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Porcine Blood as Control Materials for Animal Blood Cell Analysis

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ABSTRACT

Control material is an important component to verify the quality of animal blood cell analysis. In this study, 2 in-house control materials, denoted as normal and abnormal levels, were prepared from the porcine blood by fixing cells with 0.4% glutaraldehyde and 2.5% formaldehyde and kept at 4°C. The results obtained from weekly analyzed controls by automated cell analyzer illustrated that all of 10 hematological parameters (WBC, RBC, HB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV) of normal control were stable for a period of 5 weeks without any significantly changed from the original (p>0.05). But abnormal control demonstrated differently, the MPV value was significantly increased while MCHC value was significantly decreased (p<0.05). The other parameters were stable as found in the normal one. The hematological parameters of porcine blood were similar to those observed in various animals. The advantageous view is that a large amount of porcine blood could be conveniently collected from a slaughterhouse, therefore, it might be an unlimited sample source for preparing in-house control materials for animal blood cell analysis.

Key words: quality control material, porcine blood

INTRODUCTION

Complete blood count (CBC) is an analysis performed to check for abnormalities of the blood cells including the red and white blood cells and platelets (Lewis *et al*., 2001; College of Veterinary Medicine, Cornell University, 2007; Ruben, 2007; Foster *et al*., 2007). CBC is the fastest and helpful diagnostic tool available to the veterinarian. It is the most common blood test initially requested when a pet, whether it is a dog, cat, or even other animals, is sick (Knoll *et al*., 1996; College of Veterinary Medicine, Cornell University, 2007; Foster *et al*., 2007; Ruben, 2007). This test is also required for assessing health status of a dog at the first blood donation (Department of Veterinary Clinic & Hospital, University of Melbourne, 2007). In addition, CBC is included in blood tests of laboratory veterinary animals such as rats, mice, and other species which

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are generally used in drug efficacy studies and safety trials in medical research, in food and pharmaceutical development.

CBC can be performed by an automated cell analyzer. To ensure the correctness of the results, blood cell control materials are required to assure the quality of laboratory performance (Lewis *et al*., 2001). Theoritically, the properties of control material, such as homoginiety, viscosity and cellular properties, should be similar to patient's blood specimens. Control material should also be stable for being used in quality assurance process of blood cell analysis. Some fixatives such as glutaraldehyde (Morgan *et al*., 1978; Anutti *et al*., 1997), formaldehyde (Readon *et al*., 1991; Lewis *et al*., 2001), or urea (Springer *et al*., 1999) have been reported to be used for blood cell preservation. The mixture of glutaraldehyde and formaldehyde fixative described by Readon *et al*. (1991) is commonly used in control material preparation. Although blood cell control materials are commercially available, they are expensive and short self-life due to most of them being imported materials. In-house control materials prepared from each type of animal blood are limited by their small blood volume collected. Since porcine blood demonstrates hematological parameters similar to those of various animals such as dogs and cats (O'Brien *et al*., 1998, Ruben, 2007; Foster *et al*., 2007) and it can be collected in large amount from a slaughterhouse. Thus, in this study, we had prepared and evaluated the stability of partially stabilized porcine blood to be used as in-house control materials for quality control system of animal blood analysis by automated cell analyzer.

MATERIALS AND METHODS

A fresh porcine whole blood sample was collected from a slaughterhouse in sterile flasks containing citrate phosphate dextrose (CPD) anticoagulant (blood : CPD = 7:1). The CPD blood was divided into 3 parts. The first part was preserved by mixing 50 volume of blood with 1 volume of preservative composed of 0.4% glutaraldehyde and 2.5% formaldehyde as described by Readon *et al*. (1991). This partially preserved blood was named normal level control. To prepare abnormal level control, the second part of CPD blood was centrifuged at 800 *g* for 5 minutes and concentrated white blood cells and platelets from buffy coat region were transferred to the third part of CPD blood. After cells being well suspended, this CPD blood; or abnormal level control; was then preserved by the same process as normal level control. The controls were then aliquoted (500 μl) into microcentrifuged tube and weekly assayed (7-8 samples analysed/week) by automated cell analyzer, Coulter MAXM (Coulter, USA) for blood stability study. Ten hematological parameters were evaluated. There were the determinations of white blood cell (WBC), red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution with (RDW), platelet (PLT) and mean platelet volume (MPV).

