Harmonisation of adjusted calcium equation, is it a realistic aim: a narrative review

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Background and Objective: A prerequisite for harmonisation is the recognition and elimination of source(s) of variability in the system we aim to harmonise. The albumin-adjusted calcium equation is widely used and deeply rooted in current clinical practice. However, there are a number of well-recognised, scientifically-sound reasons and sources for variability in adjusted calcium determination. In this article, we review these factors contributing to adjusted calcium variability and explore a number of potential approaches to harmonisation.

Methods: A search of EMBASE, Medline and PubMed Database was conducted with language restriction from the earliest publication date available to January 31, 2022.

Key Content and Findings: The source of variations was summarised in three main categories related to the cumulative variation acquired from calcium/albumin measurements, the mathematical method(s) for equation derivation using these measurements, in addition to the inherent variability built-in the concept of adjusting calcium to albumin level.

Conclusions: Variability in adjusted calcium results can be reduced by adopting a world-wide unified approach to several variables included in the adjusted calcium measurement. Further work is required for linearity of the range over which adjustment is made. However, standardisation of adjusted calcium is not achievable without improving the accuracy of albumin methods. Improvement work requires the collaborative efforts of laboratory medicine professional bodies and diagnostic companies.

Keywords: Albumin adjusted calcium; corrected calcium equation; total calcium; harmonisation; standardisation

Introduction

The establishment of interchangeability (harmonisation) of biochemical test results across laboratories is an essential and important responsibility of laboratory medicine professionals. This is especially true for establishment and running effective clinical networks, where patients may receive laboratory results from multiple laboratory providers within the same network, and hence the availability of valid interchangeable test results is crucial. In the absence of harmonisation, meaningful integration of test results from different laboratory providers into an electronic patient record (EPR) is impossible. Indeed, for the last two decades, there has been a global drive to harmonise all aspects of the laboratory testing processes.

The measurement of serum or plasma calcium is a very commonly requested laboratory test. In serum, around 40% of the calcium is bound to protein (principally

albumin), about 10% is complexed with small anions with the remaining (around 50% of the calcium) being in the free ionised form. Although it is only the free ionised calcium that is biologically active, the direct measurement of this fraction is complex and generally not available on high throughput laboratory analysers. In contrast, the measurement of total calcium and albumin is straightforward, and widely available. There has, therefore, been a long-standing considerable interest in the concept of adjusting the serum/plasma calcium concentration to the albumin level to allow for a more accurate assessment of the patient's calcium status. The practice of reporting 'adjusted' calcium alongside total calcium and albumin levels is widely implemented in UK laboratories and across the world. The adjustment equations used to allow this practice, have generally been derived from the linear regression of calcium concentration on albumin concentration (1). There are, however, significant differences between the various reported adjustment equations commonly in use. This variability may clearly relate both to the differences in the analytical techniques employed for calcium/albumin measurements and to the various methods of derivation of the linear regression equation. Additionally, and perhaps more importantly, this variation can be related to the 'case-mix' of participants from whom the albumin and calcium results were used to generate the linear regression equations.

Consequently, it is unsurprising to recognise and dissect the evidence for the clinically-significant between-laboratory variation in adjusted-calcium results, which has been reported on a single serum pool (2,3). This variability has the potential to have an adverse impact on clinical outcomes and patient safety. Therefore, there is an urgent need for harmonisation of the whole process of determining and reporting the adjusted calcium levels.

In this article, we discuss the theoretical basis for adjustment of calcium level, we review the different methods for determination of adjusted calcium, the factors contributing to its variability and we explore a number of potential approaches to harmonisation. We present the following article in accordance with the Narrative Review reporting checklist (available at https://jlpm.amegroups.com/article/view/10.21037/jlpm-22-56/rc).

Methods

The focus of this review was assessing the degree of current variation in the calculation, measurement and reporting of adjusted calcium, whether adjusted calcium can potentially be harmonised and what further work may be required to achieve this aim. For this purpose, we performed a search (from the earliest publication date available to January 31, 2022) on the following electronic databases: EMBASE, Medline and PubMed. Search terms covered combinations of expressions used in association with corrected calcium equation (e.g., corrected calcium, adjusted calcium, total calcium, calcium equation, calcium formula, calcium algorithm, albumin adjusted calcium, albumin corrected calcium and protein corrected calcium). We searched for the above term in the abstracts only. The search was restricted to English language publications only and we excluded animal's studies. The initial search resulted in 141 publications, which included research publication, letters and editorials (*Table 1*).

