Short Report

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on behalf of the AACB Harmonization Committee for Common Reference Intervals

An update report on the harmonization of adult reference intervals in Australasia

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Abstract: The Australasian Association of Clinical Biochemists (AACB) has over the past 5 years been actively working to achieve harmonized reference intervals (RIs) for common clinical chemistry analytes using an evidence-based checklist approach where there is sound calibration and metrological traceability. It has now recommended harmonized RIs for 18 common clinical chemistry analytes which are performed in most routine laboratories and these have been endorsed by the Royal College of Pathologists of Australasia (RCPA). In 2017 another group of analytes including urea, albumin and arterial blood gas parameters were considered and suggested harmonized RIs proposed. This report provides an update of those harmonization efforts.

Keywords: bias; harmonization; reference intervals.

Introduction

Clinicians use reference intervals (RI) to help in their determination of the patient's clinical state – diseased or healthy. There are often sound scientific and clinical reasons for differences in reference intervals. With progression towards a national e-health framework and a single electronic health record and a need to provide clinicians with results that allow appropriate and reliable clinical interpretation, the Australasian Association of Clinical Biochemists (AACB) has over the past 5 years been actively working to achieve harmonized RI for common clinical chemistry analytes where there is sound calibration and metrological traceability.

In Australasia an evidence-based checklist approach was used to assess the feasibility of using common RIs [1].

Bias between methods could result in the misclassification of patients or the need for method-specific RI. To assess bias the AACB analyzed commutable patient-based samples on multiple platforms using multiple methods. In this study, specified performance limits based on biological variation were applied to determine whether bias would prevent the use of a common RI by assessing if all results fell within the allowable limits of agreement and if regression lines were all within allowable limits for the tested measurement procedure [2]. Of the initial 27 analytes tested, bias was not seen to prevent harmonization in 19 of those analytes [3].

A reference intervals program from the Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP) has demonstrated significant uptake of these reference intervals into routine laboratories [4]. It is relevant to note that 11 of these analytes have certified reference materials and/or reference measurement procedures listed on the Joint Committee for Traceability in Laboratory Medicine database (www.bipm.org/jctlm).

In 2015 after further stakeholder consultation a second group of six harmonized reference intervals for common chemistry analytes was proposed and endorsed for adults [5]. These included alanine transaminase and aspartate transaminase where methods do not use pyridoxal 5-phosphate as an activator and lipase excluding the Ortho Clinical Diagnostics and Siemens Dimension assays. These endorsed RIs are seen in Table 1.

Pediatric reference intervals have also been proposed [6] and in 2015 further working groups were established to look at calculated values, thyroid function tests, human growth hormone and IGF-1, pregnancy electrolytes, lipids and critical values. In 2017 a further group of analytes were discussed and considered as candidates for harmonized

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Table 1: Adult harmonized reference intervals.[a]

Analyte	Male	Female
Sodium	135–145 mmol/L	
Potassium[b]	3.5–5.2 mmol/L	
Chloride	95–110 mmol/L	
Bicarbonate	22–32 mmol/L	
Creatinine[c]	60–110 μmol/L	45–90 μmol/L
Calcium	2.10–2.60 mmol/L	
Calcium (albumin adjusted)	2.10–2.60 mmol/L	
Phosphate[d]	0.75–1.50 mmol/L	
Magnesium	0.70–1.10 mmol/L	
Lactate dehydrogenase [L to P] (IFCC)[e]	120–250 U/L	
Alkaline phosphatase[f]	30–110 U/L	
Total protein	60–80 g/L	
Total bilirubin	1–20 μmol/L	
Creatine kinase	<60 years: 45–250 U/L 60 + years: 40–200 U/L	30–150 U/L
Alanine transaminase (no pyridoxal 5-phosphate)	5–40 U/L	5–35 U/L
Aspartate transaminase (no pyridoxal 5-phosphate)	5–35 U/L	5–30 U/L
Γ-Glutamyl transferase	5–50 U/L	5–35 U/L
Serum lipase[g]	10–60 U/L	

[a]Unless otherwise specified, the intervals are for serum or plasma for adults (18 years and older). [b]This range is proposed for use for both serum and plasma. Laboratories testing only heparin plasma may choose to use a lower interval. [c]Creatinine has harmonized reference intervals for adults up to the age of 60 years. For older ages laboratories may elect to maintain these. [d]Starting at age 20 years to align with pediatric intervals. [e][L to P] (IFCC), lactate to pyruvate method (IFCC method). [f]Starting at age 22 years to align with pediatric intervals. [g]The reference interval for adult serum lipase excludes Siemens Dimension and Ortho Clinical Vitros. There are linear relationships between the 'harmonized' assay group and the Dimension and Vitros: 'Harmonized' = Dimension × 0.21 – 0.6; 'Harmonized' = Vitros × 0.27 + 12.

