Clinical Chemistry 65:4
589–592 (2019)

High-Sensitivity Generation 5 Cardiac Troponin T Sex- and Age-Specific 99th Percentiles in the CALIPER Cohort of Healthy Children and Adolescents

To the Editor:

International societies and expert groups recommend using sex-specific upper reference limits (URLs)[1] for interpretation of high-sensitivity cardiac troponin (hs-cTnI/T) assays *(1, 2)*. Despite advances in our understanding of cTn in adults, the effect of age and sex on cTn in pediatric populations is less understood. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) previously determined 97.5th and 99th percentiles for the Abbott hs-cTnI assay in a healthy pediatric cohort *(3)*. These data represented the first attempt to characterize hs-cTnI in healthy children but did not include neonates nor were sex-specific differences evident. It is unclear, however, what cTnT URLs would be in this population because cTnT is a different protein and there are differences in analytical sensitivity and specificity between hs-cTnT and hs-cTnI assays *(1–4)*. Establishing pediatric reference values for cTn is important because its measurement has several clinical indications in pediatrics, including monitoring children with postoperative myocarditis and congenital pediatric heart disease.

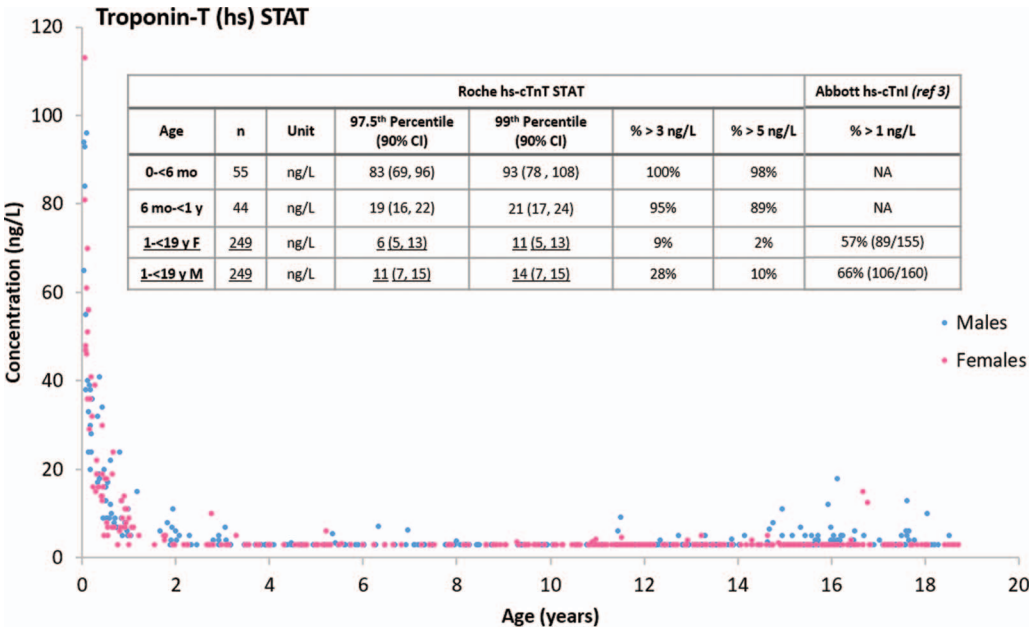
Serum samples from 598 children and adolescents were selected from the CALIPER biobank. Samples from participants <1 year were obtained from apparently healthy infants at local maternity wards or outpatient clinics. Samples were measured with the Roche hs-cTnT (Gen 5 assay) STAT assay on the cobas 8000 e602 analyzer and reported in whole numbers per laboratory practice recommendations *(2)*, with 3 ng/L used to designate undetectable concentrations *(4)*. Any result <3 ng/L was reported as 3 ng/L, consistent with prior work *(4)*. Data were visually inspected, and partitions were confirmed by the Harris and Boyd method, as previously described *(3)*. Although hs-cTnT concentrations from 0 to <1 year were normally distributed, hs-cTnT concentrations for both sexes of age 1 to <19 years were highly skewed and could not be normalized via transformation *(3)*. No outliers were identified by the Reed method *(3)*. The URLs were calculated with the nonparametric method (>120 participants) or robust method (>40 and <120 participants) *(3)*, with 90% CIs determined according to Clinical and Laboratory Standards Institute guidelines (Fig. 1).

hs-cTnT concentrations were markedly increased from 0 to <6 months and subsequently decreased and narrowed at 1 year. The sex-specific 99th percentiles from 1 to <19 years were 14 ng/L for boys and 11 ng/L for girls. Fig. 1 reports the percentage of hs-cTnT results detectable (>3 ng/L and >5 ng/L). Although the sensitivity of the Roche hs-cTnT and Abbott hs-cTnI assays in CALIPER could not be directly compared because different samples were measured, the percent detectable (hs-cTnI >1 ng/L) was markedly higher in the hs-cTnI study *(3)* as illustrated in Fig. 1.

