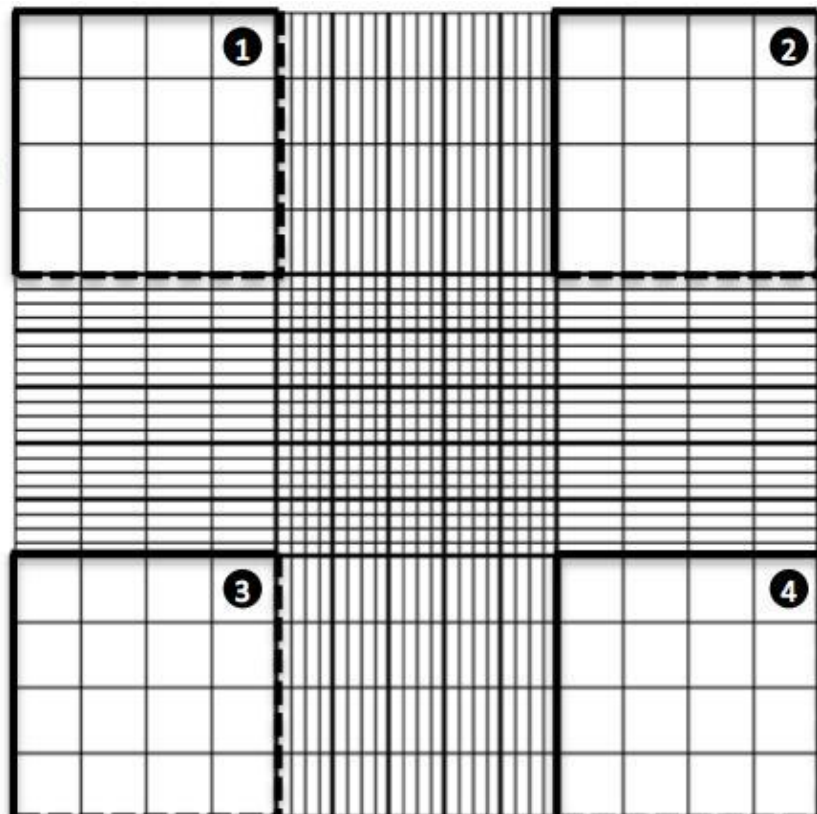


HOST-PATHOGEN
INTERACTIONS
LAB
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**TITLE: COUNTING OF CELLS USING
HEMOCYTOMETER:
Date: 15/07/2021
Name: Ipsita Nandi**

1. Trypsinize cells cultured on a 10-cm plate and resuspend rigorously in a 10 ml growth medium.
2. IMMEDIATELY thereafter (as cells tend to sink) take a 10 μ l aliquot of the resuspended cells and place it between coverslip and hemocytometer. Take care that the entire area is covered with liquid and that there are no significant amount of bubbles.
3. Using a 10X objective of the TC microscope, focus on the grid lines of the hemocytometer, which will be seen as in the scheme below.
4. Count the cells present in areas 1, 2, 3, and 4.
5. Calculate the average number of cells per area by dividing the total number of cells by 4.
6. Multiply that number by 10,000 (10^4).
7. The total no. of cells = the average no of cells $\times 10^4$ /ml.

— count
- - - don't count



Useful numbers for HeLa cell culture:

	Catalog No.	Surface area (cm ²)	Seeding density*	Cells at confluency*	Versene (mL of 0.05% EDTA). Approx. volume	Trypsin (mL of 0.05% trypsin, 0.53 mM EDTA). Approx. volume	Growth medium (mL). Approx. volume
Dishes							
35mm	150460;150318	8.8	0.3 x 10 ⁶	1.2 x 10 ⁶	1	1	2
60mm	150462;150288	21.5	0.8 x 10 ⁶	3.2 x 10 ⁶	3	3	5
100mm	150464;150350	56.7	2.2 x 10 ⁶	8.8 x 10 ⁶	5	5	12
150mm	150468;168381	145	5.0 x 10 ⁶	20.0 x 10 ⁶	10	10	30
Culture plates							
6-well	140675	9.6	0.3 x 10 ⁶	1.2 x 10 ⁶	1	1	1 to 3
12-well	150628	3.5	0.1 x 10 ⁶	0.5 x 10 ⁶	0.4 to 1	0.4 to 1	1 to 2
24-well	142475	1.9	0.05 x 10 ⁶	0.24 x 10 ⁶	0.2 to 0.3	0.2 to 0.3	0.5 to 1.0
48-well	150687	1.1	0.03 x 10 ⁶	0.12 x 10 ⁶	0.1 to 0.2	0.1 to 0.2	0.2 to 0.4
96-well	167008	0.32	0.01 x 10 ⁶	0.04 x 10 ⁶	0.05 to 0.1	0.05 to 0.1	0.1 to 0.2
Flasks							
T-25	156367;156340	25	0.7 x 10 ⁶	2.8 x 10 ⁶	3	3	3–5
T-75	156499;156472	75	2.1 x 10 ⁶	8.4 x 10 ⁶	5	5	8–15
T-175	159910;159920	175	4.9 x 10 ⁶	23.3 x 10 ⁶	17	17	35–53
T-225	159934;159933	225	6.3 x 10 ⁶	30 x 10 ⁶	22	22	45–68
*Seeding density is given for each culture vessel type as follows: Dishes and Flasks: Cells per vessel; Culture plates: Cells per well.							
†The number of cells on a confluent plate, dish, or flask will vary with cell type. For this table, HeLa cells were used.							