



Letter to the Editor

Effect of edta-anticoagulated whole blood storage on cell morphology examination. A need for standardization

Sir, With the development of sophisticated automated blood cell analyzers, the proportion of blood samples that require a blood smear examination has steadily decreased. Microscopic examination of stained blood smear, however, still remains a crucial diagnostic aid if an automated instrument produces a highly improbable or doubtful result or abnormal cell flags and it is, in general, performed from anticoagulated whole blood according to standardized procedures [1]. In a parallel way, the increasing automation of modern clinical labo-

ratories has led to the centralization of high-volume clinical testing into large centers, covering large geographical areas. In these conditions, long distance transportation of the collected blood samples has dramatically increased the time of blood storage before analysis and/or the preparation of stained smears for blood cell morphology (BCM) examination. It is well known that, within 30 minutes of collection, morphological changes of blood cells begin, and that they increase with the time and conditions of blood storage [2,3]. Accordingly, in some circumstances, transfer process of collected blood samples to a centralized laboratory may become unsuitable for BCM examination and therefore for clinical diagnosis [4]. Although

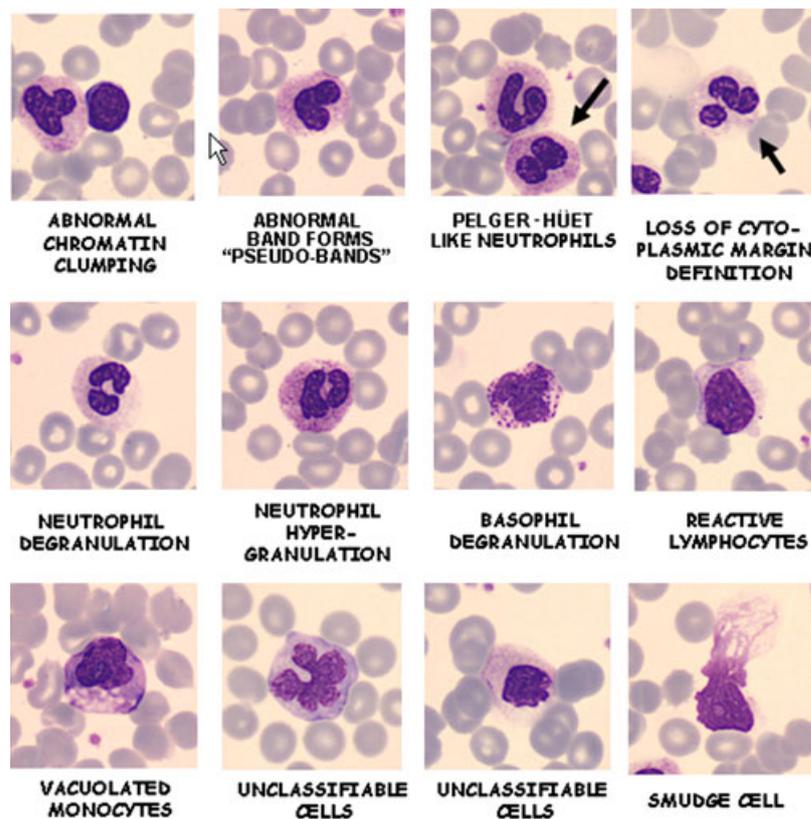


Figure 1. Most relevant morphological changes observed for blood cell leukocytes after incubation at 20 °C.

