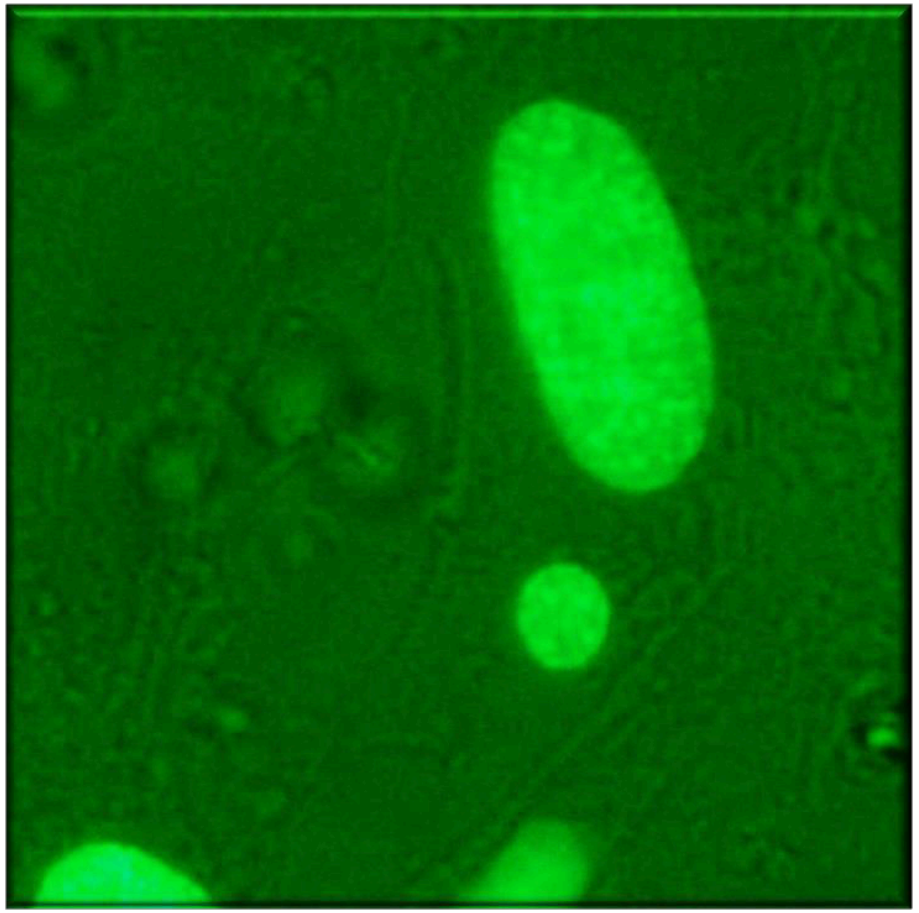
In vitro assessment of genotoxicity in living cells: the pluripotent stem cell micronucleus assay (PMNvit)

CF Lerche, BN Hölzel, P Stahlschmidt-Allner, B Allner



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TECOmedical Group

Overview

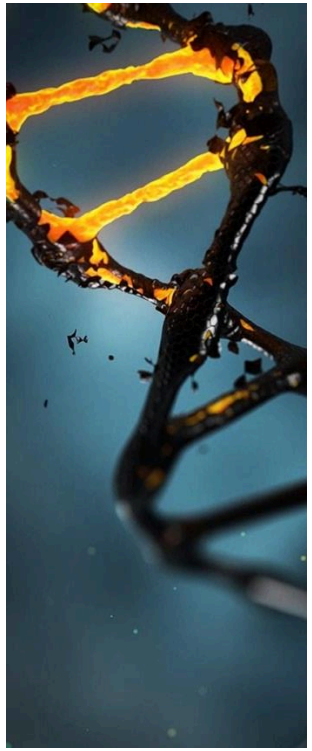
Genotoxicity testing

Prokaryotes
(*S. typhimurium, E. coli*)

Direct DNA damage
(e.g. mutations)

AMES

SOS chromotest
(umu assay)



https://sciencealert.com

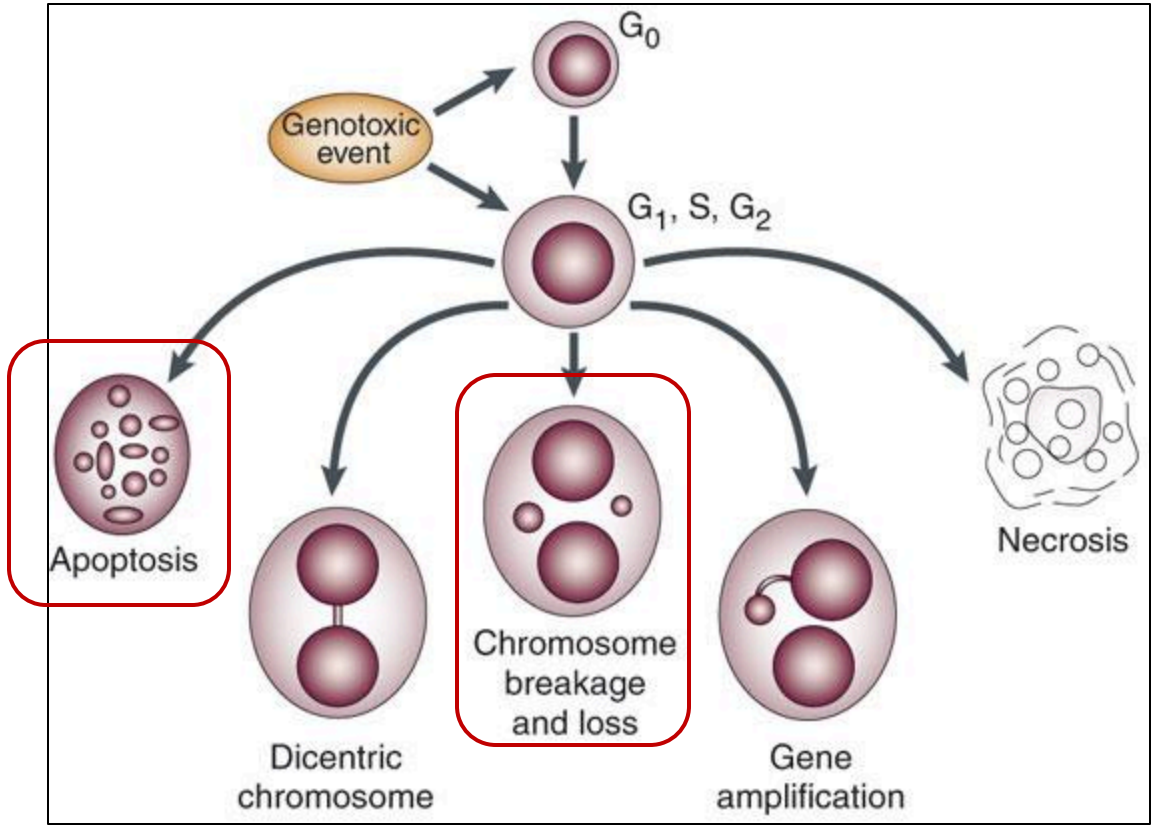
Eukaryotes / Vertebrates
(*in vivo / ex-vivo*, rodent cells)

Direct DNA damage
Double strand breakage
Mitotic apparatus failure

MLA (TK+/-), HPRT (mutations)

Comet assay,
Chromosome aberration (epigenetic)
Micronucleus assay

Micronucleus formation



Ferench, M., 2007. *Nat. Prot.*

- **MN formation:**

Chromosome breakage (clastogens)

Chromosome loss (aneugens)

- **Karryorhexis** (fragmentation of nuclei)**:**

Apoptosis (preceded by pyknosis)