The clinical value of using sex-specific 99th percentiles for hs-cTn has been recently debated *(1, 2)*. Several groups suggest that owing to intrinsic sex-specific pathophysiological differences in adults, sex-specific URLs would improve the accuracy of hs-cTn result interpretation, especially in the acute coronary syndrome setting *(1, 2, 4)*. However, the pathophysiological reasons for increased cTn in children are not the same as those in adults *(3)*. Importantly, the data illustrate a time-dependent decrease in hs-cTnT over the first year of life, with age-specific URLs being important in this population to identify myocardial injury. The higher concentrations in this age group could be a partial result of transient hypoxia at parturition and/or fetal cTn expression in skeletal muscle; however, more studies are needed to further investigate this finding. After 1 year, hs-cTnT 99th percentiles (girls = 11 ng/L; boys = 14 ng/L) are similar to what has been reported for the AACC Universal Sample Bank (USB) 99th percentiles (females = 10 ng/L; males = 16 ng/L) but lower than the US 5th-generation cTnT package insert cutoffs (females = 14 ng/L; males = 22 ng/L) for which the population selection criteria were not stated *(4)*. In this regard, the lower CALIPER 99th percentiles for hs-cTnT, especially for boys, may be preferred in the pediatric population to prevent misdiagnoses.

An important study limitation to consider is that there were fewer than 300 individuals in each subgroup after partitioning *(2)*. It has been reported that 300 samples are required to attain a 95% probability that 99% of results will not exceed the estimated URL (i.e., tolerance level) *(5)*. Thus, owing to smaller sample sizes in this study, reported 99th percentiles should be interpreted cautiously. However, at study onset, we had nearly 600 individuals, which would have been sufficiently powered to derive the 99th percentile, if there was no further partitioning required, similar to hs-cTnI *(3)*. Another limitation was the lack of imaging or other cardiac biomarkers to confirm participant cardiovascular health. However, the similarity of CALIPER and AACC USB 99th percentiles suggests

[1] Nonstandard abbreviations: URL, upper reference limit; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; cTn, cardiac troponin; CALIPER, Canadian Laboratory Initiative on Pediatric Reference Intervals.



	Roche hs-cTnT STAT						Abbott hs-cTnI *(ref 3)*
Age	n	Unit	97.5th Percentile (90% CI)	99th Percentile (90% CI)	% > 3 ng/L	% > 5 ng/L	% > 1 ng/L
0-<6 mo	55	ng/L	83 (69, 96)	93 (78 , 108)	100%	98%	NA
6 mo-<1 y	44	ng/L	19 (16, 22)	21 (17, 24)	95%	89%	NA
1-<19 y F	249	ng/L	6 (5, 13)	11 (5, 13)	9%	2%	57% (89/155)
1-<19 y M	249	ng/L	11 (7, 15)	14 (7, 15)	28%	10%	66% (106/160)

Fig. 1. Scatter plots of hs-cTnT results reported in whole numbers (ng/L) for boys (blue) and girls (pink); 97.5th and 99th percentiles and corresponding CIs for hs-cTnT in ng/L.

Percentage of detectable hs-cTnT results based on Roche package insert limit of blank (3 ng/L) and limit of detection (5 ng/L) and Abbott hs-cTnI limit of detection (1 ng/L) from the International Federation of Clinical Chemistry Committee on Clinical Applications of Cardiac Bio-Markers (http://www.ifcc.org/media/477441/high-sensitivity-cardiac-troponin-i-and-t-assay-analytical-characteristics-designated-by-manufacturer-v08232018.pdf).

that this population was, in fact, a healthy population in a cardiovascular sense. In summary, the current study provides novel and useful information regarding the effect of age and sex on hs-cTnT concentrations in healthy children and fills an important gap for laboratories offering hs-cTnT testing by providing age- and sex-specific URLs.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

M.K. Bohn, statistical analysis; V. Higgins, statistical analysis.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: P.A. Kavsak, Abbott, Beckman, Roche, Siemens.
Stock Ownership: None declared.
Honoraria: P.A. Kavsak, Beckman, Roche, Siemens.
Research Funding: Foundation Grant from the Canadian Institutes of Health Research (CIHR), Roche Diagnostics Canada. hsTroponin T STAT kits provided at no charge by Roche.
Expert Testimony: None declared.
Patents: McMaster has filed patents with P.A. Kavsak listed as an inventor in the acute cardiac biomarker field.

