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Original Article

Establishment of internal quality control system in anti-mitochondrial antibody subtype M2 detection by using human serum as quality control material

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Abstract: Internal quality control (QC) material is important for day-to-day checking of analytical processes in a clinical laboratory. For anti-mitochondrial antibody subtype M2 (AMA-M2) detection, there are no commercial QC materials available. In this study, pooled serum was evaluated as internal QC material for AMA-M2 detection. Remnant serum samples of primary biliary cirrhosis (PBC) patients requested for AMA-M2 were collected after test. The collected samples were pooled and homogenized. After heat-inactivation the pooled serum was divided in aliquots as internal QC material. The stability of the internal QC material and the precision of the AMA-M2 detecting system were evaluated, and a 20-day daily internal QC test was assessed. The quality of pooled serum was reliable, and the validity period could last for more than 16 months. The precision of AMA-M2 signal intensity detection was stable with a mean value 112.7 and coefficients of variation (CV) 9.7% in repetitive tests of one same sample, and a mean value 113.4 and CV 11.8% in different sample. Considering the 20-day daily internal QC evaluation period, the CV of the internal QC results was 8.1% which achieved the goal of internal quality control. The results suggest that the home-made AMA-M2 internal QC material was stable and reliable. The AMA-M2 signal intensity detection system has high precision. The home-made AMA-M2 internal quality control system has a good application value for its stability, precision, and cost effectiveness.

Keywords: Internal quality control, AMA-M2, pooled serum, primary biliary cirrhosis

Introduction

Anti-mitochondrial antibody (AMA) is a group of serum signature antibodies of primary biliary cirrhosis (PBC) [1, 2]. Among them, anti-mitochondrial antibody subtype M2 (AMA-M2) has peculiar sensitivity and specificity for PBC diagnosis, with the specificity up to 95%, and has become one of the most important diagnostic methods of PBC [3].

Currently, whole set of automatic detection system, including analysis equipment, reagent and calibration product which are manufactured by the same manufacturer, are widely adopted in clinical laboratory for clinical examination because of high precision, accuracy, and stability. However, there is no such complete set of detection system for AMA-M2 detection. Self-built testing system is often used in clinical laboratory for AMA-M2 detection, but there is a lack of standardized technical platform and equipment among different laboratories. ISO15189 standards require the laboratory to confirm the performance of the self-built testing system to ensure the accuracy and reliability of the test results [4]. Therefore, internal quality control (QC) of AMA-M2 detection is vital to obtain an accurate testing result.

Internal QC material is important for day-to-day checking of analytical processes in a clinical laboratory, and validation of internal quality control system makes laboratory results obtained from patients' samples reliable. However, for most autoimmune markers such as AMA-M2, there are no commercial QC materials. Even for some autoimmune markers, there are com-

moditized QC materials, but not suitable for daily QC activities due to a high cost. To conduct day-to-day checking for internal quality control system, a large amount of QC material is required. The QC materials must be stable, homogenous, and cost effective, with a long validity period.

Human serum is suitable for preparing control materials in clinical immunological laboratories. There have been some reports of evaluations of pooled serum as QC material for tumor markers and biochemical markers, but not for autoimmune markers [5, 6]. Therefore, the aim of this study was to evaluate pooled serum as internal QC material for AMA-M2 detection. Remnant serum samples of PBC patients requested for AMA-M2 were collected after test. The collected samples were pooled and homogenized. After heat-inactivation the pooled serum was divided into 81 aliquots as internal QC material. The stability of the internal QC material and the precision of the AMA-M2 detecting system were evaluated, and a 20-day daily internal QC tests was assessed.

Materials and methods

Serum collection

From August 2016 to February 2017, remnant serum samples requested for AMA-M2 with a signal intensity of AMA-M2 of about 4 times of cut off value were collected after the test results were obtained. Only patients whose test results for HBsAg and antibodies against HIV, HCV, TP were all negative were selected. Information of patients was immediately removed after serum collection for confidentiality protection. The collected samples were pooled and homogenized. The pooled serum comprised remnant serum samples from 18 patients with a total volume of about 45 mL.

Heat inactivation and preparation of aliquots

Since the pooled serum could have an unacceptable risk of transmitting etiological agent and causing infection, heat inactivation was used in this study. The pooled serum was incubated at 56°C for 30 minutes. According to the main study design, a total of 81 aliquots were made and the volume of each aliquot was 500 μL. Ten aliquots were used to study the stability, twenty aliquots to study the CV of determination in different aliquot, one aliquot to study the CV of repetitive determination in a single QC material, and fifty aliquots for daily internal QC use. All aliquots were stored at -20°C.

