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Evaluation and additional recommendations for preparing a whole blood control material*

Avaliação e recomendações adicionais para a preparação de material sangüíneo integral para controles

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Abstract

Objective The assessment of an easy to prepare and low cost control material for Hematology, available for manual and automated methods.

Material and Method Aliquots of stabilized whole blood were prepared by partial fixation with aldehydes; the stability at different temperatures (4. 20 and 37 °C) during periods of up to 8-9 weeks and aliquot variability with both methods were controlled.

Results Aliquot variability with automated methods at day 1, expressed as CV% (coefficient of variation) was: white blood cells (WBC) 2.7, red blood cells (RBC) 0.7, hemoglobin (Hb) 0.6, hematocrit (Hct) 0.7, mean cell volume (MCV) 0.3, mean cell hemoglobin (MCH) 0.6, mean cell hemoglobin concentration (MCHC) 0.7, and platelets (PLT) 4.6. The CV (coefficient of variation) percentages obtained with manual methods in one of the batches were: WBC 23, Hct 2.8, Hb 4.5, MCHC 5.9, PLT 41. Samples stored at 4ºC and 20ºC showed good stability, only a very low initial hemolysis being observed, whereas those stored at 37ºC deteriobed a rapidly (metahemoglobin formation, aggregation of WBC and platelets, as well as alteration of erythrocyte indexes).

Conclusions It was confirmed that, as long as there is no exposure to high temperatures during distribution, this material is stable, allowing assessment, both esternal and internal, for control purposes, with acceptable reproductivity, both for manual and auttomatic methods.

Blood. Quality control. Laboratories.

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Resumo

Objetivo *Avaliar material de controle para hematologia, de fácil preparação e baixo custo, que poderá ser usado por métodos manuais e automatizados.*

Material e Método *Alíquotas de sangue estabilizado foram preparadas por fixação parcial com aldeídos. Foram estudadas a estabilidade a diferentes temperaturas (4, 20, 37ºC) durante períodos de até 8-9 semanas e a variabilidade das alíquotas para ambos os métodos.*

Resultados *A variabilidade entre alíquotas, expressada em CV% (coeficiente de variação) com métodos automatizados no primero dia, foi: glóbulos blancos (WBC) 2,7, glóbulos vermelhos (RBC) 0,7, hemoglobina (Hb) 0,6, hematócrito (Hct) 0,7, volume corpuscular médio (MCV) 0,3, Hb corpuscular médio (MCH) 0,3, concentração de Hb corpuscular médio (MCHC) 0,7 e plaquetas (Plt) 4,6. O CV% obtido com métodos manuais para um dos grupos foi: WBC 23, Hct 2,8, Hb 4,5, MCHC 5,9, Plt 41. As amostras conservadas a 4 e 20ºC foram estáveis, observando-se leve hemólise inicial, enquanto que as amostras conservadas a 37ºC mostraram uma rápida decomposição (formação de metaemoglobina, agregação de glóbulos brancos e plaquetas e alteração de índices).*

Conclusões *Confirmou-se que, se não houver exposição a altas temperaturas durante a distribuição, este material é estável, permitindo avaliação, tanto externa como interna para controle e com reprodutividade aceitável, tanto para métodos manuais como automáticos.*

Sangue. Controle de qualidade. Laboratórios.

INTRODUCTION

The implementation of internal quality control (IQC) or external quality assessment (EQA) programs calls for large quantities of stable control materials. In Hematology, except for the standard solution of hemoglobincyanide, there is no reference material for the performance of several tests. This situation is critical for cell counts. There is little possibility of obtaining mono- or multiparametric control materials for hemocytometry, especially due to the multiple problems affecting blood preservation[1,3,9]. Among these problems, the aggregation of white blood cells (WBC) and platelets, as well as the increase in the volume of red blood cells (RBC) are significant due to the uptake of sodium and water, which occurs by depression of the Na-Ka-ATPase pump at the low temperature used for blood preservation (4ºC)[8]. On the other hand, there are the metahemoglobin formation and accumulation of lactate due to glycolisis, which not only decrease the pH, but produce an osmotic effect[2]. The depletion of ATP leads to the spheroechinocyte transformation of RBC, while the loss of 2.3-DPG produces an osmotic effect due to the replacement of 2.3-DPG by chloride ions.