RESULTS AND DISCUSSION

Complete blood count (CBC) is a test required for assessing health status of pets as well as laboratory veterinary animals. This test is frequently performed by an automated cell analyzer in which normal and abnormal control materials are required to verify the quality of an operation. Mean and standard deviation of 10 hematological parameters of partially preserved porcine blood analyzed from Coulter MAXM for 6 weeks are shown in Table 1 and Table 2. The results revealed that the normal level blood was stable for 5 weeks ($p>0.05$). Some parameters obtained from the 6th week were significantly different from the originals (Table 1). Except for MPV and MCHC, the other 8 hematological

parameters of abnormal level blood were also stable for 5 weeks as normal level blood (Table 2). MPV value was significantly increased while MCHC value was significantly decreased (p<0.05). Increase in MPV might be due to platelet swelling during storage or interfering by other cell degradation from the processes of abnormal control preparation. Therefore, this process needs to be improved.

The drifts of mean values assayed on the 1st-6th week from those of original base line were shown in Figure 1 (for normal level) and Figure 2 (for abnormal level). This drift (times of SD_{week0}) is calculated from $mean_{weekN}$ subtracted by $mean_{week0}$ and divided by SD_{week0}. All parameters showed less than 2SD drift except for the mean platelet count on the 6th week. This also indicated that the controls was stable for

Table 1 Hematological parameters of normal level control material (n = 56).

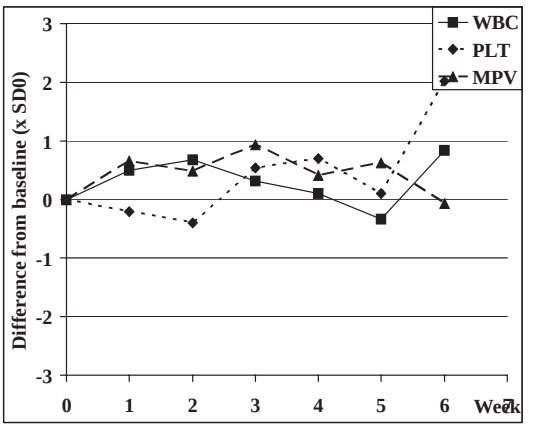
		Hematological parameters									
		WBC	RBC	HB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV
		$x10^9$/L	$x10^{12}$/L	g/dL	%	fL	pg	g/dL	%	$x10^9$/L	fL
Week-0	**Mean**	17.61	5.23	9.50	28.58	54.56	18.29	33.46	23.38	274.63	7.85
(Baseline)	**SD**	0.48	0.15	0.28	0.84	0.66	0.36	0.59	1.98	15.94	0.36
Week-1	**Mean**	17.85	5.27	9.50	28.70	54.40	17.98	33.04	22.61	271.25	8.09
	SD	0.18	0.05	0.19	0.60	1.05	0.35	0.51	2.25	33.26	0.25
Week-2	**Mean**	17.94	5.33	9.59	29.04	54.38	18.00	33.09	21.95	268.25	8.03
	SD	0.39	0.08	0.24	0.71	0.98	0.36	0.60	2.45	22.80	0.24
Week-3	**Mean**	17.76	5.30	9.58	28.68	54.11	18.09	33.48	21.66	283.13	8.19
	SD	0.31	0.08	0.18	0.58	0.89	0.44	0.73	2.05	23.77	0.31
Week-4	**Mean**	17.66	5.30	9.63	28.55	53.84	18.18	33.69	22.64	285.75	8.00
	SD	0.47	0.06	0.21	0.91	1.78	0.38	0.74	2.79	24.16	0.34
Week-5	**Mean**	17.45	5.31	9.56	28.71	54.03	18.04	33.33	21.80	276.25	8.08
	SD	0.55	0.07	0.17	0.76	1.24	0.38	0.63	2.92	17.24	0.20
Week-6	**Mean**	18.01	5.35	9.64	28.94	54.10	18.05	33.35	20.50*	306.75*	7.83
	SD	0.24	0.04	0.20	0.31	0.46	0.47	0.61	0.51	12.71	0.50