Necrosis

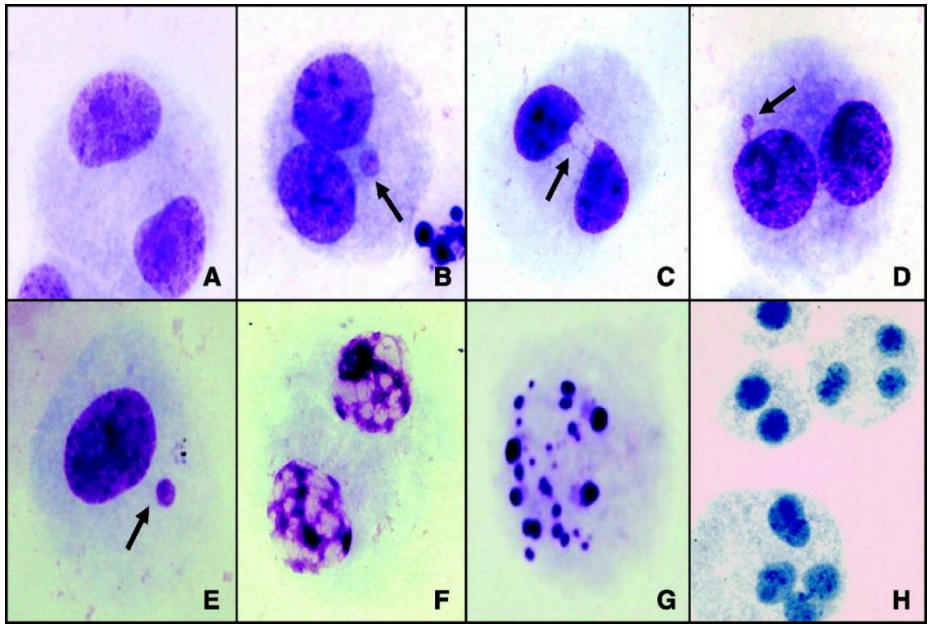
Micronucleus assay

***In vivo (ex-vivo)*:** OECD TG 474 (mammalian erythrocytes)

***In vitro*:** OECD TG 487 (human peripheral blood lymphocytes, rodent immortalized cell lines e.g. CHO, V79)

- Exposure to test compounds
- Transfer of cells to microscope slides
- Fixation and nuclear staining
  (e.g. Giemsa, acridine orange)
  - Microscopic scoring of MN-frequency

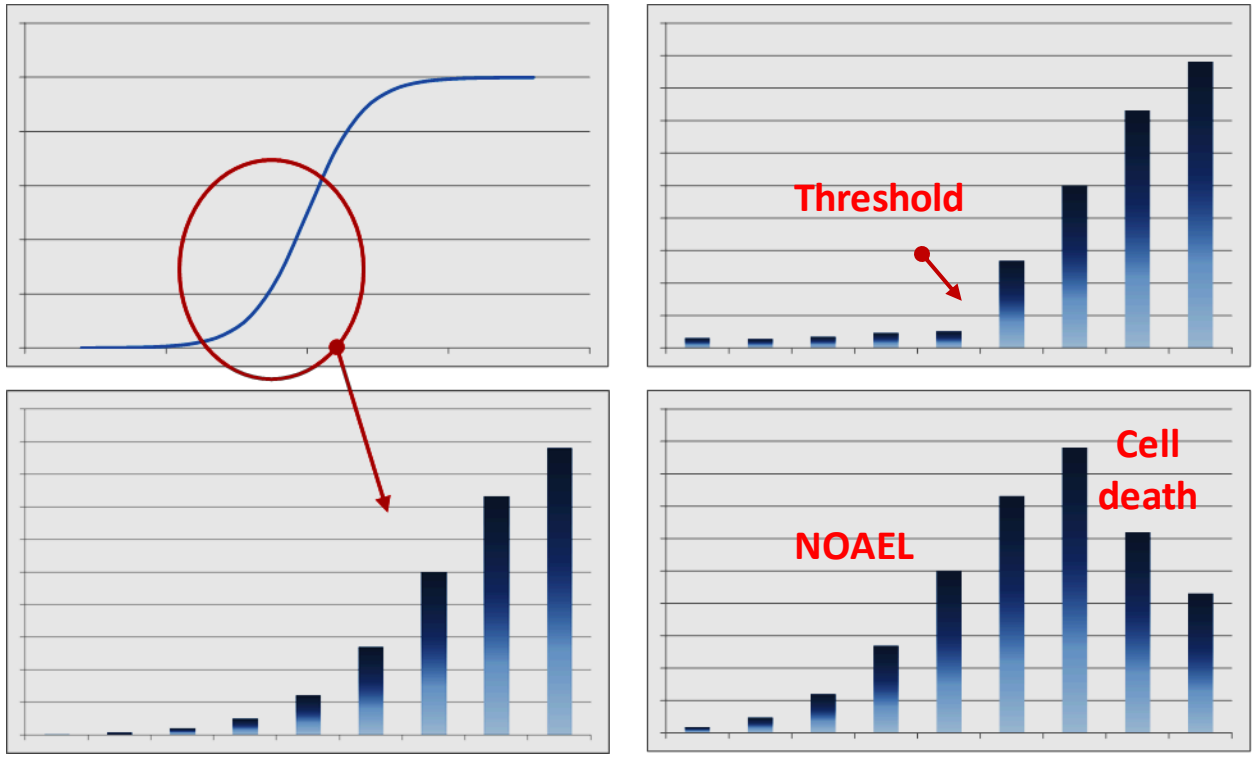
Alternatively: Flow cytometry methods
(e.g. Torous et al. 2005, De Boeck et al. 2005)



El-Zain *et al.* 2008. *Cancer Epidemiol. Biom. Prev.*

Micronucleus Frequencies and Carcinogenesis

Dose-response, threshold dose and LOAEL



Micronuclei, nucleoplasmic bridges, and nuclear buds in lymphocytes as predictors of cancer risk

El-Zain *et al.* 2008. *Cancer Epidemiol. Biom. Prev.*, 17(5): 1111-1119.

Bonassi *et al.* 2007. *Carcinogenesis*, 28 (3): 625–631.

Chromotripsis

e.g. Stephens *et al.* 2011. *Cell*, 144(1): 27 – 40.

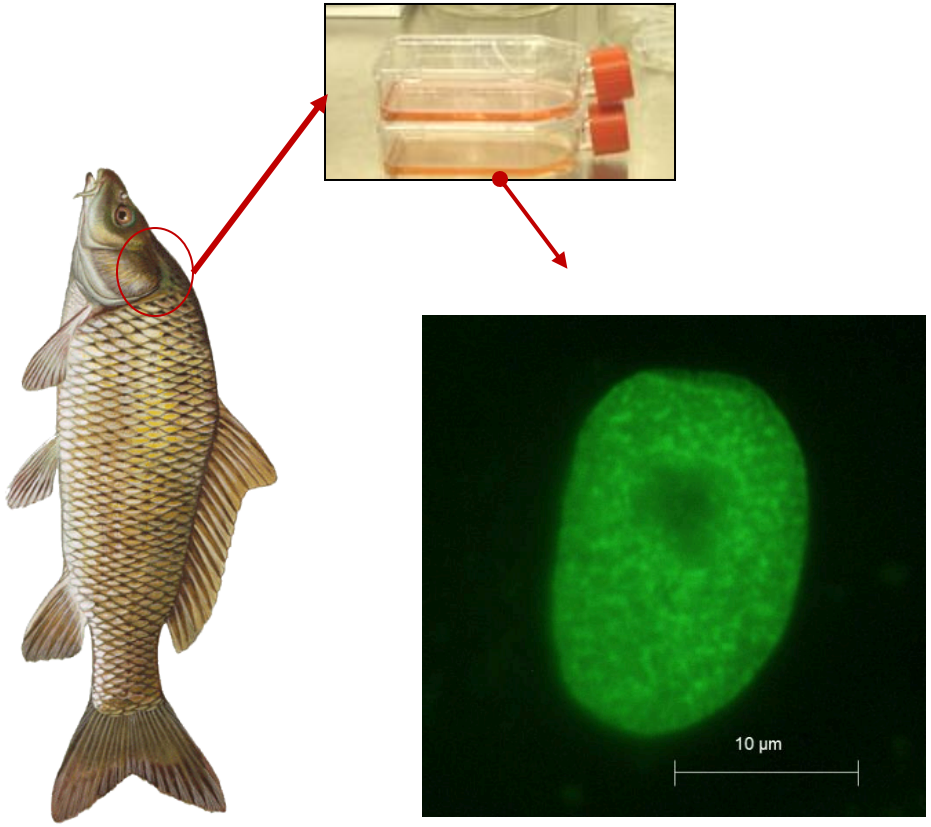
↓

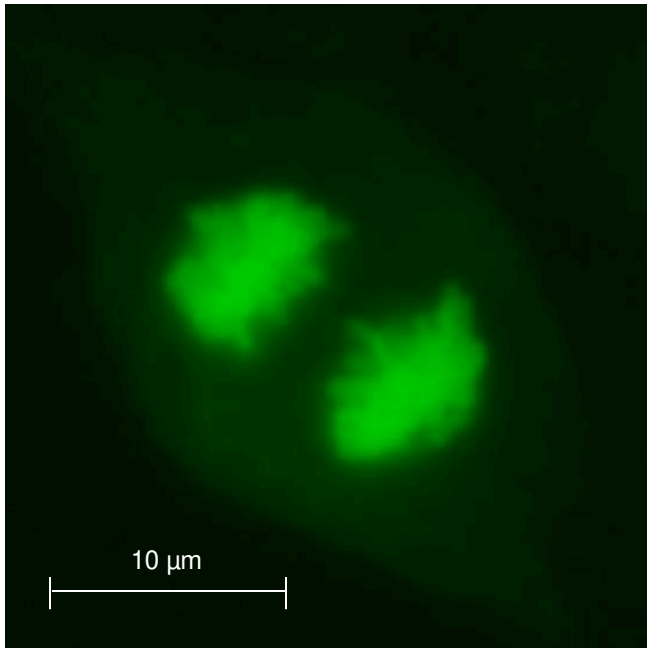
Micronuclei

e.g. Zhang *et al.* 2015. *Nature* 522: 179–184.

