Count the number of cells with Trypan Blue exclusion

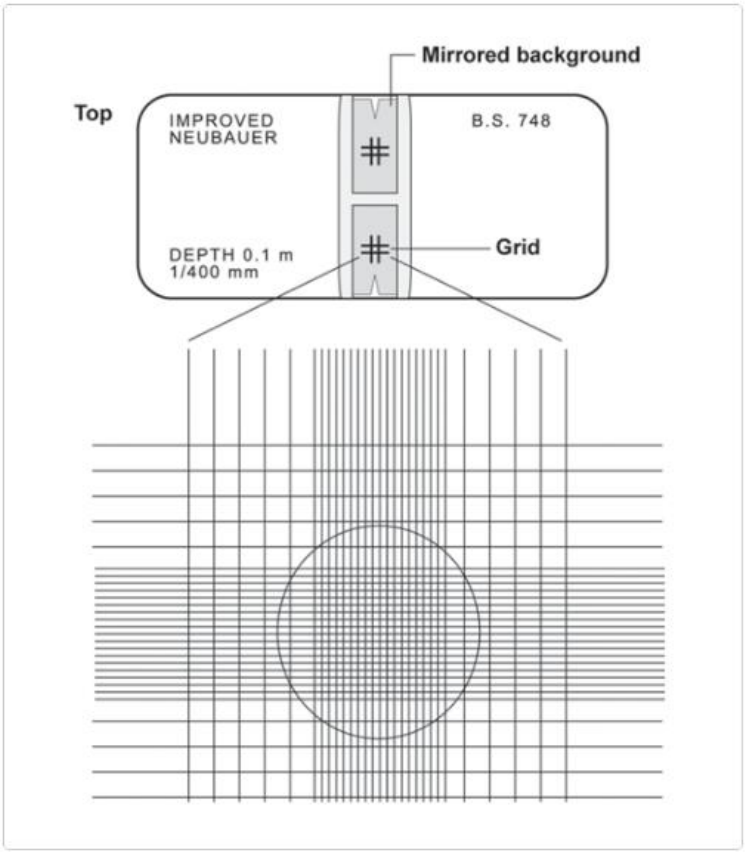
The following protocol describes a procedure to determine the cell viability accurately. Cell viability is calculated as the number of viable cells divided by the total number of cells within the grids on the hemacytometer. If the cells take up trypan blue, they are considered non-viable.

1) Clean the chamber and cover slip with alcohol. Dry and fix the coverslip in position.
2) Prepare a 0.4% solution of trypan blue in buffered isotonic salt solution, pH 7.2 to 7.3 (i.e., phosphate-buffered saline).
3) Add 0.1 mL of trypan blue stock solution to 1 mL of cell suspension.
4) Add 10 µL of the cells to the hemacytometer. Do not overfill.
5) Examine immediately under a microscope at low magnification.
6) Count the cells in the large, central gridded square (1 mm^2). The gridded square is circled in the graphic below. Multiply by 10^4 to calculate the number of cells per mL.

   [Note]:

   1. **Prepare duplicate samples and average the count.**
   2. **Count the number of blue staining cells and the number of total cells.**

   % viable cells = [1.00 – (Number of blue cells ÷ Number of total cells)] × 100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Grid