Nordic collaboration within biological dosimetry

Carita Lindholm
Daniela Stricklin
Alicja Jaworska

Radiation and Nuclear Safety Authority (STUK)
Swedish Defence Research Agency (FOI)
Norwegian Radiation Protection Authority (NRPA)

Mass casualty scenarios and biodosimetry

- large number of exposed individuals with wide range of doses
- rapid and reliable dose assessment required
- physical dosimetry and clinical analysis (blood cell counts) may not give sufficient support for medical decision making
- essential to identify individuals with no or low exposure
- capacity of small biodosimetry laboratories exceeds easily: collaboration and networking are key issues

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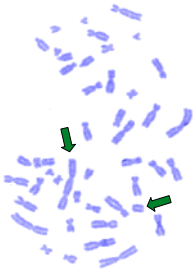
Classic chromosome aberration assay

- based on dicentric chromosomes observed in blood lymphocytes
- sensitive: 100-200 mGy (low-LET); 10-20 mGy (high LET), with
- demanding, analysis requires excessive training
- upper dose limit 6-7 Gy
- dependent on mitogen sensitive cells (T-lymphocytes) reaching mitosis (high doses may block cell cycle)



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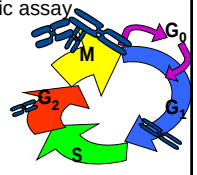
NKS-project 2006-2007: Biodosimetry application in emergency preparedness (BIODOS)

- **prematurely condensed chromosome** (PCC) assay

Advantage of PCC with respect to dicentric assay

- scoring of radiation-induced damage in pre-mitotic cell cycle stages
- ability to assess very high doses
- potential for more rapid scoring



Cell cycle

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Induction of prematurely condensed chromosomes (PCC)

- original procedure: fusion of interphase lymphocytes and mitotic Chinese hamster ovary cells; mitotic factors induce the nucleus to condense into chromosomes within 1-2 hours
  - relatively low yield of PCC, inconsistent assay
- more recently, chemically (okadaic acid and calyculin A) induced chromosome condensation of stimulated cells; requires 48 h culture
  - increased yield of PCC
- induction of PCC in unstimulated cells within hours facilitated by cyclin B kinase and calyculin A / okadaic acid
  - inadequate condensation
- the need for systematic evaluation of the different assays

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PCC induction and analysis methods

Okadaic acid and Calyculin A

- Giemsa-staining
  - excess fragments
  - ring chromosomes
- Fluorescence in situ hybridization (FISH) with chromosome probes
  - exchange type aberrations

Protein kinase/Cyclin B + OA or CalA

- FISH
  - evaluation of painted chromosome “areas”

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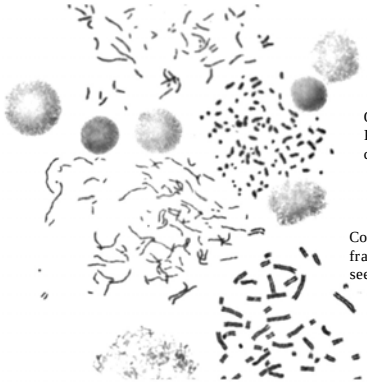
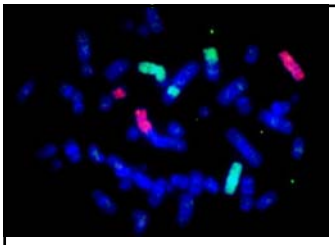
Analysis Approach	Consideration
Excess fragments	Reliability and accuracy
Ring chromosomes	Time required for analysis
Aberrations detected by FISH	Technical characteristics
“Spot” technique (FISH)	

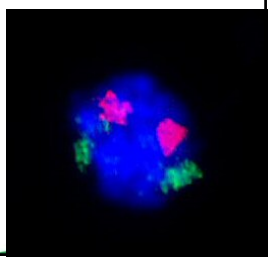


Okadaic acid induced PCC in cells in different cell cycle stages

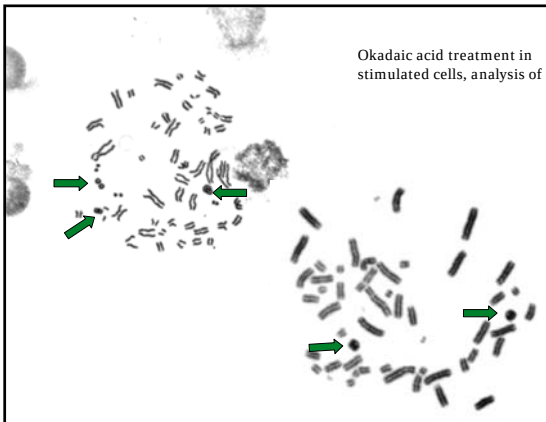
Counting of excess fragments (>46 elements seen)



Chromosome aberration analysis in FISH painted stimulated cells



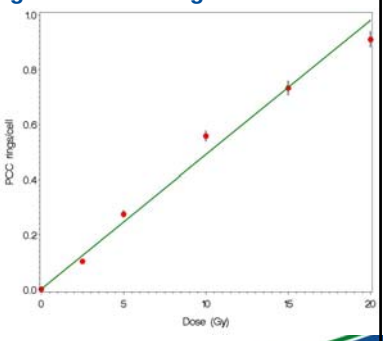
”Spot analysis” after FISH painting in cyclin B kinase + CalA treated unstimulated cells

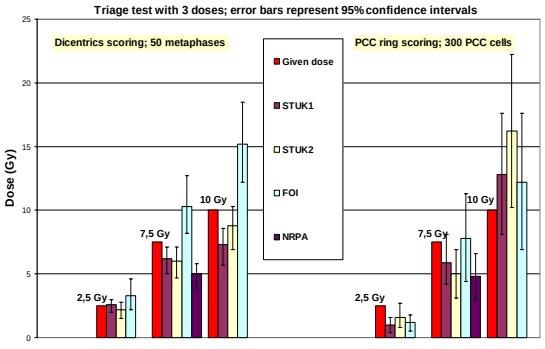


Okadaic acid treatment in stimulated cells, analysis of rings

Curve fitting of the PCC ring data

- The data fit Poisson distribution
- Data best described by linear relationship:
  Y=C + α • D
- where:
  Y = freq. of PCC rings
  C = 0,002 (±0,002)
  α = 0,049 (±0,006)
  D = dose (Gy)





Conclusions of BIODOS

- Okadaic acid treatment of lymphocyte cultures
- Evaluation of ring chromosomes
- Linear fit of data 0 - 20 Gy
- The PCC assay may be most applicable at doses above 5 Gy
- For emergency preparedness applications, the dicentric assay and PCC assay cultures could be run in parallel and evaluated in triage mode
- PCC ring assay requires less training than the classical dicentric assay
- Essential to maintain the analysis routine by arranging and participating in intra- and intercomparisons

Mass casualty exercise (BIOPEX) NKS 2008

Carita Lindholm, Wendla Paile, Marjo Perälä, Armi Koivistoinen, Daniela Stricklin, Eva Arvidsson, Alicja Jaworska

- Main aim is to evaluate the applicability of the PCC ring assay in comparison to the dicentric assay
- Simulated triage exercise involving a large number of exposed casualties = 60 blood samples
- *In vitro* exposure with ^{60}Co
  - wide range of doses, including non-uniform exposures
- Parallel cultures for both PCC ring and dicentric assays
- Dose estimation will be performed using the PCC ring curve and the routinely used dicentric curve

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