Another search for the analytical technologies and reference interval for albumin and calcium measurement was conducted (e.g., albumin methods, standardisation, calcium method, standardisation) (calcium reference interval, calcium reference range). Restricting the search to English language only, the secondary search resulted in 35 publications. Titles and abstracts were first screened, followed by full text review of relevant studies. Reference lists were also hand searched for potential inclusion. Following the initial screening phase, publications evaluated the full texts to assess their eligibility for inclusion in the review. The publications of interest were classified into the following categories: (I) adjusted calcium concept studies; (II) calcium and albumin methods/performance/standardisation; (III) linearity studies; (IV) calcium reference range studies.

Albumin adjusted calcium—a brief history

The adjustment of calcium is based on the generation of a regression equation of calcium concentration on albumin concentration (4). This commonly involves the retrieval of albumin and calcium results from a laboratory information system. The regression coefficient of calcium on albumin is then calculated using the intercept from the regression at zero albumin concentration as the non-protein bound calcium (*Figure 1*).

The adjustment equation is then usually given as:

$$
\begin{aligned}
\text{Adjusted calcium}\,(\text{mmol/L}) = {} & \text{total calcium}\,(\text{mmol/L}) \\
& -\left[\text{slope} \times \text{albumin concentration}\,(\text{g/L})\right] \quad [1] \\
& +\left(\text{mean total calcium} - \text{intercept calcium}\right)
\end{aligned}
$$

Table 1 The literature search process

Terms	Specifications
Date of search	31 January 2022
Databases and other sources searched	EMBASE and Medline
Search terms used	calcium.af AND (total.ti,ab OR serum.ti,ab OR ionised.ti,ab OR ionized.ti,ab) AND (corrected.ti,ab OR correction.ti,ab OR adjust*.ti,ab) AND (equation*.ti,ab OR formula*.ti,ab OR estimation.ti,ab OR algorithm*.af) AND ("comparative stud*" OR "validation stud*" OR "evaluation stud*" OR "intermethod comparison*" OR "case control stud*" OR review*).af; 141 results
Timeframe	Earliest publication date available to 31 January, 2022
Inclusion criteria	Human studies, English language only
Selection process	The generated list was screened for relevance by N Jassam. The list of ultimately included publications was decided by both authors with no disagreement
Any additional considerations, if applicable	Publications for ionised calcium were excluded after the revision for suitability first
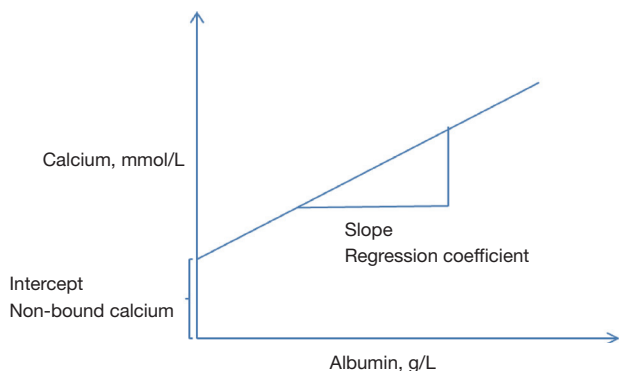
Figure 1 The regression of calcium on albumin.

The mean total calcium was generally taken as the mid-point of the total calcium reference interval, which was 2.40 mmol/L at the time of derivation of Payne's equation.

This equation is usually re-arranged mathematically to give rise to the most common format of adjusted calcium equation which is:

$$\text{Adjusted calcium}\,(\text{mmol/L}) = \text{total calcium}\,(\text{mmol/L})$$
$$- \text{slope}\big[\text{measured albumin}\,(\text{g/L})\quad [2]$$
$$- \text{derived albumin value}\,(\text{g/L})\big]$$

where 'derived albumin value' refers to the albumin concentration around which the calcium will be adjusted.