Table 1. List of changes in blood cell morphology observed in MGG stained smears after incubation at 4° and 20° C during 4h, 12h, and 24h

Blood Cell	Changes	4 °C			20 °C		
		4 h	12 h	24 h	4 h	12 h	24 h
Neutrophils	Abnormal chromatin homogeneity/clumping			+	+	+	++
	Excessive separation of nuclear lobes						+
	Cytoplasmic fragmentation		+	++	+	+	++
	Degranulation or Pelguer-Huet forms			++	+	+	++
	Cytoplasmic vacuolization/hipergranulation			+			+
Monocytes	Abnormal chromatin homogeneity						
	Irregular nuclear lobulation/multilobulation			++			+
	Cytoplasmic margin definition			+			+
	Cytoplasmic fragmentation (smudge cells)	+	+	++	+	+	+
Lymphocytes	Cytoplasmic vacuolization			++			++
	Abnormal chromatin homogeneity						
	Nuclear lobulation/budding				+	+	+
	Reactive lymphocytes				+	+	+
	Cytoplasmic margin definition						
Eosinophils	Cytoplasmic fragmentation (smudge cells)		+		+	+	+
	Cytoplasmic vacuolization						
	Degranulation		+	+		+	++
	Cytoplasmic margin definition			+			+
	Cytoplasmic fragmentation (smudge cells)						
Basophils	Cytoplasmic vacuolization		+	++			
	Degranulation	+		++		+	++
	Cytoplasmic margin definition	+					
Platelets	Cytoplasmic vacuolization						
	Large platelets	+	+	+	+	++	++
	Platelet clumps	+	+	+	+	+	+
Erythrocytes	Degranulation				+	+	+
	Echinocytic forms					+	++
	Spherocytic forms						

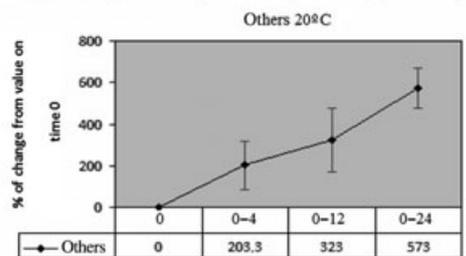
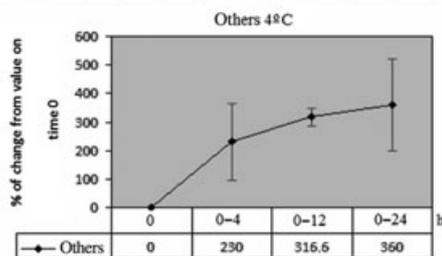
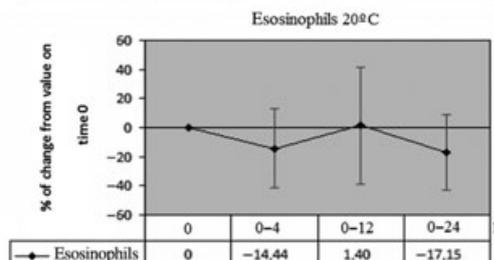
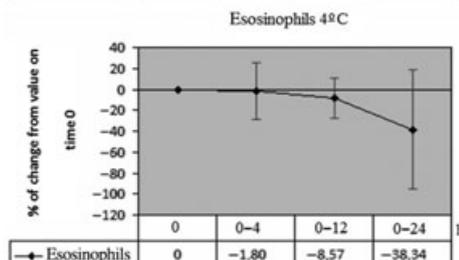
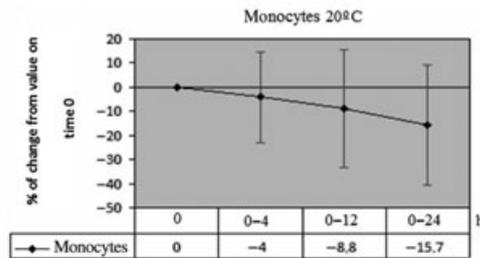
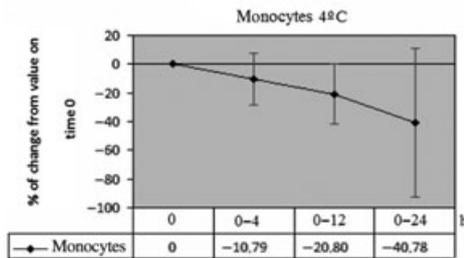
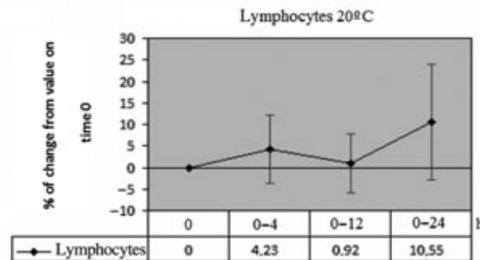
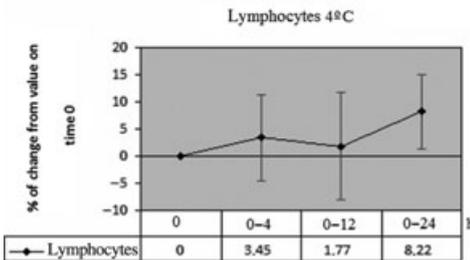
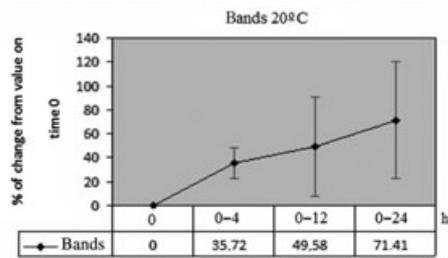
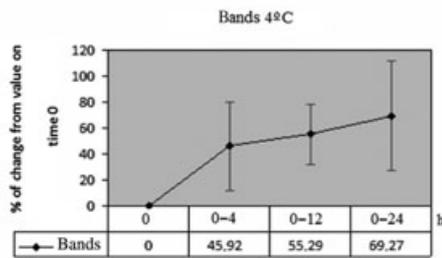
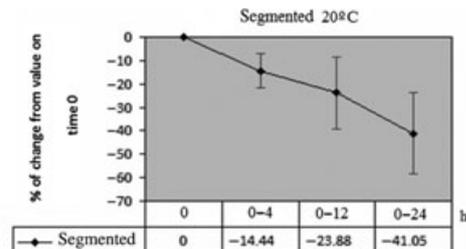
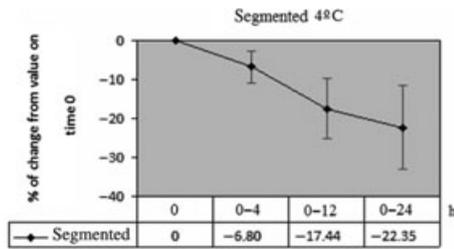
+: Morphological changes reported by less than 50% of the experts.

