for many analytes, and in some instances were considerable. The results of the EQA evaluation generally mimicked the similarities and differences in reference limits among the five manufacturers' assays. In addition, the majority of transferred reference intervals were validated through the analysis of CALIPER reference samples.

Conclusions: This study greatly extends the utility of the CALIPER reference interval database which is now directly applicable for assays performed on five major analytical platforms in clinical use, and should permit the worldwide application of CALIPER pediatric reference intervals.

Introduction

Clinical interpretation of laboratory test results is heavily dependent on the availability of reliable reference intervals. In simplistic terms, reference intervals represent the range of results that are commonly observed in a population of healthy individuals. More specifically, current guidelines define a reference interval as the range that encompasses the central 95% of the distribution of test results from reference individuals sampled from a healthy reference population [1]. Comparison of a given test result to an appropriate reference interval gives meaning to that result, enabling proper clinical assessment and patient care. The process of establishing accurate and reliable reference intervals is complex, and highly dependent on selecting an appropriate reference population [2]. Factors such as age, sex, sexual development, ethnicity, and geographic location may profoundly affect the reference concentration of a given analyte. As a result, partitioned reference intervals accounting for the influence of these covariates are required for

Abbreviations: CALIPER, Canadian Laboratory Initiative in Pediatric Reference Intervals; CLSI, Clinical Laboratory Standards Institute; Q–Q, quantile–quantile; EQA, External quality assessment; CEQAL, Canadian External Quality Assessment Laboratory.

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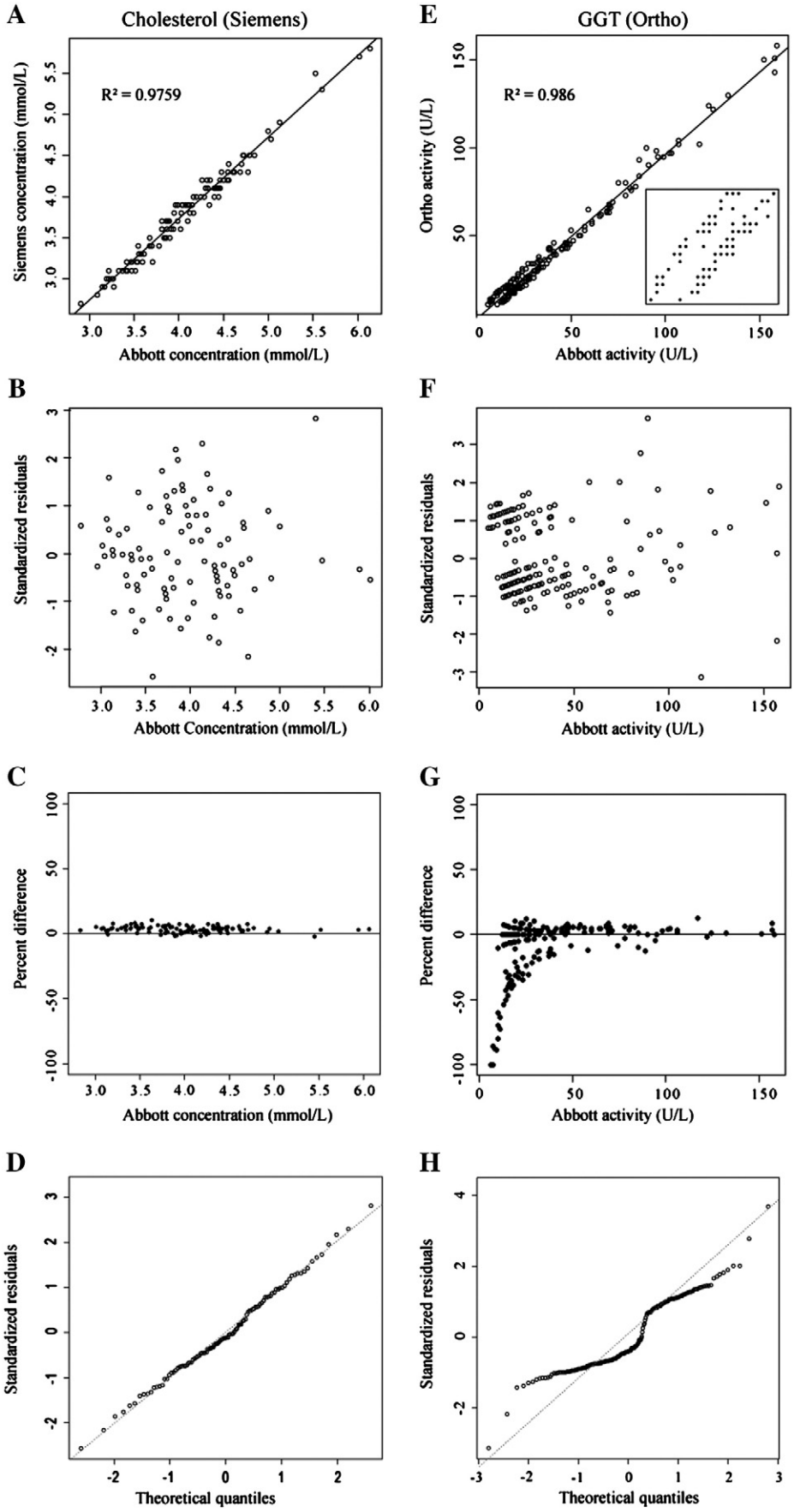
A
Cholesterol (Siemens)
R² = 0.9759
Siemens concentration (mmol/L)
Abbott concentration (mmol/L)
E
GGT (Ortho)
R² = 0.986
Ortho activity (U/L)
Abbott activity (U/L)
B
Standardized residuals
Abbott Concentration (mmol/L)
F
Standardized residuals
Abbott activity (U/L)
C
Percent difference
Abbott concentration (mmol/L)
G
Percent difference
Abbott activity (U/L)
D
Standardized residuals
Theoretical quantiles
H
Standardized residuals
Theoretical quantiles

many analytes. This is particularly important for pediatric populations, as the concentrations of many routinely measured analytes vary significantly with growth and development [3–7]. It is well documented that the use of inappropriate pediatric reference intervals — those that do not account for the effect of age, sex or ethnicity on analyte concentrations — can result in misdiagnosis and misclassification of disease [8–11]. However, significant challenges have precluded establishing covariate-stratified pediatric reference intervals for many analytes [12]. These hurdles include difficulties in recruiting a large number of healthy participants and issues in collecting adequate blood volumes, particularly from very young children.

The CALIPER (*CA*nadian *L*aboratory *I*nitiative in *PE*diatric *R*eference Intervals) Project is a collaboration between multiple pediatric centers across Canada, that aims to address current gaps in pediatric reference intervals [13,14]. As part of this project, we recently reported a comprehensive database of age- and sex-specific reference intervals for 40 biochemical markers (serum chemistry, enzymes, lipids, and proteins), that was established from a multiethnic population of healthy children and adolescents [3]. This study was a significant step forward in developing appropriate pediatric reference intervals for many analytes. However, the CALIPER reference intervals were established using Abbott ARCHITECT assays, meaning that the database is currently only applicable to laboratories using the Abbott ARCHITECT platform. Consequently, the utility of the CALIPER pediatric reference interval database was limited to a subset of pediatric centers.

Given the complexity, challenges, and cost of establishing reference intervals, Clinical Laboratory Standards Institute (CLSI) guidelines emphasize the importance of transferring reference intervals established in one laboratory (donor) to other (receiving) laboratories [1]. This process involves two main steps: transference and validation. First, a method comparison study is performed in which the comparability of the analytical systems used in the two laboratories is established [15]. Provided that an appropriate distribution of values is assessed and the assay results are highly correlated, the mathematical relationship between the two analytical systems is used to calculate the new reference interval for the receiving laboratory [1]. Second, the receiving laboratory validates the transferred reference interval. This may entail a subjective assessment or an analysis of specimens obtained from reference individuals in the receiving laboratory's own population. The transference approach has been used to establish pediatric reference intervals for the Dade Behring Dimension RxL analyzer [16], and to demonstrate that reference intervals for immunoassays on the Siemens ACS:Centaur are equivalent to those on the Siemens ACS:180 system [17].

In order to broaden the utility of the CALIPER reference interval database [3], we performed transference studies to validate this database for assays performed on four analyzers commonly used in clinical chemistry laboratories. These included the Beckman Coulter DxC800, Ortho Vitros 5600, Roche Cobas 6000, and Siemens Vista 1500 platforms. We report assay-specific pediatric reference intervals stratified by age and sex, for several biochemical markers. This expanded database will be of global benefit by facilitating the use of CALIPER reference intervals at pediatric centers worldwide.

Materials and methods

Method comparison sample analysis

This study was approved by the Institutional Review Board (IRB) at the Hospital for Sick Children (Toronto, Canada) along with the review boards of collaborating hospitals. Approximately 200 pediatric pooled serum specimens, prepared from left-over serum collected at the Hospital for Sick Children (Toronto, Ontario), were analyzed on the following platforms: Abbott ARCHITECT c8000 (at Eastern Health Authority, St. John's, Newfoundland), Beckman Coulter DxC800 (at Sainte-Justine Hospital, Montreal, Quebec), Ortho Vitros 5600 (at the Children's and Women's Health Centre, Vancouver, British Columbia), Roche Cobas 6000 (at Royal University Hospital, Saskatoon, Saskatchewan), and Siemens Vista 1500 (at The Ottawa Hospital, Ottawa, Ontario). Specimens were selected to ensure that a broad concentration/activity range would be covered for each analyte under study. Chemistry (bilirubin direct, bilirubin total, calcium, total carbon dioxide, creatinine, magnesium, iron, phosphate, urea, and uric acid), enzyme (ALP, ALT, amylase, AST, GGT, LDH, and lipase), lipid/lipoprotein (ApoB, cholesterol, HDL-cholesterol, and triglycerides) and protein markers (albumin, C3, C4, CRP, haptoglobin, IgA, IgG, IgM, prealbumin, total protein, and transferrin) were assessed. It should be noted that some assays were either not available on a given platform or were not offered at a given testing site (thus not all analytes were tested on all instruments). Supplemental data Tables 1–5 summarize the analytical parameters and calibration/traceability information for all of the assays performed on each platform.

Transference protocols and statistical analysis

An overview of the data analysis procedure and the criteria for transference, which is based on CLSI C28-A3 and EP9-A2 guidelines [1,15], is presented in Supplemental data Fig. 1. Statistical analysis was performed using Excel (Microsoft) and R [18]. Concentrations/activities obtained with the Abbott ARCHITECT assays were plotted against the corresponding concentrations/activities obtained with each of the four other manufacturers' assays. In most instances, visual examination of the data did not reveal any obvious outliers. In rare cases, gross outliers (identified by visual inspection) were removed. Results below the lower end of the reportable range were excluded. In addition, isolated extreme high and extreme low data points along the regression line were removed (based on CLSI guidelines) in order to avoid overestimation of the quality of the correlation [1]. Simple linear regression assumes that the X variable is known (without error). According to CLSI EP9-A2, if the data yield an $r^2 \geq 0.95$ 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 [15]. Thus, we opted to use this regression method to determine the line of best fit and its corresponding equation in cases where $r^2 \geq 0.95$. If $r^2 < 0.95$, linear regression does not give a valid estimate of the slope and y intercept (as the error in X is not adequately compensated by the range of the data). Thus, we opted to use Deming regression, which allows both methods to have measurement error, in instances where $r^2 < 0.95$. In cases where $r^2 < 0.70$, we felt that the data was inadequately correlated and would not reliably transfer the Abbott ARCHITECT reference intervals. Consequently, the corresponding reference intervals were deemed non-transferable.

