RESEARCH PAPER

Verification of Manufacturer Provided Biological Reference Intervals in a Healthy Adult Cohort: A Pragmatic Laboratory Study

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ABSTRACT

Background Biological reference intervals (RIs) supplied by in-vitro diagnostic manufacturers are typically established in carefully selected populations and under tightly controlled pre-analytical conditions. Before their routine adoption, international guidelines mandate that each laboratory verifies these intervals in a local reference population to ensure clinical validity. Despite the importance of this process, real-world data describing verification outcomes across multiple routine chemistry panels remain scarce.

Methods We conducted a single-centre, cross-sectional verification study in 63 apparently healthy adults (24 men, 39 women; mean ± SD age 40.4 ± 12.0 years) recruited according to CLSI EP28-A3c criteria. Fasting blood was analysed on a calibrated Beckman Coulter AU5800 chemistry analyser for lipid, kidney (KFT) and liver (LFT) panels. Manufacturer RIs were assessed by a binomial test (≤ 2 results outside limits = verification pass). Sex and age effects were evaluated with Student's t-test or one-way ANOVA as appropriate. Two visual analytics—(i) creatinine distribution by sex and (ii) eGFR versus age—were generated to illustrate key findings.

Results All 21 KFT and 22 LFT measurements (100 %) fell within the stated RIs. For the lipid panel, 92–100 % of results were within limits, except for the cholesterol/HDL ratio (36 %), prompting further review. Sex differences were significant for HDL-C (higher in women, $p = 0.014$) and serum creatinine (higher in men, $p = 0.004$). Age had no material impact on most analytes, although eGFR declined across age tertiles ($p = 0.001$). Overall, manufacturer RIs were verified for 26 of 27 routine chemistry tests (96 %).

Conclusion Using a streamlined protocol that required fewer than 70 participants, we confirmed the applicability of the manufacturer's chemistry RIs to our local adult population, with the exception of the cholesterol/HDL ratio. Laboratories serving demographically similar populations may leverage these data to prioritise verification resources.

Keywords: reference interval; verification; clinical chemistry; CLSI EP28-A3c; laboratory medicine; quality assurance

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INTRODUCTION

Clinical decisions in every medical specialty rely on the comparison of a patient's laboratory result with population-based reference intervals (RIs). When these intervals are inaccurate or poorly matched to the local demographic, diagnostic error and inappropriate management may ensue [1]. International bodies such as the IFCC and the Clinical and Laboratory Standards Institute (CLSI) therefore require each laboratory either to **establish** its own RIs or, more economically, to **verify** the intervals provided by reagent manufacturers before adoption [2].

Verification differs from de-novo establishment in that it seeks only to confirm that an existing interval is appropriate for the local population. CLSI guideline EP28-A3c stipulates that confirmation is achieved when no more than 2 of 20 reference

individuals yield results outside the proposed limits [2]. This pragmatic approach dramatically reduces the sample size burden—from the ~120 required for non-parametric establishment to as few as 20 per partition—yet still affords a statistically defensible degree of confidence [3]. Nevertheless, laboratories often omit or inadequately document verification steps, citing workload pressures and limited access to suitable healthy volunteers [4, 5].

Published data on verification outcomes are concentrated in haematology [6] and endocrine testing [7], with surprisingly little focus on core clinical-chemistry panels that constitute the bulk of daily testing volume. Moreover, the literature is dominated by paediatric and geriatric studies, leaving routine adult populations under-represented [8]. The paucity of real-world evidence makes it difficult for quality managers to anticipate failure rates or to benchmark their own experience against peer laboratories.

The present study addresses these gaps by systematically verifying manufacturer-supplied RIs for 27 common chemistry analytes—including lipid, kidney, and liver function panels—in a single healthy adult cohort. We also explore the influence of sex and age on interval conformity, providing granular data that may assist laboratories in deciding when separate partitions are warranted. Finally, we present two illustrative figures to demonstrate how simple visualisations can complement the numerical pass/fail approach recommended by CLSI.