The hematological parameters from blood obtained with EDTA change within a few days[4], and therefore the preparation of artificial or stabilized material for quality control is necessary for IQC and even more for EQA. Several variants have been employed in the preparation of control materials for hemocytometry, among them the replacement of WBC by erythrocytes fixed in a sterile medium, suitable for preservation, which at their pH and osmolarity simulate plasma conditions[11, 12].

With a view to making a control material available, aliquots of whole stabilized blood were prepared according to a simple, low cost method[13]. In this study additional data for evaluation, preparation (homogeneity and thermic stability), as well as performance when manual and automated methods are used, are described.

MATERIAL AND METHOD

Two batches were prepared (batch 1: 10-01-93 and batch 2: 12-03-93), using blood of the same ABO group, from a blood bank, obtained in CPD (sodium citrate, citric

acid, sodium dihydrogen phosphate, dextrose) or ACD (sodium citrate, citric acid, dextrose), as recommended by Lewis[10] for the preparation of quality material, with no more than 48 hours since collection, and with negative serology for HBsAg and HIV. The whole blood was filtered through a 40µ filter (SQ40SK, Pall)[14], stabilized with aldehydes (formaldehyde 37-40% and glutaraldehyde 50%) with antibiotics (penicillin 50-100 mg/L of material gentamycin 50-100 mg/L of material) and anfotericine B 2mg/L added. Aliquots of 2 ml were prepared from each batch (200 from batch nº 1 and 500 from batch nº 2). Then, some aliquots were randomly distributed among four laboratories to be quantified according to manual methods (5 parameters) and automated methods (8 parameters). On the other hand, during the fractioning process a sampling of each 20 fractionated aliquots was made, in order to ensure the uniformity of the aliquots. Different groups of aliquots were maintained at different temperatures (4ºC, 20ºC and 37ºC) for different times (8 and 40 days), in order to establish their performance under the extreme conditions to which the material may be submitted during distribution, if the cold chain is not assured. Automated determinations were made in a Coulter JT, and the manual ones were performed by three operators. The manual methods employed were: for RBC, WBC and platelets: total count by visual method in a Neubauer chamber, for Hb estimation: cyanmethemoglobin method, and for hematocrit: a micromethod, according to the conventional methodology described by Dacie and Lewis[6]. Data were processed with Statgraphics Plus software.

RESULTS

Table 1 shows aliquot homogeneity during the whole fractionating process. It can be observed that data, analyzed by ANOVA (analysis of variance) test were not statistically different from those of the original material (first aliquot), and between aliquots. Therefore, this material was considered homogeneous and adequate for the study to be carried out.

In relation to the effect of temperature, samples maintained at 4ºC over 40 days, with the exception of a very low initial hemolysis, showed no other changes. This result has been obtained repeated by in several batches since then. However, in those aliquots maintained at 20ºC and 37ºC a rapid decrease in the number of WBC and platelets with aggregation, metahemoglobin formation, and alteration of erythrocyte indexes: an increase of MCV and MCH, as well as a decrease in MCHC (mean cell hemoglobin concentration) with the rise in temperature, were observed.

On the other hand, both batches maintained at 4ºC for 8-9 weeks were followed up by automated methods. Table 2 shows the intra-assay CV% (coefficient of variation) at day 1, and the inter-assay CVs carried out up to 56 and 63 days.

In order to discover the performance of this material to be used with both manual and automated methods, data were compared by means of ANOVA (Table 3).

DISCUSSION

The finding of Reardon et al.[13], which persuitted the production of a stable easily-prepared, low cost material, met a need of the hematological laboratories. Later, the same method, with a modification in relation to the antibiotics employed (penicillin and gentamycin) was included in the manual by Lewis[10]. The present authors introduced

Table 1- Homogeneity of aliquots during the fractionating process*.