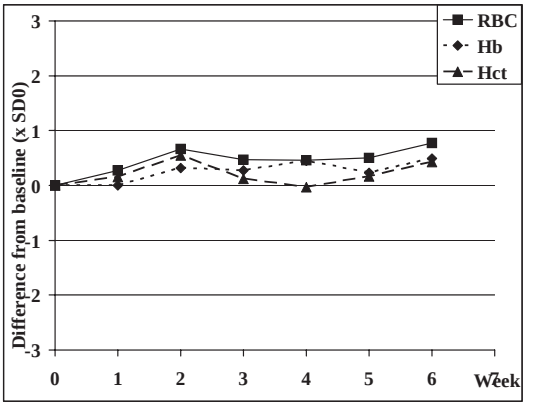
* p-value < 0.05 by t-test, when comparing to baseline

Table 2 Hematological parameters of abnormal level control material (n = 49).

		Hematological parameters									
		WBC	RBC	HB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV
		$x10^9$/L	$x10^{12}$/L	g/dL	%	fL	pg	g/dL	%	$x10^9$/L	fL
Week-0	**Mean**	22.80	5.23	9.96	29.96	57.20	19.04	33.24	20.00	386.86	6.99
(Baseline)	**SD**	0.79	0.25	0.40	1.54	1.50	0.34	0.65	2.79	28.20	0.52
Week-1	**Mean**	23.11	5.44	10.21	30.84	56.49	18.74	33.11	20.30	403.43	7.29
	SD	0.47	0.15	0.35	1.36	1.74	0.36	0.76	3.22	37.95	0.48
Week-2	**Mean**	23.11	5.20	9.69	29.79	57.33	18.64	32.56*	18.34	388.43	7.61*
	SD	0.56	0.22	0.40	1.37	1.14	0.31	0.70	2.24	24.72	0.45
Week-3	**Mean**	23.34	5.41	10.14	30.67	56.67	18.76	33.07	18.36	397.86	7.61*
	SD	0.40	0.22	0.39	1.18	1.67	0.38	0.72	2.23	41.05	0.42
Week-4	**Mean**	23.39	5.20	9.69	29.73	57.09	18.60	32.54*	18.13	405.43	7.94*
	SD	0.51	0.18	0.26	1.11	1.44	0.43	0.69	2.12	18.25	0.69
Week-5	**Mean**	23.37	5.36	9.96	30.47	56.83	18.63	32.80*	18.16	405.57	7.64*
	SD	0.82	0.20	0.38	1.00	1.35	0.28	0.74	2.24	26.48	0.36
Week-6	**Mean**	23.06	5.41	10.27	30.94	57.20	18.97	33.13	17.00	424.57	7.33
	SD	0.57	0.22	0.35	0.98	0.57	0.31	0.44	0.12	21.77	0.10

* p-value < 0.05 by t-test, when comparing to baseline





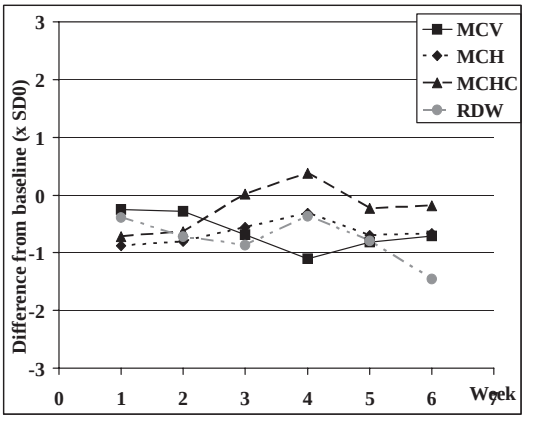
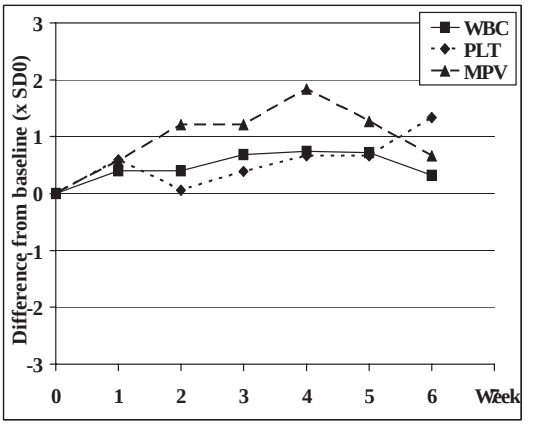
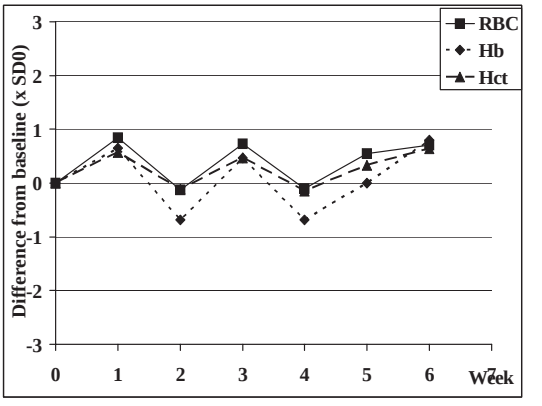


