Measuring the Biological Activity of Recombinant Human Erythropoietin (rHuEPO) by R-500, Automated Reticulocyte Analyzer, *in vivo*

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***Objective**: Improve laboratory methods in testing biological activities of rHuEPO by using R-500, automated reticulocyte analyzer, instead of traditional microscopic counting method.*

***Methods:** Testing 20 rHuEPO samples by two methods respectively.*

***Result**: There was no significant difference between the two testing methods statistically.*

***Conclusion**: R-500 automated reticulocyte analyze method can replace traditional microscopic counting method as an available laboratory evaluation in measuring the biological activities of rHuEPO.*

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Key Words rHuEPO, Reticulocyte, Biological Activity *in Vivo*, R-500, Automated Reticulocyte Analyzer

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INTRODUCTION

Recombinant Human Erythropoietin (rHuEPO) is a kind of active glycoprotein which is used to stimulate the production of red blood cells. It is mainly prescribed to treat chronic renal failure related anemia. The biological activity of rHuEPO *in vivo* is one of the most important indexes of quality control,which is analyzed by reticulocyte testing method. We use the R-500, automated reticulocyte analyzer, instead of traditional microscopic counting method, so as to simplify the operation procedure and specify the testing results.

MATERIALS AND METHODS

Laboratory animals

BALB/c Mice, females, 6-8 weeks, ordered from National Institute for the Control of Pharmaceutical and Biological Products(NICPBP).

National standard

rHuEPO national standard, Lot.980528, NICPBP.

Reagents

Ethylenediamine tetra-acetate Dipotassium (K_2EDTA), Bovine serum albumin.

R-500 reticulocyte analyze method

The mice were divided into 6 groups: 3 groups using national standard, 3 groups using sample, with at least 4 mice in each group. Each group accepted 3 different dosage respectively including 1.8, 3.6 and 7.2IU/ mouse, which were subcutaneously injected continuously for three days. We collected veno-medial canthus blood samples on the 4_{th} day, using K_2EDTA. We counted RET% by using the Sysmex R-500. Using RET% of different dosage in the standard product group and the sample group, we calculated the valence of products by dosage-reaction parallel line method.

Table 1 *Reproducibility of R-500*

	RET%		
	Test Low	Test Middle	Test High
	4.78	6.54	8.59
	4.69	6.35	8.85
	4.77	6.50	8.62
	4.83	6.32	8.90
X–SD	4.77–0.06	6.43–0.11	8.74–0.16
CV(%)	1.26	1.71	1.83
CV(%)-Average		1.60	

