Asian Journal of Medicine and Biomedicine
eISNN: 2600-8173
https://journal.unisza.edu.my/ajmb

PENERBIT Universiti Sultan Zainal Abidin

Asian Journal of Medicine and Biomedicine, Vol 3:2.

Original Article

Open Access

Outcome of Comparison Study Of Various Equations For Serum Osmolality

Saraswathy A[1], Pavai S[2], Nadiah Z[3], Putri Junaidah MY[2]

[1]Endocrine Unit, Specialised Diagnostic Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia
[2]Department of Pathology, Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur, Malaysia
[3]Department of Pathology, Hospital Sungai Buloh, Jalan Hospital, 47000 Sg Buloh, Selangor, Malaysia

saraspine@gmail.com

Abstract

Background: Serum osmolality measured by cryoscopic technique in laboratory is the reference method.In clinical settings,serum osmolality measurement is not feasible at bedside. In normal subjects, sodium, potassium glucose, and urea are the primary circulating solutes. These solute concentrations can be used to predict measured osmolality if no other solutes present at high millimolar concentrations. Many equations of serum osmolality have been proposed. The osmolal gap (OG) is the difference between measured osmolality and calculated osmolality. The major use of OG is to screen for presence of exogenous toxic substances and to screen alcohol intoxication cases. **Aim/Objective**: The purpose of this study was to compare the calculated osmolality using various formulae with the measured osmolality to determine which calculated formula fit best with measured osmolality. **Materials and Methods**: Serum osmolality results from January 2015 to December 2015 were extracted from the laboratory information system (LIS). Serum osmolality performed simultaneously with renal and liver function tests, serum electrolytes and plasma glucose were included. Serum osmolality measured for patients with the history of drug abuse and poisoning were excluded from the study. 405 serum osmolality results were chosen and divided into two groups. Group 1 included 205 data with normal serum osmolality, renal, liver function tests and plasma glucose level less than 7.8 mmol/L. For the second group (n=200), data with low serum osmolality (n=90) and high serum osmolality (n=80) and normal serum osmolality (n=30) were included. Group 1 data was to identify which equation correlated with the measured osmolality and the Group 2 data to study the performance of equation that correlated with the measured osmolality.
Results: Only four out of 19 formulae were identified as optimal by having the mean OG $\leq$ 2 mOsm/kg. The Smithline-Gardner formula (2Na+ Glu + BUN) showed the smallest osmolal gap with mean bias 0.3 mOsm/kg. The Dorwart-Chalmers formula incorporated in most autoanalysers for calculation of osmolality underestimated compared to measured osmolality. **Conclusion:** We recommend Smithline-Gardner formula for calculation of osmolal gap, as the OG gap is close to zero, simple, easy to calculate at bedside and easily incorporated in the Laboratory Information System.

Keywords: measured osmolality; calculated osmolality; osmolal gap; regression analysis
*Author for Correspondence
Received (April 6th, 2019), Accepted (Oct 2nd, 2019) & Published (Oct 31st, 2019)
Cite as: Saraswathy, A., Pavai, S., Nadiah, Z. & Putri Junaidah, M.Y. (2019). Outcome of Comparison Study of Various Equations for Serum Osmolality. *Asian Journal of Medicine and Biomedicine* , *3*(2), 4-9.

