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CELL COUNT- 01 - Manual cell count with Trypan Blue

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Marco Cosentino[1], Elisa Storelli[1], Alessandra Luini[1], Massimiliano LM Legnaro[1], Emanuela Rasini[1], Marco Ferrari[1], Franca Marino[1]

[1]Center for Research in Medical Pharmacology, University of Insubria (Varese)

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Protocol status: Working
We use this protocol and it's working

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Abstract

Published work using this protocol:

- Kustrimovic N., Comi C., Magistrelli L., Rasini E., Legnaro M., Bombelli R., Aleksic I., Blandini F., Minafra B., Riboldazzi G., Struchio A., Mauri M., Bono G., Marino F., Cosentino M. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naïve and drug-treated patients (2018). Journal of neuroinflammation, 15(1), 205. https://doi.org/10.1186/s12974-018-1248-8

- Kustrimovic, N., Rasini, E., Legnaro, M., Bombelli, R., Aleksic, I., Blandini, F., Comi, C., Mauri, M., Minafra, B., Riboldazzi, G., Sanchez-Guajardo, V., Marino, F., & Cosentino, M. (2016). Dopaminergic Receptors on CD4+ T Naive and Memory Lymphocytes Correlate with Motor Impairment in Patients with Parkinson's Disease. Scientific reports, 6, 33738. https://doi.org/10.1038/srep33738

- Cosentino M., Ferrari M., Kustrimovic N., Rasini E., Marino F. (2015). Influence of dopamine receptor gene polymorphisms on circulating T lymphocytes: A pilot study in healthy subjects. Human immunology, 76, 10, 747-752. https://doi.org/10.1016/j.humimm.2015.09.032

- Boydum A.Isolation of mononuclear cells and granulocytes from human blood. Scand.J.Clin.Lab. Invest. 21 (Suppl.97): 77-89, 1968

- Alex Mabou Tagne, Franca Marino, Massimiliano Legnaro, Alessandra Luini, Barbara Pacchetti and Marco Cosentino. A Novel Standardized Cannabis sativa L. Extract and Its Constituent Cannabidiol Inhibit Human Polymorphonuclear Leukocyte Functions. Int J Mol Sci2019 Apr; 20(8): 1833. Published online 2019 Apr 13. doi: 10.3390/ijms20081833.

- Angela Scanzano, Laura Schembri, Emanuela Rasini, Alessandra Luini, Jessica Dallatorre, Massimiliano Legnaro, Raffaella Bombelli, Terenzio Congiu, Marco Cosentino, Franca Marino. Adrenergic Modulation of Migration, CD11b and CD18 Expression, ROS and interleukin-8 Production by Human Polymorphonuclear Leukocytes. Inflamm Res. 2015 Feb;64(2):127-35. doi: 10.1007/s00011-014-0791-8. Epub 2015 Jan 6.

Materials

Instruments needed:
- Bürker chamber
- Optical microscope
- Plastic labware

Materials:
Trypan blue catalog number: T8154-100mLSigma Italy

Safety warnings

Cancerogenic solution, be careful when handling it!

1 Use Trypan Blue solution for checking cell viability.

Mix 10 µL of cell suspention with an equal amount of Trypan Blue solution (dilution factor = 2).

Document

NAME

SOLUTION- 09 - Trypan Blue solution

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PREVIEW

2 Take 10 µL of the mixture and place it inside a Bürker chamber and view under an optical microscope using 40X magnification.

3 Count the cells in each square found in the four corners and in the central square (see figure 1 below), including those
that lie on the bottom and left-hand perimeters, but not those that lie on the top and right hand perimeters (see figure
2 below).

Total number of cells per ml = mean number of cells x dilution factor x 10^4 (hemacytometer volume).