Parameter	Original material**	Aliquots N:25 Mean ± SEM	CV	Statistical significance (p)
Red blood cells (x 10^{12}/L)	4.18	4.15 ± 0.005	0.7	NS
Hemoglobin (g/L)	124	123.5 ± 0.21	0.7	NS
Hematocrit (L/L)	0.38	0.37 ± 0.003	0.7	NS
White blood cells (x 10^9/L)	7.00	6.85 ± 0.024	1.8	NS
Platelets (x 10^9/L)	232	228 ± 2.17	4.9	NS

* Automated duplicate measurements
** Mean of duplicate values
Batch nº 2: date 12-03-93
Mean±SEM = Mean ± mean standard error
CV = coeficient of variation
NS = Non significant (p>0.05)

Table 2 - Intra- and inter-assay variability for different parameters measured in the control material stored at 4ºC*.

Variability	Intra-assay		Inter-assay	
	Batch 1	Batch 2	Batch 1	Batch 2
Days	1	1	56	63
Number of aliquots	10	10	52	44
Red blood cells	0.7	0.6	1.1	1.9
White blood cells	2.7	2.5	7.7	4.7
Hemoglobin	0.6	0.7	1.3	4.3
Hematocrit	0.7	0.8	1.8	2.1
Mean cell volume	0.3	0.4	2.5	0.8
Mean cell hemoglobin	0.6	0.5	1.7	2.8
Mean cell hemoglobin concentration	0.7	0.8	3.4	1.5
Platelets	4.6	3.9	14.7	13.6

* Automated duplicate measurements. Data expressed as CV% (coefficient of variation)
Batch nº 1: date 10-01-93
Batch nº 2: date 12-03-93

Table 3 - Hematological parameters of a stable whole blood control material, determined by manual and automated methods.

Parameters	Manual methods*			Automated methods			
	n	$\bar{X} \pm SD$	CV%	n	$\bar{X} \pm SD$ (p)**	CV%	Statistical significance
Hemoglobin (g/L)	46	129.3 ± 5.8	4.5	44	123.6 ± 5.30	4.3	<0.00001
Hematocrit (L/L)	45	0.36 ± 0.01	2.8	44	0.38 ± 0.008	2.1	<0.00001
Mean cell hemoglobin concentration (g/L)	45	357 + 21	5.9	44	321 + 0.5	1.5	<0.00001
White blood cells ($x10^9$/L)	46	5.50 ± 1.27	23.1	44	7.1 ± 0.33	4.7	<0.00001
Platelets ($x10^9$/L)	46	222 ± 91	41.0	44	184 ± 25	13.6	<0.009

* Manual duplicate measurements were carried out by three operators.
** Between manual and automated methods ($p<0.05$).
Batch nº 2: date 12-03-93
n = Number of aliquots
$\bar{X} \pm SD$
CV% - Coeficiente of Variation

another slight modification with the addition of antimycotic anfotericine B. This material was also studied by Cruz R. et al.[5], who pointed out that the high cost of commercial controls should stimulate the use of more economical control materials prepared in the laboratories.

The data here given, relating to two batches which were followed up over a period of 60 days, are similar to those obtained by Reardon et al.[13] Additional preparations confirmed the present authors' data. In this experience using automatic methods, the intra-assay CVpercentages, from different batches, were low and very similar. On the other hand, the inter-assay CV percentages were higher, particularly for platelets.

Another issue to be raised out is the usefulness for both manual and automated methods. Although significant differences were obtained for all the parameters with both methods, the CV percentages fell within the expected range for each one of the parameters and methods employed (Table 3), as was shown in a survey carried out among laboratories in seven Latin American countries[7]. In the same survey it was observed that nearly 19-75% of clinical laboratories used manual methods and this is not always taken into account by control material manufacturers. Furthermore, in the present authors' experience not all commercial materials can be employed in manual methods.

The collaboration of the present autors with the External Quality Assessment Scheme in Hematology of the Fundación Bioquímica Argentina, in preparing a control material for cell counts, which at the present time has more than 2,000 registered laboratories, is a difficult challenge to meet. This goal is important, specially in view of the economical problems faced by Third World countries, and their need for an analytical quality assessment[7].

The present authors have confirm that, as long as samples are not exposed to high temperatures during distribution, this is a stable material for internal quality control and external quality assessment with acceptable reproducibility for both manual and automated methods.

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