Introduction

Osmolality is a measure of the number of dissolved solute particles per kilogram of solvent. It is determined by the number and not by the nature of the particles in solution. Dissolved solutes increase the osmotic pressure and decrease freezing point of the solution. In normal serum, the osmolality depends mainly on the concentration of the five major osmotic solutes; Na^+, Cl^-, $HCO3^-$, which are of ionic nature and glucose and urea that are non-ionic.[1 2] Since the sodium ions can be assumed to be counterbalanced by an anion, the dependence of serum osmolality on electrolyte concentration may be considered to be a function of sodium, glucose and urea.[2] Serum osmolality is a useful initial test for investigating the cause of body fluid imbalance and in identifying a raised osmolal gap for suspected poisoning. However, in a clinical setting, the routine measurement of serum osmolality is not feasible at bedside in the intensive care unit. In these situations, calculation of serum osmolality is often favoured than directly measuring serum osmolality. Osmolal gap (OG) is the difference between the measured osmolality and calculated osmolality based on the major commonly measured osmotically active particles. [3 4] The major use of OG is to screen for the possible presence of exogenous toxic substances in patients in an emergency department or intensive care unit.[5-10] Osmolal gap can also be used to screen for cases of alcohol intoxication where ethanol testing may not be immediately available.[11] For the calculation of serum osmolality different formulae, up to 36 have been published and there is no consensus over the most accurate one to be used in routine practice. Most of the published formulae are based on sodium, urea and glucose.[5 12]

In 1975, Dorwart and Chalmers reviewed 13 formulae and concluded that only four formulae, that utilised sodium, glucose and urea, had the highest correlation and the lowest standard deviation (SD) of difference when compared with the measured osmolality.[13 14] A simplified formula was proposed by them based on linear regression analysis: Calculated osmolality = 1.86(Sodium) + Glucose + Urea + 9. This formula is widely used to calculate the osmolality and has been incorporated in commercial analysers so that it can be reported with sodium, urea and glucose.[13 14] However, there are issues of using Dorwart-Chalmer's formula to calculate osmolality; it tends to underestimate true osmolality of sample, whereby the measured osmolality exceeds the calculated osmolality.[14 15]Therefore, it is no longer recommended but still used to calculate serum osmolality automatically by the analyser. [14 15] Smithline and Gardner used a factor of 2 as the osmotic coefficient of sodium chloride instead of 1.86.[16] This formula has been shown not to overestimate plasma osmolality as the other cations mainly potassium, calcium and magnesium as well as the constant of +9 are excluded from the calculation.[16] In 1984, Bhagat et al improvised the Dorwart's formula with the inclusion of potassium to propose a complex formula: Calculated osmolality = 1.86(Sodium+ Potassium) + Glucose + Urea + 10.[15] However, this simple Smithline formula [2(Sodium) + Glucose + Urea] showed the best predictive performance of osmolar gap with lowest bias and good precision.[17] Nowadays, it is the most commonly used in clinical settings for the calculation of serum osmolality.[17 18]

The best way for calculation of osmolality at bedside should be quick and convenient to be used in clinical practice.[16] However, there are only a few studies that determine which one of them provides the best results. Extended effort on validation equations also continue to be expanded over the years in the aim to achieve ultimate formula for calculated osmolarity. The Royal College of Pathologists of Australasian Quality Assurance Program (RCPAQAP) Chemical Pathology Calculated Results Program Survey revealed that 26 laboratories used simplified Bhagat formula and 12 laboratories use Smithline-Gardner formula for evaluation of calculated osmolarity. Fazakes et al suggested that a mean difference of $\leq$ 2 mOsm/kg between the calculated and measured osmolality would be desirable and a value above 5 mOsm/kg significantly compromise the usefulness of the formula.[12] Using the data from RCPAQAP Liquid Serum Chemistry, Choy et al showed that the Smithline-Gardner formula provided the smallest osmolal gap which is close to zero with an SD of less than 4.[16] It was shown that this equation performed well across different analytical platforms.[16] This equation was proposed to be adopted as it is simple and can be used for rapid mental calculation at the bedside and automated laboratory reporting whenever measured osmolality is requested.[16] The purpose of this study was to compare the calculated osmolality using various formulae with the measured osmolality in order to determine which calculated formula fit best with actual measured osmolality.

