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Consensus Recommendations, Special Reports or Practice Guidelines

# Best practice guidelines on reference interval harmonization in Canada: *Evidence-based recommendations from the CSCC working group on reference interval harmonization (CSCC WG-hRI)*

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ABSTRACT

Unnecessary variation in reference intervals across clinical laboratories increases the risk of inconsistent or misinformed clinical decision-making. Development of harmonized or common reference intervals for assays that demonstrate minimal bias across measurement procedures and laboratories is an important step towards standardized quality healthcare. The aim of this document is to recommend evidence-based harmonized reference intervals for routine clinical laboratory tests that can be implemented in hospital and community settings across Canada. The approach that was taken for these recommendations included several important steps. Candidate analytes for harmonization were selected based on documented traceability and external quality assessment performance. Two years of patient test result data for 16 routine clinical chemistry analytes were extracted from four provincial community laboratories across Canada. A robust indirect statistical algorithm was applied to assess the feasibility of harmonization and harmonized reference intervals were established for appropriate analytes. Derived harmonized reference intervals were compared to existing data from healthy individuals from Canadian and international studies. All recommended harmonized reference intervals were verified across nine Canadian laboratories that included all main manufacturers using serum and plasma samples collected from 60 healthy volunteers. Based on our findings, evidence-based harmonized reference intervals are recommended for 13 analytes, including: albumin (bromocresol green method only), alanine aminotransferase (ALT) with and without pyridoxal 5′-phosphate, alkaline phosphatase (ALP), calcium, carbon dioxide (total), chloride, creatinine, lactate dehydrogenase (LDH), phosphate, potassium (serum only), magnesium, total protein, and thyroid stimulating hormone (TSH). These recommendations will support national harmonization of laboratory reference intervals with the goal of improving and standardizing clinical decision-making and patient care across Canada.

## 1. Background

Reference intervals are health-associated targets for interpreting laboratory test results and alerting clinicians of the potential need for follow-up. Represented as the 2.5th and 97.5th percentiles in a reference population, reference intervals guide clinical decision-making, influencing the diagnosis, prognostication, and monitoring of many diseases [1]. Patients, clinicians, and other healthcare professionals often assume laboratory test results and their interpretations are consistent across various clinical laboratories [2]. However, this assumption of uniformity in care is not always met due to multiple contributing factors. Although variations in analytical methodology are often attributed as the cause of variable test results and corresponding reference intervals, international assay standardization efforts have achieved excellent analytical standardization and comparability across measurement procedures and manufacturer platforms for key analytes. Reference interval harmonization for analytes with established traceability is the next desirable step in laboratory standardization efforts. A survey circulated in 2017 to assess reference intervals used across Canadian laboratories demonstrated significant variation that often exceeded test result variation from a commutable sample [3]. This suggests unwarranted differences in reference intervals across Canadian clinical laboratories that cannot be explained by differences in analytical measurement. Harmonization is an essential step towards more consistent, accurate, and standardized clinical decision-making. With broad access to electronic medical records, merging healthcare systems, and multidisciplinary care of patients, these efforts are more important than ever.

## 2. Aim and target audience

The aim of the Canadian Society of Clinical Chemists (CSCC) Working Group on Reference Interval Harmonization (hRI-WG) is to develop evidence-based, harmonized/common reference intervals and to support their implementation in clinical laboratories across Canada. In this guidance document, we provide five recommendations for adult reference interval harmonization in Canadian clinical laboratories (Box 1) [4]. These recommendations are based on findings from our group's recent publication where a cross-Canada study comprising both outpatient populations and healthy Canadians was completed [4]. In Phase I of this harmonization effort, we assessed feasibility of reference interval harmonization for 16 analytes. Of these, harmonized reference intervals are recommended for 13 analytes: albumin (bromocresol green (BCG)

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method only), alanine aminotransferase (ALT) with and without pyridoxal 5′-phosphate, alkaline phosphatase (ALP), calcium, carbon dioxide (total), chloride, creatinine, lactate dehydrogenase (LDH), phosphate, potassium (serum only), magnesium, total protein, and thyroid stimulating hormone (TSH). The evidence and rationale for these recommendations are outlined below. Canadian laboratories are encouraged to adopt these evidence-based recommendations and support national harmonization of laboratory reference intervals to improve clinical decision-making and patient care across Canada.