In the early 1970s, at least three well known equations for the 'adjustment' of total calcium for serum albumin were published: Payne *et al.*, 1973 (4), Berry *et al.*, 1973 (5), Orrel, 1971 (6).

The equation generated by Payne, in particular, has been widely used and is given as:

$$\text{Adjusted calcium}\,(\text{mmol/L}) = \text{total calcium}\,(\text{mmol/L})$$
$$- 0.2\big[\text{measured albumin}\,(\text{g/L}) - 40\big]^{[3]}$$

However, it quickly became apparent that different equations gave rise to different values for the adjusted calcium concentration (7).

Sources of variability in adjusted calcium determination

The documented between-laboratory variation in adjusted calcium results (2,3) relates to a number of factors:
- ❖ Variation acquired from calcium and albumin measurement;
  - ◆ Differences between assays in total calcium measurement;
  - ◆ Differences between assays in albumin measurement.
- ❖ Variation built in the method of equation derivation;
  - ◆ Differences in the derivation of the linear regression equation of calcium on albumin;
  - ◆ Differences in the albumin concentration range over which adjustment is made.

❖ Variation intrinsic to the adjusted calcium concept.
  ◆ Differences in the case-mix of the population from which the linear regression equation is derived.

We will consider each of these points in turn.

Variation acquired from calcium and albumin measurement

Total calcium measurement

The analytical performance of commercially available and commonly used calcium assays has been widely considered acceptable for various analytical platforms such as Abbott Architect, Roche Modular, Roche Cobas and Artho Vitros (8). In contrast, the Siemens' calcium assays demonstrated significant concentration dependent bias related to the use of non-commutable calibrator (NIST SRM 915) (9).

Milan's Model for precision specifications recommended a total acceptable variation for calcium measurements between 0.91–1.36% (10). While there has been a substantial improvement in the imprecision of calcium measurement, it is debatable whether current assays are sufficiently precise for clinical purposes. Using commutable samples, it has been found that total calcium varies by a 3% difference at concentrations <2.20 mmol/L and a 5% difference at concentrations >2.55 mmol/L (3). This finding was further confirmed by other studies, where commutable samples and reference methods were built in the study design. A large calcium total error of 6.5% has been reported, which is exceeding that of acceptable biological variation, across a larger number of various analytical platforms (11,12). Although total calcium has a well-established traceability system, the findings from these studies prompted the authors to call for further optimisation of calcium assays.

The total variation of adjusted calcium is even more pronounced and is significantly wider than that of total calcium. Data from a recent UK audit [2020] showed between laboratories variation of 11%, this is equivalent to a variation of 0.24 and 0.29 mmol/L at the lower and upper limits of the reference range (2.2–2.6 mmol/L) respectively (2).

Albumin measurement

In diagnostic laboratories, serum albumin concentrations are most commonly measured using dye-binding assays, with either Bromocresol Purple (BCP) or Bromocresol Green (BCG). The poor performance of albumin methods is well documented and repeatedly reported as analytically and clinically unsatisfactory (13-15). This has been attributed to a number of factors including a suboptimal standardisation method, between methods imprecision and the poor specificity of BCG methods (15-17).

BCG reacts not only with albumin, but also with α-globulins, haptoglobin and immunoglobulins, resulting in overestimation of albumin concentrations in disease processes that elevate these globulin concentrations in serum. Using albuminaemic commutable samples, BCG method reported results of 14 g/L higher than the nephelometric method results of 0.27 g/L (17).

It has been well recognised that there is significant analytical bias between BCG and BCP methods (15,16). As the difference between albumin methods could be as much as 10 g/L (18). Irrespective of analytical platform, BCG methods in particular, had a larger and more varied mean biases (5–13.9%), with all methods failed to meet the minimum performance criteria for bias based on biological variation limits (2.1%) (19). In one study, the positive bias of BCG methods reduced adjusted calcium concentration by 0.15 mmol/L across the entire range (15). A multi-platform comparative study demonstrated that BCG methods bias is concentration dependent as it varied from 5% at albumin concentration >32 g/L to 10% at albumin concentration <25 g/L (16,18).