MATERIALS AND METHOD

Serum osmolality results done during the period of January 2015 to December 2015 were extracted from the laboratory information system (LIS). Serum osmolality that was performed simultaneously with renal and liver function tests, serum electrolytes and plasma glucose were included for the study. Serum osmolality measured for patients with the history of drug abuse and poisoning were excluded from the study. The study protocol was approved by the Medical Ethics Committee, UMMC (UMMC Medical Ethics: 20161230-4721). Whole blood samples were collected into 3.5-mL BD Vacutainer serum separator tube II Advance and 2.0 mL BD Vacutainer Sodium Fluoride Na2 EDTA tube and centrifuged at 3073 rpm (1900 rcf) for 5 minutes to acquire serum and plasma respectively. A 20 µL aliquot of serum sample was immediately transferred to sampler tip for osmolality determination. Measurement of osmolality was performed by freezing-point depression using Micro-Osmometer (Micro-Osmometer Model 3320) that has been calibrated with Clinitriol 290 Reference Solution.

Serum was analyzed for sodium and potassium by using a direct ion selective electrode. The blood urea nitrogen by using Roch-Ramel enzymatic reaction using urease and glutamate dehydrogenase. The plasma glucose was analyzed by using hexokinase enzymatic method. Analysis of the serum chemistry tests were performed using Siemens Advia® 2400 Chemistry Analyzer (Siemens Healthineers Global). All serum constituents were reported in standard international units. All calculations are referred as calculated osmolality (mmol/L), Serum measurements directly done via freezing point depression will be referred as measured osmolality (mmol/kg). The osmolal gap was calculated as measured osmolality (mmol/kg) minus calculated osmolality (mmol/L) and reported in standard international units which is mOsm/kg. 405 serum osmolality results were chosen to study the relationship between the measured and calculated serum osmolality. The clinical diagnosis and other relevant information for these data were recorded from the case notes. The results were divided into two groups. The Group 1 (n = 205) consisted of data with normal serum osmolality, electrolytes, urea and also plasma glucose level less than 7.8 mmol/L. The first group data were to identify which equation correlated well with the measured osmolality. The second group (n = 200) which comprised of data with low serum osmolality (n=90) and high serum osmolality (n=80) and normal serum osmolality (n=30) were included. The Group 2 data was to study the performance of the formula which correlated well with the measured osmolality based on the Group 1.

RESULTS

The results were presented as the mean.Statistical analysis for the comparison of multiple methods which is an extension of the Bland-Altman plot for more than 2 methods and Passing- Bablok regression analysis were conducted using MedCalc for Windows Version 15.0 (Medcalc Software, Ostend,Belgium). Serum sodium, potassium, blood urea nitrogen, plasma glucose and measured osmolality levels of the group 1 were (mean ± SD) 139.0 ± 1.99 mmol/L, 4.0 ± 0.36 mmol/L, 5.0 ± 1.17 mmol/L, 6.0 ± 0.99 mmol/L, and 288.0 ± 4.68 mOsm/kg respectively. Calculated osmolality using 19 different formulae (Table 1) and the osmolal gap (OG) are shown in Table 2. Of the 19 formulae, only Formula 1, 13, 17 and 19 were identified as optimal by having the mean OG ≤ 2 mOsm/kg (Table 2). The smallest OG was seen with Smithline-Gardner formula (Formula 1) with the mean bias of 0.3mOsm/kg. Comparison of the median of measured and calculated osmolality was performed by Wilcoxon test for paired samples in group 1. Based on Wilcoxon test for 2 paired samples, the four formulae showed the osmolal gap of ≤ 2 mOsm/kg shown in Table 2. The best result was achieved with the use of Formula 1 (Table 2).

The Bland-Altman plot and Passing–Bablok regression analysis were done for the Formula 1, 13, 17 and 19.The Bland-Altman plot showed more than 95% of the results were within the confidence interval (mean + 1.96SD).Passing–Bablok regression analysis for the Formula 1, 13, 17 and 19 yielded the equations shown in Table 3.The confidence intervals for the slope and the intercept include the values 1 and 0, respectively except for the Formula 13. This indicates that the calculated osmolality by Formula 1,17 and 19 correlated well with the measured osmolality.