Acknowledgments: The authors thank all participants, their families and CALIPER volunteers who made this study possible. The authors also thank the technical and managerial staff at Mount Sinai Hospital (Toronto, ON) for sample analysis.

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Previously published online at
DOI: 10.1373/clinchem.2018.299156

Room Temperature Detection of Plasma Epstein–Barr Virus DNA with CRISPR–Cas13

To the Editor:

Detection of plasma Epstein–Barr virus (EBV)[1] DNA is useful for screening and monitoring nasopharyngeal carcinoma (NPC) and other EBV-associated diseases *(1–3)*. However, quantitative PCR requires complex and expensive instrumentation, restricting its use in point-of-care testing and remote regions *(4)*. Thus, a simple, portable, and inexpensive EBV DNA detection assay is needed.

Prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated adaptive immune systems contain programmable endonucleases that can be used for CRISPR-based diagnostics *(4, 5)*. Recently, the CRISPR effector Cas13a was combined with recombinase polymerase amplification (RPA) to establish a molecular detection platform, termed specific high-sensitivity enzymatic reporter unlocking (SHERLOCK), providing rapid and analytically sensitive nucleic acid detection *(4, 5)*.

To establish a SHERLOCK platform for the detection of EBV DNA, we first purified *Leptotrichia wadei* Cas13a (LwCas13a) protein. According to a previous study *(5)*, the SHERLOCK platform works at 37 °C. We optimized the RPA primers and occasionally observed that the SHERLOCK platform could work at 25 °C.

The SHERLOCK detection assay was carried out according to a previous study with modifications *(5)*. A single 100-μL combined reaction assay consisted of 0.48 μmol/L forward primer, 0.48 μmol/L reverse primer, 1× RPA rehydration buffer (TwistDx), varying amounts of input DNA, 22.5 nmol/L CRISPR RNA (crRNA), 45 nmol/L LwCas13a recombinant protein, 2.5 μL of murine RNase inhibitor (New England Biolabs), 125 nmol/L substrate reporter (RNase alert v2), 2 mmol/L ATP, 2 mmol/L GTP, 2 mmol/L UTP, 2 mmol/L CTP (Yeasen), 1 μL of T7 polymerase (New England Biolabs), 14 mmol/L MgAc (TwistDx), and 5 mmol/L $MgCl_2$ (Invitrogen). EBV DNA standards were purchased from BDS Biological Technology. The RPA primers for the room temperature SHERLOCK assay were designed to target the *Bam*HI-W region. RPA primer *Bam*HI-W-F: AATTCTAATACGACTCACTATAGGCCTAAGAAGGCACCGGTCGCCCAGTCCTACC; RPA primer *Bam*HI-W-R: TGAACCGCTTACCACCTCCTCTTCTTGCTGGA; crRNA: GGGGAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUCUACGGACUCGUCUGGGUUCUUGGCCC. Reactions were allowed to proceed for 2 h at 25 °C.

Because this was the first time that the SHERLOCK platform was reported to have been carried out at room temperature, which could make the SHERLOCK platform simpler, less expensive, and more convenient, we aimed to detect plasma EBV DNA with the room temperature SHERLOCK platform. The clinical serum samples used for this study were from Sun Yat-Sen University Cancer Center, and this study was approved by the Ethics Committee of Sun Yat-Sen University Cancer Center. Plasma cell-free DNA was isolated with the QIAamp Blood Mini Kit (Qiagen). Forty-eight EBV-positive plasma DNA samples from patients with NPC and 50 EBV-negative control plasma DNA samples were analyzed with the room temperature SHERLOCK platform. Cell-free EBV DNA was detectable in 96% (46 of 48) of the EBV-positive NPC samples and 0% (0 of 50) of the controls. The results showed a Pearson correlation coefficient of $R^2 = 0.9314$, $P < 0.0001$ (Fig. 1) with the quantitative PCR data, supporting that the performance of the room temperature SHERLOCK platform is comparable with that of quantitative PCR.

Because the SHERLOCK platform could be carried out at room temperature, the instruments used

[1] Nonstandard abbreviations: EBV, Epstein–Barr virus; NPC, nasopharyngeal carcinoma; CRISPR, clustered regularly interspaced short palindromic repeats; RPA, recombinase polymerase amplification; SHERLOCK, specific high-sensitivity enzymatic reporter unlocking; LwCas13a, *Leptotrichia wadei* Cas13a; crRNA, CRISPR RNA.