In contrast, literature data show that the BCP methods had better selectivity for albumin and less between-methods bias (16). Nonetheless, these BCP methods had their own share of criticism as having been frequently found negatively biased in uremic renal failure patients. However, recent manufactures claim indicated that a new version of BCP, so called the modified BCP (mBCP), do not suffer this uraemia interference (13).

Furthermore, the uncertainty associated with albumin measurement, even after eliminating the bias component, is two times higher than the minimum goal for albumin derived from biological variation model (<2.33%) (20). Although nephelometric-based method and ERM-DA470k/IFCC materials were accepted by the Joint Committee on Traceability in Laboratory Medicine (JCTLM) as a reference measurement system for albumin, albumin standardisation and traceability transfer are still suffering from clinically unacceptable uncertainty and they definitely require further optimisation on an international scale (20,21).

Several studies have confirmed that adjusted calcium is

substantially influenced not only by the choice of BCG or BCP methods but also by the specific implementation of a given analytical platform. Subsequently, variability generated from the choice of the albumin method is probably the largest attributing factor to variability in adjusted calcium results. It undoubtedly has implications on the patient's calcium classification status and has to be regarded as a major source of adjusted calcium variability (16,22).

Variation built in the method of equation derivation

Differences in the derivation of the linear regression equation of calcium on albumin

The fact that adjusted calcium is a mathematical equation based on the regression of albumin on calcium would give rise to multiple sources of variations related to method of calculations, statistics used, analytical and pre-analytical factors.

Different analytical techniques and platforms are available for the measurement of calcium and albumin. Reports demonstrated that the regression coefficients values are influenced by methodology used to measure calcium and albumin (23,24). Barth *et al.* and Ashby *et al.* demonstrated differences in regression of calcium on albumin with data collected from different analytical platforms even when the same analytical method had been used (23,25). This difference indicated that variability between local operational protocols and quality indices might partly contributed to the variability of regression coefficient between laboratories (26). This finding has been reproduced world-wide and it was the underlying reason for the recommendation that laboratories derive an equation using their own local platform and methods (27-29).

The most widely used Payne's adjusted calcium equation, has been calculated using a mid-point of the local calcium reference range 2.2–2.60 mmol/L, that is 2.40 mmol/L (30). This adjusted calcium equation was reproduced by many laboratories world-wide using the same recommended value of 2.40 mmol/L rather than the mid value of the locally derived reference range. Evidence of calcium reference ranges variation exists. Among harmonisation initiatives, the Australian Association of Clinical Biochemistry (AACB) proposed a harmonised calcium reference interval wider than that of the UK Pathology Harmony (31,32). In turn, another UK-based perspective study of 300 healthy individuals from the Yorkshire region, showed that calcium intervals for Abbott Architect, Beckman DXI and Roche

were all in good concordance with the UK Pathology Harmony reference interval of 2.2–2.6 mmol/L with the exception of Siemens Advia (2.12–2.51 mmo/L) (33). This difference was attributed to the use of a non-commutable calibrator on Siemens calcium assays (9). Other large national studies, the "Nordic Reference Interval Project (NORIP), the Nationwide Turkish Reference Intervals study and NUMBER reference intervals (Netherlands)" in which harmonisation of measurement methods with commutable calibrator was ensured before the measurement of healthy volunteers samples (34-36). Nevertheless, the reported reference interval for calcium was significantly different from the other initiatives indicating impact of population on reference intervals. Yamamoto and colleagues looked at the impact of population differences across Japan on calcium reference intervals (37). This study recommended a single reference interval for calcium regardless of age or gender differences. On the contrary, Carlsson and colleagues demonstrated higher upper limit for calcium reference interval in a population of 70 years old without cardiovascular disease (CVD) or hyperparathyroidism (38). In conclusion, while there are several initiatives to harmonise the calcium reference range nationally, variations attributed to calibration differences (39), population differences and age exist. Therefore, the mid-point values for calcium reference intervals (*Table 2*) varies from 2.29–2.45 mmol/L. In no doubt, imposing a value of 2.4 on a population with a lower mid-point value will shift results of adjusted calcium away from the normal range and mask hypocalcaemia. The reverse is true if reference interval mid-point is higher than population mean. It worth mentioning that, while normalising an equation to a fixed value may correct for calibration systemic bias, proportional bias would certainly be overlooked. Therefore, the practice of replacing the use of a local population calcium mean by a fixed value will disregard calibration, methodological and population differences. Imposing a fixed value would also result in a different "derived albumin value" (Eq. [2]) than the actual "population albumin mean". If the "albumin derived value" in an adjusted calcium equation is not the same as the local "population albumin mean", the equation will misestimate calcium status in this population (22,39). This concept explains the shortfall of adjusted calcium equations when a common regression model is applied to populations with different albumin means. Example of that; BCG versus BCP (40), different albumin concentrations among different clinical settings, e.g., ambulant versus

