Clin. Lab. 2018;64:XXX-XXX

ORIGINAL ARTICLE

Performance Verification of Alternative Quality Control Materials for Urine Albumin Assessment

Hua-Bin Wang, Yi Hu, Xiao-Yun Shan

Department of Clinical Laboratory, Jinhua Central Hospital, People's Republic of China

SUMMARY

Background: **Accurate detection of urine albumin is important for evaluating the progression of diabetic kidney disease. However, two levels of daily quality control may not be practically feasible in some small clinical laboratories owing to a small number of patient samples and high costs. We aimed to prepare homemade quality control material (HQM) to measure urine albumin and then verify its performance.**

Methods: **Normal saline solution and fresh mixed urine samples from five donors with serious kidney disease were used to prepare two levels of HQM (HQM1 and HQM2). Anhydrous ethylene glycol and sodium azide were used as antifreeze and as a preservative, respectively.**

Results: **Before being separated into Eppendorf tubes, 20 tests for HQM1 and HQM2 were performed, resulting in mean ± SD of 19.52 ± 0.91 mg/L and 105.28 ± 3.71 mg/L, respectively. After having been divided, the vial-to-vial variations of HQM1 and HQM2 were small (4.93% and 3.70%, respectively). The stability of HQM1 and HQM2 stored at 2 - 8°C was about 2 months and 80 days, respectively, and when stored at -20°C, remained stable for more than 8 months. After 1 - 8 months of cryopreservation at -20°C, once opened, the HQM in every Eppendorf tube could be kept for at least five days (CV < 6.1%).**

Conclusions: **Our HQM stored at -20°C remained stable for a long time, and so could be considered as an alternative to standard QMs in the clinical laboratory.**

(Clin. Lab. 2018;64:xx-xx. DOI: 10.7754/Clin.Lab.2017.170906)

Correspondence:
Hua-Bin Wang
Department of Clinical Laboratory
Jinhua Central Hospital
Mingyue Street
Jinhua City, Zhejiang Province
China
Email: whb798183844@126.com

Manuscript accepted November 8, 2017

KEY WORDS

urine albumin, homemade quality control material, performance verification

INTRODUCTION

As an independent kidney damage marker, urine albumin excretion is closely associated with diabetic kidney disease and cardiovascular events [1-3]; so, accurate detection of urine albumin plays an important role in monitoring the progression of diabetic nephropathy and cardiovascular disease. In clinical laboratories, more than two levels of daily quality control material (QM) are necessary for reliable results [4]. However, it is often practically infeasible for some small clinical laboratories to measure two levels of urine albumin QM every day owing to the high cost of standard QM and a small number of patient samples.

At the annual meeting organized by the Jinhua Center for Clinical Laboratories in 2016, the chief of the center reported that the number of patient samples for testing urine albumin were between 8 and 50 per laboratory, and less than 33% of laboratories performed daily quality control in Jinhua city, Zhejiang province, China; therefore, on a large number of occasions, the laboratories were not able to ensure the reliability of urine albumin results. Therefore, we aimed to prepare a homemade QM (HQM) for urine albumin and to assess whether it could be applied in clinical practice.

MATERIALS AND METHOD

Materials and Preparation

Eight volunteers with high urine albumin excretion were recruited, and their first or second morning urine were collected on the same day [5]. Informed consent was obtained from the donors. Three of the eight urine samples were excluded according to the following criteria: (1) infection of the urinary system and/or (2) occurrence of hemoglobin in the urine sample. Afterwards, the samples were centrifuged at 400 x g for 10 minutes to remove any precipitation and the urine albumin concentration of fresh mixed urine from the five donors was measured. According to the value, the mixed urine was diluted to two levels of HQM (HQM1 and HQM2) with normal saline solution and anhydrous ethylene glycol (antifreeze, 33% v/v). Next, sodium azide was added to the HQM as a preservative (3 g/L). Finally, the two levels of HQM were separated into Eppendorf tubes (1 mL per tube), one of which was stored at 2 - 8°C and the other at -20°C.

Measurements

Urine albumin was determined by immunonephelometry with a Dade-Behring BNII special protein analyzer, for which the manufacturer provided Siemens original reagents and quality control materials. Before testing patient samples and the HQM, standard QM was used to provide reliable results. We measured the HQM stored at 2 - 8°C and -20°C every day to evaluate the vial-to-vial variation to ascertain its stability. Before testing, samples were allowed to return to room temperature and thoroughly mixed by inversion.