## 3. Evidence in the Canadian Population

The CSCC hRI-WG designed and executed a rigorous data-driven approach to establish harmonized reference intervals for common laboratory tests. In-depth description of methodology and results was recently published [4] (Fig. 1). Candidate analytes were reviewed for standardization or harmonization of assay principle and calibration traceability, and 16 analytes were selected based on demonstrated equivalence and therefore feasibility of reference interval harmonization for the adult population aged 19–79 years. Data mining was completed to extract retrospective outpatient laboratory data for 16 analytes from four community laboratories across Canada with different analytical assays/instrumentation: **1)** Ontario (*chemistry: Roche Cobas, immunoassay: Abbott Architect*), **2)** Ontario (*chemistry and immunoassay: Roche Cobas*), **3)** British Columbia (*chemistry: Roche Cobas; immunoassay: Abbott Architect*), and **4)** Alberta (*chemistry: Siemens Advia; immunoassay: Siemens Centaur*). Outpatient test results for a two-year period (2017–2018) were extracted for all patients aged 19 to 79 years with sample sizes of up to 14 million per analyte. Where there was repeat testing, only the first result per patient was included. Significant drift in test result measurement due to reagent reformulation, population drift, seasonal variation, and/or reagent or calibrator lot-to-lot variation/reformulation was evaluated to identify data instability. Age- and sex-specific differences were evaluated to establish partitions followed by

