SOP-E022

Operation Procedure for the Coulter Counter Model F/FN - White Blood Cell (WBC) Counts/ Red Blood Cell (RBC) Counts

Objective: To determine the concentration of white blood cells or red blood cells, in an aqueous suspension

Procedure:

1. Turn Coulter Counter ON if necessary. Counter is located in Lynn Hall, room G221.

2. Check the vacuum gauge on the right side of the instrument. The pressure must be -10 to -6 mm Hg for proper functioning.

3. Verify the following instrument settings:
   Attenuation: 0.707
   Aperture: 16
   Threshold 10

4. For **white blood cells counts** a 1:500 dilution is made. Pipet 10 ml PBS (refer to SOP#-P020 for preparation of Phosphate Buffered Saline) into a disposable blood dilution vial ("Coulter vial").

   a. With a finger, flick the tube containing the suspension several times to ensure that white cells are well suspended.

   b. Pick up 20 µl cell suspension with a Pipetman, quickly and carefully wipe off the outside of the pipet tip with a Kimwipe, (be careful not to hold the Kimwipe against the end of the tip, or the contents will be pulled out of the tip) and dispense into the 10 ml PBS.

   c. Cap the vial and invert several times to mix. Avoid foaming.

5 For **red blood cell counts**, a 1:50,000 dilution of whole blood is made.
   a. Prepare a 1:500 diluiton of whole blood as above for the white blood cell count
   b. Add 100µl of the 1:500 dilution to 10ml of PBS in a coulter vial. This is now a 1:50,000 dilution

6. For **white blood cell counts only!** Just before performing the count, add 3 drops of *Zap-O-globin* or *Criterion Sciences Manual Lyse*, to lyse any remaining red blood cells that may be present. Cap the vial and mix gently by inversion. Allow to sit for 30 seconds for the lyse to work. Do not allow to sit for more than 10 minutes, otherwise destruction of the WBC's will begin to occur. **NOTE: This step is not necessary if counting cultured cells where RBC's are not present.**

7. Before removing the vial of PBS in which the aperture tube sits, open and close the filling/flushing stopcock (the left one) by turning gently clockwise from the horizontal position to the vertical position and back to horizontal. Always turn in the same (clockwise) direction. This step helps minimize bubbles in the system and should be performed every time a vial is removed.

8. Lower the sample stand by pushing gently down and remove the vial of PBS.

9. Place the vial with the diluted sample on the stand and allow it to rise back into place. The aperture tube and external electrode should be immersed in the sample. Move the vial around till the orifice of the

aperture tube (on your left) lies against the side of the vial. This ensures a good projection of the orifice onto the screen.

10. Open the vacuum control stopcock (the top one) by turning it clockwise until it is in the vertical position. This sets up negative pressure, and the mercury column will fall and you will hear a "click". (If it does not, adjust the tissue paper in the syringe filter to increase the vacuum pressure) As soon as the "click" is heard, close the stopcock by turning it clockwise to the horizontal position. As the mercury rises, a corresponding amount of diluted sample is pulled into the aperture through the orifice. When the mercury comes in contact with the first electrode, the count begins. There is a current running through the orifice between the internal and external electrodes. When a cell passes through the orifice, it produces a voltage pulse which is registered as a count. All cells contained in the electrolyte, which pass through the orifice, are counted until the mercury comes in contact with the second electrode on the manometer.

11. While the cells are being counted, monitor the screens for irregularities. If dirt or debris partially or completely blocks the orifice, first try opening then closing the flushing/filling stopcock. This will sometimes be enough to dislodge the debris. Then you may restart the count by opening then closing the vacuum control stopcock as above. If the debris returns, lower the sample stand and gently wipe off the orifice with a small brush (located in the drawer underneath the coulter Counter) Replace the vial and restart the count as above.

12. Read the count from left to right and record, rounding off to the nearest hundred. Repeat the count from step #10, on the same sample dilution till two counts are within 200 cells for white blood cell counts or within 500 cells for red cell counts. You can only get three counts out of a vial with 10ml in it. You lose accuracy once the diluent level is no longer covering one third of the external electrode. So if more than three counts are needed you will need to make another dilution.

    WBC example: coulter counter display reads 5400
    count is $5.4x10^6$/ml

    RBC example: coulter counter display reads 5400
    count is $5.4x10^8$/ml

13. If the count is above 10,000 per cu mm, it is necessary to correct for coincidence loss, by referring to the coincidence chart in the back of the Couler Model F/FN manual. This correction must be done due to the small loss in count which occurs when two or more cells enter the aperture at the same time and are registered or counted as only one cell. For red cell counts, the result when corrected for coincidence loss, must be multiplied by 100 before reporting.

Discussion

1. If the cell count is greater than 20,000 or less than 4,000 per cu mm the count should be repeated with a new dilution. If the count exceeds 50,000 per cumm, a 1:10 dilution of the 1:500 dilution should be made, and the count repeated.

2. Before a set of counts is to be performed, it is advisable to check the background count. This consists of doing a count on the diluent used for the red and white blood cells counts, to ensure that falsely elevated counts are not being obtained due to contaminated diluent or sample vials. Background counts of 100 per cu mm or less are good, and no correction need be made on the counts. If the machine is kept clean, backgrounds can be less than 20 per cu mm.

3. In order to avoid interference during the counting procedure, keep all objects, including hands away from the Coulter Counter until the count is completed.

4. Always make sure the external electrode is submerged in the sample vial.

5. There should never be a break in the mercury in the manometer. If this does occur, the vacuum stopcock may be opened and the vacuum adjusted till the mercury falls down into the coalescing bulb. This should bring the mercury back together. Readjust the mercury level and proceed with the count.

6. When the Coulter Counter is not in use, it should be left with either Coulter Clenz or PBS in it.

7. When a series of red or white cell counts are being done, it is advisable to rinse the aperture tube with diluent after 4 or 5 samples. Rinse also when switching between red and white counts.

8. The counting cycle (amount of time cells are actually being counted by the machine) should be between 12 and 15 seconds, for accurate results. A cycle less than 15 seconds suggests a broken orifice. Cycles greater than 15 seconds usually indicate a dirty orifice or a dirty manometer and mercury. In this case, clean those parts.

9. It may be necessary to alter the vacuum. To increase the vacuum pressure push more Kimwipe into the syringe filter (which is acting as a vacuum regulator). To decrease the vacuum pressure loosen, or remove the piece of Kimwipe. It is also necessary to occasionally replace the syringe filter.

10. It is advisable to leave the Coulter Counter on all day and only turn off at the end of the day.

11. For daily shutdown you want to fill the machine with Coulter Clenz or Isoterge(Coulter) To do this immerse the aperture tube in a vial containing Cloulter Clenz. Place the free end of the tubing that is now in the PBS supply flask into a flask containing Coulter Clenz. Make sure the waste tank is empty or at least has a lot of room. Open both stopcocks (clockwise). The Clenz will run through the aperture tube through the "T" tube, and finally out into the waste flask. Leave the stopcocks open for about 10 seconds. Close the stopcocks (horizontal position). Turn off the power.

    NOTE: the clenz must be rinsed out well just prior to using by flushing the same as above only with the tubing in the PBS this time.

Created by: Gretchen Lawler/ Kathy Ragheb Date: 2-18-99

Verified by:_____ Date:_____ _____

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