Standardized residual, Bland–Altman, and quantile–quantile (Q–Q) plots were generated and used to assess the appropriateness of a linear model with normally distributed data points. The standardized residual and Bland–Altman plots were visually examined to confirm that the data points did not cluster into distinct patterns. A Q–Q plot is a tool to determine whether the residuals follow a normal distribution. This 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. Thus, a straight line of the equation $y = x$ is

Fig. 1. Statistical criteria used to assess the appropriateness of transference (representative assays shown). Representative scatter (A and E), standardized residual (B and F), Bland–Altman (C and G), and Q–Q (D and H) plots for an analyte where transference was deemed appropriate (cholesterol on the Siemens Vista platform) or inappropriate (GGT on the Ortho Vitros platform). The inset in (E) shows an enlargement of the lower end of the scatter plot (up to 25 U/L) in order to demonstrate the presence of two distinct populations of data. The Q–Q plots (D and H) show 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.

indicative of normally distributed residuals, and the Q–Q plots were visually examined to verify that the data followed this pattern. If all of 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 assay [3] to the other manufacturer's assay. Otherwise, the corresponding reference intervals were deemed non-transferable. While we examined the entire data range in the standardized residual, Bland–Altman, and Q–Q plots, it is only necessary that a linear model be found appropriate at concentrations close to the CALIPER reference limits established in our previous study [3]

Only the upper reference limit was transferred in cases where low concentrations of the analyte are not clinically relevant and in instances where the lower reference limit corresponds to the lower end of the reportable range of the corresponding Abbott ARCHITECT assay (chemistry markers: bilirubin direct and bilirubin total; enzyme markers: ALT, amylase, AST, lipase and LDH; lipid/lipoprotein markers: some partitions for ApoB; protein markers: CRP, haptoglobin, and some partitions for IgA and prealbumin). The root of the mean-squared error (RMSE) was used to determine 95% confidence intervals around each lower and upper reference limit. These were calculated as the reference limit plus or minus 1.96 * RMSE. The 95% confidence intervals were used as secondary limits with which to validate the transferred reference intervals.

Validation of transferred reference intervals using samples from the CALIPER cohort

Validation of the transferred reference intervals was performed based on CLSI C28-A3 guidelines [1]. Approximately 100 reference specimens (healthy community children and adolescents) from the CALIPER bio-bank [3] were analyzed on the Beckman Coulter DxC800, Ortho Vitros 5600, Roche Cobas 6000, and Siemens Vista 1500 analytical platforms. Specimens were selected to span as many age and gender partitions as possible. A conservative approach to outlier detection and removal was employed. The results were visually inspected for obvious outliers; that is, data points that were several-fold higher/lower than the next highest/lowest data point. Such points were considered outliers by the Tukey method and were removed. However, it should be noted that the Tukey method was not universally applied across the dataset. The percentage of values falling within the lower and upper reference limits of each partition was determined. The total proportion of samples that fell within the appropriate reference limits was then calculated (total validation across all age and gender partitions). This process was repeated for the lower and upper reference limits inclusive of the 95% confidence intervals.

External quality assessment (EQA)

As an independent means of assessing the performance of each assay, a comprehensive EQA evaluation was performed. Survey samples were prepared by CEQAL ((Canadian External Quality Assessment Laboratory), Vancouver, British Columbia) and shipped at 4 °C to each of the participating centers. The following analytes were measured 3 times over the course of a single day, with the testing spread throughout the day. Chemistry markers: bilirubin direct, bilirubin total, calcium, total carbon dioxide, creatinine, magnesium, iron, phosphate, urea, and uric acid; enzyme markers: ALP, ALT, amylase, AST, GGT, LDH, and lipase; lipid/lipoprotein markers: apoB, cholesterol, HDL-cholesterol, and triglycerides; protein markers: albumin, total protein, and transferrin. Each measurement was performed on a separate set of samples. Performance reports were obtained from CEQAL (Supplemental data File 2).

Results

Approximately 200 specimens were used to establish the relationship between the Abbott ARCHITECT assay and those from the four other manufacturers for each analyte in the CALIPER reference interval database [3]. We first inspected the scatter plots and the corresponding correlation coefficients (examples shown in Supplemental data File 1). In most instances, a linear relationship appeared evident, and the results from the other manufacturers' assays correlated well with those obtained using the Abbott ARCHITECT assay (representative example shown in Fig. 1A). A notable exception was carbon dioxide, for which no correlation was evident in any of the assay comparisons ($r^2 < 0.1$; see Discussion for details). In addition, the magnesium results from each of the four manufacturers' assays correlated only modestly with the Abbott ARCHITECT results ($r^2 = 0.65$–0.66 for the four assays). This was also the case for the Beckman Coulter phosphate assay ($r^2 = 0.68$). As a result of these poor correlations, the reference intervals for CO_2 and magnesium were not transferred in any instance, and those for phosphate were not transferred to the Beckman Coulter assay.

For assays that correlated well with the Abbott ARCHITECT ($r^2 > 0.70$), we next assessed the appropriateness of a linear model with normally distributed data points by generating and visually analyzing standardized residual, Bland–Altman and Q–Q plots (examples shown in Supplemental data File 1). In the majority of cases, the residuals were randomly distributed, the Bland–Altman plot did not show discrete groups of data points, and the Q–Q plot followed a straight line (representative example shown in Fig. 1B–D). Therefore, the linear model was deemed appropriate in the vast majority of instances, and the regression equations were used to transfer the Abbott ARCHITECT reference intervals to the other assays. However, this was not always the case. The most striking exception was GTT, which exhibited problems in all of the above-mentioned plots despite correlating extremely well with the Abbott ARCHITECT GGT assay (Fig. 1E). The residuals fell into two clusters, the Bland–Altman plot clearly demonstrated two distinct groups of data points, and the Q–Q plot did not follow a straight line (Fig. 1F–H). This suggested that two separate populations were present within the dataset, thus making a linear model with normally distributed data inappropriate. Interestingly, the same patterns were observed for the GGT assays from all four manufacturers (see Discussion for details). A similar phenomenon occurred for CRP, although this issue was restricted to the Roche Cobas assay. Consequently, the GGT reference intervals were not transferred in any instance, and those for CRP were not transferred to the Roche Cobas assay. These results demonstrate the limitation of relying solely on the correlation coefficient to assess the appropriateness of a linear model [19].

Some degree of scatter about the linear regression line was observed in each assay comparison. As a result, it is possible that the corresponding linear regression equations may not reliably transfer the Abbott ARCHITECT reference intervals to the other assays. Consequently, we calculated 95% confidence intervals around each transferred lower and upper reference limit, and used these as secondary limits in the validation process.

Pediatric reference intervals stratified by age and sex for several biochemical markers are presented for Beckman Coulter (Table 1), Ortho Vitros (Table 2), Roche Cobas (Table 3), and Siemens Vista (Table 4) assays.

In order to validate the transferred reference intervals, we analyzed approximately 100 reference samples from our healthy CALIPER children population [3] using each assay under investigation. Table 5 summarizes the total proportion of values that fell within the appropriate age and gender-specific reference limits for each assay (total validation across all partitions). The proportion of values that fell within the reference limits of individual partitions is shown in Supplemental data Tables 6–9. As per CLSI C28-A3 guidelines [1], validation was considered successful if ≥90% of values fell within the reference limits and ≥20 samples were assessed. It should be noted that this approach will only fail if a given reference interval is too narrow, and not if it is too broad.

Table 1
Age-specific and sex-specific pediatric reference intervals for biochemical markers measured with Beckman Coulter assays.[a]

Chemistry										
Analyte	Age	Female reference interval					Male reference interval			
		Lower limit	Upper limit	Lower limit Confidence Interval	Upper limit Confidence Interval		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval
Bilirubin	0 – 14 days		7		(6–9)			7		(6–9)
Direct[b]	15 days – < 1yr		3		(1–5)			3		(1–5)
(umol/L)	1 – < 9 yrs		2		(0–4)			2		(0–4)
	9 – < 13 yrs		3		(1–5)			3		(1–5)
	13 –<19yrs		4		(2–6)			4		(2–6)
Bilirubin	0 – 14 days		263		(261–266)			263		(261–266)
Total	15 days – < 1 yr		11		(9–14)			11		(9–14)
(Enzymatic)	1 – < 9 yrs		7		(4–9)			7		(4–9)
(umol/L)	9 – < 12 yrs		9		(6–12)			9		(6–12)
	12 – < 15 yrs		11		(9–14)			11		(9–14)
	15 – < 19 yrs		14		(11–16)			14		(11–16)
Calcium	0 – < 1yr	2.17	2.74	(2.06–2.28)	(2.63–2.86)		2.17	2.74	(2.06–2.28)	(2.63–2.86)
(mmol/L)	1 – < 19 yrs	2.32	2.64	(2.21–2.43)	(2.52–2.75)		2.32	2.64	(2.21–2.43)	(2.52–2.75)
Creatinine	0 – 14 days	31.9	85.8	(23.3–40.5)	(77.1–94.4)		31.9	85.8	(23.3–40.5)	(77.1–94.4)
(Jaffe)	15 days – < 2 yrs	11.8	35.6	(3.1–20.4)	(27.0–44.3)		11.8	35.6	(3.1–20.4)	(27.0–44.3)
(umol/L)	2 – < 5 yrs	20.8	41.3	(12.2–29.5)	(32.7–50.0)		20.8	41.3	(12.2–29.5)	(32.7–50.0)
	5 – < 12 yrs	30.5	57.3	(21.8–39.1)	(48.6–65.9)		30.5	57.3	(21.8–39.1)	(48.6–65.9)
	12 – < 15 yrs	43.4	75.8	(34.7–52.0)	(67.2–84.5)		43.4	75.8	(34.7–52.0)	(67.2–84.5)
	15 – < 19 yrs	46.9	78.2	(38.3–55.5)	(69.5–86.8)		58.9	99.9	(50.2–67.5)	(91.2–108.5)
Iron	0 – < 14 yrs	4.3	25.2	(2.8–5.9)	(23.6–26.8)		4.3	25.2	(2.8–5.9)	(23.6–26.8)
(umol/L)	14 –< 19 yrs	5.1	31.6	(3.5–6.6)	(30.0–33.1)		7.1	32.6	(5.6–8.7)	(31.0–34.2)
Urea	0 – < 14 days	0.48	7.50	(0–1.07)	(6.91–8.09)		0.48	7.5	(0–1.07)	(6.91–8.09)
(mmol/L)	15 days – < 1 yr	0.68	5.36	(0.09–1.26)	(4.77–5.95)		0.68	5.36	(0.09–1.26)	(4.77–5.95)

