WHAT YOU NEED
Neubauer Improved Haemocytometer
Cell / spore suspension suitably diluted
Tally counter or counter pen
Pasteur pipettes
Microscope (compound or stereo)

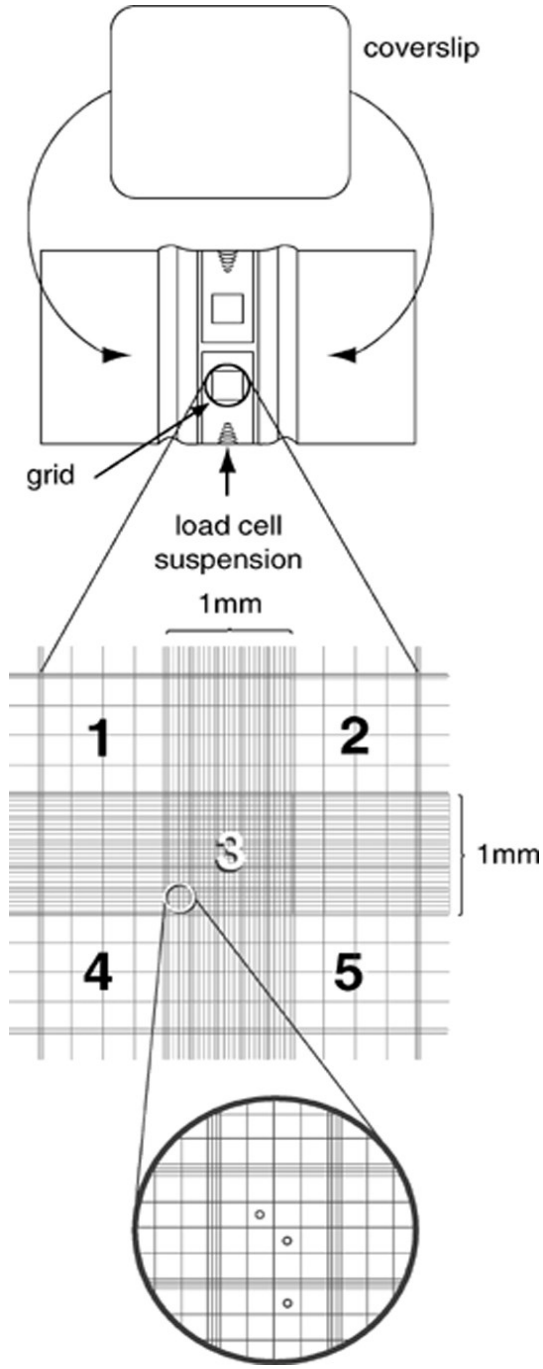
1. Preparation before using a counting chamber:

1. The haemocytometer must be handled carefully. To avoid getting fingerprints on the ruled areas, the counting chamber should be held by the sides and bottom only.

2. **USE HAEMOCYTOMETER COVERSLIPS, RATHERT HAN A REGULAR COVERSLIP AS THIS WILL NOT GIVE ACCURATE RESULTS.**

3. Take extra care when focusing the microscope as the counting chamber is much thicker than a conventional slide.
   The microscope objective, as well as the chamber and cover glass, may be damaged if the user is not careful.

4. Clean the counting chamber gently with 70% alcohol and a lint-free wipe



Note:
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2. Protocol for using a counting chamber:

1. Moisten the shoulders of the haemocytometer and affix cover glass using gentle pressure and small circular motions. This ensures that the depth of the chamber is correct (0.1 mm).

2. Using a Pasteur pipette, place a drop of the cell suspension at the edge of the 'V' shape of the chamber. Allow the suspension to be drawn into the chamber by capillary action.
CARE Do not over-fill or under-fill the chamber. Fill the opposite chamber in the same manner.

3. The rulings cover 9 mm^2 in a 3x3 square. The central square of the grid is ruled into 25 groups of 16 smaller squares (area of each smaller square is 0.0025 mm^2) with each group separated by triple lines, the middle one of which is the boundary.

   The area of the central square is: 25 x 16 x 0.0025 = 1 mm^2 and the volume is: 1 mm^2 x 0.1 mm = 0.1mm^3 or 0.1 µL (1 mL = 1000 mm^3).

   The number of cells counted per mL = number of cells counted per square mm x dilution x 10,000.

4. To ensure accuracy of counts, a specific counting pattern must be determined. Here is a clear guide to using a counting chamber:
   http://www.ruf.rice.edu/~bioslabs/methods/microscopy/cellcounting.html

   Reproduced with permission, a pdf 'Using a Counting Chamber' can be found at www.kirkhousetrust.org, go to: Resources → Research Resources → Equipment Manuals → Haemocytometer.
   Counting Fungal Spores: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC202674/pdf/aem00111-0126.pdf

   Useful discussion for counting fungal spores:
   https://www.researchgate.net/post/What_are_the_general_methods_used_for_the_spore_counting_of_fungi