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ORIGINAL ARTICLE

Evaluation of Uniformity and Stability of Frozen Mixed Serum Potassium Candidate Reference Materials

Rui Zhang [1], Dan Zhang [1], Qingtao Wang [1, 2]

[1] *Department of Clinical Laboratory, Beijing Chao-yang Hospital, Capital Medical University, Beijing, People's Republic of China*
[2] *Beijing Center for Clinical Laboratories, Beijing, People's Republic of China*

SUMMARY

Background: **We tested the uniformity and stability of candidate reference materials (RMs) for serum potassium and aimed to design RMs with better quality that can meet all clinical test requirements and effectively solve the quantity traceability transfer problem.**

Methods: **Three levels of frozen mixed serum potassium candidate RMs were prepared and packed in freezing tubes. RMs were determined in triplicate in 10 vials randomly selected from each level. A one-way analysis of variance was used to evaluate the uniformity using a ratio of the mean squares among groups to mean squares within groups $F < F_{0.05}$ as the criteria. Stability was studied by synchronization; the short-term stability of the serum potassium in the transport conditions was observed for 30, 15, and 7 days at refrigeration (2 - 8°C), room temperature (18 - 25°C), and 37°C, respectively. By linear regression analysis of variance, the straight line was used as an empirical model. The criterion for judging is $|b1| < t_{0.95, n-2} \cdot s_{b1}$.**

Results: **Based on the statistical analysis using SPSS 17, the F values for the homogeneity tests of each level of the frozen mixed serum potassium RMs were 0.247, 0.117, and 0.162. These values were less than $F_{0.05\ (9,\ 20)} = 2.39$. When the short-term stability of the serum potassium was observed for 30, 12, and 4 days at 2 - 8°C, room temperature, and 37°C, respectively, $|b1| < t_{0.95, n-2} \cdot s_{b1}$, and instability was not observed.**

Conclusions: **The three levels of the frozen mixed serum potassium RMs have good uniformity. At 2 - 8°C, room temperature, and 37°C, the stability can be ensured for at least 30, 12, and 4 days, respectively. The serum potassium candidate RMs are sufficiently stable under these transportation conditions.**

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Correspondence:
Qingtao Wang
Department of Clinical Laboratory
Beijing Chao-Yang Hospital
Capital Medical University
No. 8, Gongtinan Road
Chao-Yang District, Beijing, 100020
PR China
Phone: +86 10-85231660
Fax: +86 10-85231660
Email: wqt36@163.com

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INTRODUCTION

A reference material (RM) is a material or substance used to calibrate measurement systems, evaluate measurement procedures, or assign values to materials [1-3]. It is characterized by its uniformity, stability, and accuracy, of which, the first two are essential properties of certified reference materials (RMs). The homogeneity of RMs is used to describe their spatial distribution characteristics. Stability tests determine sufficient stable storage conditions for the candidate RMs. To be of practical value, the substance must remain stable for an acceptable period under actual storage, transportation, and use conditions [1-3]. Standard materials with good uniformity and high stability can improve the quality of analysis and accuracy of measurement.

The accurate measurement of serum potassium has great clinical significance in the diagnosis and monitoring for different diseases [4-6]. The frozen mixed human RM for serum potassium can be used to effectively solve traceability problems [7-9] and achieve accuracy comparable to the measurement results of serum potassium. Although there are some currently available materials for potassium such as SRM956 from NIST in US, and ERMDA250 and 251 from LGC in UK, these international RMs cannot be extensively applied in China owing to the long transport period and high price. In this study, the homogeneity and short-term stability of frozen mixed candidate RMs for serum potassium were studied, which laid the foundation for assigning values to candidate RMs for serum potassium in the future to develop the frozen mixed RMs for serum potassium (certified RMs).

MATERIALS AND METHODS

Materials

Siemens Advia 2400 was purchased from Siemens (USA). Both low- and high-value quality controls were procured from Randox Laboratory (United Kingdom). A miniature magnetic stirrer 90-1 was purchased from Shanghai Zhen Rong Scientific Instruments (Shanghai). A Milli-Q water system was obtained from EMD Millipore (Billerica, MA, USA). Polypropylene cryogenic vials (50-mL and 2-mL) were obtained from Corning (USA). A sterile filter was obtained from Sartorius Sartobran^{TM} P-capsuli 5231307H9-SO-A, pore size 0.45 µm for the prefilter and 0.20 µm for the final filter.

