Development of prototype-reagents of sheath fluid, hemo-lysing solution and cleaning solution of flow cytometer for commercialized purpose

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Abstract

Flow cytometry is a technique for counting the cells in suspension on passing through the laser beam one by one at a high number within a short time. It is reliable, objective and can be applied to various hematological tests. However the technique needs some expensive imported reagents such as sheath fluid, hemo-lysing solution and cleaning solution. This study aims to compare these reagents between the self-preparing and commercial ones. The indicators to be compared were %F-cells, %reticulocytes, %senescent red cells, %reticulated blood platelets, %senescent blood platelets and %apoptotic leukocytes. F-cells were stained with monoclonal antibody (MoAb) to fetal hemoglobin (HbF) labeled with fluorescent dyes (FITC, RPE & TC). Reticulocytes and reticulated platelets were stained with AO & TO. Senescent red cells and senescent blood platelets were stained with annexin-V-FITC and MoAb-GPA-RPE or MoAb-CD42b-RPE as appropriate. Apoptotic leukocytes were stained with annexin-V-FITC and PI. Blood samples were collected from patients with thalassemia (n=11) and anemia (n=8). All samples were flow cytometric enumerated for those 6 parameters using self-preparing and commercial sheath fluids. Leukocyte samples were obtained from both self-preparing and commercial hemo-lysing solutions. Self-preparing and commercial cleaning solutions were also compared using the time of reducing the dirty particles until they were stable for at least 15 seconds as indicators. All procedures were counted for 25,000 cells on FACSort or FACSCalibur. All parameters obtaining from using self-preparing and commercial sheath fluids were not significantly different ($p>0.05$). Leukocyte samples obtained from self-preparing and commercial hemo-lysing solutions were not significantly different ($p>0.05$) on apoptotic leukocyte count. The time of reducing the dirty particles until they were stable for at least 15 seconds obtained from self-preparing and commercial cleaning solutions were also not significantly different ($p>0.05$). In conclusion all 3 self-preparing reagents gave not significant different results from the commercial ones. Thus self-preparing reagents for flow cytometer are encouraged to replace the commercial reagents for an economical reason. These 3 self-preparing reagents also have a potential for commercialization.

Key words: Prototype-reagent, sheath fluid, hemo-lysing solution, cleaning solution, flow cytometry.

1. Introduction

Flow cytometry is a technique for counting the cells in suspension on passing through the laser beam, one by one at a high number within a short time. It can be apply for diagnostic leukemias, human immunodeficiency virus (HIV) infection following up, reticulocyte count, F-cell count and malarial infected red cell count. There are many flow cytometers in Thailand that need expensive imported reagents such as sheath fluid, hemo-lysing solution and cleaning solution. Sheath fluid is isotonic buffer saline solution that is pumped through the flow chamber wrapping the cells to flow singularly in the middle of the stream. Cleaning solution is sodium hypochlorite solution that is used for cleaning the tubing of the machine. These reagents can be prepared in house with a simple technique. Aim of this study is preparing of these reagents in our laboratory and compares their quality with the commercial reagents. The prototype reagents are supposed to have a commercialized advantage.

Definition of the reagents

Sheath fluid is isotonic buffer saline solution that is pumped through the flow chamber wrapping the cells to flow singularly in the middle of the stream

Cleaning solution is sodium hypochlorite solution, use for cleaning the tubing of the flow cytometer.

Hemo-lysing solution is hypotonic solution use for lysis of red blood cells to obtain platelets and leukocytes.

2. Materials and method

Thalassemic (n=11) and anemic (n=8) ethylene diamine tetra acetated (EDTA) blood sample were collected from Chiang Mai University Hospital. Complete blood counts (CBC) were determined on Sysmex KX-21N and then platelet rich plasma (PRP) was obtained. The remaining packed red cells were resuspended with 0.1% bovine serum albumin in phosphate buffer saline (BSA-PBS) to be reconstituted whole blood (RWB). PRP was fixed with fixative solution (0.2% glyoxal and 0.4% paraformaldehyde in PBS) at the ratio of 1:1. The cells were washed twice with 0.1% BSA-PBS and once with annexin-V buffer (AVB) and then resuspended in AVB. The suspension was dispensed into plastic tubes for 400,000 platelets each. Reticulated platelets were stained with acridine orange (AO) or thiazole orange (TO). Senescent platelets were stained with annexin-V conjugated fluorescein isothiocyanate (FITC) and monoclonal antibody to CD42b conjugated to rhodamine phycoerythrin (MoAb-CD42b-RPE) simultaneously. RWB was fixed with 1 ml cold 0.05% glutaraldehyde in PBS at room temperature (RT), in the dark for 10 minutes. One hundred ml of fixed cell suspension was permeabilized with 400 ml cold 0.05% Triton X-100 for 10 minutes at RT. The cells were stained