Table 1: The different formulae used for calculation of osmolality

	Formula
Formula 1	2(Na) + Glu + Urea (Smithline – Gardner)
Formula 2	1.86(Na) + Glu + Urea + 9 (Dowart's Chalmer)
Formula 3	1.75(Na) + Glu + 0.5(Urea) + 10.1
Formula 4	2.63(Na) – 65.4
Formula 5	1.86(Na) + Glu + 0.5(Urea)
Formula 6	2(Na + K) + Glu + 0.5(Urea)
Formula 7	2(Na)
Formula 8	2(Na) + Glu + 0.5(Urea)
Formula 9	2(Na) + 7
Formula 10	2(Na) + Glu
Formula 11	2.1(Na)
Formula 12	2(Na) + Glu + 0.93 x 0.5(Urea)
Formula 13	0.985[2(Na + K) + Glu + 0.5(Urea)]
Formula 14	1.86(Na) + Glu + 0.5(Urea) + 5
Formula 15	2(Na) + 0.9(Glu) + 0.93(Urea) x 0.5
Formula 16	2(Na) + 0.5(Urea)
Formula 17	[1.86(Na) + Glu + 0.5(Urea)] / 0.93
Formula 18	1.9(Na + K) + Glu + 0.5(Urea)
Formula 19	1.86(Na + K) + Glu + Urea + 10 (Bhagat)
Formula 20	1.86(Na) + Glu + 0.5(Urea) + 9
Formula 21	1.85(Na) + Glu + 0.5(Urea) + 8.55
Formula 22	1.8(Na + K + iCa) + Glu + 0.47 x 0.5(Urea)
Formula 23	2(Na) + 10
Formula 24	2(Na + K) + Glu + 0.93 x 0.5(Urea)
Formula 25	1.89(Na) + 1.38(K) + 1.08(Glu) + 1.03(Urea) + 7.47
Formula 26	2(Na) + 0.9(Glu) + 0.93 x 0.5(Urea) + 8
Formula 27	0.985[1.86(Na) + 1.03(Glu) + 1.28 x 0.5(Urea)]
Formula 28	1.36(Na) + 1.6(Glu) + 0.45(Urea) + 91.75
Formula 29	0.985[2(Na) + Glu + Urea + 35.2)]
Formula 30	1.897(Na) + Glu + 0.5(Urea) + 13.5
Formula 31	1.9(Na + K) + Glu + 0.5(Urea) + 5
Formula 32	1.86(Na + K) + Glu + Urea
Formula 33	2(Na) + 1.15(Glu) + Urea
Formula 34	1.86(Na + K) + 1.15(Glu) + Urea + 14
Formula 35	1.85(Na) + 1.84(K) + 1.15(iCa) + 1.17(Mg) + Glu + 0.5(Urea)
Formula 36	1.09 x 1.86(Na) + Glu + Urea
Formula 37	0.985(Na + K + Cl + HCO3 + Lactate + Glu +Urea + 6.5)

Table 2: The Wilcoxon test for 2 paired samples between measured osmolarity and calculated osmolality for Group 1

Formula	Mean +SD	Mean Difference ± SD	95% CI	OG + SD	p (2- tailed)
Formula 1	288+ 4.1	-0.3+2.9	-0.69 to 0.12	0.3 +2.9	0.1083
Formula 2	277+3.8	-10.7+2.9	-11.13 to -10.32	11+2.9	< 0.0001
Formula 3	261+3.5	-27.3+2.9	-27.68 to -26.86	27+3	< 0.0001
Formula 4	299+5.2	11.1+3.7	10.59 to 11.62	-11+3.8	< 0.0001
Formula 5	266+3.7	-22.1+2.9	-22.53 to -21.71	22+3	< 0.0001
Formula 6	294+4	5.5+2.9	5.10 to 5.90	-5+2.9	< 0.0001
Formula 7	277+4	-10.9+3.3	-11.34 to -10.43	11+3.3	< 0.0001
Formula 8	286+4	-2.7+2.9	-3.15 to -2.33	3+3	< 0.0001
Formula 9	284+4	-3.9+3.3	-4.34 to -3.43	4+3.3	< 0.0001
Formula 10	283+4	-5.1+3.2	-5.52 to -4.65	5+3.2	< 0.0001
Formula 11	291+4.2	3.1+3.3	2.65 to 3.57	-3+3.4	< 0.0001
Formula 12	285+4	-2.9+3.0	-3.32 to -2.48	3+3	< 0.0001
Formula 13	289+3.9	1.1+1.1	0.69 to 1.48	-1+2.9	< 0.0001
Formula 14	271+3.7	-17.1+-17.7	-17.53 to -16.71	17+3	< 0.0001
Formula 15	285+4	-3.5+-3.5	-3.93 to -3.09	3+3	< 0.0001
Formula 16	280+4	-8.5+-8.4	-8.90 to -8.04	8+3.1	< 0.0001
Formula 17	286+4	-2.0+-1.9	-2.50 to -1.67	2+3	< 0.0001
Formula 18	279+3.9	-8.9+3.1	-9.21 to -8.36	9+3.1	< 0.0001
Formula 19	286	-2.0 +2.7	-2.50 to -1.71	2+2.8	< 0.0001

