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ORIGINAL ARTICLE

Preparation of Commutable Frozen Human Serum Candidate Reference Materials for Standardization of Sodium Measurements

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SUMMARY

Background: **The measurement of serum sodium is important in the diagnosis of diseases. However, the accuracy and comparability of results in clinical laboratories are not ideal. The aim of this study was to prepare the candidate reference materials (RMs) to standardize serum sodium measurements.**
Methods: **Fresh sera without hemolysis, lipemia, and choloplania were collected and packed in the cryovials. According to ISO Guide 35, the homogeneity and stability were tested. The value was assigned by transfer from the National Institute of Standards and Technology (NIST) SRM919b using the reference method of ion chromatography (IC) and uncertainty was calculated. The commutability of candidate RMs were observed between the reference method and the two analyzed systems and then distributed to 47 laboratories to apply in routine assays.**
Results: **The F values of the homogeneity test were less than $F_{0.05}$; stability can last at least 12 months, 30 days, 15 days, and 7 days at -80°C, 2 - 8°C, room temperature (20 - 24°C), and 37°C, respectively. The results of three levels of candidate RMs for sodium were (159.00 ± 2.70), (139.16 ± 2.57), and (124.71 ± 3.12) mmol/L; coordinate dots of candidate RMs were all within the 95% confidence interval range of a 25-serum regression line. More than 76.6% of laboratories were within a bias of ± 1.5% from the target values.**
Conclusions: **The homogeneity, stability, and commutability of candidate RMs all meet the requirement, and the target values are assigned accurately. These new RMs of sodium in the human serum pool can be used to set up the traceability chain to improve the comparability of measurement results.**
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KEY WORDS

sodium, candidate reference materials, homogeneity, stability, commutability, value assignment

INTRODUCTION

Sodium is an important metal ion in the human body, which plays a pivotal role in the regulation of osmotic pressure and the distribution of water within the extracellular fluid compartments of the body. Serum sodium measurement has great clinical significance in diagnosis and monitoring for different diseases [1-4]. However, the present situation for routine measurements of serum sodium is not ideal. In the Chinese external quality assessment (EQA) survey from the National Center for Clinical Laboratories in 2016, we found the return re-

sults for sodium from different laboratories varied greatly, almost up to 2-fold. To ensure accuracy, reliability, and comparability of sodium analytical results from different clinical laboratories, clinical laboratories need to be provided with suitable quality assurance tools, such as reference materials, which are typically used for method validation (including trueness determination) and method performance verification [5-9].

RMs with commutability can produce equal results across different methods, which are of great significance in the standardization of clinical laboratory. Recent studies show that the reference materials of fresh frozen serum matrix have good commutability and can be the ideal form of reference materials. In China, there are so many clinical laboratories, some of which have poor quality and need more and more matrix-matched certified RMs to standardize the routine measurement. Although there are some currently available materials for sodium such as SRM956 from NIST in US, ERM-DA250 and 251 from LGC in UK, etc., these international RMs still cannot be extensively applied in China due to the long transport period and high price. In this study, we aimed to prepare candidate RMs for serum sodium with low, normal, and high sodium concentrations (sodium-L1, sodium-L2, and sodium-L3) on the basis of established reference methods for serum sodium, which was intended to effectively solve the traceability problems [10-13] and achieve the accuracy and comparability of the measurement results of serum sodium.

MATERIALS AND METHODS

Materials

Siemens Advia 2400 was purchased from Siemens (USA). An IC (ICS1100) was purchased from Thermo Fisher Scientific (USA). A digital AE 240 dual range analytical balance was purchased from Mettler-Toledo International, Inc. (Zurich, Switzerland). A Milli-Q water system was obtained from EMD Millipore (Billerica, MA, USA). Electrolyte standard reference material (SRM919b and SRM956c) was purchased from the NIST (Gaithersburg, MD, USA). All chemicals were of analytical grade. 15-mL and 2-mL polypropylene cryogenic vials were produced by Corning. A sterile filter was from Sartorius SartobranTM P-capsuli 5231307H9-SO-A, pore size 0.45 μm for the prefilter and 0.20 μm final filter.