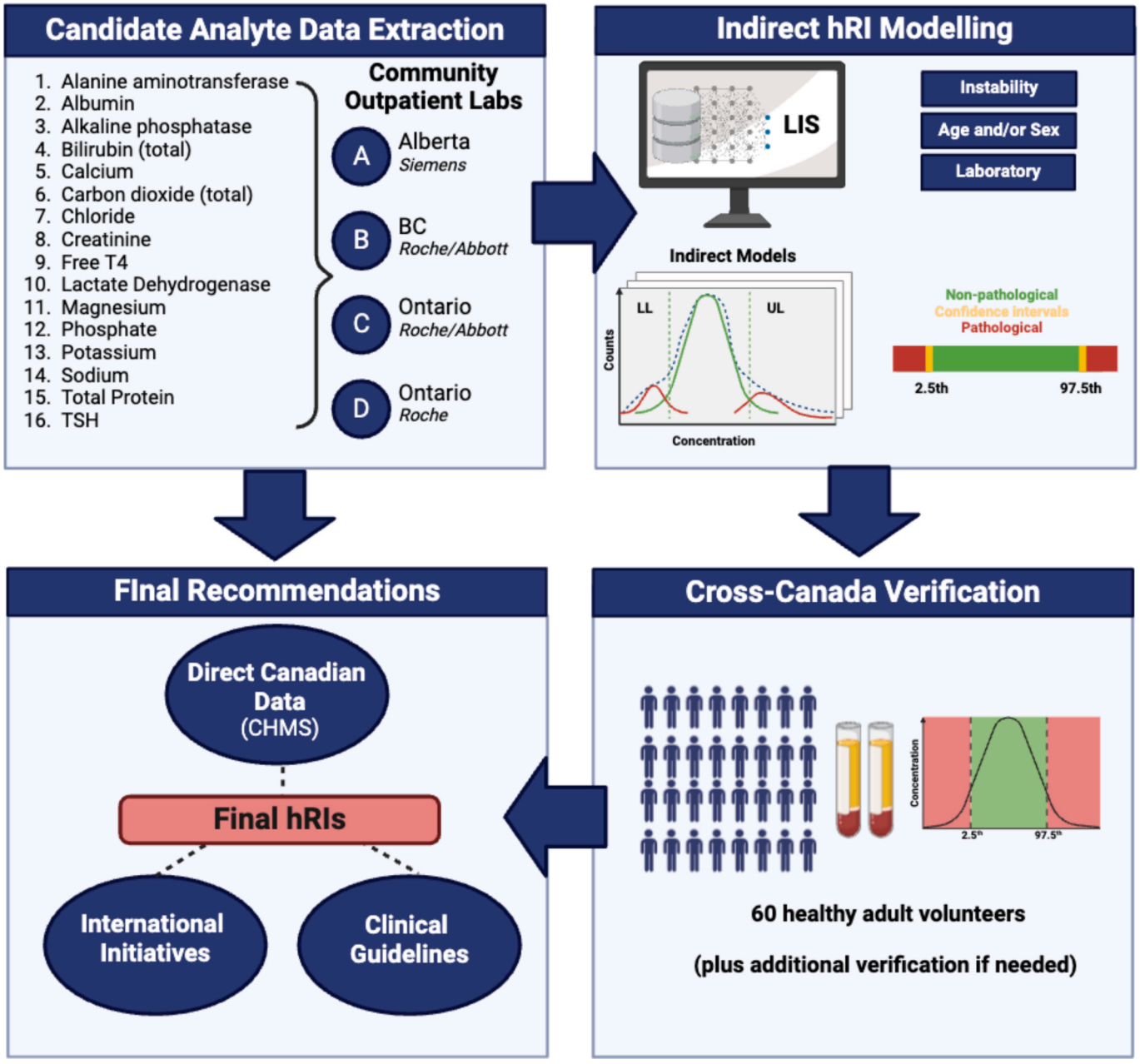


**Fig. 1.** Summary of multi-step data-driven approach for reference interval harmonization in Canada. BC: British Columbia, *CHMS: Canadian Health Measures Survey, hRIs: harmonized reference intervals, LIS: laboratory information system, LL: lower limit, T4, thyroxine, TSH: thyroid-stimulating hormone, UL: upper limit.*

outlier removal. Differences between community laboratory datasets for each analyte were then assessed. If no statistical differences were observed, data from all provinces were combined to estimate harmonized reference intervals. If statistical differences were observed, data were reviewed to determine feasibility of combination in conjunction with analyte-specific factors (e.g., method standardization, mean difference relative to measurement uncertainty etc.). Reference intervals were then estimated using the refineR algorithm for each community laboratory dataset separately and combined [5,6] (Table 1). The refineR method has been shown to achieve results comparable or superior to the direct method for routine chemistry analytes in datasets with a minimum sample size of 5000 and a non-pathological fraction of 20 % [25]. Application of indirect techniques should be carefully considered based on the statistical model and assumptions as well as the dataset population (e.g., inpatient vs outpatient), sample size, and analyte distribution to avoid development of inappropriate reference intervals, as discussed in-depth elsewhere [28,29].

Established reference intervals using indirect community laboratory data and the refineR algorithm were compared to available data collected prospectively from healthy Canadians through the Canadian Health Measures Survey (CHMS) [7–9] as well as work by harmonization initiatives globally, including Nordic Reference Interval Project (NORIP), UK Pathology, and Australasian Harmonized Reference Intervals for Adults (AHRIA) [10–14]. Relevant clinical guidelines were also reviewed [15,16].