Table 2 Variation in calcium reference intervals

Study	Calcium interval (mmol/L)	Reference
Australian adult RI	2.10–2.60	Koerbin *et al.* (31)
The Harmony, UK	2.20–2.60	Berg *et al.* (32)
Yorkshire RI, UK, Siemens Advia	2.12–2.51	Jassam *et al.* (33)
Nordic RI project (NORIP)	2.17–2.51	Rustad *et al.* (34)
Turkish nationwide RI Study	2.12–2.47	Ozarda *et al.* (35)
NUMBER RI study (Netherlands)	2.18–2.55	den Elzena *et al.* (36)
Japan RI harmonisation project	2.20–2.50	Yamamoto *et al.* (37)
Reference Intervals for 70-year-old; Abbott Architect	Male: 2.17–2.66; female: 2.18–2.70	Carlsson *et al.* (38)

RI, reference interval.

hospitalised patients (41) and albumin mean difference related to different age groups (42,43).

Many laboratories worldwide use published equation rather than a locally derived one. It's known that most of the published equations have been derived using BCG albumin methods including Payne's equation. A recent Australian survey, of the approximately 630 participated laboratories, showed a clear unequal split between the BCP and BCG users. Notably, only 260 were using the BCG dye binding method at the time of that survey. Knowing that albumin concentration can be significantly different between BCG and BCP; this renders the use of published equations inappropriate in most clinical laboratories today and is clearly a major source of inaccuracy. In fact, a recent Australian study showed that Eq. [3] above gave adjusted calcium reference range 2.14–2.67 mmol/L wider and higher than total calcium reference ranges agreed by the ACBB and UK-Pathology Harmony 2.10–2.60 and 2.20–2.60 mmol/L respectively, indicating a possible misestimation of correct calcium status (44). This overestimation of calcium status by the original equation was confirmed by others (24).

The statistical methods of the first generation of calcium equation derivation were all based on the least squares linear regression. Payne, however, argued that the use of Deming regression is a more statistically valid approach (45). Different statistical methods may introduce a small variability in the value of slope and the intercept, and while the difference may not be clinically significant, it certainly adds to the total variation.

The number of data points used to generate regression equations has varied significantly, from one hundred to thousands of data points (1,6,28,41). The number of the

data points included may have a statistical impact on the regression value and consequently on the adjusted calcium result generated. This was the underlying reason for the Association of Clinical Biochemistry (ACB-UK) recommendation for a minimum 1,000 data point for equation calculation (46).

Pre-analytical factors are also of importance. Albumin concentration in blood is affected by venous stasis and the prolonged use of tourniquets may alter total calcium concentration due to the rapid increase of protein in the blood sample (47). Equations have differed in their approach to this; for example; Orrel's but not Payne's equation excluded data from patients where blood samples were collected with tourniquets (6). Including patient samples with possible venous stasis may partly explain the difference between Payne's and Orrel's equations. Therefore, local pre-analytical protocols may affect the quality of data used for the calculation of adjusted calcium equation.

