Supplemental Digital Content, containing data, 5 tables, and 1 figure, for Pediatric Thromboelastograph 6s and Laboratory Coagulation Reference Values.

The supplemental Digital Content was not copyedited by *Archives of Pathology & Laboratory Medicine*.

Expanded methods:

Sampling process, specimens and coagulation tests (laboratory assays and Thromboelastograph 6s)

Samples were transported by the pneumatic tube system to the on-site National Association of Testing Authority (NATA) Australia accredited laboratory. The pneumatic tube system was internally validated for use of routine sample transport including coagulation samples for all pneumatic tube transportation zones. The 1mL, 3.2% sodium citrate tube was used for Thromboelastograph 6s (TEG 6s) measurement with 0.3ml of this sample added to the TEG 6s Hemostasis Analyzer (Haemonetics Corporation, Chicago IL USA). Viscoelastic hemostatic assays (VHA) point of care (POC) testing occurred in a temperature-controlled environment within two hours of blood collection according to manufacturer's instructions.

Statistical analysis

Missing values were excluded from analysis of that parameter. Values of the parameter within each age group that were more than three times the interquartile range from the median were excluded. Parameters between male and female participants was first checked using Mann-Whitney U test; no differences were present and as such gender was not further explored for discrete Reference Intervals (RIs). The Kruskal-Wallis test was used to compare the parameter between age-groups; post-hoc analysis using Dunn's test was undertaken (allowing for multiple comparisons using the Bonferroni adjustment) if the initial Kruskal-Wallis test was significant. Model-based method: Equations for the 2.5^{th} and 97.5^{th} RIs were estimated using quantile regression [1, 2]. Fractional polynomial regression was first used to model the mean; using this model, residuals were calculated and if residuals were three times the interquartile range from the median, the corresponding participant record was excluded for that parameter. Predicted values of the mean were then compared with observed values for neonates and infants; if there was a significant difference, neonates and/or infants were not included in the generation of the RIs. Interactions between gender and polynomial terms were explored, and the resulting quantile regression included the terms significant in fractional polynomial regression as well as relevant interaction terms. Generated RIs, along with 95% confidence intervals (CIs), were plotted against age, separated by gender if required.

Table 1 Expansion: Methodology for Laboratory Coagulation Parameters with different reagents

Laboratory Coagulation Parameters		
Test	**Reagent**	**Summary[a]**
Activated Partial Thromboplastin Time (aPTT-SS)	HemosIL[b] SynthASil	Incubation of plasma sample with an optimal quantity of phospholipid, a negatively charged silica contact activator and buffer initiates activation of the intrinsic coagulation pathway. After incubation at 37°C for a specific period of time calcium is added to trigger the coagulation process and time required for clot formation is measured.
Activated Partial Thromboplastin Time (aPTT-SP)	HemosIL [b] aPTT-SP	Silica contact activator is used to stimulate production of Factor XIIa by providing a surface for the function of high molecular weight kinigen, kallikrein and Factor XIIa. This contact activation is allowed to proceed at 37°C for a specific period of time. Calcium is added to trigger further reactions and time required for clot formation is measured. Phospholipids are required to form complexes which activate Factor X and Prothrombin.
Activated Partial Thromboplastin Time (aPTT)	Stago TriniCLOT HS	Citrated plasma may be partially "activated" by contact with micronized silica in the presence of a phospholipid reagent. The addition of calcium ions to this activated plasma results in generation of a fibrin clot. As fibrin is generated, optical density of the reaction mixture increases thereby decreasing light transmission. Optical data is analyzed by ACL TOP software to generate clotting data plots and times.
Activated Partial Thromboplastin Time (aPTT-P)	Stago TriniCLOT HS with Heparin resistant CaCl2 [c]	Phospholipid and micronized silica activator are added to citrated plasma and incubated for five minutes. Calcium with polybrene is then added as the start reagent initiating the generation of a fibrin clot. Polybrene neutralizes heparin in the plasma so aPTT-P can assist with detection of heparin contamination when compared to aPTT.
Prothrombin time (PT-RP8)	HaemosIL [b] RecombiPlasTin 2G	This test adds tissue thromboplastin to the patient plasma in the presence of calcium ions which initiates the activation of the extrinsic pathway. This results in the