RIs. These included albumin, urea, and arterial blood gas parameters.

Albumin

Significant differences have been shown between immunochemical and dye binding methods for albumin estimation [7]. This study, along with our own and other investigations show, in general, that bromocresol green (BCG) methods have a high bias when compared with bromophenol purple (BCP). This bias also varies with albumin concentration; however, in our hands comparing 42 serum samples, including 10 replicate samples using the Abbott Architect, Siemens Advia and Dimension, Beckman AU and Dx and Roche Cobas Modular and Integra analytical platforms and proprietary methods undertaken in 28 different laboratories, the bias study showed a difference of around 2 g/L across the range of 23–45 g/L which is shown in Figure 1.

An average difference of 2 g/L was also seen in the RCPA QAP liquid serum chemistry program based on results from over 150 laboratories and commutable samples.

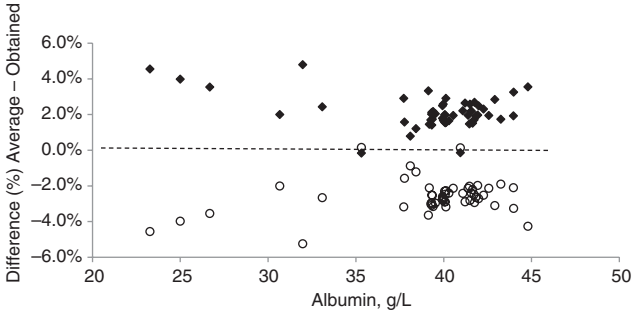

Figure 1: Percentage differences in albumin concentrations observed in the AACB bias study using the BCG and BCP dye binding methods across the concentration range of 23–45 g/L.
The black diamonds represent BCG and the open circles represent BCP. The horizontal dotted line represents zero % difference between the two methods.

The study by Bachmann et al. [7] showed that the mean biases for the BCP methods were smaller in magnitude when compared with the Roche Tina-quant reference measurement procedure (RMP). The BCG methods by contrast had larger and more varied mean biases. The study also showed that none of the BCG methods met minimum performance criteria for bias based on biological

variability over a range of concentrations that would be commonly encountered in routine laboratory practice.

The Aussie Normals study [8] demonstrated age-related changes with median and overall albumin levels being reduced in subjects over 70 years of age. Data from that study has been reformatted to highlight those differences and is shown in Figure 2. These changes are also shown in Figure 3 demonstrating minimal gender differences again using data from the Aussie Normals study reformatted to demonstrate the differences over the age range of 18–92 years using the BCG dye binding method. This transformed data and other data including that from the Sonic Health laboratory database using Bhattacharya analysis as an indirect method derived RIs supporting these observations, has been presented to stakeholders.

Of the approximately 630 laboratories in Australia slightly more than 260 are currently using the BCG dye binding method with the balance, around 370 laboratories,

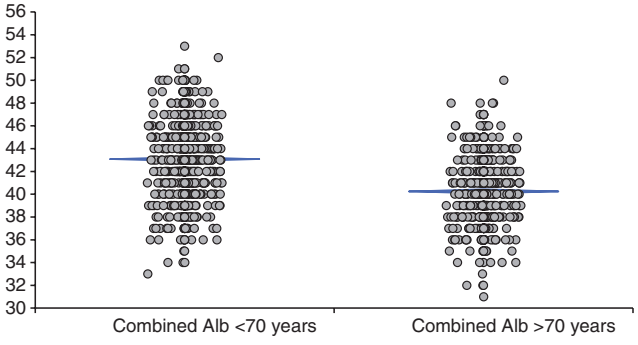

Figure 2: Age related albumin concentrations using the BCG dye binding method seen in the Aussie Normals study [8].
The x-axis represents the combined gender population by age. The y-axis is the albumin concentration in g/L. The horizontal lines are the median concentration in g/L for the population represented.

