Direct Analysis of leukocytes in Whole Blood without Lysing

Introduction

Analyzing the leukocytes in whole blood is a routine assay in clinical lab or blood bank. The concentration and viability of the leukocytes are the vital index as quality control of blood storage. Apart from leukocyte, whole blood contain a large number of platelets, red blood cells, or cellular debris, which make it impossible to analyze whole blood directly under the microscope or bright field cell counter. Conventional methods to count white blood cells involve RBC lysis process, which is time consuming.

AOPI Dual-fluoresces counting is the assay type used for detecting cell concentration and viability. The solution is combination of acridine orange (the green-fluorescent nucleic acid stain) and propidium iodide (the red-fluorescent nucleic acid stain). Propidium iodide (PI) is a membrane exclusion dye that only enters cells with compromised membranes, while acridine orange is able to penetrate all cells in a population. When both dyes are present in the nucleus, propidium iodide causes a reduction in acridine orange fluorescence by fluorescence resonance energy transfer (FRET). As a result, nucleated cells with intact membranes stain fluorescent green and are counted as live, whereas nucleated cells with compromised membranes only stain fluorescent red and are counted as dead when using the Countstar® Rigel system.

Countstar Rigel is an ideal solution for many complex cell population characterization assays, enable to rapidly analyze white-blood-cells in whole blood.

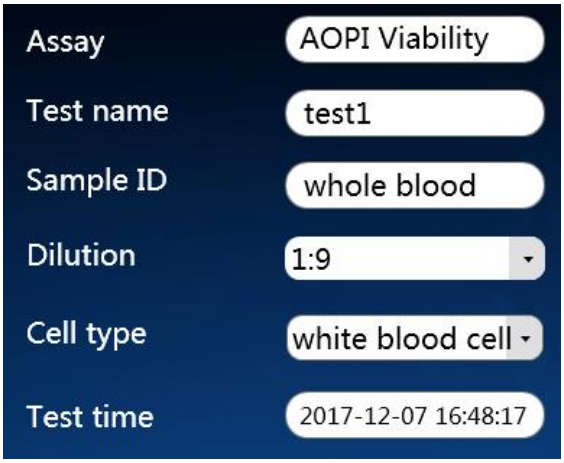
Materials and instruments：

1. 100µl whole blood samples
2. 100ml PBS
3. AO/PI Kit
4. Countstar Chamber slide
5. Countstar Rigel;
6. Pipettes (20 µl and 200 µl) and enough sample cups (approx. 100)

Experimental Procedure:

1. Take 20 µl of blood sample and dilute the sample in 180 µl of PBS.

2. Add 12μl AO/PI solution into 12μl sample, gently mixed with pipette;
3. Draw 20μl mixture into chamber slide;
4. Allow the cells to settle in the chamber for around 1 minute;
5. Insect the slide into Countstar Rigel instrument;
6. Choose the "AO/PI Viability" assay, then Enter Sample ID for this sample.
7. Select Dilution ratio, Cell Type, the click 'Run' to start the test.



Step 6: Enter Sample ID for this sample

Step 7: Select Dilution and Cell Type

Caution: AO and PI is a potential carcinogen. It is recommended that the operator wear personal protective equipment (PPE) to avoid directly contact with skin and eyes.

Result:

1. Bright Field Image of whole blood

In the bright field image of the whole blood, WBC's are not visible among the red blood cell. (Figure 1)

Figure 1 Bright field image of whole blood.

2. Fluorescence Image of whole blood

The AO and PI dye are both stains DNA in the cell nucleus of cells. Therefore, Platelets, red blood cells, or cellular debris are unable to affect leukocytes concentration and viability result. Live leukocytes (Green) and dead leukocytes (Red) are easily visualized in the fluorescence images. (Figure 2)

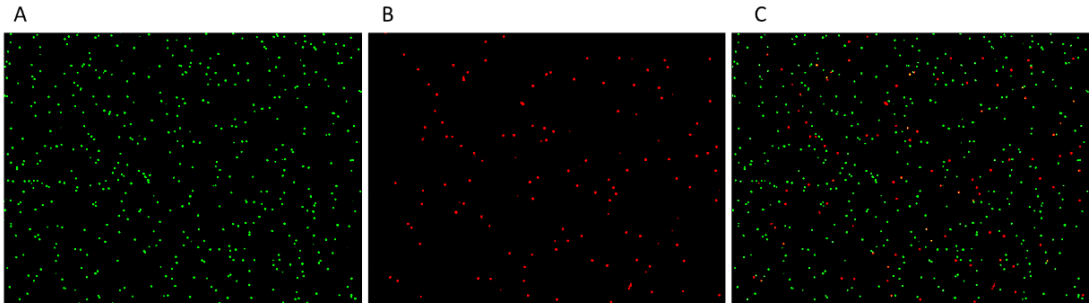


Figure 2 Fluorescence Images of whole blood. (A). Image of AO Channel; (B) Image of PI Channel; (C) Merge images of AO and PI Channel.

3. Concentration and viability of leukocytes

The Countstar Rigel software automatically count cells of three chamber sections and calculates average value of total WBC cell count (1202), concentration (1.83 x 10^6 cells/ml), and % viability (82.04%). The whole blood images and data can be easily exported as PDF, Image or Excel for additional analysis or data archiving.

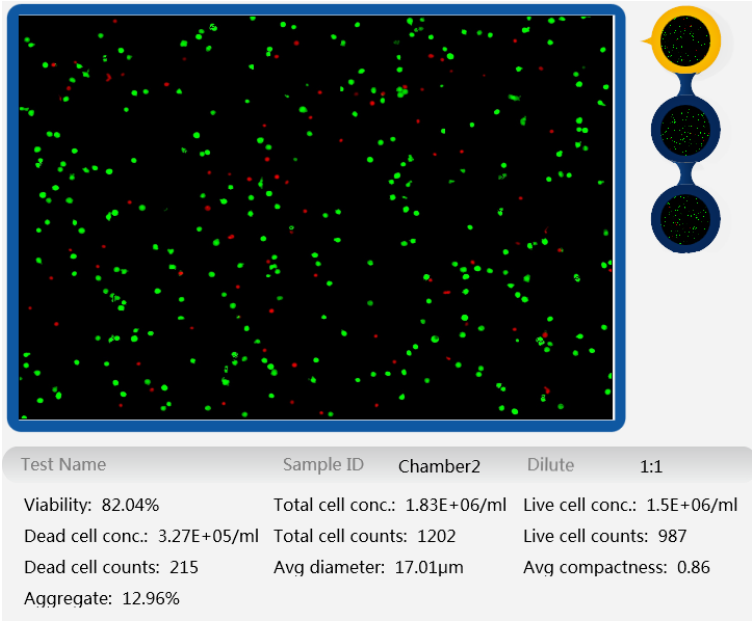


Figure 3 Screenshot of Countstar Rigel Software

4. Conclusion

Countstar Rigel and dual-fluorescent analysis enable to overcome issues related to variability in sample preparation and manual cell counting with automated. The Countstar Rigel software provide 3 chamber section of high resolution images make it feasible to accurately screen a large number of samples over time for more accurate data of assay results.