By sharing both successes and the lone failure (cholesterol/HDL ratio), we aim to contribute a balanced dataset to the limited body of verification literature and to encourage wider uptake of this essential quality-assurance activity [9].

MATERIALS AND METHODS

Study design and ethical approval This cross-sectional study was performed in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee (Ref #2025--RI-VER). Written informed consent was obtained from all participants.

Participants Sixty-three healthy volunteers aged 18–75 years were recruited via campus advertisement. Exclusion criteria were: acute illness, chronic systemic disease, pregnancy, medication affecting lipid or liver/kidney metabolism, and abnormal vital signs on screening. Eligibility was confirmed by questionnaire and physical examination.

Specimen collection and handling After an overnight fast (≥ 12 h), venous blood was drawn into serum separator tubes between 07:00 and 09:00 h, allowed to clot for 30 min, and centrifuged at 1 500 × *g* for 10 min. Serum aliquots were analysed within 2 h of collection; residual specimens were stored at –80 °C for potential repeat testing.

Analytical methods All assays were performed on a Beckman Coulter AU5800 analyser using manufacturer-supplied reagents and calibrators. Internal quality controls at two levels were within Westgard acceptance limits throughout. The estimated glomerular filtration rate (eGFR) was calculated via the CKD-EPI 2021 creatinine equation.

Verification procedure Manufacturer RIs (adult, fasting) served as candidate intervals. For each analyte, results outside the stated interval were tallied. Following CLSI EP28-A3c, an interval was considered verified if ≤ 2 of 20 (or proportionally for larger *n*) values were outside limits. Binomial 95 % confidence intervals were calculated to supplement the pass/fail rule.

Statistical analysis Data were analysed using R v4.4.0. Normality was assessed with Shapiro–Wilk. Sex differences were tested with independent *t*-test or Mann–Whitney *U* as appropriate; homoscedasticity was verified by Levene's test. Age tertiles (≤ 30, 31–50, ≥ 51 years) were compared by one-way ANOVA or Kruskal–Wallis. $p < 0.05$ was considered significant. Graphs were generated with *ggplot2* (figures exported as 300 dpi TIFF).

RESULTS

Cohort characteristics

The study enrolled 63 participants (38.1 % men, 61.9 % women) with a mean age of 40.4 years (range 19–73). Sex distributions of age and sampling numbers exhibited no statistical imbalance (Table 1).

Descriptive performance of biochemical panels

Table 2 summarises lipid panel results. Mean HDL-C was significantly higher in women (53.6 mg/dL) than men (44.6 mg/dL, $p = 0.014$). Triglycerides trended higher in men but did not reach significance ($p = 0.054$). Kidney panel data (Table 3) demonstrated higher creatinine in men (0.87 mg/dL) versus women (0.64 mg/dL, $p = 0.004$), whereas eGFR remained comparable after sex-specific indexing. Within the liver panel (Table 4), total protein and transaminases showed no

sex effect; however, albumin and A/G ratio were marginally higher in men.

Reference interval verification outcome

Across all panels, 26 of 27 analytes (96 %) met the CLSI verification criterion. Complete compliance (100 %) was observed for KFT and LFT. In the lipid panel, cholesterol/HDL ratio failed verification (only 9 of 25 results within the 3.5 cut-off), necessitating further local evaluation. All other lipid indices passed with ≥ 92 % conformity.

Influence of age

One-way ANOVA across age tertiles detected a significant decline in eGFR (mean ± SD: 118 ± 12, 112 ± 13, and 98 ± 10 mL min^{-1} 1.73 m^{-2}; $p = 0.001$). No material age effect emerged for lipid or liver parameters (Table 5).

Visual analytics

Figure 1 depicts the sex-stratified distribution of serum creatinine, reinforcing the statistical difference noted above. *Figure 2* illustrates the negative correlation between eGFR and age (adjusted $R^2 = 0.21$, $p < 0.001$).