Table 3: Passing-Bablok regression analysis between measured osmolality and calculated osmolality (Formula 1, 13, 17, 19) for Group 1

	Regression equation	Correlation coefficient , r	CI for intercept	CI for slope
Formula 1	y = 31.67 + 0.889x	0.773	0.0 to 57.2	0.8 to 1.0
Formula 13	y = 49.00 + 0.833 x	0.787	1.0 to 73.0	0.75 to 1.0
Formula 17	y = 39.0 + 0.857 x	0.762	-2.0 to 69.75	0.75 to 1.0
Formula 19	y = 45.67 + 0.833x	0.786	-2.0 to 69.75	0.75 to 1.0

Abbreviations: 95 % CI: Confidence Interval

For the group 2, serum sodium, potassium, blood urea nitrogen and plasma glucose levels were (mean ± SD) 130.0 ± 9.72 mmol/L, 4.0 ± 0.95 mmol/L, 9.0 ± 9.18mmol/L, 12.0 ± 10.10 mmol/L respectively. Of the formulae which showed OG ≤2 mOsm/kg, the Smithline-Gardner (Formula 1) and Bhagat (Formula 19) formulae are relatively simple to calculate bedside compared to Formula 13 and 17. Hence, Passing-Bablok regression analysis was performed for the comparison of measured osmolality and the calculated osmolality by the Formula 1 and 19 (Table 4). The analysis yielded the confidence interval around the fitted linear line falls within the allowable bias bands. This indicated that the calculated osmolality using Formula 1 and 19 and measured osmolality are comparable within the allowable bias. (Figure 1 A-B).

Table 4: Passing-Bablok regression analysis between measured osmolality and calculated osmolality (Formula 1 and 19) for Group 2

Formula	Regression equation	Correlation coefficient , r	CI for intercept	CI for slope
Formula 1	y = 23.59 + 0.914x	0.983	17.805 to 29.393	0.893 to 0.935
Formula 19	y = 27.20 + 0.90x	0.987	21.983 to 31.967	0.883 to 0.918

Abbreviations: 95 % CI: Confidence Interval

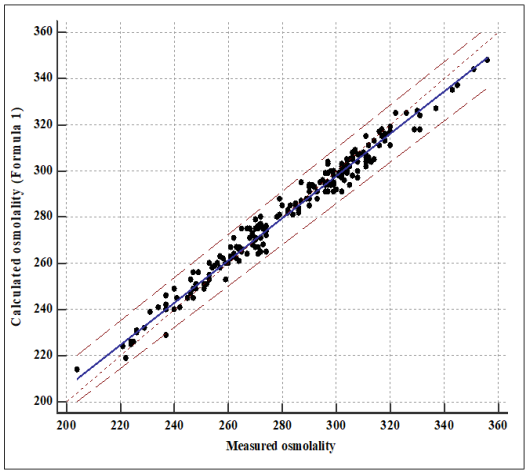

Fig 1A: Passing-Bablok regression analysis between measured osmolality and calculated osmolality (Formula 1) for Group 2