Preparation of materials

Healthy subject sera without hemolysis, lipemia, and choloplania were anonymous leftover samples which were tested and found negative for HIV1+2 antibodies, hepatitis B virus (Ag), and hepatitis C virus (Ag) obtained from the Department of Laboratory Medicine at the Beijing Chao-Yang Hospital. The three levels of serum sodium samples containing the normal concentration, the low concentration, and the high concentration, were collected and sterile-filtered to 0.20 μm. All patient samples were 1 mL dispensed aseptically into plastic screw-cap 2-mL cryovials and stored at -80°C until use, then thawed at room temperature and used immediately; any remaining sample was discarded.

Homogeneity testing of sodium candidate RMs

Base on the request of ISO Guide 35 [7], the homogeneity of the serum sodium candidate RMs were tested by measuring the concentration of sodium in triplicate in 10 vials randomly selected from each level. The measurements were performed using a sufficiently repeatable routine, the ion selective electrode (ISE) measurement method [14]. The data were processed by a one-way ANOVA approach using SPSS 17.0 software. The F value, a ratio of the mean squares among groups to mean squares within groups, was calculated to estimate whether there was a significant difference or not in the measurement data. When $F < F_{0.05}$, the materials were homogeneous.

Stability studies of sodium candidate RMs

Short-term stability of the serum sodium candidate RMs in transport conditions was observed as isochronous stability studies. The parallel samples were analyzed at different time intervals (0d, 1d, 2d, 4d, 7d, 12d, 15d, and 30d), (0d, 1d, 2d, 4d, 7d, 8d, 12d, 15d), and (0d, 1d, 2d, 4d, 7d) at different storage temperatures of 2 - 8°C, 20 - 24°C, and 37°C, respectively. The test value of samples from -80°C was as the initial value (0d). At the same time, the vials from all storage temperatures and storage times were measured in triplicate using the same method as the homogeneity testing. For the long-term stability study at -80°C, the parallel samples at 0, 1, 3, 6, and 12 months were randomly selected and measured in triplicate using IC [15-20].

By linear regression analysis of variance, the straight line was used as an empirical model. The criterion for judging is $|b1| < t0.95, n-2 \cdot s(b1)$. The s(b1) was calculated according to the following equations:

$$s^2 = \frac{\sum_{i=1}^{n}(Y_i - b_0 - b_1 x_i)^2}{n-2} \quad (1)$$

$$s(b_1) = \frac{s}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}} \quad (2)$$

As $|b1| < t0.95, n-2$ s(b1), the slope is non-significant and instability is not observed. In equation, s represents standard deviations of straight line; n-2 represents degree of freedom; b1 represents the slope of straight line; s(b1) represents standard deviations of the slope of straight line.

Determination of reference values and evaluation of uncertainty

The candidate RMs were measured using IC as a reference method [15-20]. The instrument parameters were optimized as follows: 32 mmol/L methanesulfonic acid

flow (1.0 L/min), acquisition time (25.0 min), injection volume (25.0 µL). The value was assigned by transfer from NIST SRM919b to ensure quantity transfer and traceability. NIST SRM919b as the stock solutions were prepared to weight, and calculating the final working standard solutions with known sodium concentrations about 103.80 µg/g, 117.09 µg/g, 130.60 µg/g, 139.61 µg/g, and 148.84 µg/g. The samples were pretreated by digestion with nitric acid and diluted 25-fold. The concentrations of sodium were calculated according to the calibrator curve which was established by measuring the peak area of the working standard solutions. The accuracy and repeatability of the IC reference method were assessed with SRM956c, and 5 replicates were measured each day for 3 days. The method to measure serum sodium is precise and accurate through method validation. The atomic weight of sodium is 22.9898 g/mmol. The equation from µg/g to mmol/L was described in detail in our previous study for potassium [21]. Results were expressed as "target value ± uncertainty". The final uncertainty of candidate RMs for sodium is made up of three parts: the uncertainty from the measurement of candidate RMs (u_c), the uncertainty from homogeneity test of materials (u_H) and the uncertainty from the long-term stability test of the materials (u_t).