		conversion of fibrinogen to fibrin, with formation of a solid gel and records the time taken to clot optically.
Prothrombin time (PT-READ)	HaemosIL [b] ReadiPlasTin	Thromboplastin is added to patient plasma in the presence of calcium which initiates the extrinsic pathway. Time to clot is measured optically.
Prothrombin time (PT)	Siemens Thromborel S [d]	The coagulation process is triggered by incubation of plasma with the optimal amount of thromboplastin and calcium. The time to formation of a fibrin clot is then measured optically.
Derived Fibrinogen concentration	Siemens Thromborel S [d]	Derived Fibrinogen is calculated from the delta value (change in optical transmission as PT clot forms) as referenced to a calibration curve. Plasma absorbance increases when soluble fibrinogen is rapidly converted to insoluble fibrin. The change in absorbance measured during the PT test is proportional to the concentration of fibrinogen.
Clauss Fibrinogen, Quantitative Fibrinogen Assay (QFA)	HaemosIL [b] Q.F.A Thrombin	This method is based on rate of clot formation in dilute plasma following thrombin addition. Clotting time is inversely proportional to fibrinogen level when high concentrations of thrombin are used. As fibrin is generated, optical density of the reaction mixture increases thereby decreasing light transmission. Optical data is analyzed by ACL TOP software to generate clotting data plots and times. Use of concentrated thrombin minimizes interference by therapeutic doses of heparin and raised fibrinogen degradation products. A calibration plasma traceable to the current International Fibrinogen Standard calibrates the assay on the ACL TOP.

[a] Machine**;** assays performed on ACLTOP300/500/700 analyzers (Instrumentation Laboratory, Bedford MA, USA) are based upon optical detection of clot formation in the setting of contact factors, expressed in seconds.

[b] HemosIL (Instrumentation Laboratory, Bedford MA, USA)

[c] Stago TriniCLOT HS (Diagnostica Stago, Asnières-sur-Seine, France)

[d] Siemens Thromborel S (Siemens Healthineers, Erlangen, Germany)

Expanded Results

Patient population: Surgical procedures (n=254) included ear/nose/throat/dental (n=63, 25%), general surgery (n=62, 24%), orthopedics (n=47, 19%), urology/gastroenterology (n=27, 10%), plastics (n=21, 8%), ophthalmological (n=13, 5%) and other including neurosurgical, radiological procedures (n=21, 8%) and 154 (61%) were male. We included 15 neonates, median age 24days (interquartile range [IQR] 15, 27), 56 infants (median age 4months, IQR 2, 8), 61 young children 1-5yrs of age (median 4yrs, IQR 2, 5), 62 older children (median 8, IQR 7, 9) and 60 adolescents (median 13yrs, IQR 12, 15).

Table 2: Comparison of variation of age-specific mean reference values between assay methods and allowable total error according to quality requirements [1, 3]. Bolded numbers represent Coefficients of Variation above the allowable total error.

Parameter	**Age Integer (years)**	**Coefficient of Variation between analyzer type, (%)**	**Allowable total error (%)**
Activated Partial Thromboplastin Time (aPTT)	0	1.1	4.5
	1	1.0	
	2	1.1	
	3	1.2	
	4	1.4	
	5	1.6	
	6	1.8	
	7	2.0	
	8	2.3	
	9	2.6	
	10	2.9	
	11	3.1	
	12	3.4	
	13	3.8	
	14	4.1	
	15	4.4	
	16	**4.7**	
	17	**5.1**	
Prothrombin time (PT)	0	**17.3**	5.3
	1	**17.5**	
	2	**17.8**	
	3	**18.0**	
	4	**18.3**	

	5	**18.5**	
	6	**18.8**	
	7	**19.0**	
	8	**19.3**	
	9	**19.5**	
	10	**19.7**	
	11	**20.0**	
	12	**20.2**	
	13	**20.4**	
	14	**20.7**	
	15	**20.9**	
	16	**21.1**	
	17	**21.3**	

Table 3 Expansion. Discrete Reference Intervals for Thromboelastograph 6s (TEG 6s) separating neonates and infants

Median values and reference intervals defined as the 2.5th to 97.5th percentiles in neonatal and infant age-groups for TEG 6s (Haemonetics Corporation, Chicago IL USA) variables[a]; in 4 assays - Kaolin Activated (CK), Rapid TEG (CRT), Kaolin Heparinase (CKH), Functional Fibrinogen (FF)