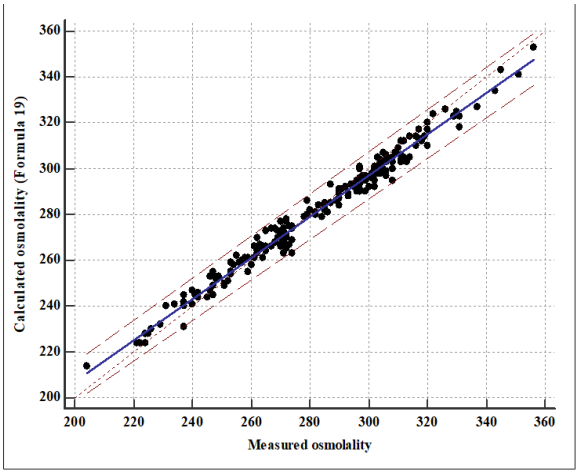

Fig 1B: Passing-Bablok regression analysis between measured osmolality and calculated osmolality (Formula 19) for Group 2

DISCUSSION

Since 1958, there has been tremendous effort in the pursuit of the formula that will give the minimum osmolal gap. The best formula will produce a gap as close to zero and with a low standard deviation. The purpose of the study was to validate 19 formulae used to predict osmolality and identify the most efficacious formula that can be applied for the calculation of osmolality.Hence, to evaluate the equation for calculating osmolality, we considered a mean difference of ≤ 2 mOsm/kg as suggested by Fazakes et al [12]. Osmolal gap ± 2 mOsm/kg was seen only for four (Equation 1, 13, 17 and 19) out of the 19 formulae being studied. Choy et al [16] applied to 34 formulae to the data from Royal College of pathologists of Australasia Quality assurance Program (RCPA QAP) Liquid Serum Chemistry and noted that only 6 formulae gave mean OG within 2 mOsm/ kg. The Smithline-Gardner formula (Formula 1) gave the lowest osmolal gap. Similar to their findings, we also observed Smithline-Gardner equation showed the minimum osmolal gap when compared to all the other formulae. When comparing these four formulae, the Formula 1 (Smithline) showed lowest mean difference of -0.3 ± 2.9 (mean ± SD) with the 95% confidence interval of -0.69 to 0.12 compared to others. The Wilcoxon test for paired samples also showed that there is statistically no significant difference between the measured and calculated osmolality ($p>0.05$).

The Bland-Altman plot for these four formulae showed that 95% of the results were within the confidence interval. This means that there is an agreement between the osmolal gap and the average of calculated and measured osmolalities of the four selected formulae. The Passing-Bablok regression analysis was performed for the four formulae which showed the least osmolal gap compared to other formulae . Of the four, only Formula 1, 17 and 19 showed the slope and intercept confidence intervals that included 1 and 0, respectively. Formula 17 ([1.86(Na) + Glu + 0.5(Urea)] / 0.93) was more complicated compared to Smithline-Gardner Formula 1 (2(Na) + Glu + Urea) and Bhagat Formula 19 (1.86(Na + K) + Glu + Urea + 10). Hence we applied Smithline-Gardner and Bhagat formulae for the group 2 patient samples to study the relationship between the calculated and measured osmolality. The Group 2 patient samples comprised of hyponatraemic, hypernatraemic and normonatraemic samples. Both formulae gave similar results as shown in Table 4.

Numerous different formulae may be used for calculation of osmolality. However, the formula and the reference intervals used may not necessarily be appropriate for all the analytical methods used.[19] In an effort to harmonise the calculation of osmolal gap in Australasia (based on the data from RCPAQAP Chemical Pathology liquid Serum Chemistry program 2014) , the Smithline-Gardner formula was recommended as it produced osmolar gap close to zero with an SD of around 4.[16] It was also demonstrated that the Smithline-Gardner formula is also adequately robust for all major analysers in laboratories across Australasia.[16] In conclusion , based on our data findings, Smithline-Gardner formula is the recommended equation for calculation of osmolality, not only fit for both healthy and hospitalized patients but also performs well across different analytical platforms. In this era of harmonization, this formula has been proposed to be adopted by all clinicians for the calculation of osmolal gap, as the OG gap is close to zero, simple to be easily calculate at bedside and can be automated into the Laboratory Information System(LIS).

