A depressing feature of this history is the cyclical rediscovery of the toxic effects of lead throughout the millennia. Although the Egyptian, Greek and Roman civilisations were aware of the link, it was forgotten and rediscovered in the Middle Ages and again in the 17th and 18th centuries.[8] Incredibly, the earliest cases of paediatric lead toxicity were described by two Brisbane physicians in 1892. One of these physicians, Dr J. L. Gibson, was later the first to identify the causative link between lead toxicity and household paint.[10,11] This discovery led to the eventual prohibition of lead-based paint in the first world.

The phenotypic presentation of lead toxicity has changed dramatically over the centuries however the adverse effects of lead are well established, even at levels previously thought to be 'low'. Our knowledge of the perils of lead continues to evolve with a recent paper concluding that low-level lead exposure is an important and usually overlooked risk factor for cardiovascular disease mortality.[12] This case raises the question of how many children are being exposed to lead unknowingly in areas thought not to pose a significant risk. The only lead screening programs we are aware of operating in Australia currently are in Port Pirie and Broken Hill.

Alarmingly, more than a century following the discovery of paediatric lead toxicity we are still being exposed to lead both from mining and smelting in some of our communities but also insidiously in homes Australia-wide.

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Is total iron binding capacity (TIBC) calculation correct?

Sir,
As part of serum/plasma investigations to determine iron status, total iron binding capacity (TIBC) is an important calculation to determine percentage transferrin (TRF) saturation. TRF saturation may be the earliest indicator of iron overload and a low TRF saturation in the setting of an equivocal ferritin level is suggestive of iron deficiency. Serum/plasma TIBC (µmol/L) values could be either obtained from a calculation or measured by the amount of iron required to saturate the specimen. When TIBC is determined by calculation, the iron transport protein, TRF concentration (g/L), is multiplied by a conversion factor. Calculation has taken molecular mass and iron binding capacity of TRF into consideration to generate TIBC results in µmol/L.

The principle author noted that their laboratory results were quite high compared to other laboratories in the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) in 2017. TIBC is reported in the General Chemistry and Therapeutic Drugs and in the Liquid Serum Chemistry programs. According to RCPAQAP, allowable limit of performance (ALP) is set as ±4.0 µmol/L up to 50.0 µmol/L; ±8% >50.0 µmol/L. The author noted when medians of TIBC were 46 g/L and 66 g/L in one interim report, the author's laboratory results were 54 g/L and 87 g/L, respectively. The same pattern was seen across other interim reports as well. Further analysis revealed differences in TIBC conversion factors. The conversion factors of 25, 23, 22 and 'other factor' were listed as possible options to obtain the TIBC from TRF level. Factor 25 has been employed by the principle author's laboratory. By 2018, of all 37 laboratories reporting TIBC in RCPAQAP, 23 used this calculation. The rest of the results were generated by direct measurements. Vitros (Ortho Clinical Diagnostics, USA), AU 5800 (Beckman Coulter, USA) and Integra (Roche, Switzerland) used factor 25; whereas factor 23 was used by Cobas c (Roche) and UniCel DxC600 (Beckman Coulter). The 'other factor' was used mainly by Architect (Abbott, USA) and by one Cobas c user. The 'other factor' group comprised the major group with 18 laboratories in 2018. This cohort created two groups generating higher and lower results. The results of the 'higher subgroup' which was only three laboratories were seen above the RCPAQAP allowable performance specification (APS) limits. The result of factor 25 was seen among 'higher subgroup' results, whereas the results of the 'lower

subgroup' were well within APS and amongst other laboratory results of factor 23 and of direct TIBC measurement. Because the factor 25 group and higher 'other factor' subgroup were in the minority, with only four results reported on RCPAQAP, these results were seen as outliers.