(*continued on next page*)

Table 1 (*continued*)

	1 – < 10 yrs	2.63	7.21	(2.04–3.22)	(6.62–7.80)		2.63	7.21	(2.04–3.22)	(6.62–7.80)
	10 – < 19 yrs	2.04	6.14	(1.45–2.63)	(5.55–6.73)		2.04	6.82	(1.45–2.63)	(6.23–7.41)
Uric acid	0 – 14 days	180	761	(156–204)	(737–786)		180	761	(156–204)	(737–786)
(umol/L)	15 days – < 1 yr	111	389	(87–135)	(365–413)		111	389	(87–135)	(365–413)
	1 – < 12 yrs	123	302	(99–147)	(278–327)		123	302	(99–147)	(278–327)
	12 – < 19 yrs	169	361	(145–193)	(337–385)		172	464	(148–196)	(440–488)
Enzymes										
Alkaline	0 – 14 days	77	237	(63–91)	(223–251)		77	237	(63–91)	(223–251)
Phosphatase	15 days – < 1 yr	116	450	(102–130)	(436–464)		116	450	(102–130)	(436–464)
(U/L)	1 – < 10 yrs	135	320	(121–149)	(306–334)		135	320	(121–149)	(306–334)
	10 – < 13 yrs	122	400	(108–136)	(386–414)		122	400	(108–136)	(386–414)
	13 – < 15 yrs	52	243	(38–66)	(229–257)		109	449	(95–123)	(435–463)
	15 – < 17 yrs	46	110	(32–60)	(96–124)		77	317	(63–91)	(303–331)
	17 – < 19 yrs	41	82	(27–55)	(68–96)		50	142	(36–64)	(128–156)
ALT[c]	0 – < 1 yr		23		(18–29)			23		(18–29)
(U/L)	1 – < 13 yrs		19		(13–24)			19		(13–24)
	13 – < 19 yrs		17		(12–23)			18		(12–23)
Amylase	0 – 14 days		10		(4–16)			10		(4–16)
(U/L)	15 days – < 13 wks		24		(18–30)			24		(18–30)
	13 wks – < 1 yr		57		(51–63)			57		(51–63)
	1 – < 19 yrs		117		(111–123)			117		(111–123)
AST	0 – 14 days		157		(152–163)			157		(152–163)
(U/L)	15 days – < 1 yr		67		(62–73)			67		(62–73)
	1 – < 7 yrs		45		(40–51)			45		(40–51)
	7 – < 12 yrs		38		(33–34)			38		(33–43)
	12 – < 19 yrs		28		(23–34)			37		(32–42)
LDH	0 – 14 days		888		(868–909)			888		(868–909)
(U/L)	15 days – < 1 yr		330		(310–351)			330		(310–351)

Table 1 (*continued*)

	1 – < 10 yrs		236		(215–256)		236		(215–256)
	10 – < 15 yrs		200		(180–221)		208		(188–229)
	15 – < 19 yrs		184		(164–205)		184		(164–205)
Lipids/Lipoproteins									
Triglycerides (mmol/L)	0 – 14 days	0.97	3.39	(0.84–1.11)	(3.25–3.53)	0.97	3.39	(0.84–1.11)	(3.25–3.53)
	15 days – < 1 yr	0.57	3.38	(0.44–0.71)	(3.24–3.51)	0.57	3.38	(0.44–0.71)	(3.24–3.51)
	1 – < 19 yrs	0.45	2.54	(0.32–0.59)	(2.41–2.68)	0.45	2.54	(0.32–0.59)	(2.41–2.68)
HDL cholesterol (mmol/L)	0 – 14 days	0.41	1.11	(0.29–0.52)	(1.00–1.23)	0.41	1.11	(0.29–0.52)	(1.00–1.23)
	15 days – < 1 yr	0.30	1.91	(0.19–0.42)	(1.80–2.03)	0.30	1.91	(0.19–0.42)	(1.80–2.03)
	1 – < 4 yrs	0.86	1.68	(0.75–0.98)	(1.57–1.80)	0.86	1.68	(0.75–0.98)	(1.57–1.80)
	4 – < 13 yrs	0.95	1.94	(0.83–1.06)	(1.83–2.06)	0.95	1.94	(0.83–1.06)	(1.83–2.06)
	13 – < 19 yrs	0.85	1.92	(0.74–0.97)	(1.81–2.04)	0.84	1.83	(0.73–0.96)	(1.72–1.94)
Proteins									
Albumin P (g/L)	0 – 14 days	29	44	(27–32)	(41–46)	29	44	(27–32)	(41–46)
	15 days – < 1 yr	26	49	(24–28)	(47–51)	26	49	(24–28)	(47–51)
	1 – < 8 yrs	37	48	(34–39)	(46–50)	37	48	(34–39)	(46–50)
	8 – < 15 yrs	39	50	(37–42)	(48–53)	39	50	(37–42)	(48–53)
	15 – < 19 yrs	37	52	(35–40)	(50–54)	40	54	(38–43)	(51–56)
Total Protein (g/L)	0 – 14 days	50	83	(45–55)	(78–88)	50	83	(45–55)	(78–88)
	15 days – < 1 yr	41	70	(35–46)	(65–75)	41	70	(35–46)	(65–75)
	1 – < 6 yrs	59	74	(54–64)	(69–79)	59	74	(54–64)	(69–79)
	6 – < 9 yrs	62	76	(57–67)	(71–81)	62	76	(57–67)	(71–81)
	9 – < 19 yrs	63	81	(58–68)	(75–86)	63	81	(58–68)	(75–86)

[a]Female-specific reference intervals are highlighted in pink, whereas male-specific reference intervals are highlighted in blue.
[b]The lower reference limits established in our previous study for total and direct bilirubin correspond to the lower end of the reportable range of the respective Abbott ARCHITECT assay. Therefore, these reference limits were not transferred to the corresponding Beckman Coulter assay.
[c]ALT, alanine aminotransferase without pyridoxal phosphate; AST, aspartate aminotransferase without pyridoxal phosphate; LDH, lactate dehydrogenase; HDL, high-density lipoprotein cholesterol; albumin P, albumin assay with bromcresol purple.

As an independent means of assessing the performance of each assay, all participating centers took part in an EQA evaluation (Supplemental data File 2). In general, the EQA results were consistent with the similarities and differences in reference limits among the five manufacturers' assays (see Discussion for exceptions and implications).

Discussion

In general, the assay-specific, age- and sex-stratified pediatric reference intervals (Tables 1–4) are similar to those established in our previous study [3]. Consistent with this observation, the results of the EQA evaluation demonstrated good agreement among the various assays for many analytes (Supplemental data File 2). This raises the question of whether the previously established CALIPER reference intervals [3], defined using Abbott assays, can simply be applied to assays from other manufacturers. However, this issue is complicated by the fact that the majority of analytes examined in this study require several age- and sex-stratified reference intervals. Most of the assays that were investigated in the current study had at least one partition whose lower and/or upper reference limit fell outside the 90% confidence intervals of the corresponding reference limit established previously [3]. We therefore opted to take the consistent approach of presenting the transferred reference limits for all partitions, as opposed to adopting the previously established CALIPER reference limits for specific partitions (in which there was no significant difference between the transferred reference limits and those established in our previous study [3]). It is important to note that, although the Abbott ARCHITECT assays were used as the comparative methods, they should not be regarded as reference methods. Therefore, deviation from the Abbott ARCHITECT reference intervals does not imply poor method performance.

The most extreme example of an assay-specific difference involved lipase, where results obtained using the Siemens Vista assay were approximately 4-fold higher than those obtained with the Abbott assay (Supplemental data File 1). The EQA evaluation yielded similar results (Supplemental data File 2). This large discrepancy is likely due to differences in the substrate employed: the Abbott assay uses 1,2-diglyceride whereas the Siemens assay uses 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester. Differences in reaction conditions may also play a role. Consistent with this large disparity, several studies have documented wide variations in lipase activity between different methods [16,20–22].

The Ortho Vitros upper reference limits for apoB (Table 2) were roughly 30% higher than those for the Abbott ARCHITECT, which was again confirmed by the results of the EQA evaluation (Supplemental data File 2). This disparity may be due to differences in standardization between the two methods. The Abbott ARCHITECT method is traceable to reference material SP3-07 [23], whereas the Ortho Vitros method is traceable to SP3-08 (Supplemental data Tables 1 and 3). These assay-specific differences are particularly noteworthy since the Canadian Cholesterol Guidelines have recommended apoB as the primary alternate target to LDL-cholesterol, with therapy targeting apoB concentrations below 0.8 g/L [24].

The assay-specific differences described above demonstrate that reference intervals established using one assay for a particular analyte may not be directly applicable to other assays. As a result, the CALIPER reference interval database, which was established using Abbott ARCHITECT assays [3], has limited usefulness for users of other methods. The pediatric reference intervals for assays from other manufacturers presented in the current study overcome this limitation, and will facilitate the implementation of the CALIPER reference interval database at pediatric centers around the world.

For many analytes, the transferred reference intervals were successfully validated for all assays examined (based on the total validation across all partitions; see Table 5). These included the chemistry markers bilirubin direct, creatinine (enzymatic), iron, and uric acid, the enzyme markers ALP, ALT (with pyridoxal phosphate), amylase, AST (with and without pyridoxal phosphate), and lipase, the lipid/lipoprotein marker apoB, and the protein markers albumin (P and G), C3, haptoglobin, IgA, and total protein. For several other analytes, the transferred reference intervals validated for all except one assay. These included urea, ALT (without pyridoxal phosphate), LDH, IgG, and prealbumin.

It should be noted that in cases where the transferred reference intervals for a specific assay did not validate, the total proportion of validated samples usually fell just slightly below the 90% cut-off. When the 95% confidence intervals around the lower and upper reference limits were taken into account, nearly all of these reference intervals validated (Table 5). While the validity of these reference intervals has not been unequivocally demonstrated as per CLSI criteria [1], we have included them in Tables 1–4. The reference intervals for a few assays did not validate even when the 95% confidence intervals around the lower and upper reference limits were taken into account. These included the Ortho Vitros LDH, IgM, total cholesterol, and HDL-cholesterol assays, the Roche Cobas triglyceride and HDL-cholesterol assays, and the Siemens Vista CRP assay. Consequently, reference intervals for these seven assays are not reported.

In rare cases, a discrepancy was apparent between the initial method comparison data and the EQA results. For example, the Ortho Vitros transferrin assay agreed very well with the Abbott ARCHITECT assay in the initial method comparison (slope 1.01 and y-intercept 0.036; Supplemental data File 1). However, the results of the EQA evaluation showed the Vitros transferrin assay running significantly higher than the Abbott ARCHITECT assay (Supplemental data File 2). This suggests that a change in method performance had occurred during the course of the study. Consistent with this, only 74% of validation samples fell within the Ortho Vitros transferrin assay reference interval, and every sample that fell outside the reference interval was above the upper limit. A new assay reagent generation, which was implemented prior to analyzing the validation and EQA samples, is the most likely explanation for the change in performance. The Siemens Vista creatinine (Jaffe) and HDL-cholesterol assays also appeared to undergo changes in performance over the course of the study (Supplemental data Files 1 and 2). Consequently, instrument-specific reference intervals for these three analytes are not reported.