Parameter	< 30 days (n=13)	1-12 months (n=55[b])	*P*[a]
Kaolin Activated (CK)			
Reaction (R) Time (min)	6.3 (4.9, 8.2)	6.4 (4.3, 9.3)	>.99
Kinetic (K) Time (min)	1.3 (0.8, 2.2)	1.3 (0.7, 2.5)	>.99
Alpha angle (deg)	73.2 (63.1, 78.3)	72.8 (62.6, 80.7)	>.99
Maximum Amplitude (mm)	62.9 (48.7, 69.5)	64.6 (55.2, 71.7)	>.99
Lysis 30 (%)	0.6 (0.0, 3.8)	0.7 (0.0, 4.7)	>.99
Rapid TEG (CRT)			
Activated Clotting Time (TEG-ACT) (sec)	97.3 (78.5, 125.3)	97.3 (69.2, 127.7)	>.99
K Time (min)	1.0 (0.8, 1.8)	1.0 (0.7, 1.8)	>.99
Alpha angle (deg)	76.1 (67.7, 79.5)	76.4 (68.1, 81.2)	>.99
Maximum Amplitude (mm)	63.8 (50.3, 69.7)	66.0 (57.5, 72.4)	.86
Lysis 30 (%)	1.3 (0.0, 3.1)	0.5 (0.0, 3.7)	.55
Kaolin Heparinase (CKH)			
R Time (min)	6.2 (4.7, 8.4)	6.5 (4.3, 9.3)	>.99
K Time (min)	1.2 (0.8, 2.1)	1.2 (0.8, 2.0)	>.99
Alpha angle (deg)	73.6 (65.8, 79.0)	73.0 (60.7, 79.4)	>.99
Maximum Amplitude (mm)	63.6 (48.7, 66.6)	64.6 (56.3, 70.2)	>.99
Functional Fibrinogen (FF)			
Maximum Amplitude (mm)	21.1 (16.6, 30.7)	22.1 (15.8, 32.4)	>.99
Functional Fibrinogen Level (FLEV)	385.1 (302.9, 560.2)	403.3 (288.2, 591.6)	>.99

[a] *P* value is adjusted using Bonferroni's correction for the comparisons between all age groups

[b] 54 infants for CK Assay, and 55 infants for CRT, CKH and FF Assays

Table 4

Reference Intervals generated using the model-based method. Equations for lower and upper limits of Reference Intervals (RIs) using quantile regression[a].

Parameter	**Lower Limit (2.5th Centile)**	**Upper Limit (97.5th Centile)**
Echis time (sec)	11.53-0.60×ln(age) +0.38×ln(age)×female + 0.11×[ln(age)]^2- 0.055×[ln(age)]^2×female	15.27-0.30×ln(age) +0.013×ln(age)×female + 0.42×[ln(age)]^2- 0.036×[ln(age)]^2×female
Rapid TEG assay: Activated Clotting Time (TEG-ACT) (sec)	69.2[b]	125.3 [b]
CKH Assay: Reaction time (min)	4.21-0.011×age-0.059×age×female	8.86-0.088×age+ 0.026×age×female
CKH Assay: Kinetics Time (min)	0.84+0.11×ln(age)- 0.090×age×female	2.09+0.051×ln(age)- 0.0012×age×female
CKH Assay: alpha angle (deg)	60.00+3.42×ln(age) - 0.15×ln(age)×female+ 0.92×[ln(age)]^2 + 0.13×[ln(age)]^2×female	78.79-0.38×ln(age) + 0.28×ln(age)×female+ 0.021×[ln(age)]^2 - 0.026×[ln(age)]^2×female
CKH Assay: Maximum Amplitude (mm)	53.55-0.94×ln(age) + 0.63×ln(age)×female	69.53-0.29×ln(age) + 0.040×ln(age)×female
Functional Fibrinogen: FLEV	317.84-65.52×sqrt(age)+ 32.22×sqrt(age)×female +20.40×[sqrt(age)]×ln(age) - 10.92×[sqrt(age)] ×ln(age) ×female	504.1+89.69×sqrt(age)- 4.79×sqrt(age)×female- 122.98×[sqrt(age)]×ln(age) - 4.78×[sqrt(age)] ×ln(age) ×female
aPTT (HemosiL[c] aPTT-SP) (sec)	28.71-0.22×age +0.20×age×female	44.32-0.30×age -0.041×age×female
aPTT (HemosIL[c] SynthASil) (sec)	25.29+1.13×ln(age)- 0.52×ln(age)×female	41.87-0.072×ln(age)- 0.44×ln(age)×female