with monoclonal antibody (MoAb) to fetal hemoglobin (HbF) labeled with fluorescent dyes (FITC, RPE or TC). Reticulocytes were stained with AO or TO. A certain volume of RWB according to its hematocrit was diluted in 1,000 ml of 0.1% BSA-AVB and 100 ml of the dilution was diluted further in 400 ml of 0.1% BSA-AVB. The senescent red cells were stained with annexin-V-FITC and MoAb-GPA-RPE . Leukocyte suspension was obtained from lysing the EDTA blood with commercial or self-preparing hemo-lysing solution. The leukocytes were washed twice with 0.1% BSA-PBS, then final washed with AVB and then resuspended with AVB. Apoptotic leukocytes were stained with annexin-V-FITC, followed by propidium iodide (PI). The cells were mixed and incubated on ice, in the dark for 30 minutes. Self-preparing and commercial cleaning solutions were also compared using the time of reducing the dirty particles until they were stable for at least 15 seconds as indicators. All procedures were counted for 25,000 cells on FACSort or FACSCalibur. All 6 parameters were assayed using self-preparing and commercial sheath fluids in comparison.

3. Results

Figures 1 to 10 show flow cytometric histogram of each parameter.

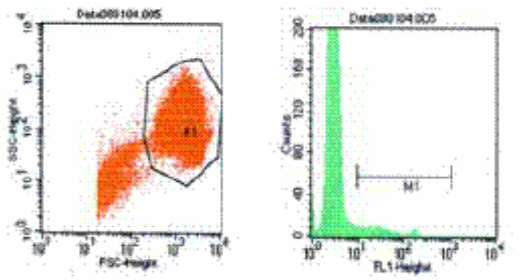

Figure 1 Histogram of reticulocytes stained with TO

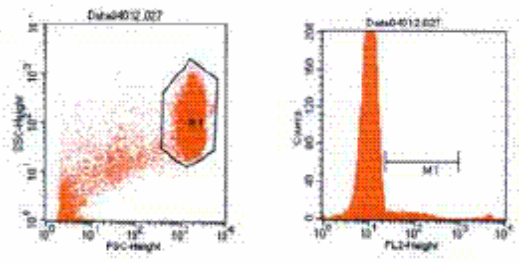

Figure 2 Histogram of reticulocytes stained with AO

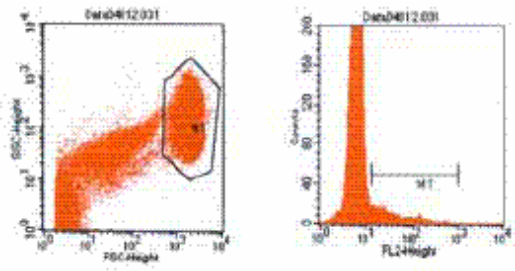

Figure 3 Histogram of F-cells stained with MoAb-HbF-RPE

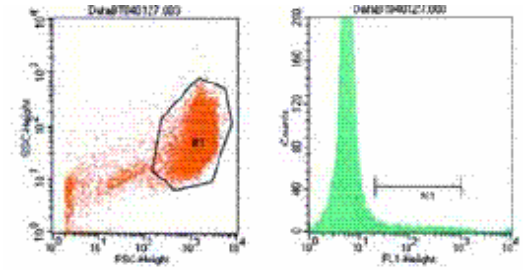

Figure 4 Histogram of F-cells stained with MoAb-HbF-FITC

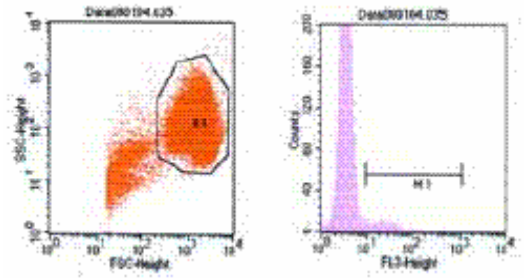

Figure 5 Histogram of F-cells stained with MoAb-HbF-TC

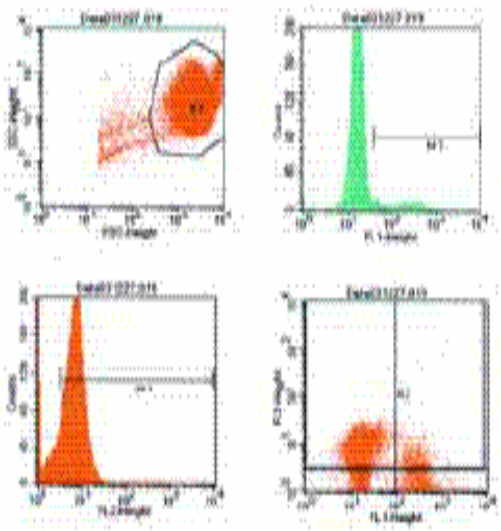

Figure 6 Histogram of senescent red cells stained with AV-FITC and MoAb-GPA-RPE

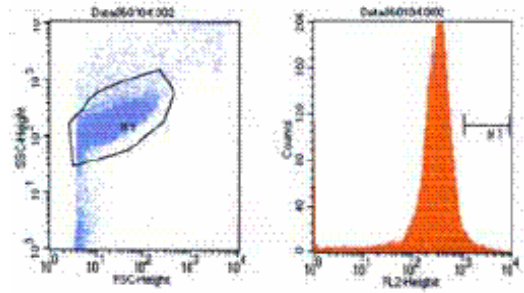

Figure 7 Histogram of reticulated platelets stained with AO

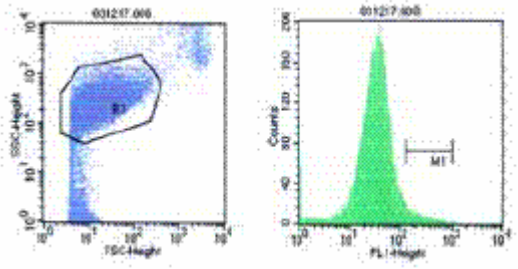

