LABORATORY PROCEDURE: **COUNTING BY HEMOCYTOMETER:** Counting mononuclear cells by method of hemocytometer

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PURPOSE: To obtain a total mononuclear cell count collected from the ficoll preparation of patient specimens.

PRINCIPLE: After ficoll preparation, cells are collected and diluted in trypan blue for a live/dead count under a hemocytometer to determine cell# per ml.

SAFETY PRECAUTIONS: All work should be performed under the biological safety cabinet observing safety regulations and using sterile technique. Personal protective equipment such as: lab coat, gloves and glasses, should be used during the procedure. Specimens should be handles as if capable of transmitting infection. All contaminated supplies should be properly disposed of in biohazard or sharps containers and liquid waste should be decontaminated with bleach for 20min before being poured down the drain.

MATERIALS AND REAGENTS:

P20, P200+ tips
Cell counter
PVC treated 96 well plate *(Falcon 353911)*
Hemocytometer / Microscope

0.4% Trypan Blue *(Invitogen 15250-061)*
1X PBS *(Cellgro 21-040-CV)*
Trypan/PBS

READENT PREPARTION: a. Trypan blue for cell counting: 3 of trypan + 5ml of 1X PBS

READENT STORAGE: **Room Temperature:** 1X PBS, Trypan blue

SPECIMAN STORAGE: Cells are counted at room temperature. Remaining cells should be at 4°C or on ice.

QUALITY CONTROL: Ensure that the Trypan/PBS does not have any debris in it. A minimum of 20 cells per square and a maximum of 100 cells per square should be read

PROCEDURE:

- Resuspend your cell pellet in up to 10mls of 1X PBS
- Do a 1:10 dilution in working stock trypan blue: Combine 10ul of the cell suspension with 90ul of working stock trypan blue. Mix well with a p200.

- Immediately place cells on a clean hemacytometer: place glass coverslip over the chambers. Then place 10ul of the cell dilution using a P20 pipetman at the edge of the chamber. The solution will pass under the glass cover by capillary action. (Do not over fill)
- Place the hemocytometer on the stage of the microscope and use a 10X magnification to count. **(If there are to many cells to count, go back and adjust dilution factor).**

- Using a cell counter count how many live lymphocytes there are in the four large corner squares of the hemocytometer. See diagram below. *(Lymphocytes can be distinguished from red blood cells by their size. Red bloods cells are smaller than lymphocytes and maybe round or oblong in shape. Live cells will be bright and glowing and have NO blue coloration)*

LIMITATIONS: Cell number on hemocytometer may be below 20 cell per square with the lowest possible dilution. If necessary, re-pellet and Resuspend in smaller volume.

CALCULATONS: Calculate the cell number by using the following formula:
(total# of cells/squares counted) X (10,000) X (Dilution factor) X (Volume)

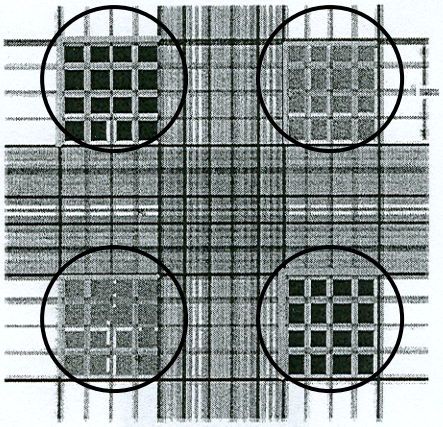
Example: **cell count**: 52,63,54,49 **Dilution factor**: 1:10 **Volume**: 6 mls
Take the average of the cell count: 61+42+46+51 / 4 = 50
(50) X (10000) X (10) X (6) =$30x10^{6}$ cells

INTERPRETATION: See Ficoll SOP

RESULTS REPORTING: The results are reported in per million of cells

TRAINING: Personnel will be trained by staff. Up to one-time visual shadowing of staff member AND up to one time hands on training with staff member AND one or more times independent performance with successful completion of SOP.

This is the grid that you see under the microscope:



The image below is a magnified view of one of the squares