aPTT (Stago[d] TriniCLOT HS with Heparin resistant CaCl2) (sec)	26.17-0.040×age+ 0.085×age×female	41.51-0.57×age+ 0.23×age×female
PT (HemosIL[c] RecombiPlasTin 2G) (sec)	10.09+0.053×age+ 0.044×age×female	12.88+0.092×age- 0.032×age×female
PT (HemosIL[c] ReadiPlasTin) (sec)	13.10+0.12×age- 0.082×age×female	19.94-0.034×age- 0.072×age×female

Thromboelastograph 6s (TEG 6s) (Haemonetics Corporation, Chicago IL USA), activated Partial Thromboplastin time (aPTT), Prothrombin time (PT), Kaolin Heparinase (CKH).

[a] Age in years and replace 'female' with 1 if the patient is female, otherwise replace 'female' with 0.

[b] No added benefit of including age or sex

[c] HemosIL (Instrumentation Laboratory, Bedford MA, USA)

[d] Stago TriniCLOT HS (Diagnostica Stago, Asnières-sur-Seine, France)

Table 5. Intraclass correlation coefficients comparing discrete and model-based methods of estimating reference intervals

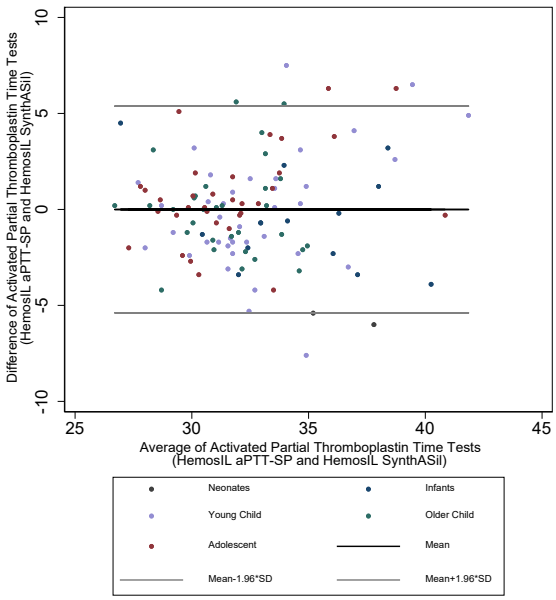
Parameter	Intraclass Correlation Coefficient (95% CI)	
	Lower Limit	**Upper Limit**
Kaolin Activated Thromboelastograph 6s		
Reaction Time	0.67 (0.60, 0.74)	0.43 (0.33, 0.53)
Kinetics Time	0.86 (0.82, 0.89)	0.83 (0.79, 0.87)
Alpha angle	0.77 (0.71, 0.81)	0.66 (0.58, 0.72)
Maximum Amplitude	0.40 (0.28, 0.50)	0.77 (0.71, 0.82)
Laboratory Coagulation Parameters		
Activated Partial Thromboplastin Time (aPTT TriniClot HS[a])	0.35 (0.23, 0.46)	0.87 (0.83, 0.90)
Prothrombin time (PT Thromborel S[b])	0.61 (0.53, 0.69)	-0.25 (-0.38, -0.13)
Thrombin Clotting Time	0.54 (0.44, 0.62)	0.81 (0.76, 0.85)
Antithrombin	0.67 (0.60, 0.74)	0.77 (0.71, 0.82)
Platelet count	0.27 (0.15, 0.38)	0.89 (0.86, 0.91)
Fibrinogen Concentration (Clauss)	0.87 (0.83, 0.90)	0.31 (0.19, 0.42)