While samples used in the validation process were selected to span as many age and gender partitions as possible, some individual partitions had fewer than the 20 samples recommended by CLSI C28-A3 [1]. This was particularly evident for analytes with a large number of partitions such as creatinine, phosphate, and ALP (Supplemental data Tables 6–9). In addition, we were unable to analyze specimens from healthy participants less than one year of age, given the major challenges associated with limited sample volumes. Nevertheless every assay had several other partitions in which greater than 20 samples were analyzed. In addition, we calculated the total proportion of the approximately 100 samples that fell within the appropriate partitioned reference interval for each assay. According to CLSI C28-A3 guidelines, if a reference interval is validated on one partition, there may not be the need to validate it on all other partitions [1]. As a result, we feel that the total proportion of validated samples across all age and gender partitions provides the best overall assessment of whether the transferred reference intervals for a given assay validated.

An important caveat to using transference to establish reference intervals is that an appropriate range of values must be used. If the range of samples is too large (such that it includes many extreme high and/or extreme low values that fall along the regression line), the quality of the correlation may be overestimated [1]. In order to avoid this pitfall, extreme high and extreme low values were removed (Supplemental data File 1). In some cases, however, the resulting range did not encompass all of the Abbott ARCHITECT partitioned reference limits established in our previous study [3]. This was most common for partitions less than one year of age, when the concentration of many analytes is significantly

Table 2
Age-specific and sex-specific pediatric reference intervals for biochemical markers measured with Ortho Vitros assays.[a]

Chemistry										
Analyte	Age	Female reference interval					Male reference interval			
		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval
Bilirubin Total[b] (umol/L)	0 – 14 days		257.8		(255.3–260.3)			257.8		(255.3–260.3)
	15 days – < 1 yr		11.4		(9.0–13.9)			11.4		(9.0–13.9)
	1 – < 9 yrs		7.0		(4.5–9.5)			7.0		(4.5–9.5)
	9 – < 12 yrs		9.4		(6.9–11.8)			9.4		(6.9–11.8)
	12 – < 15 yrs		11.6		(9.2–14.1)			11.6		(9.2–14.1)
	15 – < 19 yrs		13.9		(11.4–16.3)			13.9		(11.4–16.3)
Calcium (mmol/L)	0 – < 1 yr	2.08	2.64	(1.99–2.16)	(2.55–2.73)		2.08	2.64	(1.99–2.16)	(2.55–2.73)
	1 – < 19 yrs	2.22	2.54	(2.13–2.31)	(2.45–2.63)		2.22	2.54	(2.13–2.31)	(2.45–2.63)
Creatinine (Enzymatic) (umol/L)	0 – 14 days	30.2	77.5	(26.1–34.5)	(73.4–81.6)		30.2	77.5	(26.1–34.5)	(73.4–81.6)
	15 days – < 2 yrs	12.5	33.5	(8.4–16.6)	(29.4–37.6)		12.5	33.5	(8.4–16.6)	(29.4–37.6)
	2 – < 5 yrs	20.5	38.5	(16.4–24.6)	(34.4–42.6)		20.5	38.5	(16.4–24.6)	(34.4–42.6)
	5 – < 12 yrs	28.9	52.5	(24.8–33.0)	(48.4–56.6)		28.9	52.5	(24.8–33.0)	(48.4–56.6)
	12 – < 15 yrs	40.3	68.8	(36.2–44.3)	(64.7–72.9)		40.3	68.8	(36.2–44.3)	(64.7–72.9)
	15 – < 19 yrs	43.4	70.8	(39.3–47.5)	(66.8–74.9)		53.9	89.9	(49.8–58.0)	(85.8–94.0)
Iron (umol/L)	0 – < 14 yrs	5.2	26.6	(3.1–7.1)	(24.5–28.6)		5.2	26.6	(3.1–7.1)	(24.5–28.6)
	14 – < 19 yrs	5.9	33.0	(3.9–7.9)	(31.0–35.1)		8.0	34.1	(6.0–10.1)	(32.1–36.1)
Phosphate (mmol/L)	0 – 14 days	1.80	3.31	(1.70–1.90)	(3.21–3.41)		1.80	3.31	(1.70–1.90)	(3.21–3.41)
	15 days – < 1yr	1.56	2.67	(1.46–1.66)	(2.57–2.77)		1.56	2.67	(1.46–1.66)	(2.57–2.77)
	1 – < 5yrs	1.41	2.17	(1.31–1.51)	(2.07–2.27)		1.41	2.17	(1.31–1.51)	(2.07–2.27)
	5 – < 13 yrs	1.36	1.92	(1.26–1.46)	(1.82–2.02)		1.36	1.92	(1.26–1.46)	(1.82–2.02)
	13 – < 16 yrs	1.07	1.79	(0.97–1.17)	(1.69–1.89)		1.18	1.98	(1.08–1.28)	(1.88–2.08)
	16 – < 19 yrs	1.00	1.63	(0.90–1.10)	(1.53–1.73)		1.00	1.63	(0.90–1.10)	(1.53–1.73)

(*continued on next page*)

Table 2 (*continued*)

Urea (mmol/L)	0 – < 14 days	1.23	8.37	(0.88–1.58)	(8.02–8.72)		1.23	8.37	(0.88–1.58)	(8.02–8.72)
	15 days – < 1 yr	1.42	6.19	(1.08–1.77)	(5.84–6.54)		1.42	6.19	(1.08–1.77)	(5.84–6.54)
	1 – < 10 yrs	3.41	8.08	(3.06–3.76)	(7.73–8.43)		3.41	8.08	(3.06–3.76)	(7.73–8.43)
	10 – < 19 yrs	2.81	6.98	(2.46–3.16)	(6.63–7.33)		2.81	7.68	(2.46–3.16)	(7.33–8.03)
Uric acid (umol/L)	0 – 14 days	156	732	(136–176)	(712–752)		156	732	(136–176)	(712–752)
	15 days – < 1 yr	88	363	(68–108)	(343–383)		88	363	(68–108)	(343–383)
	1 – < 12 yrs	100	277	(80–120)	(258–297)		100	277	(80–120)	(258–297)
	12 – < 19 yrs	145	336	(125–165)	(316–356)		148	438	(128–168)	(418–458)
Enzymes										
Alkaline Phosphatase (U/L)	0 – 14 days	91	256	(74–109)	(238–274)		91	256	(74–109)	(238–274)
	15 days – < 1 yr	131	476	(113–149)	(458–493)		131	476	(113–149)	(458–493)
	1 – < 10 yrs	151	342	(133–169)	(324–360)		151	342	(133–169)	(324–360)
	10 – < 13 yrs	137	424	(119–155)	(406–441)		137	424	(119–155)	(406–441)
	13 – < 15 yrs	66	262	(48–84)	(244–280)		124	474	(106–142)	(457–492)
	15 – < 17 yrs	59	126	(41–77)	(108–143)		91	339	(73–109)	(321–356)
	17 – < 19 yrs	54	96	(36–72)	(78–114)		64	158	(46–81)	(140–176)
ALT (ACT)[c] (U/L)	0 – < 1 yr		52		(44–61)			52		(44–61)
	1 – < 13 yrs		44		(35–52)			44		(35–52)
	13 – < 19 yrs		40		(32–49)			42		(34–51)
Amylase (U/L)	0 – 14 days		8		(0–17)			8		(0–17)
	15 days – < 13 wks		18		(8–28)			18		(8–28)
	13 wks – < 1 yr		44		(34–55)			44		(34–55)
	1 – < 19 yrs		91		(81–100)			91		(81–100)
AST (ACT) (U/L)	0 – 14 days		184		(179–188)			184		(179–188)
	15 days – < 1 yr		77		(73–82)			77		(73–82)
	1 – < 7 yrs		52		(47–56)			52		(47–56)
	7 – < 12 yrs		43		(38–47)			43		(38–47)
	12 – < 19 yrs		31		(27–36)			41		(37–46)

Table 2 (*continued*)

Lipids/Lipoproteins										
Apo B (g/L)	0 – 14 days		0.86		(0.72–1.00)			0.86		(0.72–1.00)
	15 days – < 1 yr	0.14	1.70	(0–0.28)	(1.55–1.84)		0.14	1.70	(0–0.28)	(1.55–1.84)
	1 – < 6 yrs	0.47	1.25	(0.33–0.61)	(1.11–1.39)		0.47	1.25	(0.33–0.61)	(1.11–1.39)
	6 – < 19 yrs	0.32	1.11	(0.18–0.46)	(0.97–1.26)		0.32	1.11	(0.18–0.46)	(0.97–1.26)
Triglycerides (mmol/L)	0 – 14 days	0.95	3.01	(0.80–1.11)	(2.85–3.16)		0.95	3.01	(0.80–1.11)	(2.85–3.16)
	15 days – < 1 yr	0.61	3.00	(0.46–0.77)	(2.84–3.15)		0.61	3.00	(0.46–0.77)	(2.84–3.15)
	1 – < 19 yrs	0.51	2.29	(0.36–0.66)	(2.13–2.44)		0.51	2.29	(0.36–0.66)	(2.13–2.44)
Proteins										
Albumin G (g/L)	0 – 14 days	26	42	(23–29)	(39–45)		26	42	(23–29)	(39–45)
	15 days – < 1 yr	23	48	(19–26)	(45–52)		23	48	(19–26)	(45–52)
	1 – < 8 yrs	35	47	(32–38)	(44–50)		35	47	(32–38)	(44–50)
	8 – < 15 yrs	37	50	(34–40)	(47–53)		37	50	(34–40)	(47–53)
	15 – < 19 yrs	35	52	(32–38)	(49–55)		39	53	(36–42)	(50–56)
C3 (g/L)	0 – 14 days	0.53	1.24	(0.44–0.61)	(1.15–1.32)		0.53	1.24	(0.44–0.61)	(1.15–1.32)
	15 days – < 1 yr	0.54	1.62	(0.45–0.62)	(1.54–1.71)		0.54	1.62	(0.45–0.62)	(1.54–1.71)
	1 – < 19 yrs	0.86	1.54	(0.77–0.94)	(1.46–1.63)		0.86	1.54	(0.77–0.94)	(1.46–1.63)
C4 (g/L)	0 – < 1 yr	0.07	0.33	(0.05–0.09)	(0.31–0.35)		0.07	0.33	(0.05–0.09)	(0.31–0.35)
	1 – < 19 yrs	0.14	0.41	(0.12–0.16)	(0.39–0.43)		0.14	0.41	(0.12–0.16)	(0.39–0.43)
Proteins										
CRP (mg/L)	0 – 14 days		5.78		(4.60–6.96)			5.78		(4.60–6.96)
	15 days – < 15 yrs		1.21		(0.03–2.39)			1.21		(0.03–2.39)
	15 – < 19 yrs		1.87		(0.69–3.05)			1.87		(0.69–3.05)
IgA (g/L)	0 – < 1 yrs		0.19		(0.10–0.27)			0.19		(0.10–0.27)
	1 – < 3 yrs		0.85		(0.77–0.94)			0.85		(0.77–0.94)
	3 – < 6 yrs	0.15	1.48	(0.07–0.24)	(1.39–1.57)		0.15	1.48	(0.07–0.24)	(1.39–1.57)
	6 – < 14 yrs	0.38	2.29	(0.30–0.47)	(2.20–2.37)		0.38	2.29	(0.30–0.47)	(2.20–2.37)
	14 – < 19 yrs	0.45	3.01	(0.36–0.54)	(2.92–3.10)		0.45	3.01	(0.36–0.54)	(2.92–3.10)