Observation of commutability

Twenty-five fresh patient serum samples, covering a wide interval of concentration values, and three levels of frozen mixed serum sodium candidate RMs were measured using 3 assays, ICS1100, Siemens Advia 2400, and another Siemens analyzer. The measurement results were analyzed pairwise, and linear regression and 95% prediction intervals were calculated for each pair of assays. The commutability of these candidate RMs between reference method and two different analyzed systems was estimated.

Trueness verification survey

The three levels of candidate RMs were distributed to 47 clinical laboratories of hospitals to carry out the trueness verification survey. Each laboratory received three parallel samples of each level and was required to measure 5 replicates for each sample and finish analysis in three days.

RESULTS

Homogeneity

The results of homogeneity testing of the candidate RMs are provided, MS_{among} represents mean squares between groups ($V_1 = 9$), MS_{with} represents mean squares within group ($V_2 = 20$).

The F value of the homogeneity test of each level candidate reference material is in Table 1. All of them were less than $F_{0.05}$ (9, 20).

Stability

By linear regression analysis of variance of the candidate RMs for serum sodium at different temperatures and the time, $|b1| < t0.95$, n-2 s(b1), the three levels of candidate RMs can be stable for at least 12 months, 30 days, 15 days, and 7 days at -80°C, 2 - 8°C, 20 - 24°C, and 37°C, respectively (Table 2). The observation of long-term stability will be continued.

Value assignment and validation of methods

The whole set of candidate RMs for sodium was divided into 4 subsets for each level and each subset was measured in triplicate. The relative standard deviation (RSD) values were 0.53%, 0.57%, and 0.80% (Table 3). The NIST SRM 956c levels I and III were used for accuracy assessment of the applied reference method and the respective biases were 0.63% and 0.78%, respectively.

Evaluation of uncertainty

The uncertainty from homogeneity test

The value of S_H was calculated from the homogeneity results. The calculation formula:

$$_{SH} = \sqrt{\frac{S_1^2 - S_2^2}{n}}$$

n represents the number of replications (n = 3). The respective uncertainties (u_H) for sodium-L1, L2 and L3 were 0.23%, 0.30%, and 0.38% (Table 4).

The uncertainty from stability test

Evaluate the uncertainty from the long-term stability for 12 months at -80°C. The calculation formula:

$s_t = s(b_1) \cdot t$

The respective uncertainties (u_t) were 0.61% to 0.88% (Table 5).

The uncertainty (u_c) from the measurement of candidate RMs

The uncertainty (u_c) mainly includes the uncertainty of the certified reference material as a valuation transfer, method imprecision, and balance weighing for the stock standard solutions, serum RMs, and the working solutions. The uncertainty of certified reference material SRM919b (Na) was 0.0075%. The relative standard uncertainty can be calibrated to 0.010%. The uncertainty from method imprecision was the relative standard deviation (RSD) of the measurements caused by reproducibility (Table 3). All uncertainties from weighing using the balances are 0.078%. Therefore, u_c for low, normal, and high concentrations of serum sodium RMs were calculated to be 0.54% to 0.80%.

The calculation of uncertainty for the final certified values of candidate RMs

The combined standard uncertainty u associated with the three parts can be defined by:

$$u = \sqrt{u_H^2 + u_t^2 + u_c^2}$$

Results were expressed as "target value ± uncertainty

Table 1. Homogeneity test of candidate RMs for sodium.

Materials	MS_{among}	MS_{with}	F	$F_{0.05(9,20)}$
Sodium-L1	2.12	1.71	1.24	2.39
Sodium-L2	1.53	1.02	1.51	2.39
Sodium-L3	0.60	0.29	2.05	2.39

Table 2. Stability test of candidate RMs for sodium.