Figure 1 Differences of the hematological parameters of normal control material from its baseline.





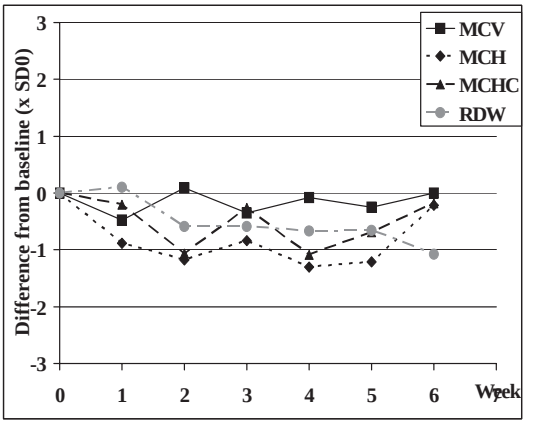


Figure 2 Differences of the hematological parameters of abnormal control material from its baseline.

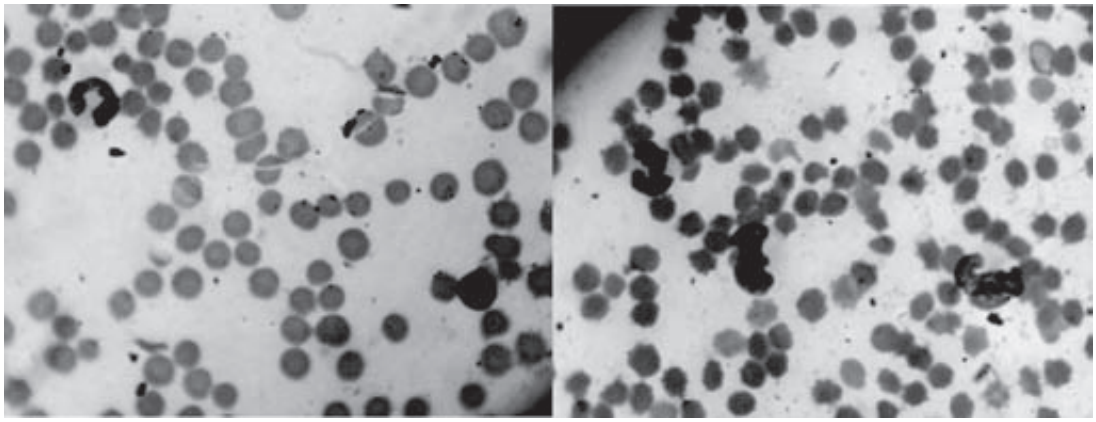


Figure 3 Blood cell morphology on preparative week (week-0, left) and on week-5 (right).

5 weeks. In addition, morphology of the preserved blood cells remained unchange during studying (Figure 3).

Since imported control materials are expensive and have short shelf-life because of the transportation. Thus, in-house control material is an alternative way to reduce laboratory cost and increase control material shelf-life. Moreover, blood collection from each animal type was limited by its volume large volume of porcine blood is available in a slaughterhouse. It has demonstrated hematological parameters similar to those of several animals such as dogs and cats (O'Brien *et al*., 1998; Ruben, 2007; Foster *et al*., 2007). The preserved porcine blood that was stable for at least a mount when stored at 4c may be used as control material. Hence, it might be an unlimited sample source for preparing in-house control materials for animal blood cell analysis.

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