Preparation of materials

1. Serum collection: The residual sera after physical examination without hemolysis, lipemia, and icterus from the Department of Laboratory Medicine at the Beijing Chao-Yang Hospital were used to prepare reference materials for potassium in full compliance with ISO Guideline 34 [10] and WHO Guidelines. These sera were tested and found to be negative for HIV1+2 antibodies, hepatitis B virus (Ag), and hepatitis C virus (Ag). The collected serum tube was numbered and the volume was recorded; the surface of the bottle was disinfected with 75% alcohol or iodine liquor and sealed. Approximately 3,000 mL of serum was collected and stored in the -80°C freezer until use.

2. Serum preparation: The mixed sera pools were prepared to the normal concentration, the low concentration, and the high concentration after proper treatment. The serum potassium, sodium, chlorine, and total protein concentrations ranged from 2.30 - 6.00 mmol/L, 130 - 134 mmol/L, 100 - 105 mmol/L, and 62 - 68 mmol/L, respectively, as determined by Siemens Advia 2400 automatic biochemical analyzer. The three levels of serum pools were filtered by 0.8, 0.45, and 0.2 micron filtration membranes, respectively, and they were then packed in 1 mL freezing tubes in a biosafety cabinet and stored at -80°C.

Homogeneity testing of candidate reference materials for serum potassium

Based on the request of the ISO Guide 35 [3], the homogeneity of the serum potassium candidate RMs were tested by measuring the concentration of potassium in triplicate in 10 vials randomly selected from each level. The measurements were performed using a sufficiently repeatable routine: the ion selective electrode measurement method [11]. The quality control product was detected simultaneously with the sample. By comparing the mean square among groups (MS_{among}) and mean square within groups (MS_{with}), we can judge whether there is significant difference between the measurements of each group (Formula (1)).

$$F = MS_{among}/MS_{with} = \frac{SS_{among}}{V_{among}} / \frac{SS_{with}}{V_{with}} \qquad (1)$$

Where SS_{among} is the sum of squares among groups, SS_{with} is the sum of squares within groups, and V is the degree of freedom.

Stability studies of candidate reference materials for serum potassium (short-term stability)

The short-term stability of the serum potassium candidate RMs in the transport conditions was observed using the same method as that for homogeneity testing. The short-term stability of serum potassium in the transport conditions was observed for 30, 15, and 7 days at refrigeration (2 - 8°C), room temperature (18 - 25°C), and 37°C, respectively. At the same time, the samples placed at different time points were removed from their respective temperature preservation conditions and were balanced at room temperature for 60 minutes. The quality control substances at low and high levels were tested simultaneously. The parallel RMs of each level were repeated 3 times, and the mean value was calculated. The linear regression equation of the relationship between the serum potassium concentration, and the time at different temperatures was obtained. The criterion for judging is $|b1| < t_{0.95,n-2} \cdot s(b1)$. The standard deviations of the linear slope are calculated as follows:

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

$$S_{b1} = \frac{s}{\sqrt{\sum_{i=1}^{n} (x - \bar{x})^2}} \qquad (3)$$

As $|b1| < t_{0.95,n-2}\, s(b1)$, the slope is nonsignificant and no instability is observed. In equation (3), *s* represents the standard deviation of a straight line; b_0 represents the straight-line intercept, b_1 represents the slope of the

Table 1. Homogeneity test results of three levels of frozen mixed serum potassium candidate reference materials.

Bottle number	Level 1	Level 2	Level 3
Total mean	3.07	5.01	6.39
K^a	10	10	10
SS_{among}	0.027	0.020	0.06
SS_{within}	0.240	0.387	0.827
V_{among}	9	9	9
V_{within}	20	20	20
MS_{among}	0.003	0.002	0.007
MS_{within}	0.012	0.019	0.041
F value	0.247	0.117	0.162
$F_{0.05\ (9,20)}$	2.39		

a - number of samples

Table 2. Short-term stability test results of the three levels of candidate reference materials for potassium.