Acknowledgement
The study was approved by University of Malaya Medical Research Ethical Committee (UMMC Medical Ethics: 20161230-4721) and is non sponsored study. The authors would like to thank the Director of Health Malaysia for his permission to publish this paper.

References

1. Dorwart WV, Chalmers L. Comparison of methods for calculating serum osmolality from chemical concentrations, and the prognostic value of such calculations. Clinical chemistry 1975;21(2):190-94.
2. Rasouli M. Basic concepts and practical equations on osmolality: Biochemical approach. Clinical Biochemistry 2016;49(12):936-41.
3. Purssell RA, Pudek M, Brubacher J, et al. Derivation and validation of a formula to calculate the contribution of ethanol to the osmolal gap. Annals of emergency medicine 2001;38(6):653-59.
4. Krahn J, Khajuria A. Osmolality gaps: diagnostic accuracy and long-term variability. Clinical chemistry 2006;52(4):737-39.
5. Glaser DS. Utility of the serum osmol gap in the diagnosis of methanol or ethylene glycol ingestion. Annals of emergency medicine 1996;27(3):343-46.
6. Erstad BL. Osmolality and osmolarity: narrowing the terminology gap. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 2003;23(9):1085-86.
7. Whittington JE, La'ulu SL, Hunsaker JJ, et al. The osmolal gap: what has changed? Clinical chemistry 2010;56(8):1353-55.
8. Holland MG, Nelsen J, Rosano TG. Osmol gap method for the detection of diethylene glycol in human serum. World journal of emergency medicine 2010;1(2):104.
9. Khajuria A, Krahn J. Osmolality revisited—deriving and validating the best formula for calculated osmolality. Clinical biochemistry 2005;38(6):514-19.
10. Wu AH, Yang HS, Thoren K. Biological variation of the osmolality and the osmolal gap. Clinical biochemistry 2014;15(47):130-31.
11. Lynd LD, Richardson KJ, Purssell RA, et al. An evaluation of the osmole gap as a screening test for toxic alcohol poisoning. BMC emergency medicine 2008;8(1):1.

12. Fazekas AS, Funk G-C, Klobassa DS, et al. Evaluation of 36 formulas for calculating plasma osmolality. Intensive care medicine 2013;39(2):302-08.
13. Martín-Calderón JL, Bustos F, Tuesta-Reina LR, et al. Choice of the best equation for plasma osmolality calculation: Comparison of fourteen formulae. Clinical biochemistry 2015;48(7):529-33.
14. Rasouli M, Kalantari KR. Comparison of methods for calculating serum osmolality: multivariate linear regression analysis. Clinical Chemical Laboratory Medicine 2005;43(6):635-40.
15. Bhagat C, Garcia-Webb P, Fletcher E, et al. Calculated vs measured plasma osmolalities revisited. Clinical chemistry 1984;30(10):1703-05.
16. Choy KW, Wijeratne N, Lu ZX, et al. Harmonisation of Osmolal Gap–Can We Use a Common Formula? The Clinical Biochemist Reviews 2016;37(3):113.
17. Osypiw J, Watson I, Gill G. What is the best formula for predicting osmolar gap? Annals of clinical biochemistry 1997;34:551.
18. Heavens KR, Kenefick RW, Caruso EM, et al. Validation of equations used to predict plasma osmolality in a healthy adult cohort. The American journal of clinical nutrition 2014;100(5):1252-56.
19. Hughes D, Doery JC, Choy KW, et al. Calculated Chemistry Parameters–do they need to be harmonised? The Clinical Biochemist Reviews 2016;37(3):131.