ESTIMATION OF CKMB FROM RIQAS CONTROL SERUM AT 37 DEGREE CELSIUS

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ABSTRACT

The quality control sera commonly available in market does not provide the value of CKMB. The CKMB kit of Randox laboratories contains two lyophilized control sera. But the stability of the control serum after reconstitution with 2ml of distilled water is 5 days at 2-8 degree Celsius, 8hrs at 25 degree Celsius and 4 weeks at -20 degree Celsius when frozen once. Hence stability after reconstitution is not sufficient to fulfill the daily need of a laboratory. In quest of a good internal quality assessment (IQA) sample trial run has been performed at 37 degree Celsius using external quality assessment (EQA) samples obtained from Randox international quality assessment sample (RIQAS). The trial run was found to be successful.

KEYWORDS

IQA, EQA, RIQAS, MI, CKMB

INTRODUCTION

The concentration of creatine kinase isoenzyme (CKMB) in myocardium is very high. After acute myocardial infarction, CKMB appears in blood within approx 4-8 hrs, peaks at 12-24 hrs and persists throughout the initial 72 hrs period (1). Because of such unique nature CKMB estimation from serum has always been considered as biochemical gold standard by WHO for detection of myocardial injury. Though now a days, cardiologists rely more on Troponin T (2), but that has not lessened the degree of importance of CKMB estimation. The diagnostic application is totally dependant on the method and authenticity of estimation. In our laboratory we used CKMB kit from Randox Laboratories (U.K). Inside the kit a lyophilized control serum of known range has been provided to prove the authenticity of the test. The lyophilized control serum after reconstitution with 2ml distilled water was found to be stable for 5 days at 2-8 degree Celsius, 8 hrs at 25 degree Celsius and 4 weeks at -20 degree Celsius when frozen once. Both the stability and quantity of the control serum was not sufficient to fulfill the need of our laboratory. Hence the necessity of a stable quality control serum of sufficient amount initiated the present study.

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MATERIALS AND METHODS

CKMB (NAC -act) kit from Randox Ltd. (U.K), contains the following reagents :-

- a. Buffer / glucose (1 x 70)ml - ready to use.
- b. Enzymes / coenzymes / substrate / antibody 19 vials (lyophilized) each to be reconstituted with 2.5ml of (a).
- c. Control serum (lyophilized) - 1 vial to be reconstituted with 2ml of water.

The Biochemistry Dept. of Peerless Hospital and B.K.Roy Research Centre are participants of an external quality assessment (EQA) scheme known as RIQAS (Randox International quality assessment scheme) whose headquarter is in U.K. The scheme is ISO 9001 and National Advisory Panel, U.K. accredited. The participants receive EQA samples for general chemistry in two cycles (13 samples in each) in a year. Reports are to be sent fortnightly to the headquarter. In the 1st half of the year samples are given odd nos. (R1, R3, R5 etc.) and in the 2nd half even nos. (R2, R4, R6 etc. R stands for RIQAS). From the general chemistry EQA samples 30 analytes may be tested. Samples are to be reconstituted with 10ml distilled water. These EQA samples were considered as test samples for serial estimation of CKMB on alternate days over a period of 45 days. In the present study a total of eight EQA samples were chosen for trial run R10,

R12, R14, R16 FROM CYCLE 23 (Oct. 2000 – March 2001) and R1, R3, R5, R7, from cycle 24 (April 2001 – Sept. 2001).

RIQAS samples were reconstituted using 10ml distilled water and preserved in aliquots of 100µl at -20 degree Celsius, to be frozen once.

CKMB was estimated by immunoinhibition assay (3) in Clinical 700 system (Beckmann)

Name – CKMB

Method – Fixed time / Two point

Factor – 1651

Standard – No

Wavelength – 340 nm

Reagent Blank – Yes

Measuring time – 600 secs

Delay time – 300 secs

Assay temp. – 37 degree C

Unit – U / l

Sample volume – 20 µl

Reagent volume – 500 µl

Aspiration volume – 450 µl

RESULTS AND DISCUSSION

The RIQAS samples were estimated on every alternate days upto day 45. The control serum provided in the kit was tested on every 5th day. The

results are shown in Table. According to RIQAS they do not provide CKMB report alongwith the 30 analytes, because the creatine kinase (CK) of the sample is of animal origin (J, Wilson, RIQAS H. Q., U. K. personal communication). There is a separate quality control programme of creatine kinase and CKMB which is of human matrix and naturally costly. The importance of CKMB control of human matrix is beyond dispute. But in the present study it has been shown that the CKMB of animal origin is also responding to the same procedure. The percentage and specificity of binding is yet to be found out. It can be concluded at present that a certain percentage of binding with the human monoclonal antibody is taking place and it is remaining constant within acceptable range over a period of at least 45 days in each of eight EQA samples. It proves that the stability of the isoenzyme in each of the samples is beyond doubt. Hence the EQA samples for general chemistry analytes may also be considered as internal quality assessment samples for CKMB at no extra cost.

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TABLE

Day	Randox Control	RIQAS Control : Cycle 23				RIQAS Control : Cycle 24			
		R 10	R 12	R 14	R 16	R 1	R 3	R 5	R 7
01	117	96	120	100	342	94	98	123	102
03		97	119	99	340	95	97	122	101
05	119	94	116	98	338	95	97	120	102
07		92	121	103	335	93	98	120	101
09	120	96	119	101	345	95	96	119	100
11		97	120	99	341	94	95	118	99
13	120	89	118	98	339	96	97	123	104
15		97	125	104	340	94	98	124	100
17	123	95	120	101	339	93	99	121	99
19		96	121	99	338	92	100	120	103
21	128	89	118	97	340	91	101	119	101
23		91	117	96	341	95	100	121	100
25	124	94	121	99	340	96	99	120	99
27		95	118	100	341	97	98	122	100
29	123	94	119	97	340	94	96	120	102
31		96	122	98	339	98	95	121	101
33	125	92	120	96	338	96	94	122	99
35		97	118	95	337	95	99	119	98
37	120	95	119	99	345	98	100	118	100
39		96	121	101	343	98	97	117	101
41	123	97	118	99	345	99	98	122	102
43		97	117	100	344	94	99	120	99
45	129	95	120	98	342	95	97	121	101
Range :	117 - 129	89 - 97	116 - 125	95 - 104	335 - 345	91 - 99	94 - 101	117 - 124	98 - 104
Mean	122.6	94.6	119.4	99.0	340.5	95.1	97.7	120.5	100.6
SD	3.5	3.0	3.1	2.8	3.3	2.4	2.6	2.3	2.1
% CV (Intra-assay)	2.1	1.2	1.0	1.1	0.8	1.2	1.1	1.0	1.1
% CV (Inter-assay)	3.4								