Differences in the albumin concentration range over which adjustment is made

The concept of linear regression of calcium on albumin is based on the assumption of a constant coefficient of calcium binding to albumin at the physiological range 30–50 g/L (48). Outside this range, in particular at a concentration <20 g/L, evidence showed that calcium binding affinity to albumin increases significantly, leading to a higher proportion of hypoalbuminaemic subjects having a calculated adjusted calcium concentration within the healthy population reference interval in spite of having a low ionised calcium concentration (49). The reverse is correct at a concentration >50 g/L with a decrease in the binding affinity of calcium to albumin. There is a broad

consensus therefore that it is not acceptable to adjust calcium for albumin concentrations over a threshold of 50 g/L. In contrast, there is no agreement on the lower threshold. A number of studies recommended linearity range of 30–50 and 32–50 g/L, below which the regression of calcium to albumin is not constant (25,49). Barth *et al.*, however, statistically proved that linearity relationship of calcium to albumin can be extended to 16–50 g/L (23). Some laboratories have made pragmatic recommendations for a wider linearity range of albumin concentrations (10–50 g/L, local data) and this approach has been endorsed by others (50). In practice, there are differences between laboratories in the albumin concentration range over which adjustment is made, a difference that would contribute to the overall variability in adjusted calcium determination.

This lack of agreement on a harmonised linear range of the calcium adjustment is further complicated by different methods for albumin measurement. Almost all linearity studies established the relationship between calcium and albumin were measured by the BCG method, including Payne's equation, with the exception of Smith et al, in which the BCP method has been used (51). Therefore, confirmatory studies in particular with BCP albumin methods are required.

Variation intrinsic to the adjusted calcium concept

Differences in the case-mix of the population from which the linear regression equation is derived

Since its inception, adjusted calcium equation has been widely criticised (52,53). The albumin adjusted calcium concept was deemed suitable for clinical practice based on the assumption of no intra-individual variability in the binding affinity of albumin to calcium (54). This assumption was challenged, as a considerable intra-individual variation has been found (55,56). Individual regression factors have been reported to vary in health between 0.013 and 0.044 and this variation is expected to be more pronounced in disease. This variation has been reported to be statistically and clinically significant. Moreover, several reports demonstrated that the regression coefficients values are influenced by the population from which the equation is derived, e.g., various patient groups (45), age (paediatric, adult, elderly). Weaving *et al.* demonstrated that albumin concentrations change with age. The authors challenged the concept of using a single regression factor to adjust calcium for various age groups (57,58). The use of an

adjusted calcium has been questioned in critically ill patients (53,59), cancer patients (60), renal failure patients; pre-dialysis (61) and end stage renal failure (62,63). A study by Payne suggested that the regression of total calcium on albumin may also differ in the pregnant population and in menopause (64-66). This illustrates the potential influence of population differences and patient mix on the adjustment equation, which would render the application of a common regression coefficient to different populations and clinical settings a source of inaccuracy. Literature evidence showed that an equation derived from dialysis patients and applied to them outperformed the conventional adjusted calcium equation (67). The poor performance of adjusted calcium equations has prompted attempts to modify existing equation by inclusion of a population specific parameter; such as the inclusion of phosphate in equations for renal failure patients (68). Another attempt showed an equation derived from/and applied to ambulant patients compared well with ionised calcium and performed better than Payne's equation (41). These studies introduce a new practice, which is called "A population specific equation", a concept that requires further exploration, nonetheless emphasizes the importance of case-mix on the adjusted calcium equation performance.

There is ample evidence in the literature demonstrating the limitations of the current format of adjusted calcium equation. Nevertheless, adjusted calcium remains in use due to the lack of a practical replacement and pragmatically due to the ease of its measurement and familiarity with its interpretation.

The way forward

Table 3 presents factors affecting adjusted calcium results. It is important that the causes of variability in adjusted calcium are identified, addressed and eliminated if possible. Considering that standardising adjusted calcium equations is not currently attainable without eliminating the albumin and calcium calibration issues, minimising other sources of variability is the only possible way forward at this point of time. Drawing on knowledge of two initiatives for harmonisation of adjusted calcium by the ACB-UK and the AACB-Australia; we conclude that a similar approach is required until the equivalence of results produced for calcium and albumin is assured by a high metrological standardisation system.