To verify the proposed harmonized reference intervals in Canadian clinical laboratories, 60 healthy Canadian adults (30 male, 30 female) were recruited with informed consent, and both serum and plasma (lithium heparin) were collected. Samples were spun, aliquoted and frozen and then sent to nine clinical laboratories across Canada using different analytical instruments. Six provinces were included, and all of the main manufacturers were represented at least once (i.e., Abbott, Beckman, Ortho, Roche, Siemens). The percentage of results falling within proposed reference intervals was then calculated for each laboratory individually. Verification results for all 16 analytes evaluated are provided in Supplementary Fig. 1–15. The number of laboratories that met 90 % verification were identified based on Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guidelines (≥90 %) [1]. For analytes where less than 90 % verification was observed across all nine laboratories, analytes were reviewed on a case-by-case basis. Four

**Table 1**
CSCC hRI-WG Recommended Harmonized Reference Intervals in Adults.

| Analyte | Calculated Harmonized Reference Interval *(see ref 4)* | | Recommended Harmonized Reference Interval | Number of Canadian Labs Achieving > 90 % Verification* | Matrices | Available Clinical Decision Limits or Direct Data in Healthy Canadians |
|---|---|---|---|---|---|---|
| **Alkaline Phosphatase** | 19 to 39 years M | 42–113 U/L | 42–113 U/L | 100 % (9/9 labs) | Serum/ Plasma | None |
| | 19 to 39 years F | 35–100 U/L | 35–100 U/L | | | |
| | 40 to 79 years | 41–120 U/L | 41–120 U/L | | | |
| **Alanine Aminotransferase** | 19 to 79 years M | <47 U/L | <47 U/L | 100 % (9/9 labs) | Serum/ Plasma | <33 U/L *(ref 16)* |
| | 19 to 79 years F | <29 U/L | <29 U/L | | | <25 U/L *(ref 16)* |
| **Calcium**[a] | 19 to 39 years M | 2.20–2.55 mmol/L | All: 2.15 to 2.55 mmol/L | 88 % (7/8 labs) | Serum/ Plasma | None |
| | 19 to 39 years F | 2.16–2.50 mmol/L | | | | |
| | 40 to 79 years | 2.15–2.51 mmol/L | | | | |
| **Carbon dioxide, total** | 19 to 79 years | 22–31 mmol/L | 22–31 mmol/L | 11 % (1/9 labs) | Serum/ Plasma | None |
| **Chloride** | 19 to 79 years | 97–107 mmol/L | 97–107 mmol/L | 88 % (7/8 labs) | Serum/ Plasma | None |
| **Creatinine** | 19 to 79 years M | 62–112 µmol/L | 62–112 µmol/L | 100 % (9/9 labs) | Serum/ Plasma | None |
| | 19 to 79 years F | 47–87 µmol/L | 47–87 µmol/L | | | |
| **Lactate Dehydrogenase** | 19 to 79 years | 122–235 U/L | 122–235 U/L | 89 % (8/9 labs) | Serum/ Plasma | None |
| **Magnesium** | 19 to 79 years | 0.73–1.00 mmol/L | 0.73–1.00 mmol/L | 100 % (9/9 labs) | Serum/ Plasma | None |
| **Phosphate** | 19 to 49 years | 0.79–1.49 mmol/L | All: 0.80–1.50 mmol/L | 100 % (9/9 labs) | Serum/ Plasma | None |
| | 50 to 79 years M | 0.74–1.44 mmol/L | | | | |
| | 50 to 79 years F | 0.88–1.53 mmol/L | | | | |
| **Thyroid Stimulating Hormone** | 19 to 79 years | 0.60–4.48 mIU/L | 0.60–4.48 mIU/L | 100 % (9/9 labs) | Serum/ Plasma | 0.40–4.00 mIU/L (*ref 14*) |
| **Total Protein** | 19 to 79 years | 62–79 g/L | 62–79 g/L | 78 % (7/9 labs) | Serum/ Plasma | None |
| **Special Considerations** | | | | | | |
| **Albumin (BCG only)** | 19 to 59 years M | 42–50 g/L | All: 40–50 g/L | 83 % (5/6 labs) | Serum/ Plasma | None |
| | 19 to 59 years F | 39–49 g/L | | | | |
| | 60–79 years | 38–48 g/L | | | | |
| **Potassium** | 19 to 79 years | 3.9–4.9 mmol/L | 3.9–4.9 mmol/L | 44 % (4/9 labs) | Serum | None |