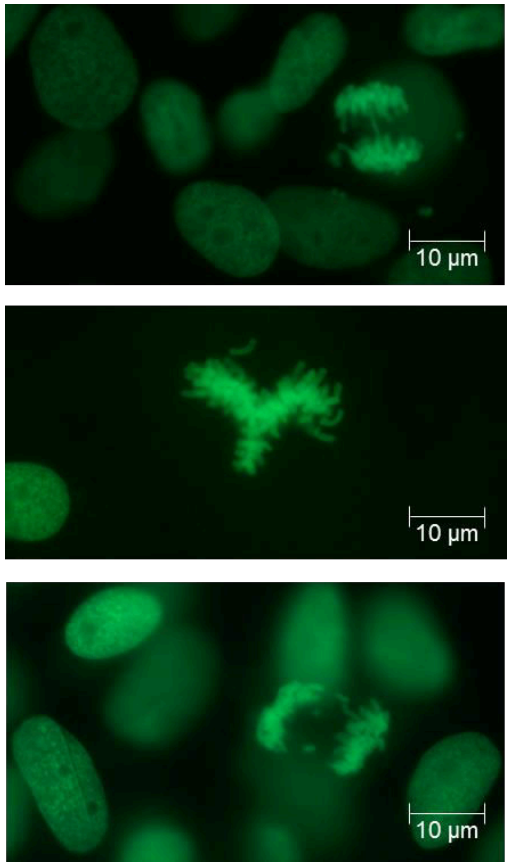
Cell line

KCB-GFP = DSM ACC3285 Budapest treaty
26°C growth temperature / no CO_2



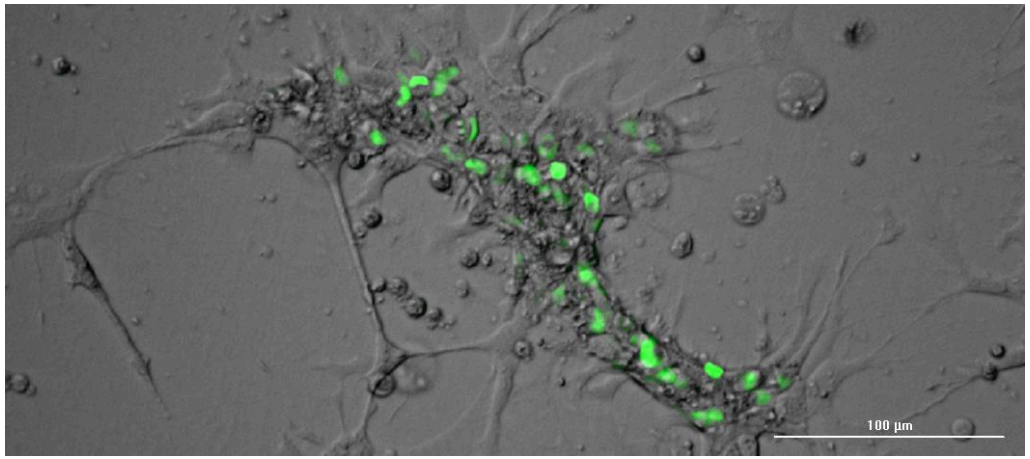


Epigenetic defects



The pluripotent stem cell micronucleus assay - PMNvit

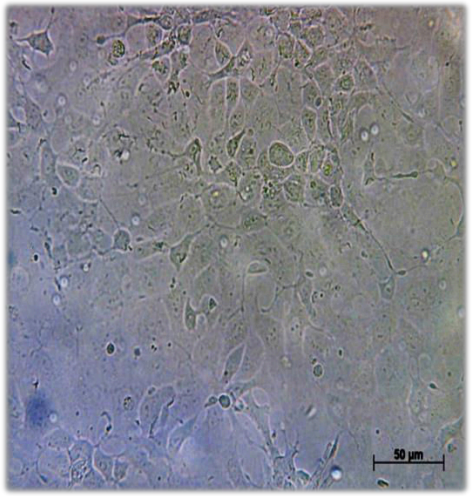
- Pre-cultivated cells stored at 4° C
- Replace medium and allow for recovery (60% monolayer confluence)
- Exposure in culture medium

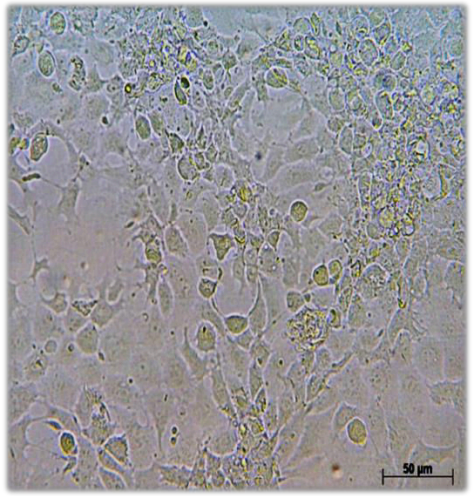


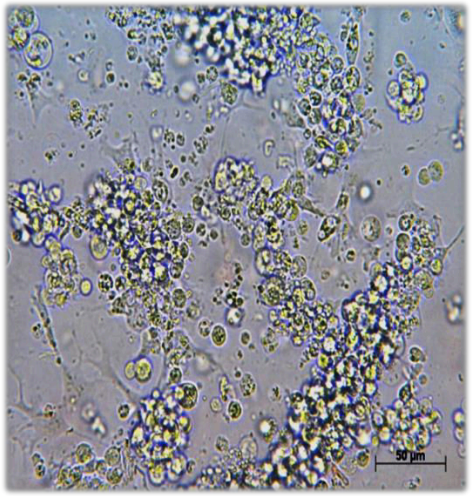
- Automatic shooting of pictures and cell counting
- Evaluation of MN frequencies (optionally also of FN frequencies)
- Passaging for downstream analysis