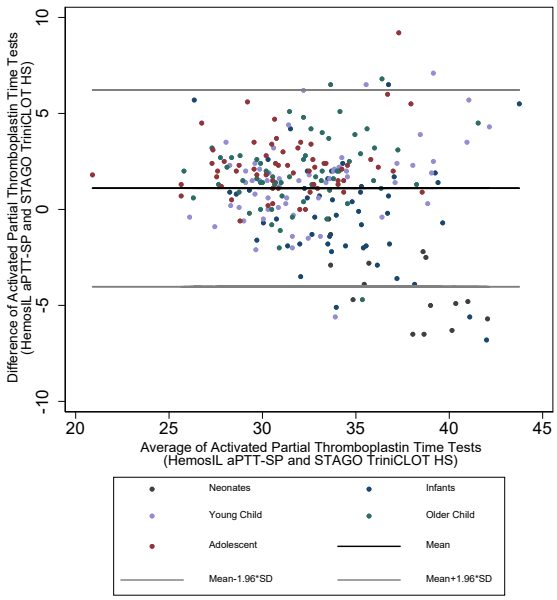
Thromboelastograph 6s (TEG 6s) (Haemonetics Corporation, Chicago IL USA), CI: confidence interval.

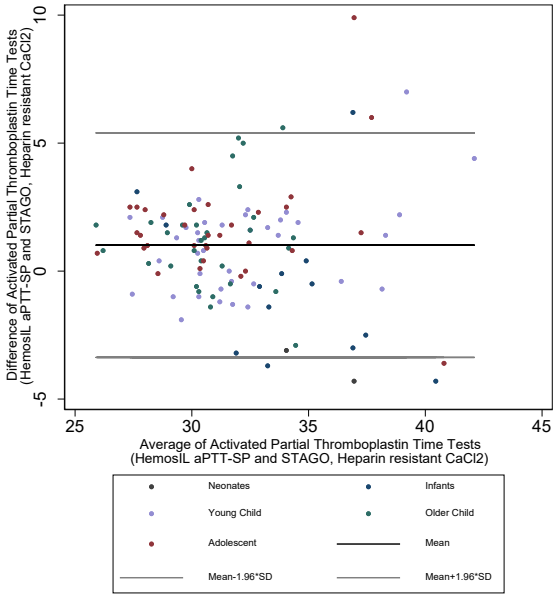
[a] Stago TriniCLOT HS (Diagnostica Stago, Asnières-sur-Seine, France)

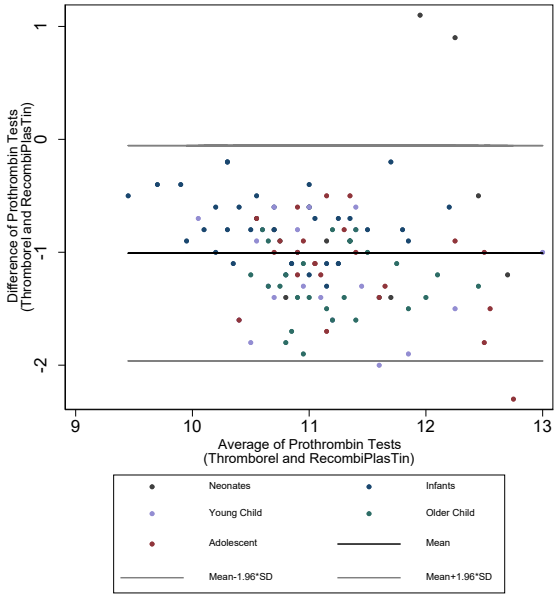
[b] Siemens Thromborel S (Siemens Healthineers, Erlangen, Germany)

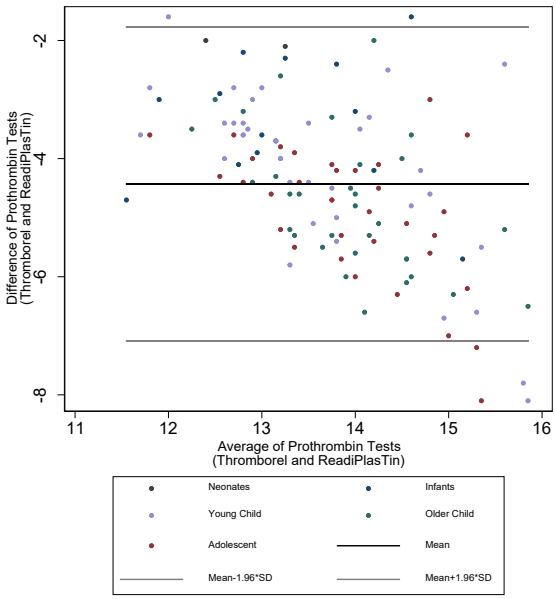
Figure 1. Bland-Altman Plots for aPTT, PT and Fibrinogen using different reagents, separated by age groups. Graphic display of agreement activated Partial Thromboplastin Time (aPTT); using HaemosIL aPTT-SP (Instrumentation Laboratory, Bedford MA, USA) [n=246], Stago TriniCLOT HS [n=246], HaemosIL SynthASil (Instrumentation Laboratory, Bedford MA, USA) [n=119] and Stago TriniCLOT HS (Diagnostica Stago, Asnières-sur-Seine, France) with Heparin resistant CaCl2 [n=119] and Prothrombin Time (PT) using Siemens Thromborel S (Siemens Healthineers, Erlangen, Germany) [n=243], HaemosIL RecombiPlasTin 2G (Instrumentation Laboratory, Bedford MA, USA) [n=119] and HaemosIL ReadiPlasTin (Instrumentation Laboratory, Bedford MA, USA) [n=119], as well as derived and Clauss methods for fibrinogen concentration (n=243).