In 1992, Beilby *et al.*[1] found that 30 Australian laboratories out of 140 laboratories enrolled in RCPAQAP used a conversion factor to produce a TIBC from TRF measurement. Five laboratories multiplied the TRF concentration by 26.1, another five laboratories used 20.2, and the remaining laboratories used factors ranging from 20.5 to 26.1. This also shows the diversity of factor used at that time for the TIBC calculation.

The conversion factor for calculation of TIBC depends on molecular mass and iron binding capacity of TRF. The literature favours factor 25 mostly.[2,3] Accordingly Yamanishi *et al.* argued that TIBC correlates well with TRF concentration, and they came up with the formula: TIBC (μmol/L) = 25.1 × TRF (g/L).[3] Kasvosve and Delanghe pointed out that even though correlation between TIBC and TRF is generally considered good, conversion factors between the two analytes found in the literature show large differences.[2] They suggested the formula TIBC (μmol/L) = TRF (g/L) × 25.2 as 1 mol of TRF (molecular mass 79,570 Da) has the capacity to bind two atoms of iron.[2] Even though 90,000 Da was considered the molecular mass of TRF during early estimates,[4,5] a more recent estimate based on the amino acid sequence gives a calculated molecular mass of 79,570 Da.[2,3,6] This explains that the uncertainty of the molecular mass of TRF has led to the use of different conversion factors. For this reason, manufacturers have recommended experimentally determined factors instead of the theoretically derived one.[7] Beilby *et al.* found that the 33 Australian laboratories quoted molecular masses to make this conversion. The values used ranged from 76,500 to 99,000, with the most frequently used values being 76,500 (8 laboratories) and 88,000 (4 laboratories).[1]

Interestingly both TRF and iron are standardised assays. The database of higher-order reference materials, measurement methods/procedures and services (JCTLM) lists ERM-DA470k as the Certified Reference Material for TRF. With the introduction of the international CRM 470, a significant reduction in inter-laboratory variation for TRF measurements was noted.[2] Likewise the National Institute of Standards and Technology (NIST) has listed SRM 937 as the standard reference material for iron. The principle author discovered that her laboratory methods for TRF and iron are traceable to those higher order reference materials.

Why should we be concerned about TIBC calculation? TIBC is important for calculation of percentage TRF saturation, another calculated parameter. It is derived from the formula: % TRF saturation = (iron/TIBC) × 100. The 'Iron Studies Standardised Reporting Protocol'[8] first published in 2013 by the Royal College of Pathologists of Australasia (RCPA) and the position statement[9] 'The Use of Iron Studies, Ferritin and Other Tests of Iron Status' published again by RCPA in 2017, emphasise the importance of TRF saturation in iron overload and iron deficiency diagnosis and monitoring. A raised percentage TRF saturation in isolation may be the earliest indicator of iron overload. TRF saturation greater than 45% is considered the cut-off for iron overload. Furthermore, the position statement points out that 'a low TRF saturation in the setting of an equivocal ferritin level is suggestive of iron deficiency'. However, if there is no agreement on which factor to use for TIBC calculation how can the percentage TRF saturation be comparable among laboratories?

Did we have to harmonise the TIBC calculation before imposing TRF saturation decision levels? APS defined for TIBC by RCPAQAP is 'minimal total error', not desirable or optimal. Accordingly, APS of TIBC is just satisfactory. The preliminary aim of employment of 'total error' quality standard is to assess the capacity of laboratories to share or harmonise reference intervals. Total error quality standard assesses the bias in a test method. Due to use of different factors for TIBC calculation even the minimal standards are not met by some laboratories. As there is a harmonisation initiative commenced by the Australian Association of Clinical Biochemists (AACB) and the RCPA for calculated parameters, is it time to harmonise TIBC calculation? The suggestion that the relationship between TIBC and TRF is not fixed, especially when results are outside the reference range, should not be ignored.[7,10,11] There should be further evaluation of the possibility of development of a universally agreed conversion factor for the determination of TIBC from TRF level.

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