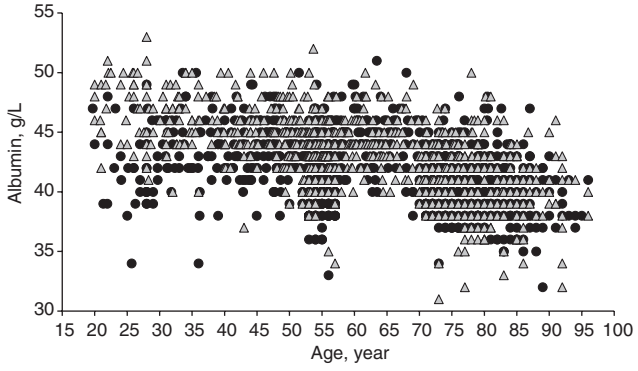

Figure 3: Albumin concentration in g/L by age and gender using the BCG dye binding method seen in the Aussie Normals study [8].
The gray triangles represent males and the black circles represent the females.

using BCP. With this and the observations previously mentioned, the AACB has not proposed a harmonized RI to date but has recommended that all laboratories should consider adopting the dye binding BCP method as their routine method of choice. The AACB has noted that not all instrument manufacturers provide a BCP method for albumin. There is an endorsed RI for total protein but further review of the observed differences in concentrations obtained between serum and lithium heparin plasma is being undertaken including the flagging rates using the harmonized intervals. Until a harmonized RI for albumin is suggested no globulin RI can be considered.

Urea

The Aussie Normals [8] and other studies including data from the Sonic Health laboratory group, a multicenter study undertaken in Turkey [9], the Southeast Asian multicenter study by Ichihara et al. [10] and NHANES 111 [11], have shown gender differences and that there is a progressive increase in concentration and range of concentrations for urea in healthy persons as the individual ages. These increases are at both the lower reference limit (LRL) and upper reference limit (URL).

Analysis of flagging rates using the patient data base from Sonic Healthcare laboratories showed that if a uniform URL of 8.5 mmol/L which is a concentration approaching most quoted URLs was used, young adults had a flagging rate of 4%–6% but elderly patients (>70 years) had a flagging rate of 20%–30%. Conversely, if flagging rates were age specific these flagging rates in the elderly were significantly reduced. If a uniform concentration, that is similar to most quoted LRL, of 2.5 mmol/L was used, very few flags resulted; however, if age related cut-offs were used then the flagging rate returned to around 2.5% across all ages.

Analysis of bias between urea methods [3] demonstrated that bias would not prevent the implementation of harmonized RIs.

The AACB has recommended age and gender related RIs for urea and these are for males: <50 years 3.5–8.0; 50–69 years 4.0–9.0; and 70+ years 4.5–10.0 mmol/L. For females the recommended harmonized RIs are: <50 years 3.0–7.0; 50–69 years 3.5–8.0; and 70+ years 4.0–9.0 mmol/L.

Arterial blood gases

Combined information on RIs in use for arterial blood gas parameters from laboratories across New South

Wales (63 laboratories) and Queensland (35 laboratories), as well as analyzer manufacturers, show good agreement. The difficulty of undertaking a direct study of blood gas and acid-base analysis is acknowledged by the AACB. The AACB has reviewed the analytical performance data from the RCPAQAP. This may be biased by the large number of analysers from one supplier and, as the product is an artificial material, have inherent technical issues including commutability. The scatter of all the results does, however, fall within the manufacturer and laboratory quoted RIs.

The 63 laboratories of NSW Health Pathology have adopted the following arterial ranges and it has been suggested that these be candidates for harmonized RIs for arterial blood gas analysis:

- pH: 7.35–7.45
- pO_2: 83–108 mmHg
- pCO_2: 35–45 mmHg
- Total CO_2: 22–28 mmol/L
- Bicarbonate: 22–28 mmol/L
- Base excess: –3 to +3 mmol/L

RIs for analytes also measured on some blood gas analyzers include the following. It should be noted that due to the sample type and use of an indirect method of determining the blood gas RIs some are different to those for the endorsed serum RIs seen in Table 1.

- Lactate: 0–2.2 mmol/L
- Sodium: 136–146 mmol/L
- Potassium: 3.2–4.9 mmol/L
- Chloride: 95–110 mmol/L
- Ionized calcium: 1.15–1.30 mmol/L

NSW Health Pathology is currently evaluating venous blood gas requests from its state-wide database to develop common RIs that can be implemented across their laboratories and which may also be candidates for Australia-wide common RIs. These actions have been endorsed by the AACB and the proposed RIs will be considered at a later date.

The AACB has now recommended harmonized RIs for 18 common clinical chemistry analytes which are performed in most routine laboratories and have been endorsed by the RCPA. The AACB continues to strive to meet the strategic priority to achieve harmonization where sound calibration and traceability are in place. These harmonized RIs are recommended based on professional opinion and consensus when compared with data from both direct and indirect RI studies with clinical relevance and flagging rates also taken into consideration. Future harmonization workshops have been scheduled where discussions relating to future analytes as candidates for harmonization will be discussed.

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