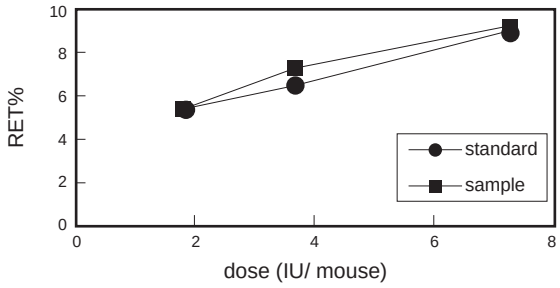

Fig. 1 *Dosage-Reacion curve of rHuEPO*

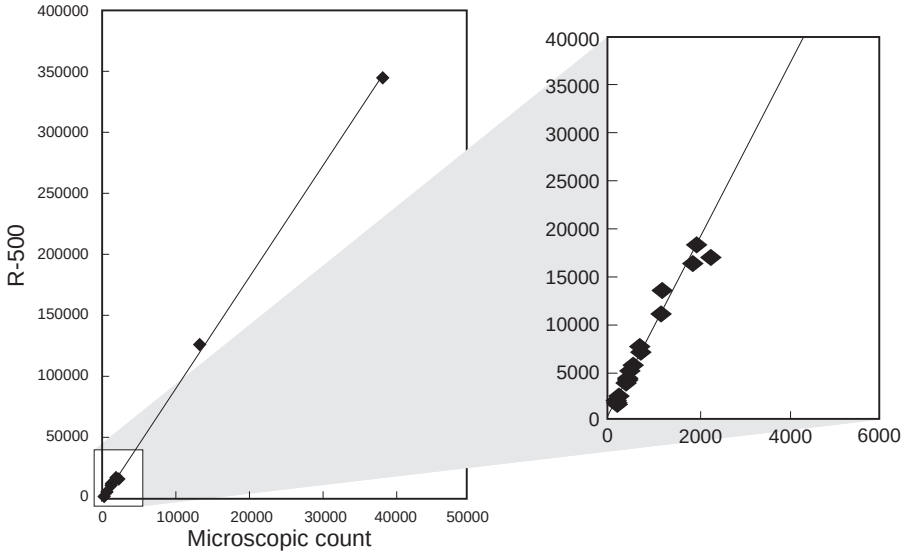

Fig. 2 *Comparison of rHuEPO activity between R-500 automatic reticulocyte analyzer method and microscopic counting method*
y=0.8934x+890.79 r=0.9997

RESULTS

Reproducibility of instrument measurement

We measured blood samples from low, medium and high dosage groups with the R-500. The results are showed in ***Table 1***. It is clear that the reproducibility is very good in the R-500 group.

Dosage-reaction curve of rHuEPO

Dosage-Reaction curve result from R-500 coincides with the microscopic counting method[1]. See in ***Fig. 1***.

Accuracy

We tested 20 rHuEPO samples using the R-500 and the microscopic counting method, respectively. See results in ***Fig. 2*** and ***Table2***. Paired t-test: ($t=1.203$, $t_{(19,0.05)}=2.093$), $P>0.05$, There was no significant difference between the two methods.

Table 2 *Comparison or rHuEPO activity between the R-500 method and the microscopic counting method*

Lot number	Eyeballing reticulocyte method(IU/bottle)	R-500 method(IU/bottle)
0102-2	132843	126556
000801	3960	4250
141M	3294	3910
141W	5136	5716
E005	386293	343855
20001212	1738	1610
000706	4158	4993
20010103	1631	1752
20001101	1646	1660
20010403	19679	18144
20010401	18766	16264
20010405	22492	16904
20010410	6425	7592
20010409	6649	7414
20010411	6724	6942
20010408	11917	13471
20010407	11453	11044
MH63509	2037	2397
000802	3433	3772
000801	1754	2100

DISCUSSION

Reticulocyte testing has been generally accepted as a mature method in measuring biological activities of rHuEPO *in vivo*. Microscopic counting is considered to be time consuming complex method, which has many errors due to personal counting. The European Pharmacopoeia (1999 edition) measured biological activities of rHuEPO *in vivo* by flow cytometer. The samples should be dilutional stained previously. The flow cytometer is a very expensive instrument for cell counting. The R-500 was developed as a special instrument for reticulocyte counting in recent years. It has been generally adopted because of its simple operation and doesn't need sample preparation. This article is the first report to animal testing especially in mice by the R-500.

Since the size of the human reticulocyte is different from mouse, we can not use the same setting of R-500 in human to test mouse samples. Otherwise the three doses of RET% in each group are paralleled and have no correlation in dosage-reaction. Compared with microscopic counting results, we choose the following setting parameters in mice testing: RBC-X=6.7ch, RET-X=27.5ch, RET-Y=167.5ch, DW/X=1.04, DW/Y=0.36, PLYE=1.

The testing results correlate between the two methods according to ***Fig. 2***, and there was no significant difference in the t-test. It was suggested that the R-500 analyzer could replace the microscopic counting method. It is a great improvement in reticulocyte testing not only because it saves time but also the testing results are more precise and reliable.

In addition, we suggested that the R-500 be improved as follows: Design different applications according to different blood samples in order to make operation faster and more simple, and to meet different laboratory needs.

Reference

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