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some instrument's algorithms are, in general, capable of detecting blood CBC changes induced by blood storage, morphological changes are always undetectable, and if no careful control of blood transportation storage exists, blood cell deterioration may be the cause of unreliable results, inaccurate diagnosis, and patient's risk. For this reason, as for coagulation tests, clear pre-analytical conditions have to be urgently defined to maintain reliable high-quality results of microscopic BCM examination after specimen collection and transfer.

Here, an international panel of 14 experts in laboratory hematology has undertaken a controlled experimental study of blood incubation at different times (4 h, 12 h, 24 h.) and temperature (4 and 20 °C) to analyze the effect of whole blood storage on stained blood smear BCM examination. A total of 490 MGG stained blood smears from five healthy adult volunteers were prepared and distributed into sets of 35 units, one for each of the experts participating in the study. Venous blood samples were collected in K2EDTA tubes as routinely used by

Figure 2. Percentage of changes observed from value on time 0. Each bar represents the 95% confidence interval. The dotted line represents the linear regression trend line. These graphics show the increase or decrease of different cell types in a manual count, taking two experimental conditions at 4 and 20 °C.



clinical laboratories and filled to the correct volume in all cases. After saving a 1-ml aliquot for immediate blood smear preparation (Time 0), the remaining blood was divided into six aliquots of 1 ml each. Three of these aliquots were kept at 4 ± 1 °C (refrigerator), and the other three were incubated at 20 ± 1 °C (incubator). After 4, 12, and 24 h, a blood smear was performed and stained for all the six aliquots. Each expert reported BCM for leukocytes, platelets, and red blood cells and performed a 200-cell differential leukocyte count (DLC) of 6 leukocyte populations (segmented neutrophils, nonsegmented neutrophils, monocytes lymphocytes, eosinophils, and basophils).