Statistical analysis

Before being separated into Eppendorf tubes, we tested the HQM1 and HQM2 20 times to calculate their means and standard deviations (SDs), and coefficients of variation (CVs). Subsequently, we use these three parameters to evaluate whether the HQMs were stable in the daily tests. After separation into Eppendorf tubes, 20 tubes of HQM1 and HQM2 were randomly selected to assess vial-to-vial variation. Every month, we also verified how long the HQM was able to remain stable once opened and stored tightly capped at 2 - 8°C. All analyses were performed using SPSS 19 (IBM, Armonk, NY, USA) and GraphPad Prism 5 (Graph-Pad Software, Inc., La Jolla, CA, USA). $p < 0.05$ was considered as statistically significant.

RESULTS

The values of HQM before division and the vial-to-vial variation of HQM after division are displayed in Table 1. The mean ± SD of the two levels of HQM before division were 19.52 ± 0.91 mg/L and 105.28 ± 3.71 mg/L. There was no statistically significant difference between the HQMs before and after division ($p = 0.186$ for HQM1 and $p = 0.079$ for HQM2).

In addition, small vial-to-vial variation was found in both HQM1 and HQM2 (CV = 4.93% for HQM1 and CV = 3.70% for HQM2).

Figure 1 shows the daily results for HQM stored at 2 - 8°C, in which the Y-axis presents the mean and SD of the HQM before division. On days 1 to 60, the HQM1 stored at 2 - 8°C remained stable (19.38 ± 1.15, CV = 5.91%). However, the values of HQM1 obviously decreased after about 60 days of preservation. Similarly, the values of HQM2 stored at 2 - 8°C took on a decreasing trend after about 72 days of preservation. By comparison, HQM1 and HQM2 stored at -20°C remained stable for at least 8 months (19.45 ± 1.11, CV = 5.72% for HQM1 and 104.88 ± 4.27, CV = 4.07% for HQM2; $p > 0.05$ compared with HQM before division).

The daily results for HQM stored at -20°C are displayed in Figure 2, in which the Y-axis also presents the mean and SD of the HQM before division. For HQM1, there were 18 values that exceeded the range of the mean ± 2 SDs and only 2 values that exceeded the range of the mean ± 3 SDs. Correspondingly, there were 16 values that exceeded the range of the mean ± 2 SDs and 2 values that exceeded the range of the mean ± 3 SDs for HQM2.

On days 30, 60, 90, 120, 180, and 240 for each level of HQM stored at -20°C, one tube was measured once a day for five days, during which once opened, it was stored tightly capped. The HQM which once opened remained stable for at least 5 days (1 mL per tube was not sufficient to run tests for a longer time period than this) even when measured after 8 months of cryopreservation (shown in Table 2). No significant statistical difference was found between the values of the opened HQM and the HQM before division ($p > 0.05$).

DISCUSSION

Urine albumin as an early biomarker to screen for kidney damage plays an important role in monitoring the progression of diabetic kidney disease and cardiovascular disease [6-8], and consequently, it is important to report reliable results of urine albumin levels for patients with these conditions. However, there is no unified

Table 1. The values and vial to vial variation of HQM.

	Before division		After division (vial to vial variation)	
	HQM1	HQM2	HQM1	HQM2
Mean ± SD	19.52 ± 0.91	105.28 ± 3.71	19.47 ± 0.96 [a]	103.96 ± 3.85 [b]
Mean ± 2SD	17.7 - 21.34	97.86 - 112.7	17.55 - 21.39	96.26 - 111.66
Mean ± 3SD	16.79 - 22.25	94.15 - 116.41	16.59 - 22.35	92.41 - 115.51
CV%	4.66%	3.52%	4.93%	3.70%

Abbreviations: HQM - homemade quality control material, Mean ± 2SD - range from mean -2SD to mean +2SD, Mean ±3SD - range from mean -3SD to mean +3SD. [a] - p > 0.05 compared with HQM1 before divided, [b] - p > 0.05 compared with HQM2 before divided.

Table 2. The stability of the opened HQM after 1 - 8 months storage.