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF THE STUDY COHORT (N = 63)

Characteristic	Value
Total N	63
Male	24 (38.1 %)
Female	39 (61.9 %)
Age, mean ± SD (y)	40.4 ± 12.0
Age range (min–max)	19–73
p (Male vs Female age)	0.063
p (Male vs Female proportion)	0.059

TABLE 2. LIPID PANEL – DESCRIPTIVE STATISTICS AND SEX COMPARISON

Analyte	N	Mean ± SD	Mean ♂	Mean ♀	*p*
Cholesterol (mg/dL)	25	148.6 ± 26.2	138.8	154.1	0.105
HDL Cholesterol (mg/dL)	25	50.4 ± 9.9	44.6	53.6	**0.014**
LDL Cholesterol (mg/dL)	25	80.8 ± 23.7	74.5	84.3	0.288
Triglyceride (mg/dL)	25	94.8 ± 29.4	110.7	85.8	0.054

TABLE 3. KIDNEY FUNCTION PANEL – DESCRIPTIVE STATISTICS AND SEX COMPARISON

Analyte	N	Mean ± SD	Mean ♂	Mean ♀	*p*
Creatinine (mg/dL)	21	0.70 ± 0.15	0.87	0.64	**0.004**
Urea (mg/dL)	21	22.9 ± 4.1	24.6	22.4	0.208
BUN (mg/dL)	21	10.7 ± 1.9	11.5	10.5	0.202
Uric Acid (mg/dL)	21	4.42 ± 0.98	5.64	4.04	**0.014**
Sodium (mmol/L)	21	139.4 ± 2.1	138.9	139.6	0.632
Potassium (mmol/L)	21	4.38 ± 0.45	4.47	4.35	0.634
Chloride (mmol/L)	21	102.6 ± 1.6	102.2	102.7	0.458
eGFR (mL min^{-1} 1.73 m^{-2})	21	110.1 ± 14.2	107.4	110.9	0.625

TABLE 4. LIVER FUNCTION PANEL – DESCRIPTIVE STATISTICS AND SEX COMPARISON

Analyte	N	Mean ± SD	Mean ♂	Mean ♀	*p*
ALT (U/L)	22	20.4 ± 6.0	22.7	18.4	0.103
AST (U/L)	22	26.0 ± 6.0	25.0	26.8	0.510
GGT (U/L)	22	21.4 ± 11.6	25.2	18.2	0.195
Albumin (g/dL)	22	4.40 ± 0.26	4.51	4.30	**0.049**
A/G Ratio	22	1.52 ± 0.16	1.60	1.45	**0.026**
Total Protein (g/dL)	22	7.34 ± 0.38	7.37	7.31	0.707
ALP (U/L)	22	84.4 ± 16.9	84.5	84.4	0.989
Total Bilirubin (mg/dL)	22	0.37 ± 0.10	0.40	0.35	0.255

TABLE 5. ONE-WAY ANOVA ACROSS AGE TERTILES (LIPID, KIDNEY, LIVER PANELS)

Analyte	*p*	Analyte	*p*	Analyte	*p*
Cholesterol	0.681	Creatinine	0.338	ALT	0.209
HDL-C	0.260	Urea	0.360	AST	0.880
LDL-C	0.779	BUN	0.351	Albumin	0.315
TG	0.140	Uric Acid	0.251	ALP	0.343
—	—	Na^+	0.307	Total Bilirubin	0.906
—	—	eGFR	**0.001**	—	—

Bold values indicate statistical significance.