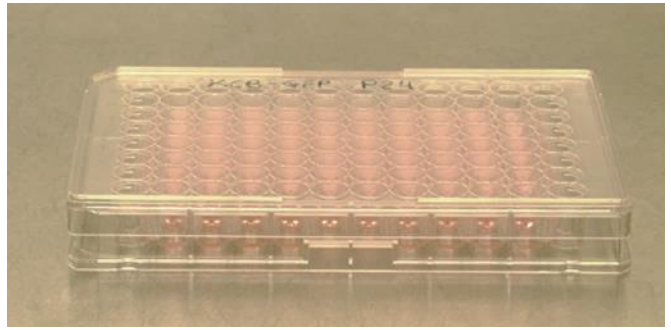
Range Finding

Normal cell layer ← **Genotoxicity testing** → Cytotoxic effect







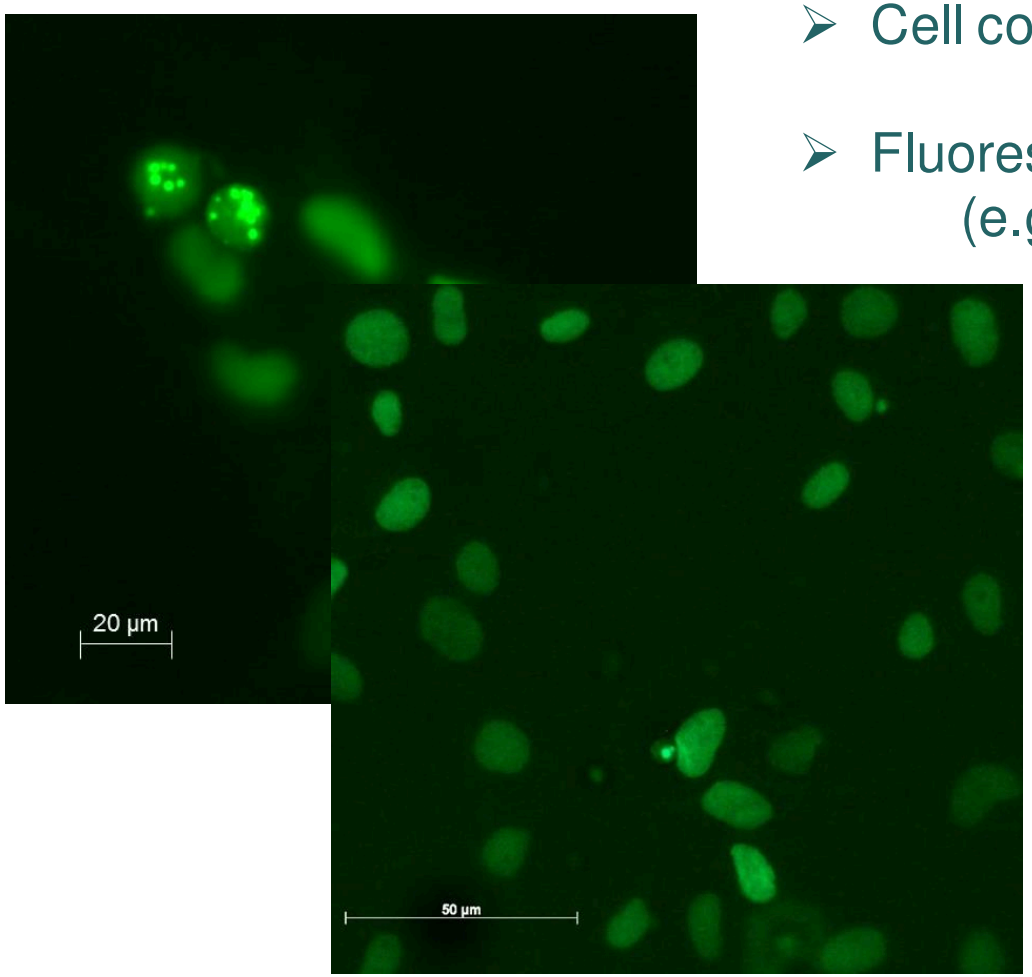


Cell count

Concrete criteria

Concentration range

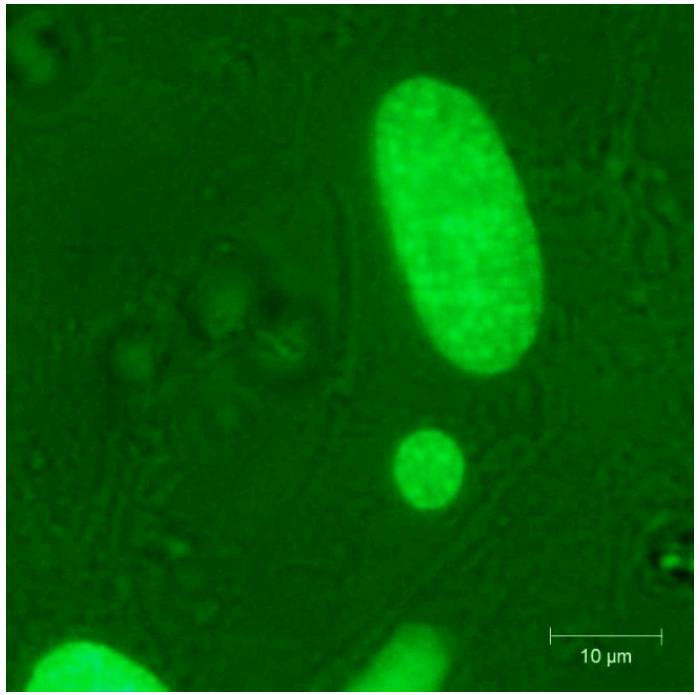
MN frequency evaluation



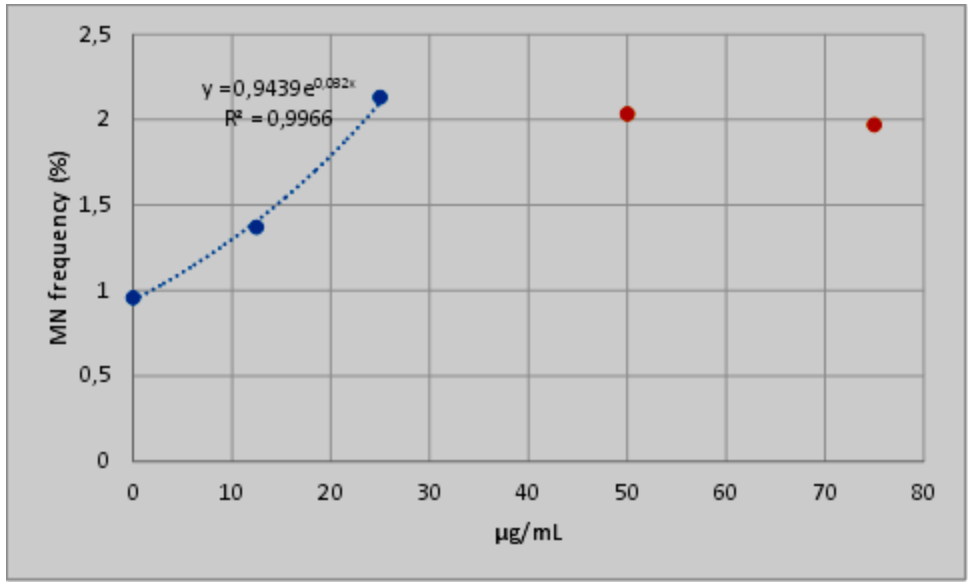
- Cell counting
- Fluorescence intensity (e.g. pyknosis)

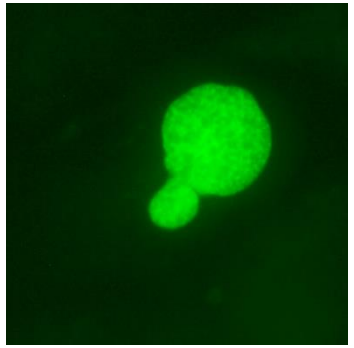
Manual evaluation:

- Micronuclei
- Fragmented nuclei

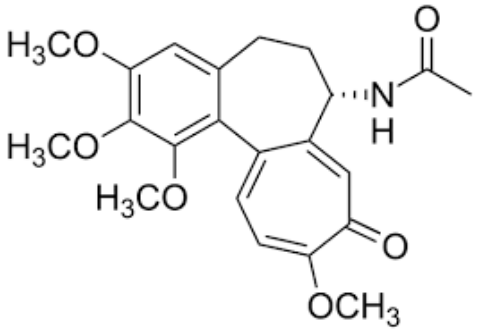


Colchicine (aneugen)