Preservation time	HQM1		HQM2	
	Mean ± SD	CV%	Mean ± SD	CV%
One month	20.03 ± 0.92 [*]	4.59%	107.61 ± 3.87 [#]	3.40%
Two months	19.56 ± 1.06 [*]	5.42%	106.02 ± 3.81 [#]	3.59%
Three months	19.84 ± 1.02 [*]	5.14%	104.76 ± 4.52 [#]	4.31%
Four months	19.28 ± 1.13 [*]	5.86%	103.98 ± 3.94 [#]	3.79%
Six months	19.39 ± 1.17 [*]	6.03%	106.27 ± 4.21 [#]	3.96%
Eight months	19.81 ± 1.19 [*]	6.01%	104.75 ± 4.17	3.98%
p	0.171		0.158	

Abbreviations: HQM - homemade quality control material. [*] - p > 0.05 compared with the HQM1 before divided, [#] - p > 0.05 compared with the HQM2 before divided.

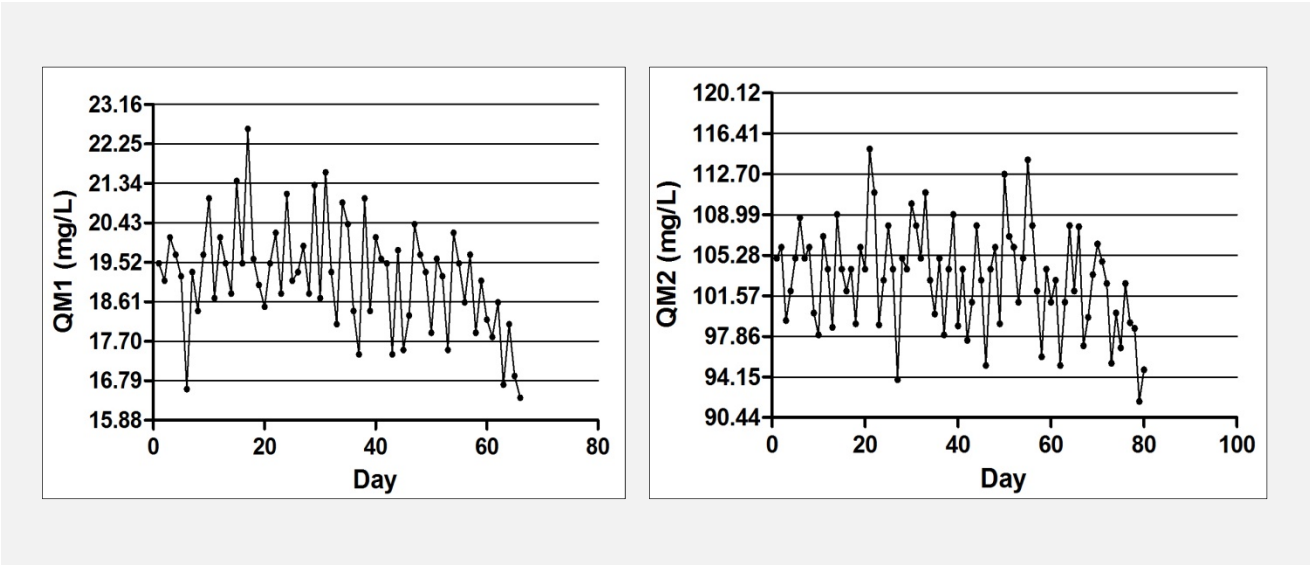

Figure 1. The daily results for HQM1 and HQM2 stored at 2-8 degrees Celsius.