Temperature	Level	T_{0d}	T_{1d}	T_{2d}	T_{4d}	T_{7d}	T_{8d}	T_{12d}	T_{15d}	T_{30d}
		Mean (mmol/L); Bias (%)								
2 - 8°C	L1	2.98	3.00	2.98	3.00	3.00	-	3.00	2.95	2.97
		0.00%	0.67%	0.00%	0.67%	0.67%	-	0.67%	-1.01%	-0.45%
	L2	4.91	4.82	4.85	4.88	4.88	-	4.82	4.87	4.88
		0.00%	-1.83%	-1.22%	-0.61%	-0.61%	-	-1.83%	-0.81%	-0.61%
	L3	6.10	6.10	6.10	6.12	6.10	-	6.13	6.10	6.13
		0.00%	0.00%	0.00%	0.33%	0.00%	-	0.49%	0.00%	0.49%
18 - 25°C	L1	2.98	2.97	2.93	2.93	2.92	2.97	2.95	3.05	-
		0.00%	-0.34%	-1.58%	-1.58%	-2.01%	-0.34%	-1.01%	2.35%	-
	L2	4.91	4.86	4.86	4.88	4.87	4.90	4.97	5.02	-
		0.00%	-1.08%	-1.08%	-0.61%	-0.71%	-0.20%	1.15%	2.17%	-
	L3	6.10	6.13	6.10	6.07	6.05	6.05	6.07	6.05	-
		0.00%	0.49%	0.00%	-0.49%	-0.82%	-0.82%	-0.49%	-0.82%	-
37°C	L1	2.98	2.97	2.94	3.05	3.05	-	-	-	-
		0.00%	-0.34%	-1.34%	2.35%	2.35%	-	-	-	-
	L2	4.91	4.91	4.97	4.97	5.12	-	-	-	-
		0.00%	0.00%	1.20%	1.20%	4.21%	-	-	-	-
	L3	6.10	6.15	6.20	6.27	6.22	-	-	-	-
		0.00%	0.82%	1.61%	2.73%	1.91%	-	-	-	-

straight line, $s(b_1)$ represents the standard deviations of the slope of the straight line, and n - 2 represents the degree of freedom.

The test value of samples from -80°C was used as the initial value (0d). The percentage deviation between the mean value and the initial value of samples at different observation times was calculated as:

$Bias\% = [(M_X - M_0)/M_0)] \times 100\%$ (4) M_0: Sample mean of immediate determination (T0d); M_X: Sample mean of different times (T1d - T30d)

Table 3. Linear regression analysis for stability of three levels of candidate RMs for potassium.

Temperature	Time	Regression equation	n	b1	s_{b1}	$t_{0.95,\,n-2}$	$s_{b1}{*}\,t_{0.95,\,n-2}$	Conclusion
2 - 8°C	L1 (30*d*)	y = -0.001x + 2.994	8	−0.001	0.001	2.447	0.002447	stable
	L2 (30*d*)	y = 0.0011x + 4.8461	8	0.000	0.001	2.447	0.002447	stable
	L3 (30*d*)	y = 0.0009x + 6.1018	8	0.001	0.001	2.447	0.002447	stable
18 - 25°C	L1 (15*d*)	y = 0.0036x + 2.9401	8	0.004	0.003	2.447	0.007341	stable
	L2 (15*d*)	y = 0.0087x + 4.8545	8	0.009	0.002	2.447	0.004894	unstable
	L2 (12*d*)	y = 0.0058x + 4.864	7	0.006	0.003	2.571	0.007713	stable
	L3 (15*d*)	y = -0.0043x + 6.1036	8	−0.004	0.0015	2.447	0.003738	unstable
	L3 (12*d*)	y = -0.0053x + 6.1069	7	−0.005	0.002	2.571	0.005142	stable
37°C	L1 (7*d*)	y = 0.0143x + 2.9561	5	0.014	0.007	3.182	0.022274	stable
	L2 (7*d*)	y = 0.0285x + 4.8955	5	0.029	0.005	3.182	0.01591	unstable
	L2 (4*d*)	y = 0.0156x + 4.9127	4	0.016	0.006	4.303	0.025818	stable
	L3 (7*d*)	y = 0.0169x + 6.1394	5	0.017	0.009	3.182	0.028638	stable