Temperature [a]	Level	n	$t_{0.95, n-2}$ [b]	$y = b_1x + b_0$ [c]	$s(b_1)$	$t_{0.95, n-2} \cdot s(b_1)$
-80°C	L1	6	2.78	y = -0.058x + 159.34	0.081	0.225
	L2	6	2.78	y = 0.004x + 138.94	0.075	0.209
	L3	6	2.78	y = 0.162x + 124.18	0.091	0.253
2 - 8°C	L1	8	2.45	y = -0.032x + 156.90	0.080	0.196
	L2	8	2.45	y = -0.041x + 138.76	0.039	0.096
	L3	8	2.45	y = -0.013x + 1 25.07	0.014	0.034
20 - 24°C	L1	8	2.45	y = 0.009x + 158.91	0.018	0.044
	L2	8	2.45	y = 0.027x + 138.83	0.019	0.047
	L3	8	2.45	y = 0.022x + 124.92	0.013	0.032
37°C	L1	5	3.18	y = 0.132x + 159.31	0.053	0. 169
	L2	5	3.18	y = 0.176x + 140.14	0.143	0.455
	L3	5	3.18	y = 0.283x + 125.43	0.177	0.563

[a] The storage times of the samples are 12 months, 30 days, 15 days, and 7 days at -80°C, 2 - 8°C, 20 - 24°C, and 37°C, respectively.
[b] t value for the probability of 0.95.
[c] Equation of linear regression.

Table 3. The certified values and reproducibility of IC measurements.

Materials	Certified value [a] (mmol/L)	Overall	
		SD [b]	RSD (%)
Sodium-L1	159.00	0.84	0.53
Sodium-L2	139.16	0.79	0.57
Sodium-L3	124.71	0.99	0.80

[a] 3 replicates processed and measured each subset in 4 subsets for each level (mmol/L).
[b] Standard deviation of the mean for that level (mmol/L).

Table 4. The uncertainty from homogeneity test.

Materials	s_H (mmol/L)	Mean [a] (mmol/L)	u_H (%)
Sodium-L1	0.37	161.37	0.23
Sodium-L2	0.42	141.00	0.30
Sodium-L3	0.49	126.58	0.38

[a] The mean value of triplicate tests of 10 vials.

Table 5. The uncertainty from the long-term stability test.

Materials	*S (b1)* (mmol/L)	*t*	s_t (mmol/L)	Mean [a] (mmol/L)	u_t (%)
Sodium-L1	0.081	12	0.97	159.49	0.61
Sodium-L2	0.075	12	0.90	138.73	0.65
Sodium-L3	0.091	12	1.09	124.62	0.88

[a] **The mean value of triplicate tests of parallel samples at 0, 1, 3, 6, and 12 months at -80°C.**

Table 6. The expanded uncertainties for the certified values of candidate RMs of sodium-L1, L2, and L3.

Uncertainty table	Sodium-L1	Sodium-L2	Sodium-L3
u_c (%)	0.54	0.58	0.80
u_H (%)	0.23	0.30	0.38
u_t (%)	0.61	0.65	0.88
u (%)	0.85	0.92	1.25
k factor	2	2	2
k **u* (%) a	1.70	1.85	2.50
U (mmol/L)	2.70	2.57	3.12
Certified value (mmol/L)	159.00 ± 2.70	139.16 ± 2.57	124.71 ± 3.12