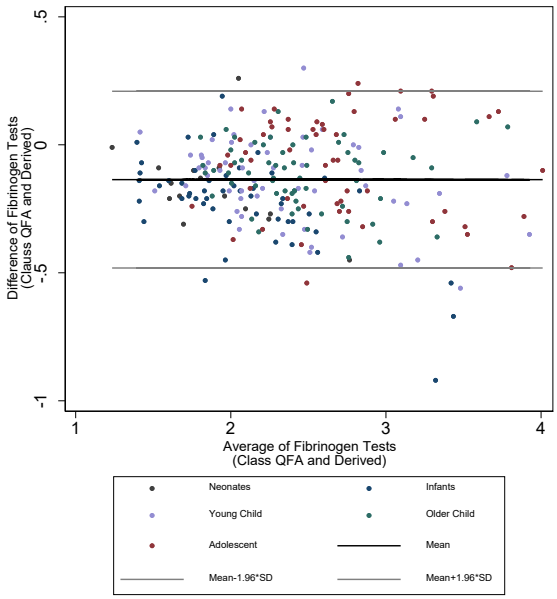

Difference of Activated Partial Thromboplastin Time Tests (HemosIL aPTT-SP and HemosIL SynthASil)
Average of Activated Partial Thromboplastin Time Tests (HemosIL aPTT-SP and HemosIL SynthASil)
Neonates
Infants
Young Child
Older Child
Adolescent
Mean
Mean-1.96*SD
Mean+1.96*SD


Difference of Activated Partial Thromboplastin Time Tests (HemosIL aPTT-SP and STAGO TriniCLOT HS)
Average of Activated Partial Thromboplastin Time Tests (HemosIL aPTT-SP and STAGO TriniCLOT HS)
Neonates
Infants
Young Child
Older Child
Adolescent
Mean
Mean-1.96*SD
Mean+1.96*SD


Difference of Activated Partial Thromboplastin Time Tests (HemosIL aPTT-SP and STAGO, Heparin resistant CaCl2)
Average of Activated Partial Thromboplastin Time Tests (HemosIL aPTT-SP and STAGO, Heparin resistant CaCl2)
Neonates
Infants
Young Child
Older Child
Adolescent
Mean
Mean-1.96*SD
Mean+1.96*SD


Difference of Prothrombin Tests (Thromborel and RecombiPlasTin)
Average of Prothrombin Tests (Thromborel and RecombiPlasTin)
Neonates
Infants
Young Child
Older Child
Adolescent
Mean
Mean-1.96*SD
Mean+1.96*SD


Difference of Prothrombin Tests (Thromborel and ReadiPlasTin)
Average of Prothrombin Tests (Thromborel and ReadiPlasTin)
Neonates
Infants
Young Child
Older Child
Adolescent
Mean
Mean-1.96*SD
Mean+1.96*SD


Difference of Fibrinogen Tests (Clauss QFA and Derived)
Average of Fibrinogen Tests (Class QFA and Derived)
Neonates
Infants
Young Child
Older Child
Adolescent
Mean
Mean-1.96*SD
Mean+1.96*SD

References

1. Hoq M, Karlaftis V, Mathews S, et al. A prospective, cross-sectional study to establish age-specific reference intervals for neonates and children in the setting of clinical biochemistry, immunology and haematology: the HAPPI Kids study protocol. *BMJ Open*. 2019;9(4):e025897. doi:10.1136/bmjopen-2018-025897
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