AMA-M2 detection

AMA-M2 detection was conducted using a EUROLINE ANA Profile (IgG) test kit (EUROIMMUN, Lubeck, Germany). Strips were automatically incubated with a EUROBlotOne (EUROIMMUN, Lubeck, Germany). The protocol for strip incubation provided by the manufacturer was as follows. Pretreatment: Put the test strip into the incubation channel and fill each channel with 1.5 ml sample buffer. Incubate for 5 minutes at room temperature on a rocking shaker. Afterwards aspirate off all the liquid. Incubation (1st step): Fill each channel with 1.5 ml diluted serum samples (1:101) and incubate at room temperature for 30 minutes on a rocking shaker. Wash: Aspirate off the liquid from each channel and wash 3 × 5 minutes each with 1.5 ml working strength wash buffer on a rocking shaker. Incubation (2nd step): Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase-labelled anti-Human IgG) into each channel and incubate for 30 minutes at room temperature on a rocking shaker. Wash: Aspirate off the liquid from each channel. Wash as described above. Incubation (3rd step): Pipette 1.5 ml substrate solution into the channels of the incubation tray. Incubate for 10 minutes at room temperature on a rocking shaker. Termination: Aspirate off the liquid from each channel and wash each strip 3 × 1 minute with distilled water. Evaluation: Place test strip on the evaluation protocol, air dry and evaluate. The dry incubated test strips were scanned using a flatbed scanner (EUROIMMUN, Lubeck, Germany), and the signal intensity of AMA-M2 was evaluated with EUROLineScan software.

Stability evaluation

Ten aliquots of pooled serum were incubated in water bath with a temperature 37°C for totally ten days. One aliquot was taken each day for AMA-M2 detection six times. According to Arrhenius equation (a formula for the temperature dependence of reaction rate), one day in 37°C water bath is equivalent to 1.6 month in 4°C refrigerator. The maximum number of days with a bias of less than 20% between the measured mean and the original mean is considered as a stable period.

Precision evaluation

One aliquot (assigned serial number U) was taken, and the signal intensity of AMA-M2 was measured 20 times, the mean (X) and standard deviation (SD) was calculated, and the coefficient of variation (CV) was calculated afterwards.

Twenty aliquots (assigned serial number A to T) were taken, and the signal intensity of AMA-M2 was measured one time each, the mean (X) and standard deviation (SD) was calculated, and the coefficient of variation (CV) was calculated afterwards.

Daily internal QC test

Another twenty aliquots were used for daily internal quality control (twenty working days). Internal QC data were recorded and presented as the mean ± standard deviation (SD). The coefficient of variation (CV) was calculated, and westgard multi-rule control method was adopted.

Results

Stability evaluation

Stability test results are presented in **Table 1**. The original mean value was 113.0, and the CVs of measured mean in different days were between -10.9% and 18.9%, still falls in a reasonable range (±20%). According to Arrhenius equation, the quality of pooled serum was considered stable and reliable, and the validity period could last for more than 16 months when kept in a 4°C refrigerator.

Precision evaluation of AMA-M2 signal intensity detection

The results of precision evaluation are shown in **Table 2**. Overall, the AMA-M2 signal intensity was stable in repetitive tests of one same sample with a mean value 112.7 and coefficient of variation (CV) 9.7%. The AMA-M2 signal intensity was also stable in different aliquots of pooled serum with a mean value 113.4 and coefficient of variation (CV) 11.8%.

Results of internal QC test during the 20-day study period

The results of internal QC tests during the 20-day study period are shown in **Table 3**. Overall, the internal QC results during the study period were stable. Considering the entire 20-day evaluation period, the CV of the internal QC results were 8.1%.

Westgard multi-rule control method: 1-2 s, 2-2 s, 1-3 s rule were adopted, setting target value to 113, 1 s value to 10, CV to 8.8%. The results of internal QC located in 1 second reach 40.0%, in 2 seconds reach 90.0% and in 3 seconds reach 100% (**Figure 1**).

Discussion

Quality control is an important part in the quality management system. The ability to quantify the sample with good accuracy and precision is essential in clinical laboratories. Thus, internal QC material used to validate the measurement is important for day-to-day checking of analytical processes.

PBC is an autoimmune liver disease caused by non-inflammatory intrahepatic bile duct destruction [7]. AMA-M2 is one of the important diagnostic indicators of PBC [8]. AMA is located in the lumen of bile duct epithelial cells and mitochondrial endometrium [9, 10]. The detection of AMA-M2 can provide important support for the pathological diagnosis of PBC when other liver diseases are excluded [9, 11, 12].