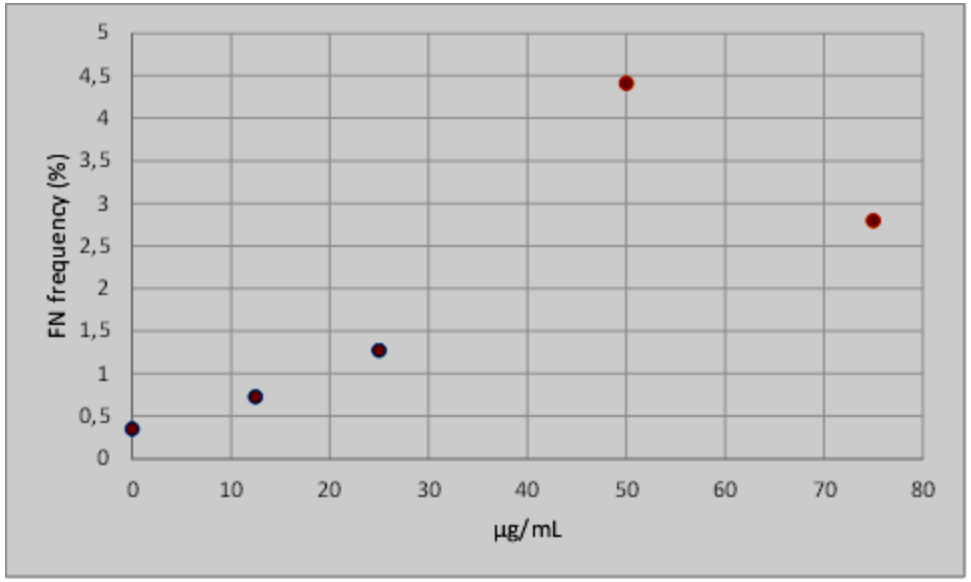




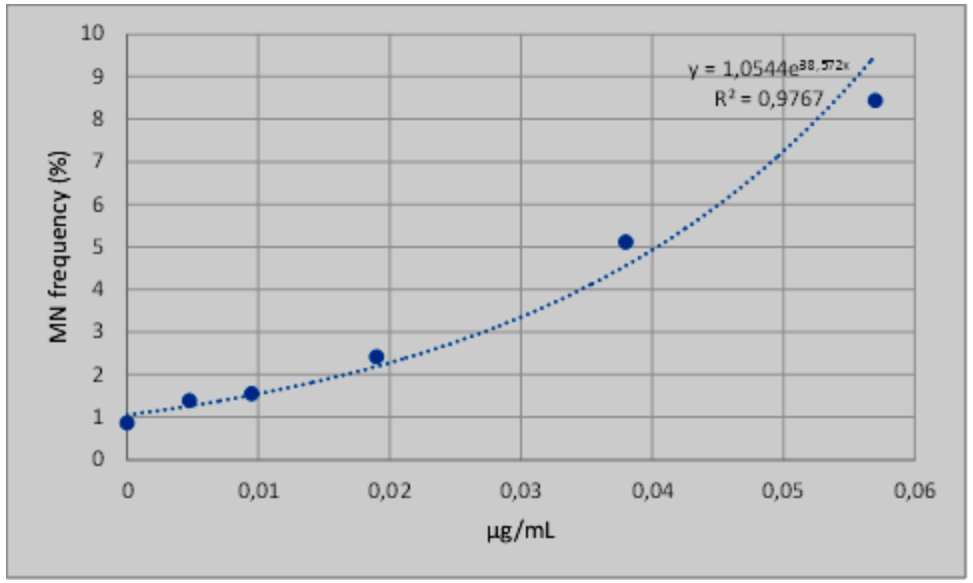
Significant (p<0.0001)

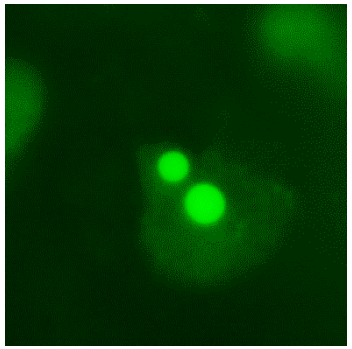


$C_{22}H_{25}NO_6$

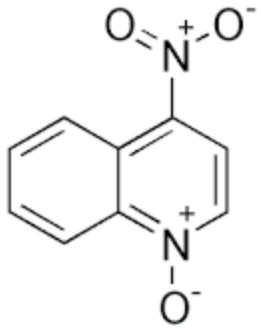


4-Nitroquinoline 1-oxide (clastogen)

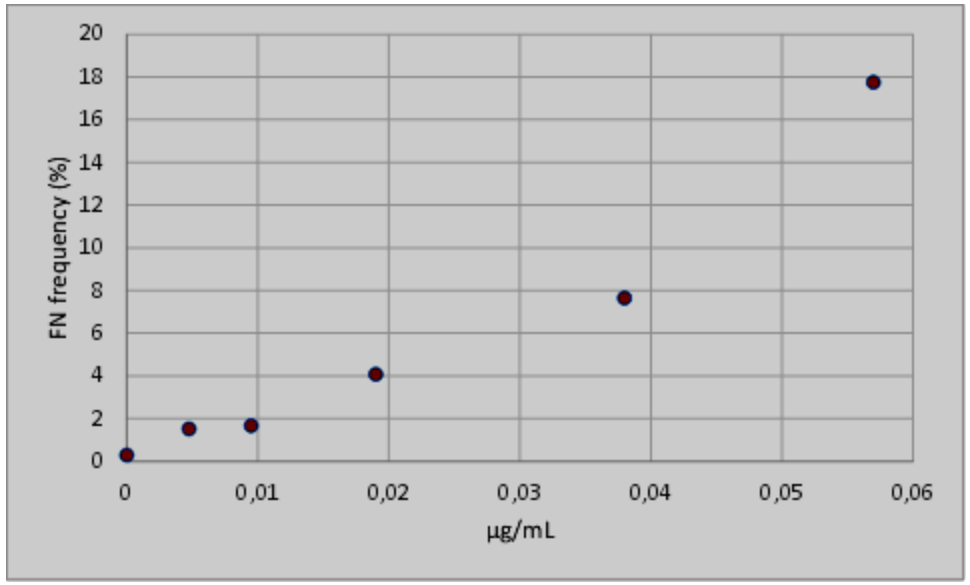




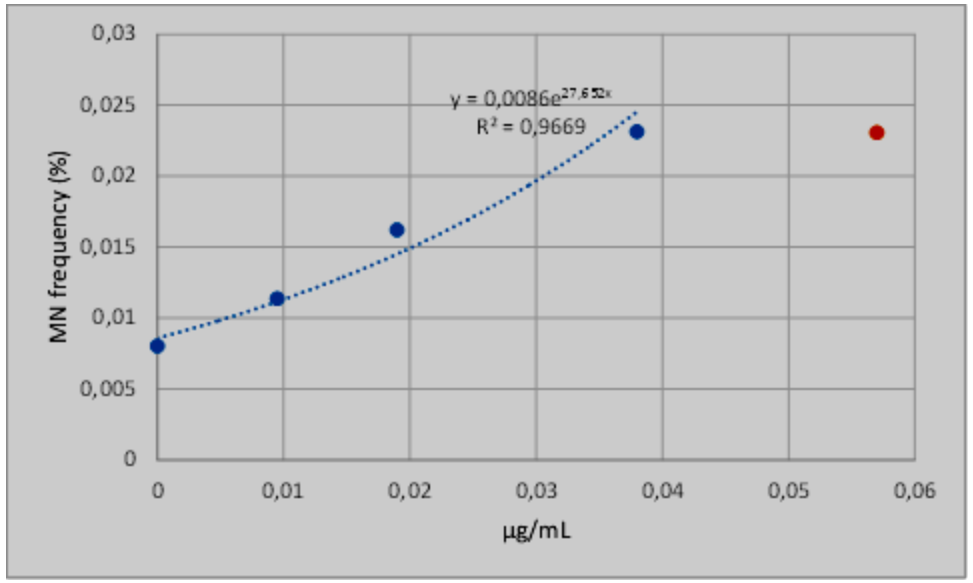
Significant (*p*<0.0001)

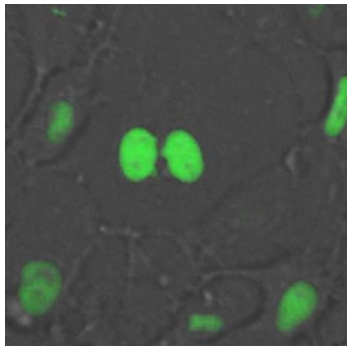


$C_9H_6N_2O_3$

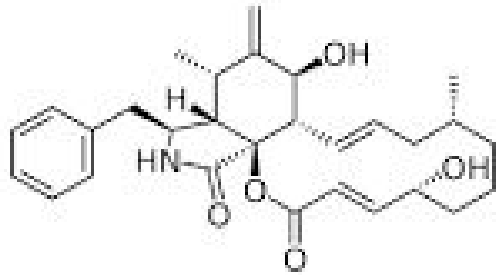


Cytokinesis blocker (Cytochalasin B)