(*continued on next page*)

Table 2 (*continued*)

IgG (g/L)	0 – 14 days	3.1	14.2	(2.26–3.89)	(13.41–15.04)		3.1	14.2	(2.26–3.89)	(13.41–15.04)
	15 days – < 1 yr	0.9	7.0	(0.08–1.71)	(6.18–7.81)		0.9	7.0	(0.08–1.71)	(6.18–7.81)
	1 – < 4 yrs	3.0	11.6	(2.22–3.85)	(10.75–12.38)		3.0	11.6	(2.22–3.85)	(10.75–12.38)
	4 – < 10 yrs	5.4	13.7	(4.54–6.16)	(12.91–14.54)		5.4	13.7	(4.54–6.16)	(12.91–14.54)
	10 – < 19 yrs	6.5	15.5	(5.73–7.35)	(14.71–16.34)		6.5	15.5	(5.73–7.35)	(14.71–16.34)
Prealbumin (mg/L)	0 – 14 days		124.8		(105.5–144.1)			124.8		(105.5–144.1)
	15 days – < 1 yr	49.8	253.2	(30.5–69.2)	(233.2–272.5)		49.8	253.2	(30.5–69.2)	(233.2–272.5)
	1 – < 5 yrs	124.8	242.5	(105.5–144.1)	(223.2–261.8)		124.8	242.5	(105.5–144.1)	(223.2–261.8)
	5 – < 13 yrs	146.2	274.6	(126.9–165.5)	(255.3–294.0)		146.2	274.6	(126.9–165.5)	(255.3–294.0)
	13 – < 16 yrs	189.0	328.2	(169.7–208.3)	(308.8–347.5)		189.0	328.2	(169.7–208.3)	(308.8–347.5)
	16 – < 19 yrs	178.3	349.6	(159.0–197.6)	(330.2–368.9)		210.4	371.0	(191.1–229.7)	(351.6–390.3)
Total Protein (g/L)	0 – 14 days	55	88	(50–60)	(83–92)		55	88	(50–60)	(83–92)
	15 days – < 1 yr	45	74	(40–50)	(70–79)		45	74	(40–50)	(70–79)
	1 – < 6 yrs	63	79	(59–68)	(74–84)		63	79	(59–68)	(74–84)
	6 – < 9 yrs	67	81	(62–72)	(76–86)		67	81	(62–72)	(76–86)
	9 – < 19 yrs	68	85	(63–73)	(81–90)		68	85	(63–73)	(81–90)

[a] Female-specific reference intervals are highlighted in pink, whereas male-specific reference intervals are highlighted in blue.[b] The lower reference limits established in our previous study for total bilirubin, apoB (0–14 days), CRP, IgA (0–<1 years and 1–<3 years) and prealbumin (0–14 days) correspond to the lower end of the reportable range of the respective Abbott ARCHITECT assay. Therefore, these reference limits were not transferred to the corresponding Ortho Vitros 5600 assay.[c] ALT ACT, alanine aminotransferase with pyridoxal phosphate; AST ACT, aspartate aminotransferase with pyridoxal phosphate; apoB, apolipoprotein B; albumin G, albumin assay with bromcresol green; C3, complement C3; C4, complement C4; hs-CRP, high-sensitivity C-reactive protein.

Table 3
Age-specific and sex-specific pediatric reference intervals for biochemical markers measured with Roche Cobas assays.[a]

Chemistry									
Analyte	Age	Female reference interval				Male reference interval			
		Lower limit	Upper limit	Lower limit confidence Interval	Upper limit confidence Interval	Lower limit	Upper limit	Lower limit confidence Interval	Upper limit confidence Interval
Bilirubin total[b] (umol/L)	0 – 14 days		250		(249 – 252)		250		(249 – 252)
	15 days – < 1 yr		10		(8 – 11)		10		(8 – 11)
	1 – < 9 yrs		5		(3 – 7)		5		(3 – 7)
	9 – < 12 yrs		8		(6 – 9)		8		(6 – 9)
	12 – < 15 yrs		10		(8 – 11)		10		(8 – 11)
	15 – < 19 yrs		12		(10 – 14)		12		(10 – 14)
Calcium (mmol/L)	0 – < 1 yr	2.16	2.74	(2.07 – 2.25)	(2.65 – 2.83)	2.16	2.74	(2.07 – 2.25)	(2.65 – 2.83)
	1 – < 19 yrs	2.31	2.64	(2.22 – 2.40)	(2.55 – 2.73)	2.31	2.64	(2.22 – 2.40)	(2.55 – 2.73)
Creatinine (enzymatic) (umol/L)	0 – 14 days	27.0	78.3	(23.3 – 30.8)	(74.5 – 82.0)	27.0	78.3	(23.3 – 30.8)	(74.5 – 82.0)
	15 days – < 2 yrs	7.9	30.6	(4.2 – 11.7)	(26.9 – 34.3)	7.9	30.6	(4.2 – 11.7)	(26.9 – 34.3)
	2 – < 5 yrs	16.5	36.0	(12.8 – 20.2)	(32.3 – 39.7)	16.5	36.0	(12.8 – 20.2)	(32.3 – 39.7)
	5 – <12 yrs	25.7	51.2	(22.0 – 29.4)	(47.4 – 54.9)	25.7	51.2	(22.0 – 29.4)	(47.4 – 54.9)
	12 – < 15 yrs	37.9	68.8	(34.2 – 41.7)	(65.1 – 72.5)	37.9	68.8	(34.2 – 41.7)	(65.1 – 72.5)
	15 – < 19 yrs	41.3	71.0	(37.6 – 45.0)	(67.3 – 74.7)	52.7	91.7	(49.0 – 56.4)	(87.9 – 95.4)
Iron (umol/L)	0 – < 14 yrs	5	25	(3 – 7)	(23 – 26)	5	25	(3 – 7)	(23 – 26)
	14 – < 19 yrs	6	30	(4 – 7)	(29 – 32)	8	31	(6 – 9)	(30 – 33)
Phosphate (mmol/L)	0 – 14 days	1.71	3.15	(1.60 – 1.82)	(3.04 – 3.26)	1.71	3.15	(1.60 – 1.82)	(3.04 – 3.26)
	15 days – < 1 yr	1.47	2.54	(1.37 – 1.58)	(2.43 – 2.65)	1.47	2.54	(1.37 – 1.58)	(2.43 – 2.65)
	1 – <5 yrs	1.33	2.06	(1.22 – 1.44)	(1.95 – 2.17)	1.33	2.06	(1.22 – 1.44)	(1.95 – 2.17)
	5 – < 13 yrs	1.28	1.82	(1.18 – 1.39)	(1.71 – 1.93)	1.28	1.82	(1.18 – 1.39)	(1.71 – 1.93)
	13 – < 16 yrs	1.00	1.70	(0.90 – 1.11)	(1.59 – 1.81)	1.11	1.88	(1.00 – 1.22)	(1.77 – 1.99)
	16 – < 19 yrs	0.94	1.55	(0.83 – 1.05)	(1.44 – 1.65)	0.94	1.55	(0.83 – 1.05)	(1.44 – 1.65)
Urea (mmol/L)	0 – < 14 days	1.08	7.86	(0.76 – 1.41)	(7.53 – 8.19)	1.08	7.86	(0.76 – 1.41)	(7.53 – 8.19)
	15 days – < 1 yr	1.27	5.79	(0.94 – 1.60)	(5.46 – 6.12)	1.27	5.79	(0.94 – 1.60)	(5.46 – 6.12)
	1 – < 10 yrs	3.15	7.58	(2.83 – 3.48)	(7.25 – 7.90)	3.15	7.58	(2.83 – 3.48)	(7.25 – 7.90)
	10 – < 19 yrs	2.59	6.54	(2.26 – 2.92)	(6.21 – 6.87)	2.59	7.20	(2.26 – 2.92)	(6.87 – 7.53)

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Table 3 (*continued*)

Chemistry										
Analyte	Age	Female reference interval					Male reference interval			
		Lower limit	Upper limit	Lower limit confidence Interval	Upper limit confidence Interval		Lower limit	Upper limit	Lower limit confidence Interval	Upper limit confidence Interval
Uric acid (umol/L)	0 – 14 days	158	748	(137 – 179)	(727 – 769)		158	748	(137 – 179)	(727 – 769)
	15 days – < 1 yr	88	370	(67 – 109)	(349 – 391)		88	370	(67 – 109)	(349 – 391)
	1 – <12 yrs	100	282	(79 – 121)	(261 – 303)		100	282	(79 – 121)	(261 – 303)
	12 – < 19 yrs	147	342	(126 – 168)	(321 – 363)		150	446	(129 – 171)	(425 – 467)
Enzymes										
Alkaline Phosphatase (U/L)	0 – 14 days	83	248	(72 – 93)	(238 – 258)		83	248	(72 – 93)	(238 – 258)
	15 days – < 1 yr	122	469	(112 – 133)	(459 – 480)		122	469	(112 – 133)	(459 – 480)
	1 – < 10 yrs	142	335	(132 – 152)	(324 – 345)		142	335	(132 – 152)	(324 – 345)
	10 – < 13 yrs	129	417	(118 – 139)	(407 – 427)		129	417	(118 – 139)	(407 – 427)
	13 – < 15 yrs	57	254	(47 – 67)	(244 – 265)		116	468	(105 – 126)	(458 – 478)
	15 – < 17 yrs	50	117	(40 – 60)	(107 – 127)		82	331	(72 – 92)	(321 – 342)
	17 – < 19 yrs	45	87	(34 – 55)	(77 – 97)		55	149	(44 – 65)	(139 – 160)
ALT[c] (U/L)	0 – < 1 yrs		25		(17 – 33)			25		(17 – 33)
	1 – < 13 yrs		19		(11 – 27)			19		(11 – 27)
	13 – < 19 yrs		17		(9 – 25)			18		(11 – 26)
AST (U/L)	0 – 14 days		155		(140 – 169)			155		(140 – 169)
	15 days – < 1 yr		63		(48 – 78)			63		(48 – 78)
	1 – < 7 yrs		41		(26 – 55)			41		(26 – 55)
	7 – < 12 yrs		33		(18 – 48)			33		(18 – 48)
	12 – < 19 yrs		23		(9 – 38)			32		(17 – 47)
LDH (U/L)	0 – 14 days		1128		(1102 – 1154)			1128		(1102 – 1154)
	15 days – < 1 yr		424		(398 – 450)			424		(398 – 450)
	1 – < 10 yrs		305		(279 – 331)			305		(279 – 331)
	10 – < 15 yrs		260		(234 – 286)			270		(244 – 296)
	15 – < 19 yrs		240		(214 – 266)			240		(214 – 266)