[a] **Uncertainty of 95% confidence interval.**

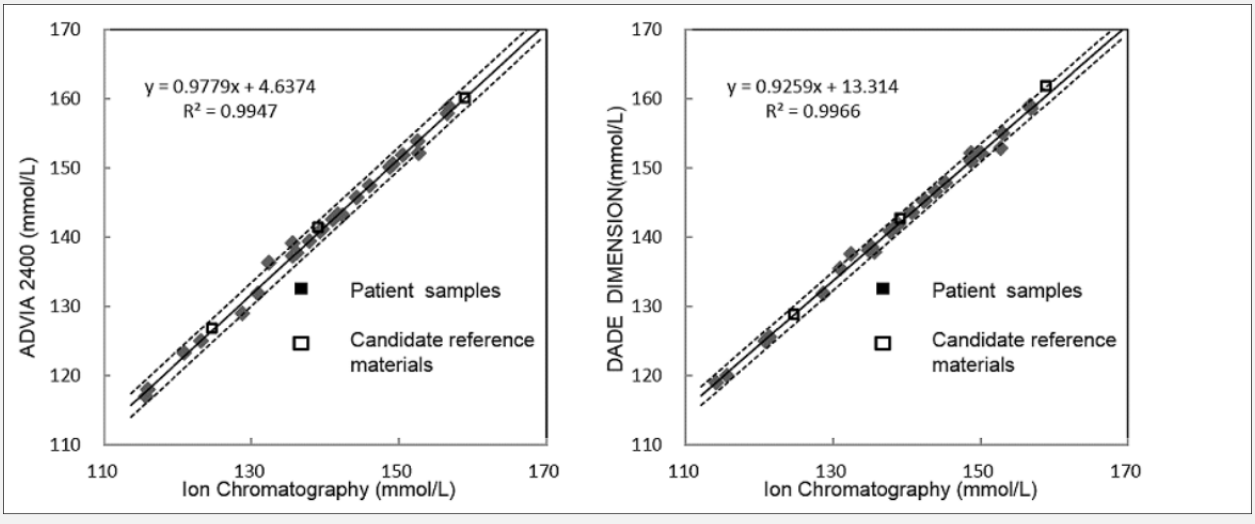

Figure 1. Commutability of candidate standard RMs for the measurement of serum sodium.

The candidate RMs together with a set of patient samples were measured for sodium with the reference method using IC and two assays including Siemens Advia 2400 and another Siemens analyzer. The solid lines are the regression lines and dashed are the limits of the prediction intervals.