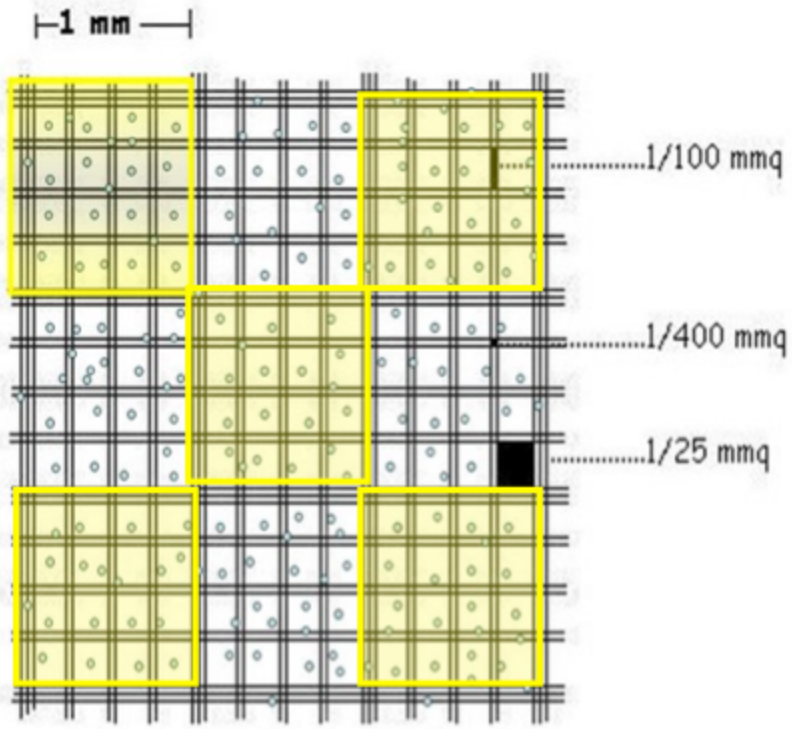

Figure 1
The gridded area of the chamber consists of nine 1 mmq squares. These squares are subdivided in three directions; 0.0625 mmq, 0.05 mmq and 0.04 mmq. The central square here in Figure 1 is further subdivided into 0.0025 mmq = 1/25 mmq squares. Count cells in 5 squares as shown.

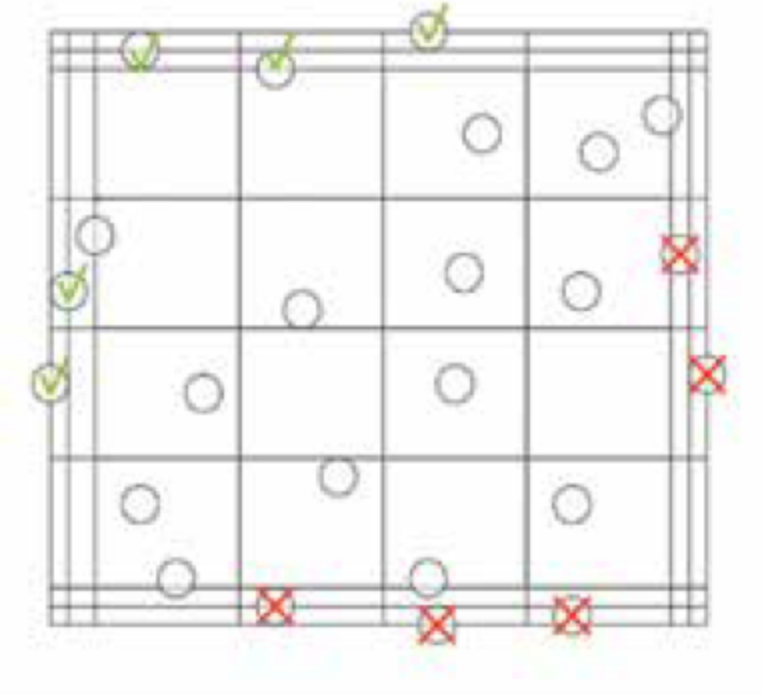

Figure 2
Concerning those cells that lay on the perimeter of the square, count following this scheme.