For example, due to the problematic standardisation of

Table 3 Causes for variability in adjusted calcium results

Variables	Factors contributing to adjusted calcium variability
Calcium	Methods: Arsenazo III, CPC, NM-PABTA
	Method traceability
	Analytical performance
	Reference interval
Albumin	Methods: BCG *vs.* BCP
	Method traceability
	Analytical performance and method selectivity
	Age and gender
Data collection and sifting criteria and equation derivation	Payne's equation *vs.* other equations
	Inclusion and exclusion criteria
	Statistical method for equation derivation
Population	Hospitalised *vs.* ambulant population
	Children, adult and elderly
	Various patient groups and case mix

CPC, cresolphthalein complexone; NM-PABTA, 5-nitro-5'-methyl-(1,2-bis (o-aminophenoxy) ethan-N, N, N', N'-tetraacetic acid. BCG, Bromocresol Green; BCP, Bromocresol Purple.

albumin methods, coupled with the widespread use of the poor specificity BCG assay, the AACB recommended that laboratories adopt the dye binding BCP method as their routine method of choice. The AACB also recommended no harmonisation for the albumin reference interval at this stage (69). Considering that, the use of assay specific reference interval is a safer practice as the current harmonised reference interval for albumin may mislead users away from the concentration difference between these methods. In the context of adjusted calcium, the use of a single and a more specific method such as the BCP will be the right move for achieving interchangeable results for adjusted calcium.

Finally, the large number of variables in the adjusted calcium derivation process makes it important that all steps of the process included in the derivation of the adjusted calcium equation are considered and described in sufficient details to laboratories professionals to fully understand the equation derivation method. Indeed, that was what the ACB-UK attempted in 2015. With the aim to reduce variability in adjusted calcium reporting, the ACB-UK

produced guidelines for standardising the adjusted calcium equation calculation (46). The guidelines described the method of equation calculation, recommended a minimum data point (1,000 data point at least) to be used for equation derivation and specified the siftings criteria for post data collection handling with a national recommendation for laboratories to locally derive and monitor the performance of their own equation. Up-to-date, no national audit has been performed to demonstrate the impact of these guidelines on the adjusted calcium variability. A final note; it is premature to embrace a common reference interval for adjusted calcium without further optimisation for albumin and calcium method standardisations, hence the UK Harmony and the AACB, recommended that the adjusted calcium reference interval should be the same as the total calcium.

These initiatives demonstrate that harmonisation of adjusted calcium measurements is a consideration, but further work is needed. International professional bodies such as the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the European Federation of Laboratory Medicine (EFLM) are in a good place to lead on production of a consensus document and drive further optimisation of the standardisation of both calcium and albumin methods. Though adjusted calcium is an old practice, there is a great role for education to disseminate knowledge about its limitations and opportunities to improve its practice. *Table 4* gives recommendations for adjusted calcium harmonisation for consideration of readers, researchers, diagnostic companies and professional bodies.

Conclusions

The accuracy of adjusted calcium results affected by several variables collectively lead to significant variation in adjusted calcium measurement. Therefore, accurate reporting and classification of calcium status requires a unified approach for the derivation of the equation, consensus over the linearity range applied, and standardisation of the measurements of albumin and calcium. Confirmatory studies are required for linearity of the range over which adjustment is made. However, the clinical relevance of albumin method on adjusted calcium cannot be overstated. Unless the lack of specificity and poor standardisation of albumin methods issue is resolved, harmonisation of adjusted calcium is hard to achieve.

Table 4 List of recommendations for adjusted calcium harmonisation

No.	Recommendation	Further work
1	Laboratories continue to derive their own equation	Descriptive guidelines cover the whole process from data collection to method of calculation
2	Agree a linearity range for albumin on calcium regression	Studies for BCP linearity range assessed preferably against ionised calcium are required
3	Promote the use of the modified-BCP method	Professional bodies: to promote the profile of the modified-BCP method in a similar manner to that used to raise the profile of the enzymatic creatinine assay
		Diagnostic companies: to improve the standardisation and precision of albumin and calcium methods
4	Harmonisation of calcium reference interval	Systematic review for calcium reference intervals in the view of harmonisation
5	To explore the age-related adjusted calcium equation and population specific adjusted calcium	Further confirmatory work is required

BCP, Bromocresol Purple.

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