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Analytical evaluation of the VITROS® 5600 Integrated System in a pediatric setting and determination of pediatric reference intervals

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ABSTRACT

Objectives: To evaluate the VITROS® 5600 Integrated System in a pediatric setting and to determine age- and gender-specific pediatric reference intervals for several common analytes.

Design and methods: The instrument was evaluated using QC material and patient samples. Reference intervals were determined using samples obtained from children attending select outpatient clinics.

Results: Imprecision analysis for 25 analytes and serum indices, the turnaround time for a simulated workload, and MicroSensor performance were assessed in our pediatric laboratory. Pediatric reference intervals for 25 analytes were also determined according to the CLSI/IFCC C28-A3 guidelines using 770 samples and over 15,000 analyses.

Conclusion: The VITROS 5600 Integrated System is suitable for use in a pediatric setting. Age- and gender-partitioned pediatric reference intervals for 25 common analytes were also determined as a pilot to the ongoing CALIPER project. These reference intervals are valuable for all VITROS® users as well as any laboratory assessing these analytes once they demonstrate the acceptability of transference to their laboratory.

Introduction

Clinical laboratories servicing pediatric centers must overcome several unique challenges in order to provide adequate services. Obvious challenges include the ability to safely and unobtrusively collect adequate specimens from infants and children as well as to perform multiple valid analyses on these limited sample volumes. The newly released VITROS® 5600 Integrated System (Ortho Clinical Diagnostics, Johnson and Johnson) incorporates both wet and dry chemistry assays with enhanced chemiluminescence immunoassays on the same platform, reducing the sample volumes required to perform multiple tests. Given this feature, this new analyzer is well suited to the pediatric setting.

Another major challenge facing pediatric laboratory medicine is the difficulty in determining appropriate reference intervals. It must be stressed that children should not be regarded as small adults, and reference intervals obtained from adults may not be suitable for children. Furthermore, as children are constantly changing and developing, a single reference interval may not be suitable for all children and laboratory results should be interpreted with reference to an age- and gender-matched healthy population. However, many common analytes that are tested today do not have known age- and gender-appropriate reference intervals [1]. Furthermore, a significant proportion of the known pediatric reference intervals were obtained using methods that are now outdated and may no longer be appropriate for laboratory results obtained using current methodologies [1]. Recently, there have been several efforts to overcome these shortcomings. In the Unites States of America, the Mayo Medical Laboratories have created a large bank of residual samples used to determine and validate pediatric reference ranges for an extensive number of analytes. In Canada a group of investigators have undertaken an ambitious project, the Canadian Laboratory Initiative on Pediatric Reference Interval Database (CALIPER; www.caliperproject.ca) to create a national database of pediatric reference intervals. In 2008, the project began collecting samples from healthy individuals ranging in age from birth to 18 years and is over half-way to achieving its target of 3600 samples. Importantly, this project will allow for the determination of reference intervals on both current and future biomarkers of pediatric disease in a method- and platform-specific manner using healthy individuals representative of the multicultural population found in Canada.

The study outlined here is a collaboration between CALIPER, The Hospital for Sick Children, and the *in vitro* diagnostics (IVD) company

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Ortho Clinical Diagnostics in which we assessed the performance of their newly released VITROS® 5600 Integrated System in a pediatric setting and used this instrument to determine age- and gender-specific pediatric reference intervals for 25 analytes using samples obtained from our select outpatient population.

Methods

Analytical system

Ortho Clinical Diagnostics provided a preproduction model of the VITROS® 5600 Integrated System. The system integrates dry chemistry (MicroSlide), wet chemistry and immunoturbidimetry with photometric detection (MicroTip), immunoassays with enhanced chemiluminescence (MicroWell), and photometric measurement of sample quality indices (MicroSensor) into a single analyzer, with a test menu of over 120 available assays.

Instrument assessment

Twenty-five selected assays (albumin [ALB], alkaline phosphatase [ALKP], alanine aminotransferase [ALT], amylase [AMYL], aspartate aminotransferase [AST], conjugated bilirubin [Bc], unconjugated bilirubin [Bu], blood urea nitrogen [BUN/UREA], calcium [Ca], chloride [Cl^-], carbamazepine [CRBM], creatinine [Crea], direct total iron-binding capacity [dTIBC], iron [Fe], free thyroxine [FT4], gentamicin [GENT], glucose [GLU], immunoglobulin M [IgM], potassium [K^+], sodium [Na^+], amino-terminal pro brain natriuretic peptide [NTBNP], total protein [TP], troponin I [TropI], thyroid stimulating hormone [TSH], and total thyroxine [TT4]) in addition to the serum indices were assessed for imprecision by analyzing QC material (Ortho Clinical Diagnostics) once a day for up to 35 days.