M: male, F: female, BCG: bromocresol green, * Verification values from serum only shown (see ref 3. for plasma verification data); [a] Verification data based on re-verification study as outlined.

analytes were selected to undergo an additional verification cycle (i.e., albumin, calcium, total bilirubin, and sodium). Specifically, an additional 30 samples were sent to eight laboratories with all major manufacturers represented for repeated evaluation. Re-verification results for these analytes are also provided in the Supplemental material. Analytes where only one or two laboratories (out of a total of nine laboratories across Canada using different analytical systems) did not meet 90 % verification were reviewed by the hRI-WG for final recommendation of harmonization as multiple variables could potentially contribute to the results of this multi-site analysis, including methodology, pre-analytical factors, and different laboratory practices.

It is important to note that the direct approach for reference interval establishment remains the gold standard per CLSI EP23-A3c guidelines. However, increasing evidence supports the utility and accuracy of modern indirect reference interval approaches, especially in outpatient settings for high volume chemistry testing [25]. In our big data approach, we evaluated rich indirect datasets for each analyte to provide a comprehensive assessment of the feasibility for harmonization. The combination of this analysis with verification of derived estimates using specimens collected from healthy individuals support the robustness of this approach.

## 4. Best practice guidelines — adult reference intervals

### 4.1. Recommendation 1

Clinical laboratories should adopt harmonized reference intervals for 13 analytes (albumin (BCG method only), alkaline phosphatase, alanine aminotransferase, creatinine, calcium, carbon dioxide, chloride, creatinine, lactate dehydrogenase, magnesium, phosphate, total protein, and thyroid stimulating hormone)

Canadian population data analysis supports reference interval harmonization for 13 analytes (12 in both serum and plasma and potassium in serum only) (Table 1). Sex-specific reference intervals were established for ALT and creatinine. Sex and age-specific reference intervals were established for ALP, calcium, and phosphate.

Harmonized reference intervals were verified using healthy Canadian adult serum and plasma samples in nine laboratories across Canada using different methods (Supplemental Fig. 1–15). Six analytes demonstrated above 90 % serum verification performance across all nine participating laboratories (i.e., ALP, ALT, creatinine, magnesium, phosphate, thyroid). Calcium, chloride, LDH, and total protein demonstrated above 90 % verification in all laboratories except one (calcium: Architect (87 %), chloride: Atellica (87 %), LDH: Beckman DxC (88 %)), or two (total protein: Architect (85 %), Vitros (85 %)). Carbon dioxide (total) failed verification criteria in both serum and plasma for all laboratories participating in the cross-Canada verification. We anticipate this is due to sample instability issues, including exposure to air in the pre-analytical process (aliquoting, freeze–thaw, shipment, etc.). Nevertheless, a harmonized reference interval is recommended based on the results of our big data analysis of community laboratory data showing significant comparability of data obtained in different populations and different analytical systems across Canada.

Verification performance did not demonstrate dependence on methodology for creatinine (i.e., enzymatic or Jaffe) or ALT (i.e., activated, or non-activated with pyridoxal-5′-phosphate (P-5′-P)). The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference measurement procedure for ALT includes P-5′-P supplementation; however, many laboratories continue to use reagents without P-5′-P supplementation that lack traceability to the IFCC reference system [17]. Major differences between ALT assays were not observed in our verification study with > 95 % verification across all nine laboratories/methods/instrumentation evaluated (N = 3 with P-5′-P supplementation). The ALT sex-specific upper limits recommended in this guideline of 47 U/L (males) and 29 U/L (females) are supported by recent studies in other populations. Specifically, Valentini et al. completed a study of 21,296 healthy individuals using an IFCC-traceable ALT with P-5′-P supplementation resulting in upper limits of 42 U/L (males) and 30 U/L (females), similar to our findings [18]. It is also important to note that the American College of Gastroenterology proposed clinical decision limits in 2017 of 33 U/L in males and 25 U/L in females [16]. Clinical laboratories are encouraged to discuss the differences between reference intervals and clinical decision limits with clinical colleagues.