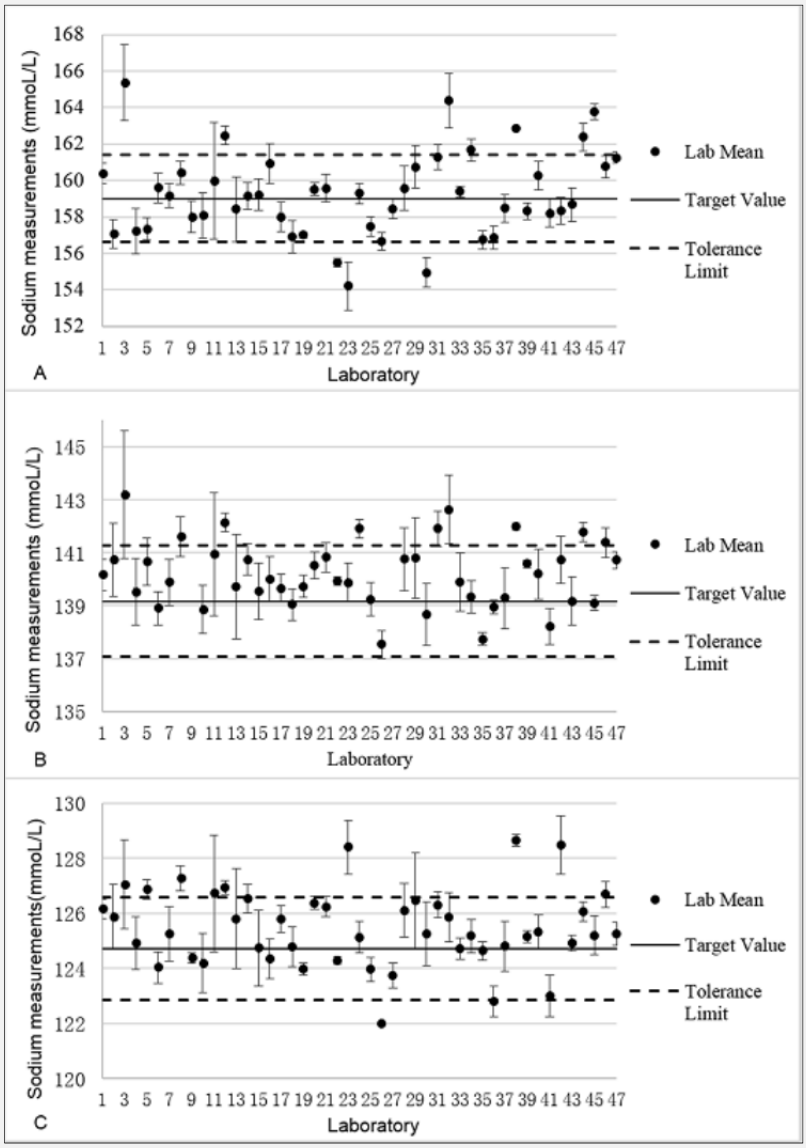

Figure 2. A: Mean values and range of the results for sodium-L1 from 47 laboratories with different commercial systems. B: Mean values and range of the results for sodium-L2 from 47 laboratories with different commercial systems. C: Mean values and range of the results for sodium-L3 from 47 laboratories with different commercial systems.

Each sample of three parallel samples for each level was measured in 5 replicates each day for three days. The X-axis displays the participant laboratory numbers. The Y-axis displays the mean results for serum sodium measurements.

(U)". The calculation of the extended uncertainty U was listed in Table 6 (confidence interval 95%, k = 2).

Commutability study

The measurement results of the reference method (X) and evaluated method (Y) were analyzed pairwise using linear regression. The three levels of candidate standard RMs for serum sodium with measurement results were all inside the 95% prediction intervals (see Figure 1).

Trueness verification survey

The trueness verification survey of 47 clinical laboratory detection assays, allowing a bias of ± 1.5% from the target value, 78.7% of the participants passed the acceptability criterion for sodium-L1, 76.6% for sodium-L2, and 78.7% for sodium-L3 (Figure 2 B1-3).

DISCUSSION

Accurate results over time and location are achieved by standardizing measurements and establishing traceability to a reference system. Reference materials play a key role for establishing traceability. Homogeneity, stability, and characterization are main features and basic requirements of RMs. The reference materials with good homogeneity and stability can improve the quality of analysis and measurement. At present, human serum-based materials are considered the appropriate RMs for ion. Fresh serum pools were collected to ensure the aseptic filtration by membrane without adding any antibiotics and preservatives.

A homogeneity test should consider the measurement method, sampling method, sampling quantity and sampling volume, etc. Henriksen et al. [8] found that sodium in the human serum-based EQA samples was not homogeneous owing to the instability of the instrument at that time. During the measurements, a sufficient repeatability of the method should be considered. We studied the homogeneity test by using a biochemical analyzer with a high degree of automation which was suitable for large quantities of samples to be measured simultaneously in a short period. The study results showed that all of the calculated F values were smaller than the critical value of 2.39 as an acceptance criterion for the F test. Therefore, no inhomogeneity was observed for this batch of RMs for sodium.

A distinction is made between the stability under specified storage conditions (long-term stability) and transport conditions (short-term stability). The isochronous short-term stability was studied for sodium in serum stored at 2 - 8□, 20 - 24□, and 37□. We think candidate RMs can be transported at ambient temperature within 15 days without the need for an extra fee for cold-chain transportation. The long-term stability at -80°C was observed for 12 months. No obvious trend was observed during the stability monitoring period. The stability monitoring is on-going and the stability status will be updated continuously.

According to ISO Guide 35 [7], the characterization of the candidate reference materials may take place in different ways. There are two mainstream approaches: characterization by a single method and characterization by multiple methods and/or multiple laboratories. The appropriate reference method should be selected for the characterization of RMs. At present, there are some reference methods to determine sodium in biological matrices such as flame atomic emission spectrometry (FAES), isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS), and inductively coupled plasma optical emission spectrometry (ICP-OES). In this study, IC was selected as an ideal sodium candidate reference method to measure the concentrations of candidate RMs for simple sample pretreatment, low operation cost, and high precision and sensitivity. The instrument parameters were optimized before measurement. The contributing factors to the uncertainty for the final certified values including the characterization measurements, homogeneity test, and long-term stability test were calculated more comprehensively. The combined standard uncertainty u for candidate RMs ranged from 0.85% to 1.25%. Short-term stability is only relevant as an uncertainty component when the stability of RMs is affected by the specified transport conditions in excess of the storage conditions [7].