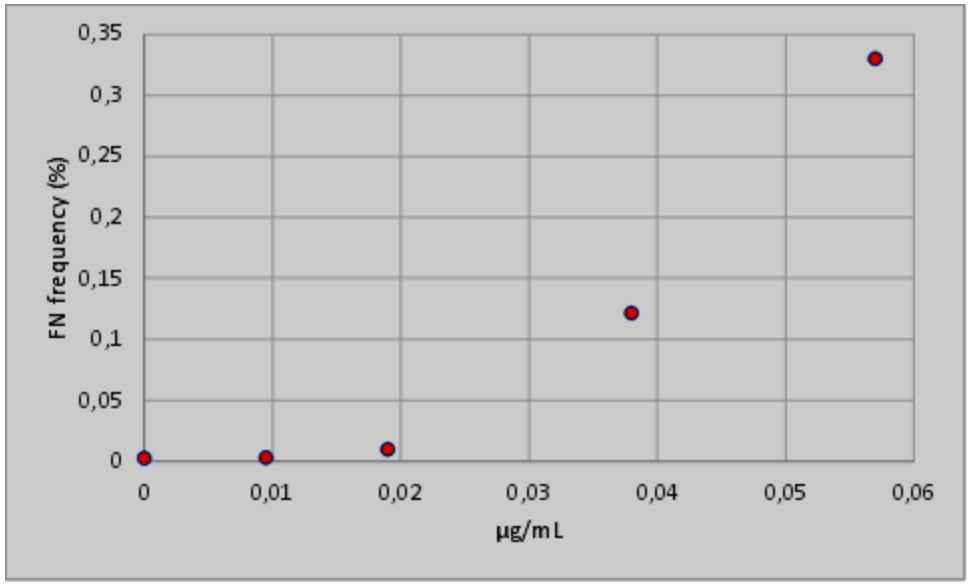




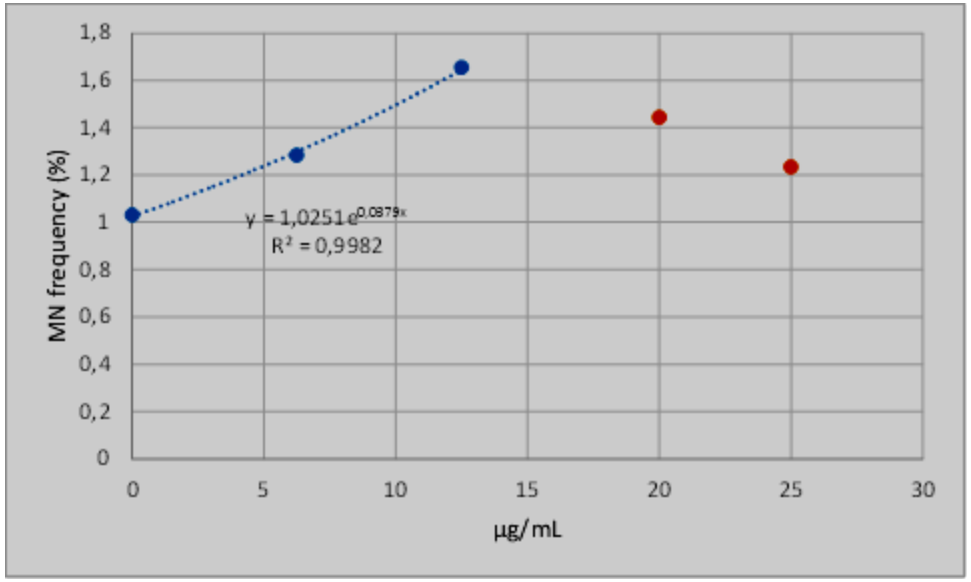
Significant (*p*=0.0012)



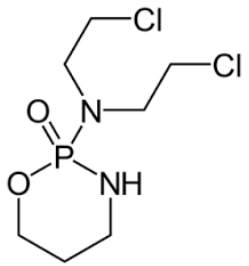
$C_{29}H_{37}NO_5$



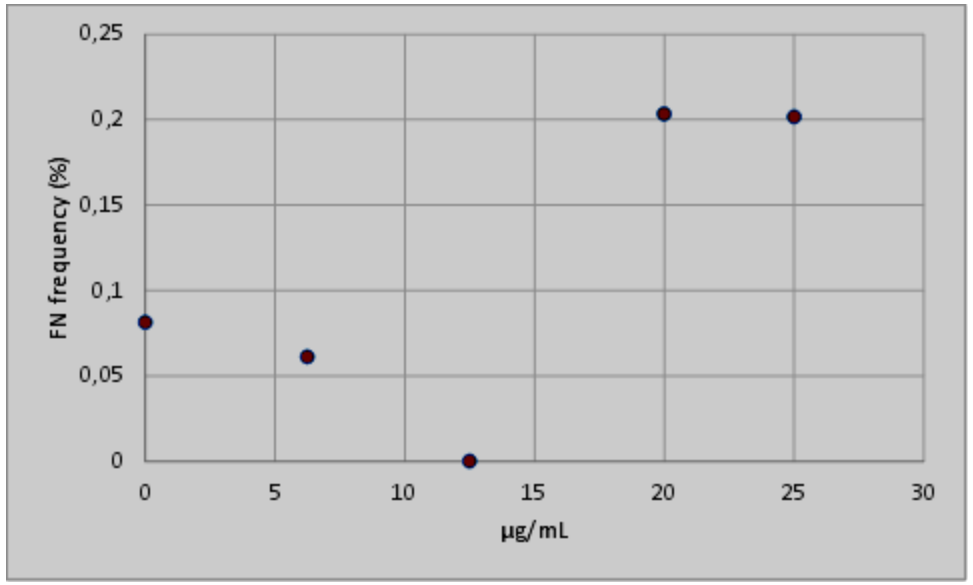
Cyclophosphamide (metabolic activation)



Significant (*p*=0.0046)