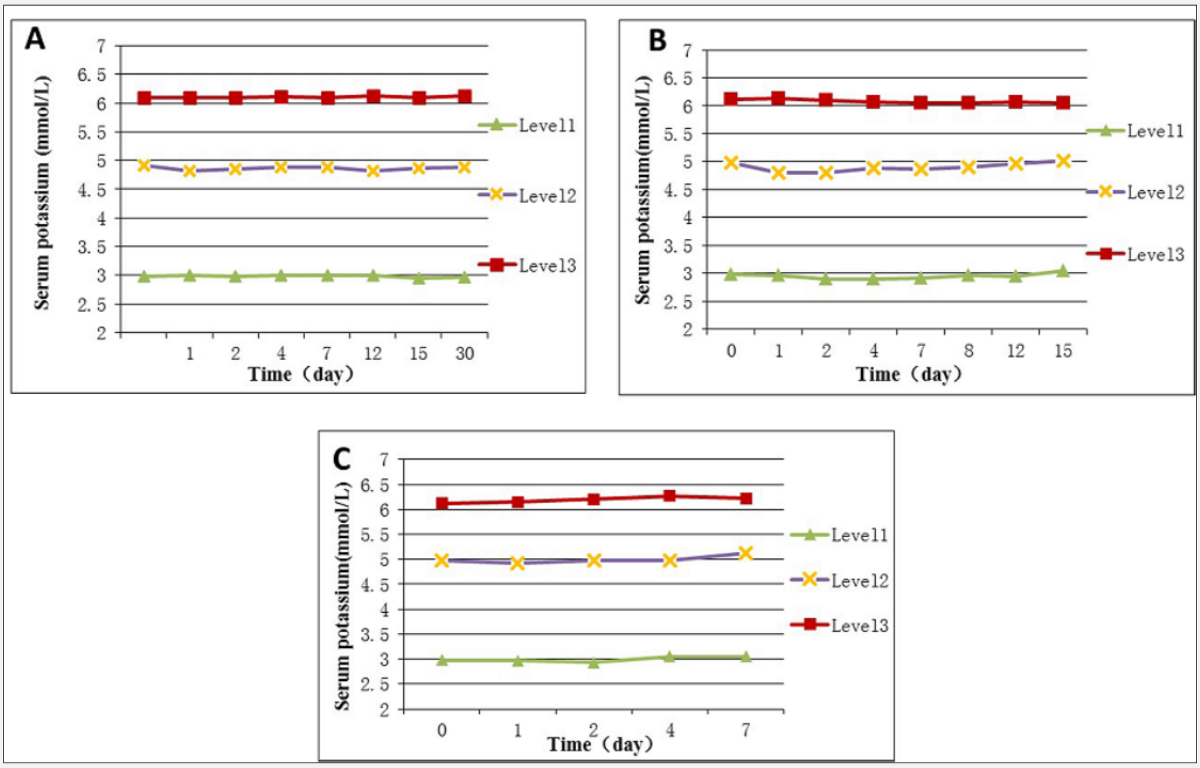

Figure 1. A. Change in the trend of frozen serum potassium at different storage times at 2 - 8°C. B. Change in the trend of frozen serum potassium at different storage times at 18 - 25°C. C. Change in the trend of frozen serum potassium at different storage times at 37°C.