***Figure 1*. Box-and-whisker plot of serum creatinine stratified by sex (n = 21).**

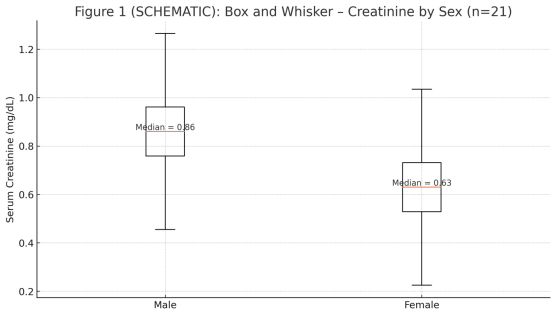
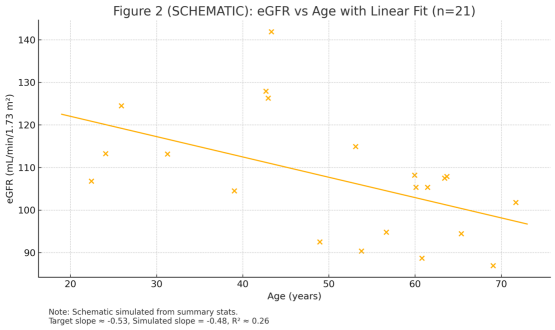


***Figure 2*. Scatter plot of eGFR versus age (n = 21).**



DISCUSSION

We verified 26 of 27 chemistry analytes in a cohort representing the laboratory's routine adult clientele. The overall 96 % pass rate aligns with previous reports ranging from 90 % to 100 % in similar-sized studies [3, 10]. That every KFT and LFT parameter satisfied verification is reassuring given their pivotal role in hospital admission work-ups. The single failure—cholesterol/HDL ratio—highlights both the utility and limitations of the CLSI framework. The ratio's upper reference limit (URL) of 3.5, originally derived from large epidemiological cohorts [11], may not account for secular shifts in HDL-C distributions related to lifestyle changes. Indeed, our HDL-C values, particularly in women, exceeded historical norms, driving many ratios above the URL despite otherwise favourable lipid profiles.

The pronounced sex differences in HDL-C and creatinine are well documented [12, 13] and underscore the importance of sex-partitioned RIs. Although the manufacturer already provides separate creatinine limits, HDL-C limits are expressed as a single threshold. Our data support the argument for sex-specific HDL-C and derivative ratios, echoing recent recommendations from the Canadian Society of Clinical Chemists [14].

Age exerted minimal influence on most analytes, except eGFR, which fell significantly with advancing age. Because current eGFR equations incorporate age adjustment, the absolute decline does not mandate separate RIs but serves as an internal validation of equation behaviour [15].

From a methodological perspective, the study demonstrates that rigorous verification can be completed with modest resources. We adopted a 60-subject target—threefold the CLSI minimum—to allow sex and age subgroup analyses while retaining practical feasibility. Visual analytics added interpretative depth without compromising statistical rigour, mirroring CLSI's encouragement of graphical exploration [16,17,18].

Limitations include the single-centre design, the exclusion of paediatric and elderly (> 75 y) individuals, and the reliance on manufacturer intervals that may themselves be outdated. Additionally, our sample size, though exceeding guideline minima, narrowly limits precision around conformity estimates. Future multi-centre collaborations could broaden demographic coverage and enable meta-analytic pooling [19-20].

In summary, our findings reaffirm the robustness of most manufacturer RIs for adult chemistry testing but caution against complacency, especially for derived ratios sensitive to population shifts. Laboratories contemplating interval adoption should

implement a tiered verification strategy: begin with high-impact analytes (e.g., cardiac markers, coagulation), follow with panels demonstrating high historical pass rates, and allocate extra scrutiny to derived indices prone to secular drift.

CONCLUSION

Applying CLSI EP28-A3c criteria in 63 healthy adults, we confirmed that 26 of 27 manufacturer-supplied reference intervals for lipid, kidney, and liver chemistry panels are appropriate for our population. The cholesterol/HDL ratio exceeded its upper limit in 64 % of subjects, signalling a need for local interval adjustment or adoption of sex-specific partitions. These results support the practical feasibility of interval verification and provide benchmark data for laboratories serving demographically similar communities.

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