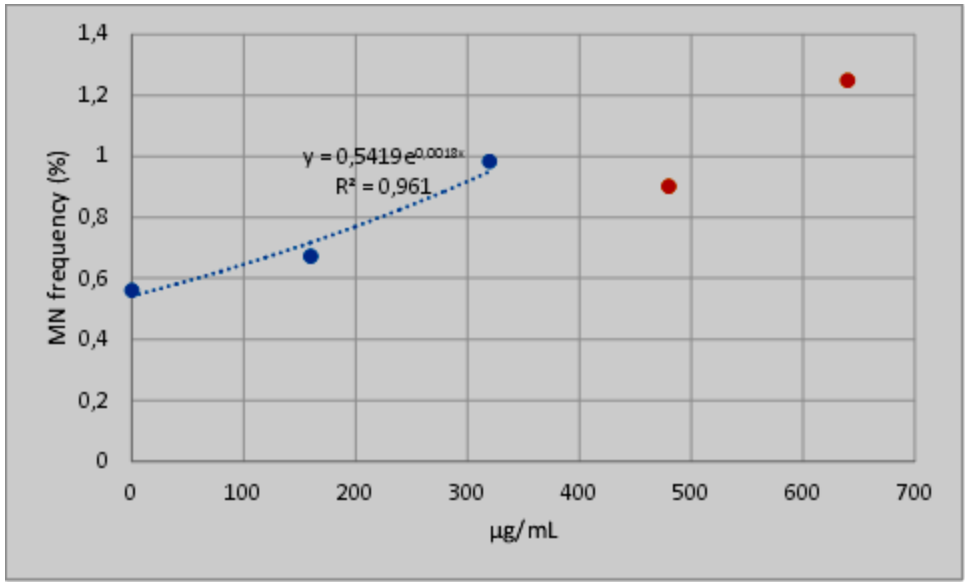


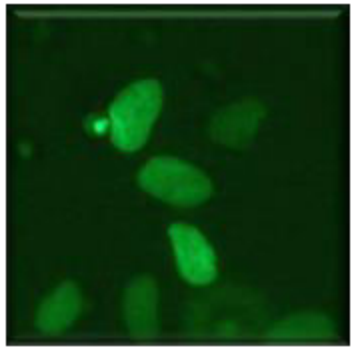
$C_7H_{15}Cl_2N_2O_2P$



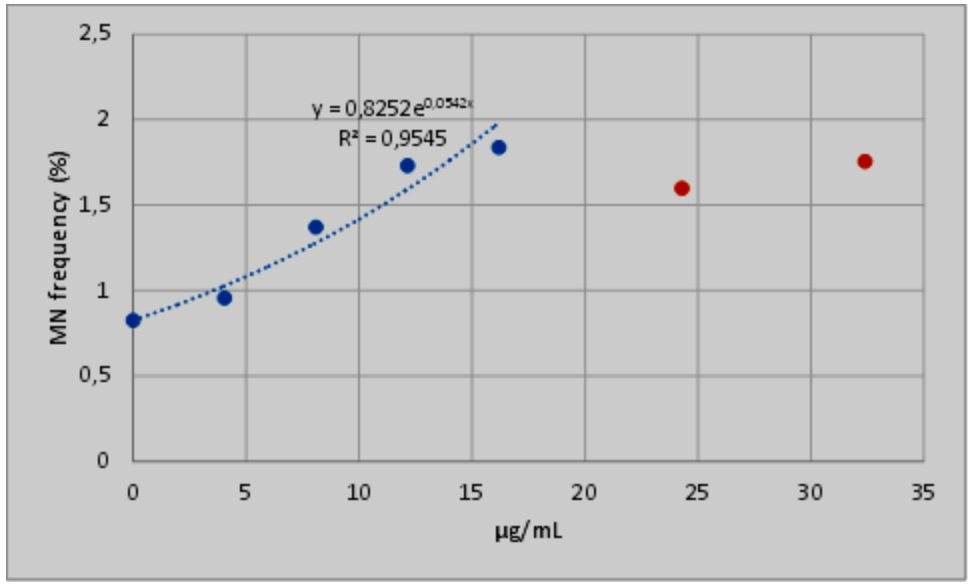
Ethyl methanesulfonate & diethylstilbestrol

Ethyl methanesulfonate (EMS)





Diethylstilbestrol (DES)

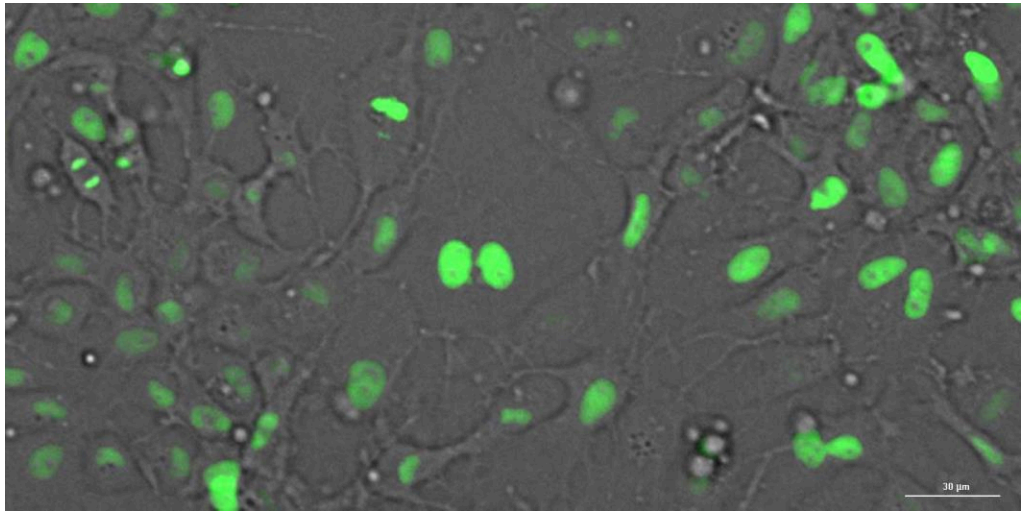


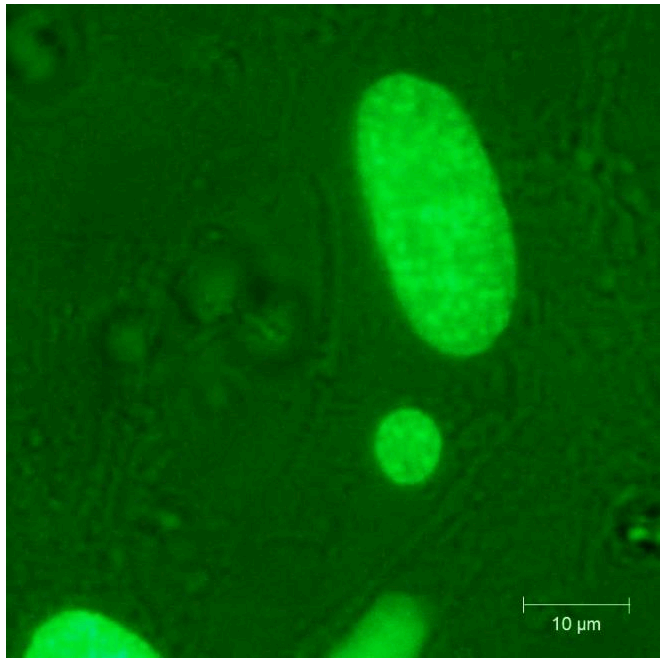
Not significant (*p*=0.11)

Significant (*p*=0.0086)

PMNvit pre-validation

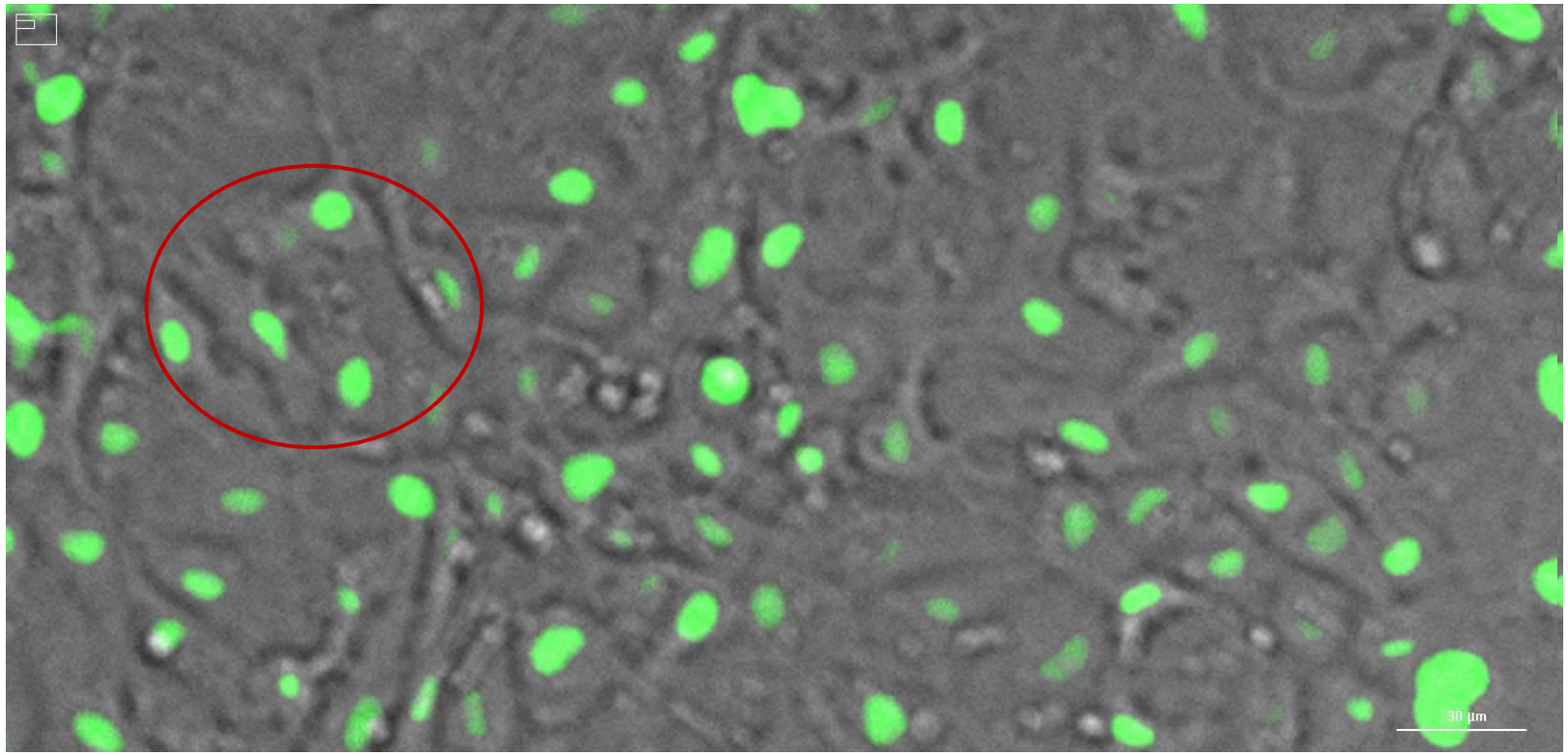
- Aneugens (*e.g.* colchicine)
- Natural clastogens (*e.g.* 4-NQO)
- Cytokinesis blocker
- Clastogens after metabolic activation (*e.g.* Cyclophosphamide)