TSH also demonstrated excellent verification of above 90 % across all methods/laboratories. Our recommended harmonized reference interval is 0.60 to 4.48 mIU/L. This is similar to the 2014 recommendations by the American Thyroid Association of 0.40 to 4.00 mIU/L [15]. Additional considerations in TSH result interpretation may vary dependent on the clinical setting, including rates of referral, reflex algorithms, and concordance with free thyroxine and free triiodothyronine measurements [19]. Clinical laboratories are encouraged to discuss with their key stakeholders (e.g., endocrinologists and family physicians).

Lastly, age- and sex-specific reference intervals for calcium, phosphate, and albumin (BCG) were merged into one recommended reference interval due to overlapping confidence intervals and lack of clinically significant differences between partitions based on discussion by hRI-WG members and clinical specialists (Table 1). Sex-specific reference intervals are recommended for ALT and creatinine.

**Box 1**
**: CSCC recommendations for Reference Interval Harmonization (Phase I) in Canada**

1. Clinical laboratories should adopt harmonized reference intervals for 13 analytes (albumin (BCG method only), alkaline phosphatase, alanine aminotransferase, creatinine, calcium, carbon dioxide, chloride, lactate dehydrogenase, magnesium, phosphate, total protein, and thyroid stimulating hormone)
2. Clinical laboratories should adopt separate reference intervals for potassium for serum and plasma.
3. Clinical laboratories should adopt separate reference intervals for albumin measured by bromocresol green and bromocresol purple methods.
4. Harmonized reference intervals for free thyroxine, total bilirubin, and sodium are not recommended at this time.
5. Clinical laboratories should consider verifying proposed harmonized reference intervals on their local analytical platform and population prior to implementation.

### 4.2. Recommendation 2

Clinical laboratories should adopt separate reference intervals for potassium for serum and plasma.

A harmonized reference interval for serum potassium (3.9–4.9 mmol/L) was derived using retrospective data from community laboratories. However, in the cross-Canada verification study, paired plasma specimens demonstrated significantly lower results relative to sera, verifying only 30–70 % across participating laboratories [4]. This is not surprising given known matrix effects on potassium concentrations including extra proteins (e.g. fibrinogen and clotting factors) in plasma, and potential platelet rupture in serum [20]. Thus, due to a lack of data availability, we are not able to recommend a harmonized reference interval for plasma potassium at this time. It is noted that common laboratory practice does differentiate serum and plasma potassium as separate tests, reporting them separately for reliable routine monitoring over time.

### 4.3. Recommendation 3

Clinical laboratories should adopt separate reference intervals for albumin measured by bromocresol green and bromocresol purple methods.

Harmonized reference intervals for albumin were derived using retrospective data from community laboratories that used the BCG method only. In the cross-Canada verification studies, laboratories using the bromocresol purple method failed to meet the verification criterion (Supplemental Table 1). This was not unexpected, as albumin measured by BCG is known to be affected by acute-phase globulins [21]. In the next phase, the CSCC hRI-WG will evaluate harmonized reference intervals for the bromocresol purple method.

### 4.4. Recommendation 4

Harmonized reference intervals for free thyroxine, total bilirubin, and sodium are not recommended at this time.

Approximately 1.6 million free thyroxine results were extracted from provincial community laboratories to assess feasibility of reference interval harmonization. Significant differences were observed between all community laboratories, with the exception of Ontario (Architect) and British Columbia (Architect), which measured free thyroxine on the same platform. Reference intervals derived using indirect refineR analysis of data from each community laboratory were markedly different. The upper limit ranged from 15.2–19.2 pmol/L; while the lower limit ranged from 9.2–12.6 pmol/L across laboratories. Results from the healthy verification cohort also demonstrated significant variation across nine laboratories, suggesting reference interval harmonization is inappropriate [4]. Our findings are supported by studies from the IFCC Committee on Standardization of Thyroid Function Tests (C-STFT) wherein data across 13 free thyroxine assays demonstrated a maximum inter-assay discrepancy of 30 % in a high concentration range and 90 % in a low concentration range [22,23]. The feasibility of "method-specific" harmonized reference intervals across laboratories should be considered.