Figure 8 **Histogram of reticulated platelets stained with TO**

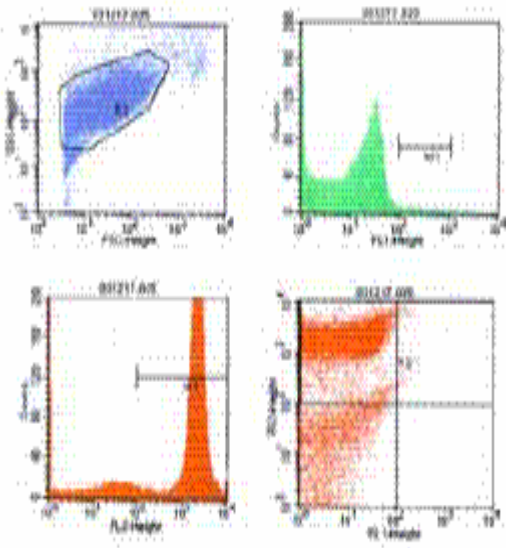

Figure 9 **Histogram of senescent platelets stained with AV-FITC and MoAb-CD42b-RPE.**

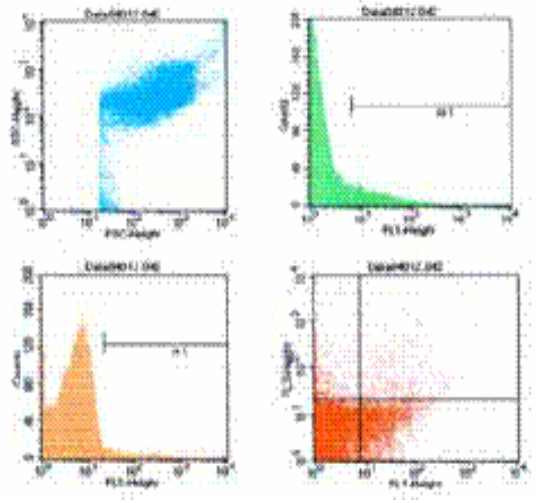

Figure 10 **Histogram of apoptotic leukocytes stained with AV-FITC, followed by PI**

Tables 1 to 3 show non significant difference of all indicating parameter for quality comparison.

Table 1 **Comparison of the values of all parameters obtaining from self-preparing and commercial sheath fluids (p>0.05)**

Parameter	Commercial Sheath Fluid (X±SD)	Self-Preparing Sheath Fluid (X±SD)
%F-cells (FITC)	10.1±7.5	9.6±7.6
%F-cells (RPE)	11.9±12.8	11.7±12.9
%F-cells (TC)	11.4±14.3	10.6±13.6
%Reticulocytes (AO)	3.2±3.4	3.5±4.3
%Reticulocytes (TO)	3.6±2.6	3.5±2.7
%Senescent red blood cells	4.9±5.0	4.7±5.0
%Reticulated blood platelets (AO)	1.8±1.3	1.8±1.2
%Reticulated blood platelets (TO)	2.3±1.9	2.3±1.8
%Senescent blood platelets	1.2±0.8	1.2±0.8
%Apoptotic leukocytes	4.4±6.6	4.4±6.6

Table 2 Comparison of the percentages of apoptotic leukocyte obtaining from self-preparing and commercial hemo-lysing solutions (p>0.05)

Parameter	Hemolysng solution	
	commercial (X±SD)	self-preparation (X±SD)
Comm.-sheath fluid	7.5±11.7	5.6±11.9
Self-sheath fluid	6.1±7.9	3.4±5.0

Table 3 Comparison of the cleaning time obtaining from self- preparing and commercial cleaning solution (p>0.05)

parameter	Cleaning solution	
	Commercial (X±SD)	Self-preparation (X±SD)
Clean time (sec.)	28.5±7.6	25.8±5.2

4. Conclusion

The over all result obtaining from using the 3 self-preparing reagents shows no significant difference from using the commercial reagents. Therefore, these self-preparing reagents for flow cytometer are encouraged to replace the commercial reagents for an economical reason. The prototypes of these reagents may have a potential in commercialized advantages.

5. Acknowledgement

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