The MicroSensor technology was evaluated for concordance of hemolysis readings to a previous VITROS® System (5,1 FS) and to visual grading using a sample integrity chart on 250 surplus patient samples. Hemolysis readings between the VITROS® 5600 Integrated System and the VITROS® 5,1 FS Chemistry System were deemed concordant if the hemolysate readings were within 50 U of each other. The VITROS® 5600 Integrated System and visual grading results were deemed concordant if the VITROS® 5600 Integrated System reading (0–25, 25–50, 50–100, 100–200, 200–400, and 400–800 U) was within the range bracketed by the visually assigned hemolysate value (0–0.25, 0.25–0.5, 0.5–1.0, 1.0–2.0, 2.0–4.0, and 4.0–8.0 g/L).

The time required to measure indices by the VITROS® 5600 Integrated System and the VITROS® 5,1 FS Chemistry System was compared by analyzing 20 samples for hemolysis, icterus, and turbidity and by comparing the average time elapsed between the 'metering' time and the 'print' times recorded by each instrument.

The turnaround time required to complete a simulated workload (specified by Ortho Clinical Diagnostics) was determined by first ordering the tests on the instruments and then determining the time elapsed from the initialization of metering to the completion of all results. The time elapsed to the completion of first test, the printing of the first sample results, and the completion of sampling were recorded. The turnaround time for the VITROS® 5600 Integrated System was compared to that of the current instrumentation setup at The Hospital for Sick Children which consists of a VITROS® 5,1 FS Chemistry System (Orthos Clinical Diagnostics), COBAS INTEGRA® 400 Plus (Roche Diagnostics), and IMMULITE® 2500 (Siemens Healthcare Diagnostics).

Reference population specimen analysis

Surplus specimens collected from the ethnically diverse population of Toronto were used in this analysis. Samples were obtained from children deemed to lack metabolic diseases attending various outpatient clinics such as dentistry, orthopedic, or plastic surgery. A waiver was obtained from the institutional review board (IRB) of The Hospital for Sick Children prior to initiation of the study on the condition that identifying information is stripped from the specimens rendering them no longer traceable to patient medical records.

Serum and plasma (lithium heparin) specimens were collected in plastic vacutainer tubes with or without a gel separator or in heparinized syringes. Serum specimens were allowed to clot for 20 minutes prior to centrifugation. All samples were stored frozen at −80 °C and subjected to a single freeze–thaw cycle at the time of analysis.

All of the technological capabilities of the instrument were utilized to analyze 25 common analytes (α1-antitrypsin [AAT], conjugated bilirubin [Bc], unconjugated bilirubin [Bu], blood urea nitrogen [BUN/UREA], calcium [Ca], creatine kinase MB form [CK-MB], creatinine [Crea], ferritin [FER], follicle-stimulating hormone [FSH], free triiodothyronine [FT3], free thyroxine [FT4], high sensitivity C-reactive protein [hsCRP], luteinizing hormone [LH], magnesium [Mg], amino-terminal pro brain natriuretic peptide [NTBNP], prealbumin/transthyretin [PALB], phosphate [Phos], total bilirubin [TBIL], testosterone [Testo], Transferrin [TRFRN], troponin I [TropI], thyroid stimulating hormone [TSH], total triiodothyronine [TT3], total thyroxine [TT4], and uric acid [URIC]).

Prior to reference interval testing, a familiarization phase was completed that consisted of calibrating and establishing the quality control limits for each assay. Specimen analyses were performed over a 6-month period with daily, weekly, and monthly preventative maintenance being performed as specified by the manufacturer. Specimen analyses were performed only after quality control analysis found the instrument to be functioning within acceptable limits. All specimens were subject to MicroSensor analysis to evaluate for hemolysis, icterus, and turbidity. Specimens exceeding the interferent values stated by the manufacturer were excluded from the study. Calibrators were obtained from the instrument manufacturer and thus the traceability information can be obtained from the manufacturer's package insert.

Statistical analysis

Data were analyzed in accordance with CLSI/IFCC C28-A3 guidelines on defining, establishing, and verifying reference intervals in the clinical laboratory [2]. Subjects were partitioned based on sex and age. Five age groups were chosen arbitrarily: <1 year; ≥ 1 year and ≤ 5 years; >5 years and ≤ 10 years; >10 years and ≤ 15 years; and >15 years and $\leq$ 20 years. Outliers within each partition were identified using the Dixon method. Briefly, a value was considered to be an outlier if the absolute difference between it (the extreme value) and the adjacent value was greater than or equal to one-third of the range of all values [2]. The appropriateness of merging the groups was assessed by using the Harris and Boyd method in a systematic fashion [2]. Priority was given to gender merging and then the acceptability of merging adjacent age groups was assessed only if the adjacent groups were of the same gender(s) (male with male, females with females, or gender combined with gender combined). Due to the inability to obtain sample sizes of 120 or greater for every partition, the Robust method was utilized to calculate each reference interval as per CLSI guidelines [2]. The ranges represent the central 95% of the population tested, thus the lower and upper reference intervals represent the 2.5th and 97.5th percentile, respectively.

Results

Instrument evaluation

Between-day imprecision for 25 selected assays as well as the serum indices was tested on the VITROS® 5600 Integrated System. The