Table 3 (*continued*)

Analyte	Age	Female reference interval				Male reference interval			
		Lower limit	Upper limit	Lower limit confidence Interval	Upper limit confidence Interval	Lower limit	Upper limit	Lower limit confidence Interval	Upper limit confidence Interval
Lipids/Lipoproteins									
Cholesterol (mmol/L)	0 – 14 days	1.25	3.24	(1.13 – 1.37)	(3.11 – 3.36)	1.15	2.83	(1.03 – 1.28)	(2.71 – 2.96)
	15 days – < 1 yr	1.70	6.07	(1.58 – 1.82)	(5.95 – 6.19)	1.70	6.07	(1.58 – 1.82)	(5.95 – 6.19)
	1 – < 19 yrs	2.91	5.36	(2.79 – 3.04)	(5.23 – 5.48)	2.91	5.36	(2.79 – 3.04)	(5.23 – 5.48)
Proteins									
Albumin P (g/L)	0 – 14 days	28	41	(27 – 29)	(40 – 42)	28	41	(27 – 29)	(40 – 42)
	15 days – < 1 yr	25	46	(24 – 26)	(45 – 47)	25	46	(24 – 26)	(45 – 47)
	1 – < 8 yrs	35	45	(34 – 36)	(44 – 46)	35	45	(34 – 36)	(44 – 46)
	8 – < 15 yrs	37	47	(36 – 38)	(46 – 48)	37	47	(36 – 38)	(46 – 48)
	15 – < 19 yrs	35	49	(34 – 37)	(47 – 50)	38	50	(37 – 39)	(49 – 52)
Haptoglobin (g/L)	0 – 14 days		0.12		(0.01 – 0.22)		0.12		(0.01 – 0.22)
	15 days – < 1 yr		2.38		(2.27 – 2.48)		2.38		(2.27 – 2.48)
	1 – < 12 yrs		1.76		(1.65 – 1.86)		1.76		(1.65 – 1.86)
	12 – < 19 yrs		1.93		(1.82 – 2.03)		1.93		(1.82 – 2.03)
IgA (g/L)	0 – < 1 yrs		0.14		(0.04 – 0.24)		0.14		(0.04 – 0.24)
	1 – < 3 yrs		0.80		(0.70 – 0.90)		0.80		(0.70 – 0.90)
	3 – < 6 yrs	0.11	1.42	(0.01 – 0.21)	(1.32 – 1.52)	0.11	1.42	(0.01 – 0.21)	(1.32 – 1.52)
	6 – < 14 yrs	0.34	2.2	(0.24 – 0.44)	(2.12 – 2.32)	0.34	2.22	(0.24 – 0.44)	(2.12 – 2.32)
	14 – < 19 yrs	0.40	2.93	(0.30 – 0.50)	(2.83 – 3.03)	0.40	2.93	(0.30 – 0.50)	(2.83 – 3.03)
IgG (g/L)	0 – 14 days	3.20	12.05	(2.65 – 3.75)	(11.49 – 12.60)	3.20	12.05	(2.65 – 3.75)	(11.49 – 12.60)
	15 days – < 1 yr	1.48	6.31	(0.92 – 2.03)	(5.76 – 6.86)	1.48	6.31	(0.92 – 2.03)	(5.76 – 6.86)
	1 – < 4 yrs	3.17	9.94	(2.62 – 3.72)	(9.39 – 10.49)	3.17	9.94	(2.62 – 3.72)	(9.39 – 10.49)
	4 – < 10 yrs	5.01	11.65	(4.45 – 5.56)	(11.10 – 12.20)	5.01	11.65	(4.45 – 5.56)	(11.10 – 12.20)
	10 – < 19 yrs	5.95	13.08	(5.40 – 6.50)	(12.53 – 13.63)	5.95	13.08	(5.40 – 6.50)	(12.53 – 13.63)
IgM (g/L)	0 – 14 days	0.03	0.32	(0 – 0.16)	(0.20 – 0.45)	0.03	0.32	(0 – 0.16)	(0.20 – 0.45)
	15 days – < 13 wks	0.10	0.67	(0 – 0.23)	(0.55 – 0.80)	0.10	0.67	(0 – 0.23)	(0.55 – 0.80)

(*continued on next page*)

Table 3 (*continued*)

Proteins										
Analyte	Age	Female reference interval					Male reference interval			
		Lower limit	Upper limit	Lower limit confidence Interval	Upper limit confidence Interval		Lower limit	Upper limit	Lower limit confidence Interval	Upper limit confidence Interval
IgM	13 wks – < 1 yr	0.14	0.82	(0.02 – 0.27)	(0.69 – 0.94)		0.14	0.82	(0.02 – 0.27)	(0.69 – 0.94)
(g/L)	1 – < 19 yrs	0.45	1.78	(0.32 – 0.57)	(1.66 – 1.91)		0.36	1.44	(0.24 – 0.49)	(1.32 – 1.57)
Prealbumin	0 – 14 days		0.11		(0.08 – 0.14)			0.11		(0.08 – 0.14)
(g/L)	15 days – < 1 yr	0.04	0.24	(0 – 0.07)	(0.20 – 0.27)		0.04	0.24	(0 – 0.07)	(0.20 – 0.27)
	1 – < 5 yrs	0.11	0.23	(0.08 – 0.14)	(0.19 – 0.26)		0.11	0.23	(0.08 – 0.14)	(0.19 – 0.26)
	5 – < 13 yrs	0.13	0.26	(0.10 – 0.17)	(0.23 – 0.29)		0.13	0.26	(0.10 – 0.17)	(0.23 – 0.29)
	13 – < 16 yrs	0.17	0.31	(0.14 – 0.21)	(0.28 – 0.34)		0.17	0.31	(0.14 – 0.21)	(0.28 – 0.34)
	16 – < 19 yrs	0.16	0.33	(0.13 – 0.20)	(0.30 – 0.37)		0.20	0.35	(0.16 – 0.23)	(0.32 – 0.39)
Total	0 – 14 days	51	80	(49 – 54)	(79 – 83)		51	80	(49 – 54)	(79 – 83)
Protein	15 days – < 1 yr	43	69	(40 – 45)	(66 – 71)		43	69	(40 – 45)	(66 – 71)
(g/L)	1 – < 6 yrs	59	73	(57 – 62)	(70 – 75)		59	73	(57 – 62)	(70 – 75)
	6 – < 9 yrs	62	75	(59 – 65)	(72 – 77)		62	75	(59 – 65)	(72 – 77)
	9 – < 19 yrs	63	78	(60 – 66)	(76 – 81)		63	78	(60 – 66)	(76 – 81)

[a] Female-specific reference intervals are highlighted in pink, whereas male-specific reference intervals are highlighted in blue.[b]The lower reference limits established in our previous study for total bilirubin, haptoglobin, IgA (0–<1 years and 1–<3 years) and prealbumin (0–14 days) correspond to the lower end of the reportable range of the respective Abbott ARCHITECT assay. Therefore, these reference limits were not transferred to the corresponding Roche Cobas assay.[c]ALT, alanine aminotransferase without pyridoxal phosphate; AST, aspartate aminotransferase without pyridoxal phosphate; LDH, lactate dehydrogenase; albumin P, albumin assay with bromcresol purple.

Table 4
Age-specific and sex-specific pediatric reference intervals for biochemical markers measured with Siemens Vista assays.[a]

Chemistry										
Analyte	Age	Female reference interval					Male reference interval			
		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval
Bilirubin Direct[b] (umol/L)	0 – 14 days		8		(6 – 10)			8		(6 – 10)
	15 days – < 1 yr		3		(1 – 5)			3		(1 – 5)
	1 – < 9 yrs		1		(0 – 3)			1		(0 – 3)
	9 – < 13 yrs		3		(1 – 5)			3		(1 – 5)
	13 – < 19 yrs		4		(2 – 6)			4		(2 – 6)
Bilirubin Total (umol/L)	0 – 14 days		239		(235 – 243)			239		(235 – 243)
	15 days – < 1 yr		9		(5 – 14)			9		(5 – 14)
	1 – < 9 yrs		5		(1 – 9)			5		(1 – 9)
	9 – < 12 yrs		7		(3 – 12)			7		(3 – 12)
	12 – < 15 yrs		10		(5 – 14)			10		(5 – 14)
	15 – < 19 yrs		12		(7 – 16)			12		(7 – 16)
Calcium (mmol/L)	0 – < 1 yr	2.13	2.67	(2.02 – 2.24)	(2.57 – 2.78)		2.13	2.67	(2.02 – 2.24)	(2.57 – 2.78)
	1 – < 19 yrs	2.27	2.58	(2.17 – 2.38)	(2.47 – 2.68)		2.27	2.58	(2.17 – 2.38)	(2.47 – 2.68)
Creatinine (Enzymatic) (umol/L)	0 – 14 days	27.2	77.6	(18.3 – 36.2)	(68.6 – 86.5)		27.2	77.6	(18.3 – 36.2)	(68.6 – 86.5)
	15 days – < 2 yrs	8.4	30.7	(0 – 17.4)	(21.8 – 39.7)		8.4	30.7	(0 – 17.4)	(21.8 – 39.7)
	2 – < 5 yrs	19.6	36.0	(7.9 – 25.8)	(27.1 – 45.0)		16.9	36.0	(7.9 – 25.8)	(27.1 – 45.0)
	5 – < 12 yrs	25.9	50.9	(16.9 – 34.8)	(42.0 – 59.9)		25.9	50.9	(16.9 – 34.8)	(42.0 – 59.9)
	12 – < 15 yrs	37.9	68.3	(29.0 – 46.9)	(59.3 – 77.2)		37.9	68.3	(29.0 – 46.9)	(59.3 – 77.2)
	15 – < 19 yrs	41.2	70.5	(32.3 – 50.2)	(61.5 – 79.4)		52.4	90.8	(43.5 – 61.4)	(81.8 – 99.7)
Iron (umol/L)	0 – < 14 yrs	5	25	(3 – 6)	(23 – 26)		5	25	(3 – 6)	(23 – 26)
	14 – < 19 yrs	5	31	(4 – 7)	(29 – 32)		7	31	(6 – 9)	(30 – 33)
Phosphate (mmol/L)	0 – 14 days	1.27	3.22	(1.57 – 1.87)	(3.07 – 3.37)		1.72	3.22	(1.57 – 1.87)	(3.07 – 3.37)
	15 days – < 1 yr	1.84	2.58	(1.33 – 1.62)	(2.44 – 2.73)		1.48	2.58	(1.33 – 1.62)	(2.44 – 2.73)

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Table 4 (*continued*)