The most relevant morphological changes observed for blood cell leukocytes after incubation are represented in Figure 1. Some of these changes mimic those observed in myelodysplastic syndromes (MDS), and if the observer is not aware about blood storage, this may be a cause of misdiagnosis. This is the case for Pelger-Huet-like figures (arrow) or loss of cytoplasmic margin definition (arrow). Although only MGG stain was used for this study, these changes in morphology over time will also be seen when using other stains, including Wright's stain. The results of BCM evaluation are summarized in Table 1. Many of these changes appeared after 4 hours of incubation at both 4 or 20 °C, and those reported by less than 50% of the experts are given as (+), and those reported by more than 50% of experts as (++). The most relevant changes were cytoplasmic fragmentation, degranulation, and/or Pelger-Huet forms for neutrophils, vacuolization for monocytes, and echinocytes for red blood cells. Platelet morphological changes were less evident, but characterized by an increase in size. Lymphocytes exhibited very few changes even after 24 of incubation at 20 °C.

The results of variations in the values (in percentage) of the leukocyte populations observed in percentage of changes observed from value on time 0 of the blood samples incubated 4, 12, and 24 hours (at 4 and 20 °C) are graphically represented in Figure 2. Accordingly, the most relevant observation was a significant decrease in segmented neutrophils that was always associated with an increase in nonsegmented neutrophils (band forms), some of them with additional changes such as a rounding of the nucleus forming homogeneous round masses or a single mass, here called 'unclassified cells'. Our study demonstrates that whole blood storage causes important changes of BCM sensitive to time of storage and to temperature. As a general hematological study, modern clinical laboratories include the complete blood cell count (CBC) determination as well as the automated differential leukocyte count (DLC). All these parameters are, in almost all laboratories, determined on EDTA-anticoagulated whole blood, shortly after blood collection, and the results are known to be

affected by a variety of factors, including sampling technique, storage, pre-analytical handling and analytical conditions. Nevertheless, examination of BCM using stained blood smears still remains a crucial diagnostic aid if an automated instrument produces a highly improbable, a doubtful result or abnormal cell flags. Because centralization of clinical laboratories has dramatically increased during the last 10 years, a very high number of blood samples are daily transferred to long distances for performing the measurements, including blood smear BCM examination and DLC when necessary. For this reason, reliable specimen collection and transfer between laboratories are a key element for high-quality BCM results.

Standardization of blood specimen collection, storage, and transmission to the laboratory for hematological tests was published by the ICSH in 2002 [5]. Here, it is recommended that because morphological changes of cells in anticoagulated blood samples begin within 30 min of collection, the tests should be set up within a maximum of 4 h, if the blood is left at room temperature (18–25 °C), and within 6 h if the specimen is kept at 4 °C. Here, we provide further support to these recommendations by an experimental evidence study of the effects of blood storage on BCM and is a strong argument for setting-up the limits required for blood specimen's transportation. So, we can conclude that if EDTA-anticoagulated whole blood has to be used for performing blood smears, the quality of BCM cannot be guaranteed after 6 h of blood drawing even if specimens are kept at 4 °C.

Acknowledgements

This study has been performed in collaboration with the European Organization for External Quality Assurance Providers in Laboratory Medicine (EQALM). We are indebted to Assumpta Pujades and Daniel Matamala for their helpful contribution to technical tasks and statistical evaluation of data, respectively.

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doi: 10.1111/ijlh.12170

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