



HEALTH EFFECTS LABORATORY Standard Operating Procedure

CELL COUNTING

Written by: ZUM

Valid from: DD.MM.YYYY

Code: HEL11C001

QA (name and sign): ESI

Last review: DD.MM.YYYY

Version: 1.0

Approved (name and sign): MDU

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SCOPE

Cells in culture are proliferating depending on their generation type they divide and grow in dishes or flasks. This SOP describes procedures for counting cells.

RESPONSIBILITY

Study Personnel are responsible of being familiar with counting procedures.

MATERIAL AND REAGENTS

(Vendors and products are suggestions and not mandatory)

Cells

Cell culture medium (Dulbecco MEM - D6046, Sigma or RPMI1640 - R8758, Sigma; according to SOP for specific cell type)

Supplements of culture medium required for the specific type of cell line (e.g. fetal bovine serum, antibiotics, non essential aminoacids, sodium pyruvate, etc.)

Trypsin-EDTA (59429C, Sigma)

Pipettes (5, 10, 25 ml)

Pasteur pipettes

Pipette tips

Counting Chamber Slides and trypan blue (0.4%) kit (C10228, Invitrogen)

EQUIPMENT

HEL11E020 Laminar Flow Hood

HEL11E023 Light Microscope Leica

HEL11E022 Countess / Bürker chamber

HEL11E044 Finn pipettes

HEL11E017 / 018 CO₂ incubator

METHODOLOGICAL PROCEDURE

All the procedures before taking a cell suspension aliquot for counting should be done in the laminar flow hood to maintain the sterile conditions (if cells are needed to grow again later).

Before counting make sure to resuspend cells well using pipette.

Cell counting using Bürker chamber

1. Place a cover slip on the Bürker chamber and tighten the screws carefully (the cover may break if you screw too tight)
2. Add an aliquot of cell suspension using Pasteur pipette (appr.1-3µl) into the Bürker chamber (under the cover slip) and use a microscope to count the cells.
3. Bürker chamber contains a network of 9 large squares divided into 9 small squares, count cells in minimum 3 large squares and calculate the average value of number of cells per one square (nc).
4. Some cells may be placed on the edge (lines). Count only cells on top and left edge for each square to avoid counting cells twice.

Calculation:

- a. Number of cells (c) per 1 ml medium



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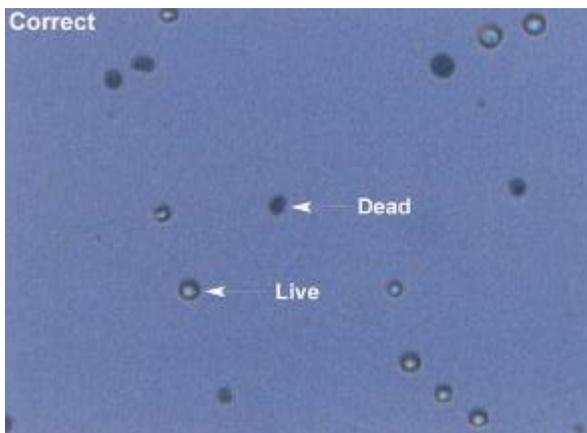
$$c = nc \times 10^4$$

- b. The total number of cells in suspension (ct) is calculated as
 $ct = c \times \text{volume of suspension in ml}$

Cell counting using Countess

1. Mix 10 μl of cell suspension with 10 μl trypan blue (0.4%)
2. Add 10 μl of the mixture into the chamber port on Countess™ counting chamber slide.
3. Insert the slide into the slide port of the Countess™ instrument
4. Press "Zoom" button and use the focus knob to optimize the image for analysis:
Viable cells have bright centers and dark edges. Dead cells have uniform blue color throughout the cell with no bright centers (see images of correct and incorrect focusing).
5. Use the grid to navigate the slide and check the image in several fields.
6. If desired, lock focusing using Focus lock knob.
7. Press "Count cells" button to obtain results.
8. Zoom (or check zoom) for each count.

Correct image: Viable cells have bright centers and dark edges.



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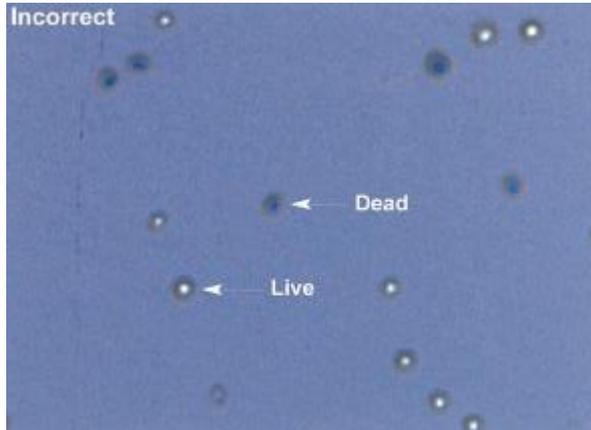
Code: HEL11C001

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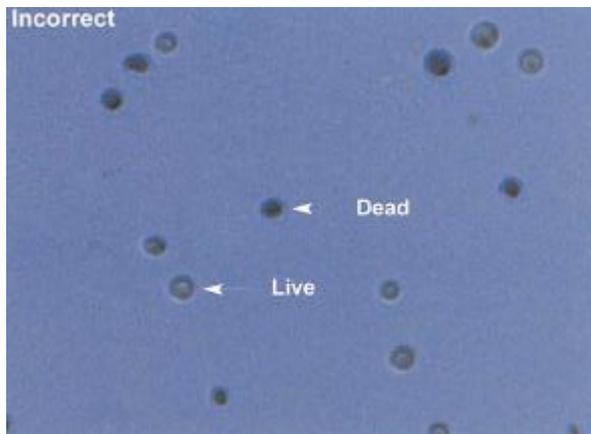
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Incorrect image: Dead cells have bright, blue centers and are counted as live.



Incorrect image: Live cells have dark centers and are counted as dead.



CRITICAL POINTS

For accuracy of cell number, counting must be performed by one person within one experiment, in case of using Bürker chamber.

Note:

See also SOP HEL11E022 Automated Cell Counter Countess™

REFERENCES

J.Michl: Bunečné kultury. Academia Praha, 1977

Cell and culture procedure: Laboratory procedures. Eds. A Doyle, JB Griffiths and DG Newell. Wiley Publishers & sons, LTD. 1996



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Countess™ Automated Cell Counter manual (Invitrogen)