Verification criteria for total bilirubin and sodium were also not met. Despite availability of a reference system for total bilirubin, inter-method differences have been reported and there are ongoing efforts to establish a revised reference measurement procedure [24]. The impact of applying common reference limits for total bilirubin has been reported in specific populations such as neonates [26,27]. While the consequences in adult populations are not well described, these studies underscore the analytical differences that persist across manufacturers for total bilirubin and the inappropriateness of reference interval harmonization.

For sodium, 50 % of laboratories achieved greater than 90 % verification. There are many potential contributing factors to the poor verification rates, including the relatively narrow harmonized reference interval derived by indirect data (138–145 mmol/L). This may be a result of the lack of variability in sodium data in healthy populations (narrow physiological range and reporting in whole numbers) combined with a large sample size. Additional contributing factors could include calibration differences across different ion selective electrodes and pre-analytical factors that differentiate outpatient settings from inpatient settings, including delayed time to centrifugation.

### 4.5. Recommendation 5

Clinical laboratories should consider verifying proposed harmonized reference intervals on their local analytical platform and population prior to implementation.

We strongly recommend laboratories verify performance of recommended harmonized reference intervals prior to implementation using healthy adult specimens as per CLSI EP28 A3c guidelines on their own assay/analytical platform, with their own pre-analytic conditions [1]. While samples collected from the clinical laboratory's local healthy population are recommended, the CSCC hRI-WG can support clinical laboratories considering verification studies by providing access to a set of healthy adult specimens, if needed, and depending on availability at the time of request. Clinical laboratories may also consider using retrospective data analysis to assess current and future flagging rates to anticipate practice changes.

The CSCC hRI-WG is developing an hRI implementation tool kit (on the CSCC website) which will include: checklists, communication templates with rationale for change for clinicians and other healthcare stakeholders, as well as additional data analysis and knowledge translation tools. We appreciate that the implementation of these recommendations in local settings may present challenges, particularly given the familiarity clinicians may have with long-standing reference intervals at a specific institution. Inter-institutional harmonization of reference intervals for analytes where sound traceability is in place will be an important step towards improved consistency and accuracy of test interpretation across Canada. The CSCC hRI-WG will assist by providing consultations, help with data analysis, and actively participating and promoting the implementation process in various regions across Canada.

## 5. Synopsis

In this best practice guideline, the CSCC hRI-WG evaluated 16 analytes for reference interval harmonization. We recommend implementing derived common/harmonized reference intervals for 13 of the 16 analytes. Recommendations are based on data-driven evidence from Canadian laboratories. Implementation of these recommendations is an essential step towards more improved and consistent laboratory data interpretation and clinical decision making in healthcare across Canada. Future directions for the CSCC hRI-WG include evaluating the next phase of analytes for consideration of harmonized reference intervals by applying the same established framework. The CSCC hRI-WG will also initiate efforts to support implementation of these recommendations across Canada.

## CRediT authorship contribution statement

**Mary Kathryn Bohn:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dana Nyholt:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Cynthia Balion:** Writing – review & editing, Methodology, Investigation, Conceptualization. **George Cembrowski:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Christine Collier:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Vincent De Guire:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Victoria Higgins:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Benjamin Jung:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Olivia Landon:** Writing – review & editing, Project administration, Formal analysis, Data curation. **Zahraa Mohammed-Ali:** Writing – review & editing, Methodology, Investigation, Conceptualization. **David Seccombe:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Jennifer Taher:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Albert K.Y. Tsui:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Allison A. Venner:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Nicole White Al-Habeeb:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Khosrow Adeli:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2025.110986.

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