Currently, the AMA-M2 is often detected by the automatic immunoblot (EUROBlotone) in the clinical laboratory with a EUROLINE ANA Profile (IgG) test kit. The test kit uses linear immunoblot method to fix the AMA-M2 antigen on the acetate film, and positive serum can bind to the AMA-M2 antigen. After adding the enzyme conjugate and substrate solution, positive serum is shown as a gray band. The dry test strips can be scanned with a flatbed scanner and the signal intensity of AMA-M2 can be evaluated. It may have weak positive or negative results in the actual work. Furthermore, it is difficult to avoid false negative results caused by the invalidation of antigen in the strips. The reliability of the results is greatly reduced without quality control. In addition, it is very difficult to deal with the critical value. Therefore, it is necessary to replace original qualitative quality control by quantitative quality control and establish quality control chart.

In the present study, pooled serum was used as internal QC material in AMA-A2 detection.

Table 1. Stability evaluation of the internal QC material

	Days under going 37 °C water bath incubation										Grand mean
	1	2	3	4	5	6	7	8	9	10	
Six times of AMA-M2 signal intensity detection	130	128	139	112	112	109	84	107	105	104	113.0
	130	112	140	118	116	109	115	116	105	101	
	130	132	137	116	114	110	116	109	106	100	
	89	151	135	112	112	116	116	109	106	100	
	128	113	130	114	112	115	109	109	106	101	
	111	161	125	113	114	100	113	120	104	98	
Mean	119.7	132.8	134.3	114.2	113.3	109.8	108.8	111.7	105.3	100.7	
CV[a]	5.9%	17.6%	18.9%	1.0%	0.3%	-2.8%	-3.7%	-1.2%	-6.8%	-10.9%	

[a]CV = (Mean-Grand mean)/Grand mean.

Table 2. Precision evaluation of AMA-M2 signal intensity detection

	One aliquot measured for 20 times																				Mean ± SD	CV
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
Signal intensity of AMA-M2	112	118	116	112	147	113	112	116	114	112	112	114	84	115	116	116	109	105	105	106	112.7±10.9	9.7
	Twenty aliquots measured for one time respectively[a]																				Mean ± SD	CV
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T		
Signal intensity of AMA-M2	130	128	106	112	112	109	84	107	105	104	101	105	116	115	109	116	118	148	112	130	113.4±13.4	11.8

[a]Twenty aliquots were assigned from A to T.

Table 3. Internal QC tests during the 20-day study period

	Days of ongoing internal QC tests																				Mean ± SD	CV
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
Signal intensity of AMA-M2	130	100	138	130	132	139	124	129	132	120	126	128	122	116	115	112	112	122	125	113	123±10	8.1

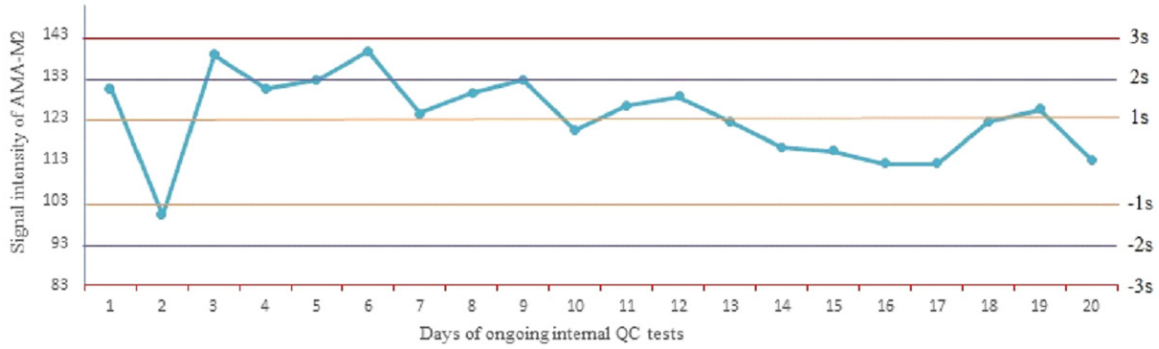

Figure 1. Quality control profile of 20 days internal QC test. Westgard multi-rule control method: 1-2 s,2-2 s,1-3 s rule were adopted, set target value to 113, 1 s value to 10, CV to 8.8%.

From the results of precision evaluation, the AMA-M2 signal intensity was stable in repetitive tests of one same sample or different aliquots of pooled serum. The CVs were located in the scope of ISO15189 quality system certification requirements. In the internal QC test during the 20-day study period, practical CV was set upper limit as 8.8% which achieved the goal of internal quality control. The analysis results show that all internal quality control results were in control. Therefore, the home-made AMA-M2 internal quality control system has a good application value and development prospect for its stability, precision, and cost effectiveness.

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Disclosure of conflict of interest

None.

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