Uncertainty analysis of cell counting by metabolic assays

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MOTIVATION

In Biological Research
and
In Diagnostic and Clinical tests

is essential the quantification of

cell number

?
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Quantification of **cell number** is required to understand:

→ How a tumor is growing
→ If a drug is having effect on cells
→ If a cell responds to treatments
→ If a biomaterials is compatible for regenerative medicine
MOTIVATION

Reliable **cell number** measurement

cell number SI Traceability

Today: lack of traceability!!
1. [...] lack of adequate standards and comparable measurements for: in vitro diagnostics [...], toxicology for drug safety, medical device materials biocompatibility, [...];

2. [...] NMI has identified the most significant measurement requirements as:
   - Cell quantification
   - Cell viability/proliferation
   - Cell viability/apoptosis ratio

3. [...] Relevant quantitative measurements include: quantifying cells according to cell density, [...], vital cells.
AIM of the study

To evaluate the uncertainty of a cell counting procedure

To overcome the lack of traceability in cell number quantification
CELL COUNTING METHODS

Day 1
Day 7
Day 14

No detachment
No direct count

Need of alternative method
CELL COUNTING METHODS:
CellTiter Blue® metabolic assay (CTB®)

www.promega.com
CELL COUNTING METHODS:
CellTiter Blue® metabolic assay

CellTiter Blue Emission Spectra

Fluorescence Intensity [A.U.]

Wavelength [nm]
CELL COUNTING PROCEDURE: Calibration

1. Seeding of nominal cell number $N_c$ (*direct cell count*) and unknown $N$ in a measured volume, $V$
CELL COUNTING PROCEDURE: Calibration

2. Cells incubation (37°C, pH7) with CTB® for a given reaction time, $t$
CELL COUNTING PROCEDURE: Calibration

3. Fluorescence Intensity $I_f$, measurement of $N_c$

Excitation wavelength: 525 nm

Emission wavelength: 580-670 nm

GloMax®- Multi Microplate Multimode reader (Promega Corp.)

$K$ calibration constant

$I_{f,b,t}$

$I_{f,sc,t}$
CELL COUNTING PROCEDURE: Measurement

4. Fluorescence Intensity. $I_f$, measurement of unknown $N$

Excitation wavelength 525 nm

Emission wavelength 580-670 nm

$I_{f,b,t}$

$I_{f,s,t}$
CELL COUNTING PROCEDURE: Measurement

5. Calculation of unknown N

\[ N = K \left( I_{f,s,t} - I_{f,b,t} \right) V \]
RESULT: UNCERTAINTY ANALYSIS OF CELL NUMBER $N$

$$N = K(I_{f,s,t} - I_{f,b,t})V$$

- Reaction time
- Culture volume
- Cell metabolism

- Repeatability 0.5%
- Reproducibility 1%
- Micropipette uncertainty 1%

• Instrument calibration (regression) 2.2%
RESULT: UNCERTAINTY ANALYSIS OF CELL NUMBER \( N \)

\[ N = K (I_{f,s,t} - I_{f,b,t}) V_{LF} \]

Combined uncertainty of \( N = 3.2\% \)
Direct count = 5%
CONCLUSION

• First approach of uncertainty evaluation
• Metabolic assays for cell counting
• Biological research and clinical tests
Thanks for your attention!
Cell number (N) 8910
Combined standard uncertainty 286 3.2%