Chemistry										
Analyte	Age	Female reference interval					Male reference interval			
		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval
Phosphate (mmol/L)	1 – < 5 yrs	1.33	2.09	(1.18 – 1.47)	(1.94 – 2.23)		1.33	2.09	(1.18 – 1.47)	(1.94 – 2.23)
	5 – < 13 yrs	1.28	1.83	(1.13 – 1.43)	(1.69 – 1.98)		1.28	1.83	(1.13 – 1.43)	(1.69 – 1.98)
	13 – < 16 yrs	0.99	1.71	(0.84 – 1.14)	(1.56 – 1.86)		1.10	1.90	(0.95 – 1.25)	(1.75 – 2.05)
	16 – < 19 yrs	0.92	1.55	(0.78 – 1.07)	(1.40 – 1.70)		0.92	1.55	(0.78 – 1.07)	(1.40 – 1.70)
Urea (mmol/L)	0 – < 14 days	1.0	8.3	(0.5 – 1.4)	(7.8 – 8.7)		1.0	8.3	(0.5 – 1.4)	(7.8 – 8.7)
	15 days – < 1 yr	1.2	6.0	(0.7 – 1.6)	(5.6 – 6.5)		1.2	6.0	(0.7 – 1.6)	(5.6 – 6.5)
	1 – < 10 yrs	3.2	8.0	(2.7 – 3.6)	(7.5 – 8.4		3.2	8.0	(2.7 – 3.6)	(7.5 – 8.4)
	10 – < 19 yrs	2.6	6.9	(2.1 – 3.0)	(6.4 – 7.3)		2.6	7.6	(2.1 – 3.0)	(7.1 – 8.0)
Uric Acid (umol/L)	0 – 14 days	132	654	(110 – 154)	(633 – 676)		132	654	(110 – 154)	(633 – 676)
	15 days – < 1 yr	71	320	(49 – 92)	(298 – 342)		71	320	(49 – 92)	(298 – 342)
	1 – < 12 yrs	81	242	(59 – 103)	(221 – 264)		81	242	(59 – 103)	(221 – 264)
	12 – < 19 yrs	123	295	(101 – 144)	(273 – 317)		125	388	(103 – 147)	(366 – 409)
Enzymes										
Alkaline Phosphatase (U/L)	0 – 14 days	81.5	248.7	(69.9 – 93.1)	(237.1 – 260.3)		81.5	248.7	(69.9 – 93.1)	(237.1 – 260.3)
	15 days – < 1 yr	121.7	472.6	(110.1 – 133.3)	(461.0 – 484.2)		121.7	472.6	(110.1 – 133.3)	(461.0 – 484.2)
	1 – < 10 yrs	141.8	336.4	(130.2 – 153.4)	(324.8 – 348.0)		141.8	336.4	(130.2 – 153.4)	(324.8 – 348.0)
	10 – < 13 yrs	128.1	419.6	(116.5 – 139.7)	(408.0 – 431.2)		128.1	419.6	(116.5 – 139.7)	(408.0 – 431.2)
	13 – < 15 yrs	55.5	255.2	(43.9 – 67.1)	(243.6 – 266.8)		114.9	471.3	(103.3 – 126.5)	(459.7 – 482.9)
	15 – < 17 yrs	48.7	116.3	(37.1 – 60.3)	(104.7 – 127.9)		80.9	333.2	(69.3 – 92.5)	(321.6 – 344.8)
	17 – < 19 yrs	43.1	86.1	(31.5 – 54.7)	(74.5 – 97.7)		53.2	149.1	(41.6 – 64.8)	(137.5 – 160.7)
ALT (ACT)[c] (U/L)	0 – < 1 yr		41		(32 – 50)			41		(32 – 50)
	1 – < 13 yrs		32		(24 – 41)			32		(24 – 41)
	13 – < 19 yrs		29		(20 – 38)			31		(23 – 40)

Table 4 (*continued*)

Enzymes										
Analyte	Age	Female reference interval					Male reference interval			
		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval
Amylase (U/L)	0 – 14 days		9.6		(6.6 – 12.6)			9.6		(6.6 – 12.6)
	15 days – < 13 wks		20.5		(17.5 – 23.4)			20.5		(17.5 – 23.4)
	13 wks – < 1 yr		46.6		(43.7 – 49.6)			46.6		(43.7 – 49.6)
	1 – < 19 yrs		93.8		(90.9 – 96.8)			93.8		(90.9 – 96.8)
AST (ACT) (U/L)	0 – 14 days		185		(167 – 203)			185		(167 – 203)
	15 days – < 1 yr		73		(55 – 91)			73		(55 – 91)
	1 – < 7 yrs		46		(28 – 64)			46		(28 – 64)
	7 – < 12 yrs		37		(19 – 55)			37		(19 – 55)
	12 – < 19 yrs		25		(7 – 43)			36		(18 – 54)
LDH (U/L)	0 – 14 days		1255		(1230 – 1279)			1255		(1230 – 1279)
	15 days – < 1 yr		470		(446 – 495)			470		(446 – 495)
	1 – < 10 yrs		337		(312 – 362)			337		(312 – 362)
	10 – < 15 yrs		287		(262 – 312)			298		(273 – 323)
	15 – < 19 yrs		265		(240 – 289)			265		(240 – 289)
Lipase (U/L)	0 – < 19 yrs		194		(151 – 238)			194		(151 – 238)
Lipids/Lipoproteins										
Apo B (g/L)	0 – 14 days		0.72		(0.66 – 0.78)			0.72		(0.66 – 0.78)
	15 days – < 1 yr	0.18	1.34	(0.12 – 0.24)	(1.28 – 1.40)		0.18	1.34	(0.12 – 0.24)	(1.28 – 1.40)
	1 – < 6 yrs	0.43	1.00	(0.37 – 0.49)	(0.94 – 1.06)		0.43	1.00	(0.37 – 0.49)	(0.94 – 1.06)
	6 – < 19 yrs	0.32	0.90	(0.26 – 0.38)	(0.84 – 0.96)		0.32	0.90	(0.26 – 0.38)	(0.84 – 0.96)
Cholesterol (mmol/L)	0 – 14 days	1.08	3.09	(0.90 – 1.26)	(2.91 – 3.28)		0.98	2.69	(0.80 – 1.16)	(2.50 – 2.87)
	15 days – < 1 yr	1.54	5.97	(1.35 – 1.72)	(5.78 – 6.15)		1.54	5.97	(1.35 – 1.72)	(5.78 – 6.15)
	1 – < 19 yrs	2.77	5.24	(2.58 – 2.95)	(5.06 – 5.43)		2.77	5.24	(2.58 – 2.95)	(5.06 – 5.43)

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Table 4 (*continued*)

Analyte	Age	Female reference interval					Male reference interval			
		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval
Lipids/Lipoproteins										
Triglycerides (mmol/L)	0 - 14 days	0.99	3.12	(0.80 – 1.1 9)	(2.92 – 3.32)		0.99	3.12	(0.80 – 1.1 9)	(2.92 – 3.32)
	15 days - < 1 yr	0.64	3.11	(0.44 – 0.84)	(2.91 – 3.31)		0.64	3.11	(0.44 – 0.84)	(2.91 – 3.31)
	1 - < 19 yrs	0.53	2.38	(0.33 – 0.73)	(2.18 – 2.57)		0.53	2.38	(0.33 – 0.73)	(2.18 – 2.57)
Proteins										
Albumin P (g/L)	0 - 14 days	30	43	(28 – 32)	(42 – 45)		30	43	(28 – 32)	(42 – 45)
	15 days - < 1 yr	27	48	(25 – 28)	(47 – 50)		27	48	(25 – 28)	(47 – 50)
	1 - < 8 yrs	37	47	(35 – 39)	(46 – 49)		37	47	(35 – 39)	(46 – 49)
	8 - < 15 yrs	39	49	(37 – 41)	(48 – 51)		39	49	(37 – 41)	(48 – 51)
	15 - < 19 yrs	37	51	(36 – 39)	(49 – 53)		40	53	(38 – 42)	(51 – 54)
C3 (g/L)	0 - 14 days	0.54	1.26	(0.43 – 0.64)	(1.16 – 1.36)		0.54	1.26	(0.43 – 0.64)	(1.16 – 1.36)
	15 days - < 1 yr	0.55	1.66	(0.44 – 0.65)	(1.56 – 1.76)		0.55	1.66	(0.44 – 0.65)	(1.56 – 1.76)
	1 - < 19 yrs	0.87	1.58	(0.77 – 0.98)	(1.47 – 1.68)		0.87	1.58	(0.77 – 0.98)	(1.47 – 1.68)
C4 (g/L)	0 - < 1 yr	0.09	0.30	(0.06 – 0.11)	(0.27 – .32)		0.09	0.30	(0.06 – 0.11)	(0.27 – 0.32)
	1 - < 19 yrs	0.14	0.36	(0.12 – 0.17)	(0.34 – 0.39)		0.14	0.36	(0.12 – 0.17)	(0.34 – 0.39)
Haptoglobin (g/L)	0 - 14 days		0.18		(0.04 – 0.32)			0.18		(0.04 – 0.32)
	15 days - < 1 yr		2.27		(2.13 – 2.41)			2.27		(2.13 – 2.41)
	1 - < 12 yrs		1.69		(1.55 – 1.84)			1.69		(1.55 – 1.84)
	12 - < 19 yrs		1.85		(1.71 – 1.99)			1.85		(1 .71 – 1.99)
IgA (g/L)	0 - < 1 yrs		0.33		(0.20 – 0.47)			0.33		(0.20 – 0.47)
	1- < 3 yrs		0.98		(0.85 – 1.12)			0.98		(0.85 – 1.12)
	3 - < 6 yrs	0.30	1.59	(0.17 – 0.44)	(1.45 – 1.72)		0.30	1.59	(0.17 – 0.44)	(1.45 – 1.72)
	6 - < 14 yrs	0.53	2.37	(0.39 – 0.66)	(2.24 – 2.51)		0.53	2.37	(0.39 – 0.66)	(2.24 – 2.51)
	14 - < 19 yrs	0.59	3.08	(0.45 – 0.72)	(2.94 – 3.21)		0.59	3.08	(0.46 – 0.72)	(2.94 – 3.21)

(*continued on next page*)

Table 4 (*continued*)