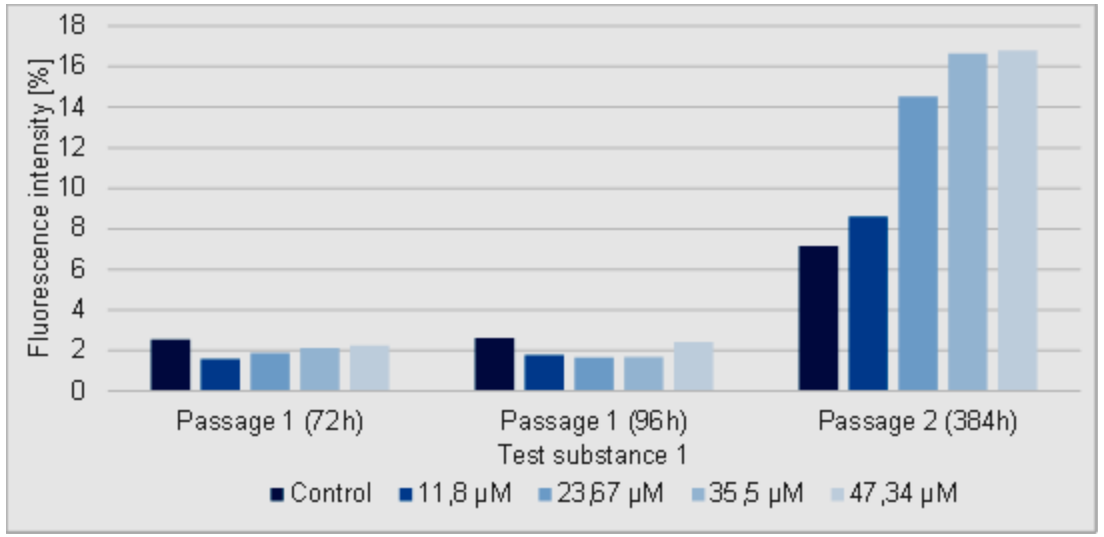
Further applications I

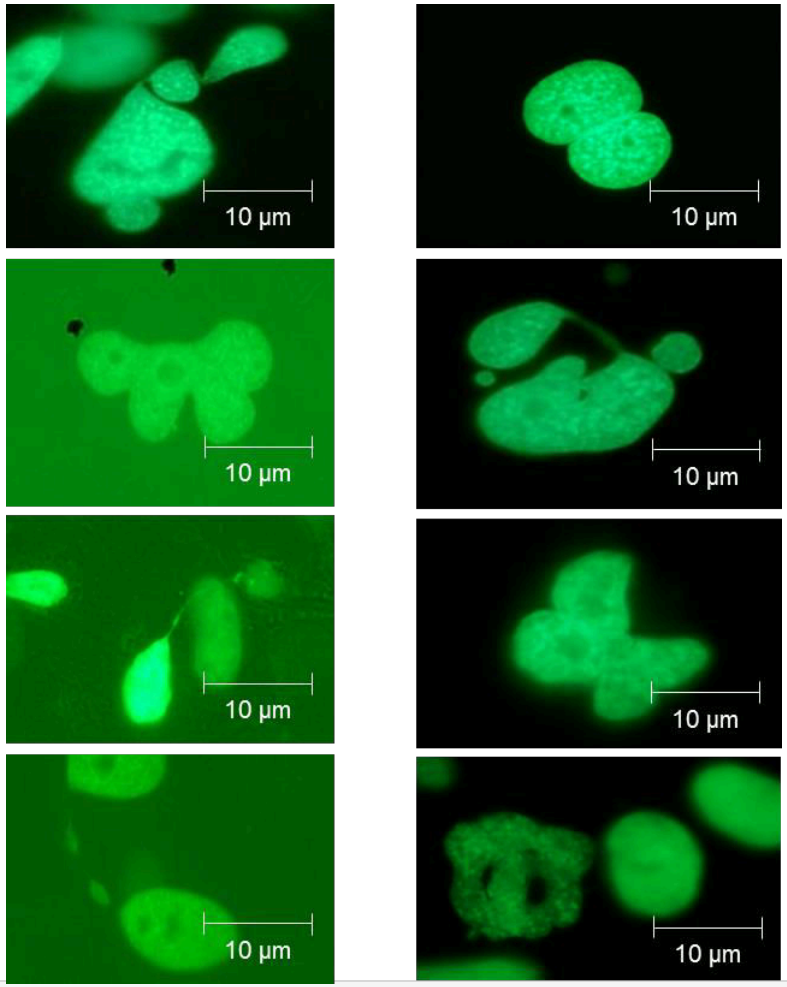
Background research



Further applications II

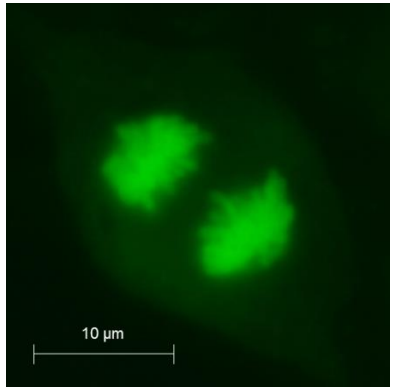
Downstream analysis



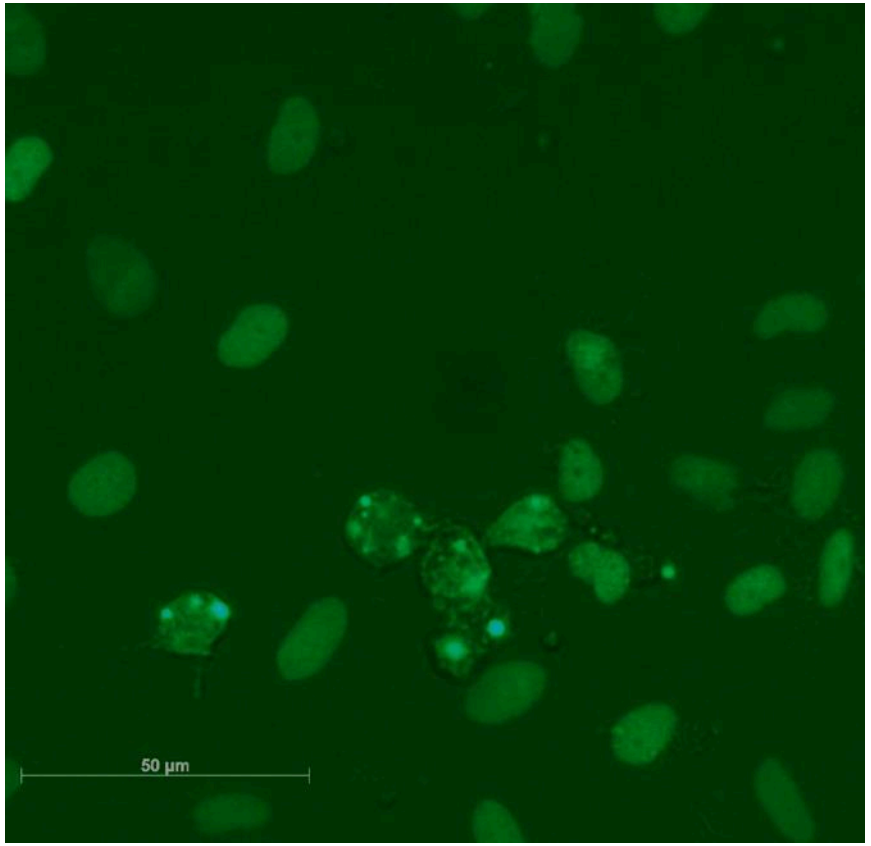


- Fluorescence intensity
- Elongated / deformed nuclei
- Proliferation rate

Perspectives



- Automatic assessment of MN (software)
- Round robin tests



- Inter-laboratory comparison - RWTH Aachen University (metabolic activation)
- Validation and certification

Acknowledgements

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J Hescheler, K Pfannkuche, D Derichsweiler

Bundesministerium für Wirtschaft und Technologie

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ZIM ZF4066801MD5