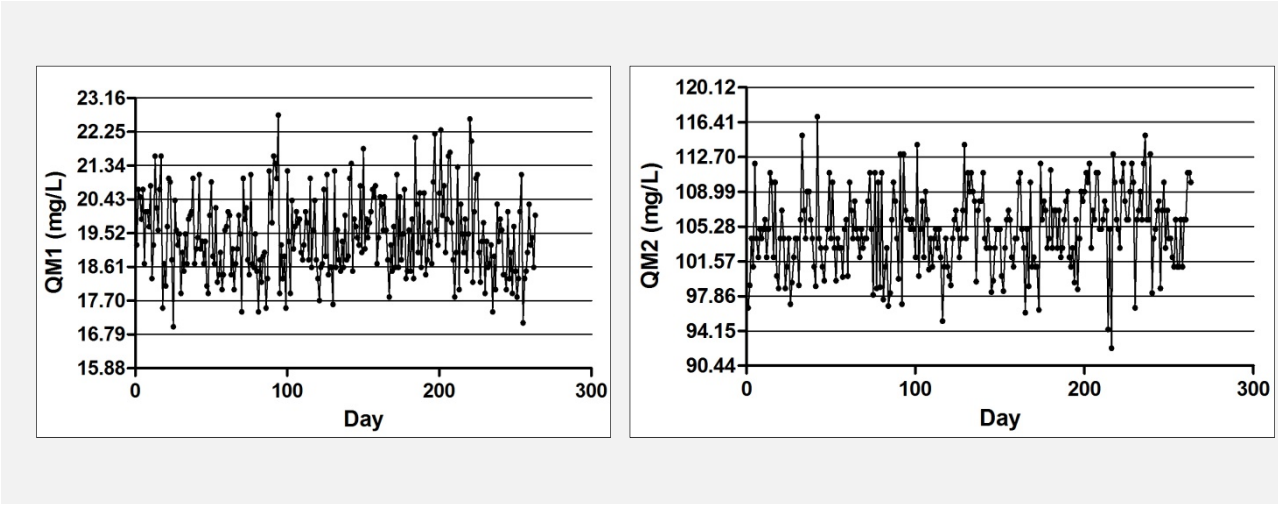

Figure 2. The daily results of HQM1 and HQM2 stored at -20 degrees Celsius.

standardization of urine albumin measurement based on a complete reference system in the world so far [9], thus the accuracy and comparability between clinical laboratories are not always satisfactory. The external quality assessment organized by the National Center for Clinical Laboratories showed high variation (60% for low levels of urine albumin and 45% for high levels of urine albumin).

In clinical laboratories, it is important to perform more than two levels of daily QM for reliable results [4]. However, it is not always practically feasible for some small laboratories because of small numbers of urine albumin samples to be tested and expensive imported QMs. Thus a useful and affordable QM for urine albumin is needed for such resource-poor settings in China. Therefore, we prepared the HQM of urine albumin in the expectation that it could be used as an alternative QM.

The performance verification of HQM included stability after storage over a long period of time, vial-to-vial variation, and stability once opened. We used fresh mixed urine samples, normal saline solution, anhydrous ethylene glycol, and sodium azide to prepare two levels of HQM. The anhydrous ethylene glycol prevented freezing of the solution and reduced the effects of multi-gelation. Afterwards, the HQM was separated into Eppendorf tubes (1 mL per tube) and its performance was verified. We found that the vial-to-vial variation of the HQM was small for both HQM1 and HQM2 (4.93% and 3.70%, respectively), and there was no statistical difference between the HQM in each Eppendorf tube. The stability of HQM stored at 2 - 8°C decreased after about 2 months of storage. Compared with HQM stored at 2 - 8°C, both HQM1 and HQM2 stored at -20°C remained stable for a longer period of time. Although the measures of daily HQM were finished after 263 days owing to the number of HQMs prepared at the beginning of the experiment, we consider that the HQM stored at -20°C would probably remain stable for more than 8 months. Therefore, -20°C is a better storage temperature than 2 - 8°C for the HQM.

CONCLUSION

In this study, we found that once opened and tightly capped while stored, the HQM stored at -20°C remained stable for at least five days (1 mL per tube was not sufficient to run tests for a longer time period than this). After 30, 60, 90, 120 ,180, and 240 days of cryopreservation, the CVs were 4.59%, 5.42%, 5.14%, 5.86%, 6.03%, and 6.01% for HQM1 and 3.40%, 3.59%, 4.31%, 3.79%, 3.96%, and 3.98% for HQM2, respectively. There was also no significant difference between the values of the opened HQM and the HQM before division ($p > 0.05$).

It is worth mentioning that although the normal saline solution and anhydrous ethylene glycol were used to adjust the concentration of urine albumin in the HQM, it is difficult to prepare the quality control materials which have the same urine albumin concentration every time. Although a comparison with the imported QM was not included, this study shows that the HQM of urine albumin prepared in this way is feasible for some resource-poor settings such as small clinical laboratories in China. The HQM showed good stability after 8 months of cryopreservation (CV < 6.1%). In addition, it is necessary to confirm the stability after longer time periods of storage in a further study.

Acknowledgement:
The authors wish to thank the colleagues for their great help in testing the homemade quality control materials.

Contribution:
Study design: Hua-Bin Wang, Xiao-Yun Shan. Experiment performed: Yi Hu, Manuscript writing: Hua-Bin Wang.

Ethical Approval:
All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation.

Funding:
This research received the grant from Science Technology Department of Jinhua City, Zhejiang province, China (2017-4-066).

Declaration of Interest:
None of the authors declared any potential conflicts of interest.

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