The commutability experiment was designed according to the CLSI EP-14 guideline [22]. In this protocol, it is suggested to use at least 20 representative native patient samples as the standard of comparison. In this study, we selected 25 patient samples and, because the comparative method is the reference method, it has little or no matrix effect with processed calibrator or control samples [22]. So, we performed a linear regression analysis using the means of the ISE method results and the means of the IC method results (from 25 patient specimens). The representative set of patient samples were measured using two measurement procedures, using the formula to compute the two-tailed 95% prediction interval y value at a given x value about the least-square linear regression line [22]. Measurement results obtained with RMs were then compared against the 95% prediction interval. The study results showed that the preparation of candidate RMs using fresh frozen mixed human serum all fall within the prediction interval and behave in the same manner as native patient samples. At the same time, we find the positive systematic bias comparing the routine methods with the reference method using IC (Figure 1). Commutability of reference materials is a critical property to ensure they are suited for application [23]. The commutable candidate RMs were applied in a trueness verification survey of 47 clinical laboratory detection assays. Clinically significant bias was observed in 21.3% of the laboratories for sodium-L1 and sodium-L3, and 23.4% of the laboratories for sodium-L2. The positive bias to the target values assigned by the IC reference method was most frequently observed. The economic and practical RMs we prepared can help clinical laboratories to link themselves for the real-time monitoring of test quality, which will accelerate the standardization of different detection systems for sodium measurements.

CONCLUSION

In the preparation of candidate RMs for serum sodium, procedures of sample preparation, certification (value assignment), homogeneity test, stability study, and evaluation of the uncertainty and commutability study were studied in detail. The F value of homogeneity testing was less than $F_{0.05}$. The candidate RMs were stable enough under the observation period at different storage temperatures. The certified reference values assigned by IC and the relative expanded uncertainties were calculated to be 1.70%, 1.85% and 2.50% for sodium-L1, sodium-L2 and sodium-L3, respectively. The three levels

of new commutable RMs can be used by field laboratories to monitor performance and provide reliable data for improving the performance of laboratory electrolyte measurement.

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Declaration of Interest:
The authors declare no conflict of interest.

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Supplemental Digital Content