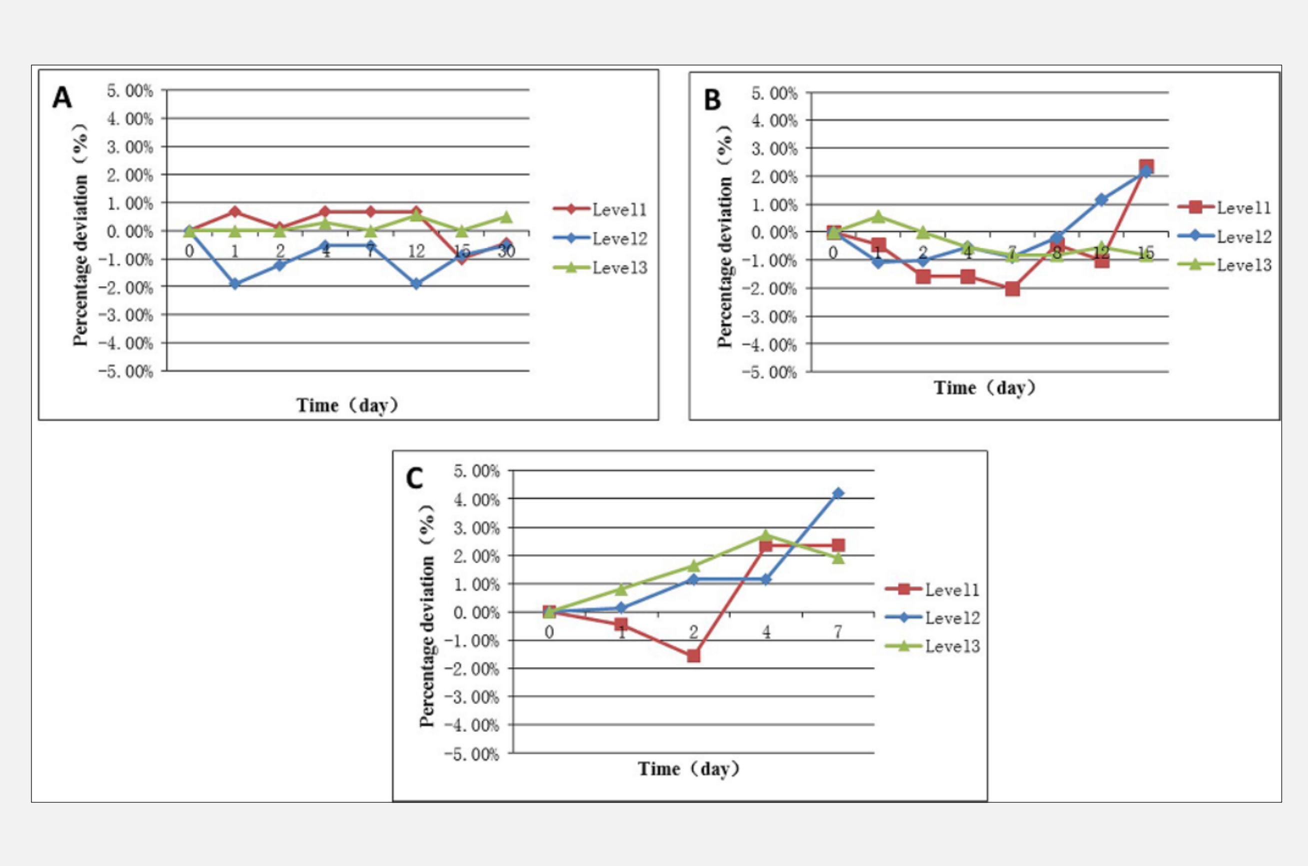

Figure 2. A. The percentage deviation between the mean value and the initial value of RMs for potassium at different time under 2 - 8°C. B. The percentage deviation between the mean value and the initial value of RMs for potassium at different time under 18 - 25°C. C. The percentage deviation between the mean value and the initial value of RMs for potassium at different time under 37°C.

Statistical analysis

The data were processed by a one-way ANOVA approach using SPSS 17.0 software. The ratio of the MS_{among}/MS_{with} was calculated (F value), which was compared with $F_{0.05}$ to determine whether the results of uniformity among the bottles meet the requirements. The linear regression analysis was used in stability testing; the criterion for judging is $|b1| < t_{0.95,\, n-2\, sb1}$.

RESULTS

Homogeneity test results

The F values of the homogeneity test of each level frozen mixed serum potassium candidate RMs were 0.247, 0.117, and 0.162; all of these were less than $F_{0.05}$ (9, 20) = 2.39. It indicates that the three levels of the frozen mixed serum potassium candidate RM have good uniformity. The F value of the homogeneity test of each level candidate RM is shown in Table 1.