Proteins										
Analyte	Age	Female reference interval					Male reference interval			
		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval		Lower limit	Upper limit	Lower limit confidence interval	Upper limit confidence interval
IgG (g/L)	0–14 days	3.20	13.72	(2.56 – 3.84)	(13.08 – 14.36)		3.20	13.72	(2.56 – 3.84)	(13.08 – 14.36)
	15 days – < 1 yr	1.15	6.90	(0.51 – 1.79)	(6.26 – 7.53)		1.15	6.90	(0.51 – 1.79)	(6.26 – 7.53)
	1 – < 4 yrs	3.16	11.21	(2.52 – 3.80)	(10.58 – 11.85)		3.16	11.21	(2.52 – 3.80)	(10.58 – 11.85)
	4 – < 10 yrs	5.35	13.25	(4.71 – 5.99)	(12.61 – 13.88)		5.35	13.25	(4.71 – 5.99)	(12.61 – 13.88)
	10 – < 19 yrs	6.47	14.95	(5.83 – 7.11)	(14.31 – 15.59)		6.47	14.95	(5.83 – 7.11)	(14.31 – 15.59)
IgM (g/L)	0 – 14 days	0.07	0.35	(0 – 0.18)	(0.25 – 0.45)		0.07	0.35	(0 – 0.18)	(0.25 – 0.45)
	15 days – < 13 wks	0.14	0.68	(0.04 – 0.24)	(0.58 – 0.79)		0.14	0.68	(0.04 – 0.24)	(0.58 – 0.79)
	13 wks – < 1yr	0.18	0.82	(0.07 – 0.28)	(0.72 – 0.92)		0.18	0.82	(0.07 – 0.28)	(0.72 – 0.92)
	1 – < 19 yrs	0.47	1.75	(0.37 – 0.57)	(1.64 – 1.85)		0.39	1.42	(0.29 – 0.49)	(1.32 – 1.52)
Prealbumin (g/L)	0 –14 days		0.10		(0.05 – 0.14)			0.10		(0.05 – 0.14)
	15 days – < 1yr	0.01	0.24	(0 – 0.06)	(0.19 – 0.28)		0.01	0.24	(0 – 0.06)	(0.19 – 0.28)
	1 – < 5 yrs	0.10	0.23	(0.05 – 0.14)	(0.18 – 0.27)		0.10	0.23	(0.05 – 0.14)	(0.18 – 0.27)
	5 – < 13 yrs	0.12	0.26	(0.08 – 0.17)	(0.22 – 0.31)		0.12	0.26	(0.08 – 0.17)	(0.22 – 0.31)
	13 – < 16 yrs	0.17	0.32	(0.12 – 0.21)	(0.28 – 0.37)		0.17	0.32	(0.12 – 0.21)	(0.28 – 0.37)
	16 – < 19 yrs	0.16	0.35	(0.11 – 0.20)	(0.30 – 0.39)		0.19	0.37	(0.15 – 0.24)	(0.32 – 0.41)
Total Protein (g/L)	0 – 14 days	54	86	(51 – 57)	(83 – 89)		54	86	(51 – 57)	(83 – 89)
	15 days – < 1yr	45	73	(41 – 48)	(70 – 77)		45	73	(41 – 48)	(70 – 77)
	1 – < 6 yrs	63	78	(60 – 66)	(75 – 81)		63	78	(60 – 66)	(75 – 81)
	6 – < 9 yrs	66	80	(63 – 69)	(77 – 83)		66	80	(63 – 69)	(77 – 83)
	9 – < 19 yrs	67	84	(64 – 70)	(81 – 87)		67	84	(64 – 70)	(81 – 87)
Transferrin (g/L)	0 – < 9 wks	0.97	2.29	(0.79 – 1.15)	(2.11 – 2.47)		0.97	2.29	(0.79 – 1.15)	(2.11 – 2.47)
	9 wks – < 1 yr	1.01	3.39	(0.83 – 1.18)	(3.21 – 3.57)		1.01	3.39	(0.83 – 1.18)	(3.21 – 3.57)
	1 – < 19 yrs	2.25	3.54	(2.07 – 2.42)	(3.36 – 3.71)		2.25	3.54	(2.07 – 2.42)	(3.36 – 3.71)

[a]Female-specific reference intervals are highlighted in pink, whereas male-specific reference intervals are highlighted in blue.
[b]The lower reference limits established in our previous study for total and direct bilirubin, apoB (0–14 days), haptoglobin, IgA (0–<1 years and 1–<3 years) and prealbumin (0–14 days) correspond to the lower end of the reportable range of the respective Abbott ARCHITECT assay. Therefore, these reference limits were not transferred to the corresponding Siemens Vista assay.
[c]ALT ACT, alanine aminotransferase with pyridoxal phosphate; AST ACT, aspartate aminotransferase with pyridoxal phosphate; LDH, lactate dehydrogenase; apoB, apolipoprotein B; HDL, high-density lipoprotein cholesterol; albumin P, albumin assay with bromcresol purple; C3, complement C3; C4, complement C4.

Table 5
CALIPER sample validation of assay-specific pediatric reference intervals.[a]

		Siemens Vista	Ortho Vitros	Roche Cobas	Beckman Coulter
Chemistry	Bilirubin, direct	96 (99)	ND[b]	ND	99 (100)
	Bilirubin, total	88 (96)	91 (94)	91 (95)	87 (94)
	Calcium	77 (99)	94 (100)	84 (98)	59 (93)
	CO2	NT[d]	NT[d]	NT[d]	NT[d]
	Creatinine (enzymatic)	92 (98)	91 (96)	90 (94)	ND
	Creatinine (Jaffe)	MPC[c]	ND	ND	87 (96)
	Magnesium	NT	NT	NT	NT
	Iron	91 (93)	93 (96)	98 (99)	94 (98)
	Phosphate	86 (98)	89 (96)	88 (96)	NT
	Urea	89 (97)	92 (95)	92 (98)	95 (99)
	Uric acid	95 (97)	94 (97)	90 (96)	94 (98)
Enzymes	Alkaline phosphatase (ALP)	91 (94)	92 (95)	91 (92)	94 (94)
	ALT ACT (with pyridoxal phosphate)	95 (99)	99 (100)	ND	ND
	ALT (without pyridoxal phosphate)	ND	ND	86 (98)	97 (99)
	Amylase	99 (99)	90 (95)	ND	97 (97)
	AST ACT (with pyridoxal phosphate)	97 (100)	92 (96)	ND	ND
	AST (without pyridoxal phosphate)	ND	ND	94 (100)	99 (99)
	Gamma-glutamyltransferase (GGT)	NT	NT	NT	NT
	Lactate dehydrogenase (LDH)	98 (100)	72 (86)	90 (98)	91 (95)
	Lipase	100 (100)	ND	ND	ND
Lipids/lipoproteins	Apolipoprotein B (Apo B)	94 (98)	91 (99)	ND	ND
	Cholesterol	89 (92)	85(89)	91 (93)	ND
	HDL-cholesterol (HDL)	MPC	69 (86)	85 (89)	85 (95)
	Triglycerides	84 (91)	89 (92)	85 (88)	87 (90)
Proteins	Albumin G	ND	98 (100)	ND	ND
	Albumin P	99 (100)	ND	97 (100)	99 (100)
	Complement C3 (C3)	98 (99)	92 (97)	ND	ND
	Complement C4 (C4)	88 (93)	88 (90)	ND	ND
	C-reactive protein (CRP)	85(89)	84(91)	NT	ND
	Haptoglobin	97 (97)	ND	98 (98)	ND
	Immunoglobulin A (IgA)	92 (95)	90 (91)	92 (93)	ND
	Immunoglobulin G (IgG)	95 (98)	85 (91)	93 (98)	ND
	Immunoglobulin M (IgM)	87 (92)	83 (83)	88 (91)	ND
	Prealbumin	85 (98)	90 (94)	93 (95)	ND
	Total protein	97 (99)	99 (100)	98 (100)	94 (100)
	Transferrin	97 (99)	MPC	ND	ND

[a] The percentage of samples that fell within the appropriate partitioned upper and lower reference limits is shown for each analyte measured with the four assays. The number in parentheses represents the percentage of samples that fell within the appropriate partitioned upper and lower reference limits inclusive of the 95% confidence intervals.
[b] ND, not determined.
[c] MPC, method performance change.
[d] NT, not transferable based on statistical criteria.

different than that of older children. The most extreme example is that of total bilirubin in children 0–14 days of age, in whom the upper reference limit is 284 μmol/L [3]. Since the method comparison curves for total bilirubin only encompassed concentrations up to 71 μmol/L, significant extrapolation of the regression equations was needed in order to transfer this reference limit to the other total bilirubin assays (Supplemental data File 1). This extrapolation issue occurred less frequently for partitions greater than one year of age, and the degree of extrapolation was typically lower compared to partitions less than one year of age. In addition, validation samples were assessed for all partitions over one year of age (Supplemental data Tables 6–9), allowing assessment of the validity of the transferred reference intervals.

The major advantage of using transference to determine reference intervals is that it obviates the need to collect and test samples from reference individuals in each partition [1]. The data presented in this study suggest that the transference approach can be used effectively to generate reference intervals for many assays. However, transference cannot be recommended in all cases. First, method performance must be stable over time in order to generate meaningful reference intervals. The EQA evaluation performed in this study provided an effective means of identifying large changes in assay performance. Second, analytes with poor stability may not be appropriate candidates for transference, especially when performing studies between distant laboratories. For example, CO_2 may be lost from a sample during storage, transport, and anaerobic handling [25]. The poor stability of CO_2 likely accounts for the lack of correlation between the Abbott ARCHITECT CO_2 results and those obtained with assays from the other manufacturers (Supplemental data File 1). Third, transference may not be ideal when analyte concentration/activity correlates only modestly between assays. For example, the Abbott ARCHITECT magnesium results did not correlate well with those obtained with any of the other assays ($r^2 = 0.65$) as a result of a large amount of scatter (Supplemental data File 1). A similar issue was encountered with the Beckman Coulter phosphate assay. Given the modest correlation, it is likely that the corresponding regression equations would not reliably transfer the Abbott ARCHITECT reference intervals to the other assays. We selected an r^2 cut-off of 0.7 ($r = 0.84$) to consider an analyte for transference, as we felt that data were insufficiently correlated below this threshold. Finally, even if the concentration/activity of a given analyte correlates well between assays, distinct populations may be present within the method comparison data set, making transference potentially inappropriate. Inspection of the Bland–Altman, standardized residual, and Q–Q plots revealed that this was the case for GGT, despite an $r^2 > 0.98$ (Fig. 1F–H). For example, when comparing the Abbott ARCHITECT GGT results to those obtained with the Ortho Vitros assay, two subsets of data separated by roughly 5–7 U/L were clearly evident, especially at low GGT activities (Fig. 1E). Interestingly, a similar phenomenon was observed when the Abbott ARCHITECT assay was compared to each of the other four assays, but was not evident when these four assays were compared to each other. Since analysis of the method comparison specimens was split between two separate Abbott ARCHITECT analyzers, it is possible that the two subsets of GGT data reflect an instrument-specific bias between these instruments.

Conclusions

The current CALIPER study establishes the relationship between Abbott ARCHITECT assays and four other commonly used assays for a wide spectrum of biochemical markers. This information was used to transfer the age- and sex-stratified pediatric reference intervals established in our previous study [3] to Beckman Coulter, Ortho Vitros, Roche Cobas, and Siemens Vista assays. The transferred reference intervals were subjected to a thorough validation analysis as recommended by CLSI C28-A3 [1], and all participating laboratories took part in a comprehensive EQA evaluation. The assay-specific, age- and sex-stratified pediatric reference intervals presented in this study expand the utility of the CALIPER reference interval database and should facilitate the broad application of CALIPER reference intervals at pediatric centers worldwide. It is important to note that the current study provides transference data to specific assays, and does not validate reference intervals for individual analyzers, specific populations, or geographic locations. Individual analyzers may have instrument-specific biases compared to those used in our study. Local populations may have different ethnic composition, environmental conditions, and lifestyles compared to the multiethnic CALIPER population [3]. As a result, assay-specific CALIPER reference intervals reported in the present study should also be validated locally, using reference specimens from healthy children in the local population as recommended by CLSI [1].

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