Lab coade	Instrument	L1			L2			L3			Evaluation
		Mean value	High limit	Low limit	Mean value	High limit	Low limit	Mean value	High limit	Low limit	
1	HITACHI DDP	160.37	161.39	156.62	140.16	141.25	137.08	126.17	126.58	122.84	
2	Beckman Synchron	157.07	161.39	156.62	140.73	141.25	137.08	125.87	126.58	122.84	
3	Roche	165.35	161.39	156.62	143.19	141.25	137.08	127.04	126.58	122.84	deviation
4	Hitachi	157.23	161.39	156.62	139.52	141.25	137.08	124.91	126.58	122.84	
5		157.33	161.39	156.62	140.67	141.25	137.08	126.87	126.58	122.84	deviation
6	HITACHI 7600	159.58	161.39	156.62	138.89	141.25	137.08	124.03	126.58	122.84	
7		159.15	161.39	156.62	139.87	141.25	137.08	125.25	126.58	122.84	
8	Siemens ADVIA	160.40	161.39	156.62	141.60	141.25	137.08	127.27	126.58	122.84	deviation
9	Beckman Synchron	158.00	161.39	156.62	127.53	141.25	137.08	124.37	126.58	122.84	deviation
10	Beckman Synchron	158.06	161.39	156.62	138.85	141.25	137.08	124.19	126.58	122.84	
11	Beckman Synchron	159.98	161.39	156.62	140.94	141.25	137.08	126.72	126.58	122.84	deviation
12	HITACHI 7600	162.47	161.39	156.62	142.13	141.25	137.08	126.93	126.58	122.84	deviation
13	AU 5800	158.41	161.39	156.62	139.71	141.25	137.08	125.79	126.58	122.84	
14		159.13	161.39	156.62	140.73	141.25	137.08	126.53	126.58	122.84	
15	AU 5800	159.20	161.39	156.62	139.53	141.25	137.08	124.73	126.58	122.84	
16	Roche Cobas c501/cobas c 311	160.93	161.39	156.62	140.00	141.25	137.08	124.33	126.58	122.84	
17	Beckman Synchron	158.00	161.39	156.62	139.65	141.25	137.08	125.79	126.58	122.84	
18	Beckman AU	156.90	161.39	156.62	139.04	141.25	137.08	124.77	126.58	122.84	
19	Medica	157.01	161.39	156.62	139.73	141.25	137.08	123.97	126.58	122.84	
20	Siemens ADVIA 1650/1800/240	159.52	161.39	156.62	140.51	141.25	137.08	126.37	126.58	122.84	
21	Siemens ADVIA 1650/1800/240	159.57	161.39	156.62	140.82	141.25	137.08	126.23	126.58	122.84	
22		155.47	161.39	156.62	139.93	141.25	137.08	124.29	126.58	122.84	deviation
23		154.20	161.39	156.62	139.87	141.25	137.08	128.40	126.58	122.84	deviation
24	HITACHI 7180	159.27	161.39	156.62	141.90	141.25	137.08	125.13	126.58	122.84	deviation
25	Beckman AU	157.45	161.39	156.62	139.24	141.25	137.08	123.96	126.58	122.84	
26	HITACHI 7600	156.67	161.39	156.62	137.53	141.25	137.08	122.00	126.58	122.84	deviation
27	Siemens ADVIA 1650/1800/240	158.43	161.39	156.62	127.47	141.25	137.08	123.73	126.58	122.84	deviation
28	AU 5800	159.57	161.39	156.62	140.76	141.25	137.08	126.11	126.58	122.84	
29	Hitachi	160.73	161.39	156.62	140.80	141.25	137.08	126.47	126.58	122.84	
30	Beckman Synchron	154.93	161.39	156.62	138.66	141.25	137.08	125.25	126.58	122.84	deviation
31	Roche	161.25	161.39	156.62	141.90	141.25	137.08	126.30	126.58	122.84	deviation
32		164.39	161.39	156.62	142.63	141.25	137.08	125.85	126.58	122.84	deviation
33	Siemens	159.38	161.39	156.62	139.89	141.25	137.08	124.72	126.58	122.84	
34		161.67	161.39	156.62	139.32	141.25	137.08	125.18	126.58	122.84	deviation
35		156.73	161.39	156.62	137.72	141.25	137.08	124.63	126.58	122.84	
36		156.87	161.39	156.62	138.93	141.25	137.08	122.80	126.58	122.84	
37	Beckman Synchron	158.46	161.39	156.62	139.28	141.25	137.08	124.80	126.58	122.84	
38	IMS-972	162.85	161.39	156.62	141.98	141.25	137.08	128.65	126.58	122.84	deviation
39	IMS-972	158.31	161.39	156.62	140.58	141.25	137.08	125.15	126.58	122.84	
40	Roche	160.27	161.39	156.62	140.20	141.25	137.08	125.33	126.58	122.84	
41		158.20	161.39	156.62	138.20	141.25	137.08	123.00	126.58	122.84	
42	Beckman Synchron	158.33	161.39	156.62	140.73	141.25	137.08	128.47	126.58	122.84	deviation

Supplemental Digital Content (continue).

43	**Beckman Synchron**	**158.67**	**161.39**	**156.62**	**139.17**	**141.25**	**137.08**	**124.91**	**126.58**	**122.84**	
44		**162.37**	**161.39**	**156.62**	**141.77**	**141.25**	**137.08**	**126.05**	**126.58**	**122.84**	**deviation**
45		**163.77**	**161.39**	**156.62**	**139.10**	**141.25**	**137.08**	**125.20**	**126.58**	**122.84**	**deviation**
46	**Siemens**	**160.75**	**161.39**	**156.62**	**141.39**	**141.25**	**137.08**	**126.69**	**126.58**	**122.84**	**deviation**
47		**161.23**	**161.39**	**156.62**	**140.72**	**141.25**	**137.08**	**125.25**	**126.58**	**122.84**	