Stability test results

Potassium candidates were observed for 1, 2, 4, 7, 12, 15, and 30 days at 2 - 8°C, and 1, 2, 4, 7, 8, 12, 15 days at room temperature, and 1, 2, 4, 7 days at 37°C. The mean values of samples at different observation times and their percentage deviations from the initial values are shown in Table 2. Figure 1A - C shows the trend of stability of three levels of the potassium candidate RM under different conditions. The linear regression analysis results are shown in Table 3. When the short-term stability of the serum potassium was observed for 30, 12, and 4 at 2 - 8°C, room temperature, and 37°C, $|b1| < t_{0.95,\, n-2}\, s_{b1}$, and no instability was observed. Time and percentage deviations were used as trend diagrams, as shown in Figure 2A - C. It showed that during the abovementioned observation period, the percentage deviations were small and the change trend was stable. Therefore, the candidate RMs for frozen human serum potassium at three concentrations were stable at least 30, 12, and 4 days at refrigeration (2 - 8°C), room temperature, and 37°C, respectively.

DISCUSSION

An accurate and traceable RM is important to ensure the accuracy and comparability of measurement results in time and space [12,13]. Homogeneity is the basic characteristic of the standard material. The homogeneity of the sample is related to the properties of the material and processing flow. The influence of the measurement method, sampling method, sampling quantity, measurement sequence, and data analysis of measurement results should be considered in a homogeneity test. According to the standard substances/calibrated sample producer capacity accreditation criteria, homogeneity testing is closely related to the precision (repeatability) of the measurement method, and therefore, homogeneity testing requires that the measurement method be under the optimal repeatability conditions; the standard deviation of the measurement method should be small, and the same operator should complete testing within a short time using the same instrument in the same laboratory. In this study, the ion selective electrode method was used for homogeneity testing. Through calibration and performance verification, the results obtained by the selected method showed its high precision and reproducibility, and it was suitable for the simultaneous measurement of a large number of samples in a short period of time.

The certified values of RMs were directly affected by the stability of the RMs, which was taken seriously by World Health Organization (WHO). The revised guidelines of WHO 2004 point to the use of statistical analysis to help determine storage conditions, transport conditions, and how products are used in the laboratory. The stability of the product was evaluated by determining its activity or titer at different temperatures and at different times. Short-term stability studies are usually carried out at different temperatures - refrigeration (2 - 8°C), room temperature, and 37°C - that may be encountered during transportation and use.

The stability study includes two basic experimental designs: classical stability studies and synchronous stability studies. The measurements of the two stability studies can be carried out under the condition of repeatability in the synchronous stability study, which only requires one calibration for one measurement. Therefore, the uncertainty of the synchronization stability research is usually lower than that of classical methods for the less difference between the measurement of repeatability and reproducibility.

The purpose of this study was to verify the short-term stability of serum potassium candidate RMs and to explore the effect of storage time under different transport conditions on the stability of serum potassium. The method of synchronous stability is adopted to avoid discreteness of time points. A straight line is used as an empirical model for stability evaluation. Linear regression analysis was carried out on the test data of three levels of candidate RMs at different concentrations. No instability was observed when the samples were under refrigeration (2 - 8°C), room temperature, and 37°C for 30, 12, and 4 days, respectively. However, at room temperature for 15 days and 37°C for 7 days, $|b1| > t_{0.95,\, n-2} \cdot s_{(b1)}$ and instability was observed.

The innovation of this study was that we also analyzed the trend in the percentage deviation between the results of each concentration level and the real-time (initial value) value at different times and different conditions. Through the percentage deviation diagram, the changing trend of RMs for serum potassium with time under different temperature conditions could be shown more intuitively. The percentage deviation was smaller, and the trend in the change was stable for 30 days under the condition of refrigeration (2 - 8°C). The 15th day at room temperature and 7th day at 37°C showed larger deviations than others, which is consistent with the results of the linear regression analysis. Therefore, the refrigeration (2 - 8°C) condition has the least effect on the stability of the RMs, and it is the best storage condition for transportation. The observation period of the RMs for serum potassium under different transport conditions (short-term stability) and storage conditions (long-term stability) can be explored in a follow-up study, and the stability of reference materials for serum potassium can be evaluated comprehensively.

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

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