Project FM 8823 – Effect of GSM-signals on isolated, human blood. Genotoxicity

aim of the project:

- to investigate whether high frequency electromagnetic fields (HF-EMF) used in mobile telecommunication can lead to DNA or chromosomal damage.

- to determine potencial genotoxic effects of RF-fields in stimulated peripheral lymphocytes in a multicentered study with high statistical validity.

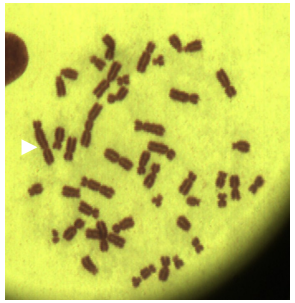
frame given by the BfS:

- **Literature research**
- **use of stimulated, peripheral lymphocytes**
  exposure of G1, S, G2-phase and (mitosis)
- **donors:** male, non-smokers, healthy
  2 collectives of age with 10 donors under 18 years and 10 donors between 50 - 60 years
- **exposure:** whole blood stimulated with PHA in appropriate medium
  1800 MHz GSM-signal (intermittent 5 min on, 10 min off), (“sham” and exposed to SAR of 0.2, 2, 10 W/kg and positive controls)

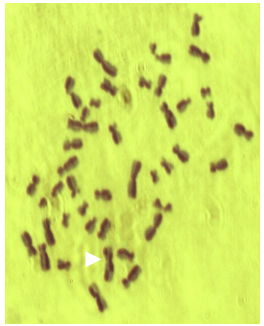
Frame given by the BfS:

Methods:

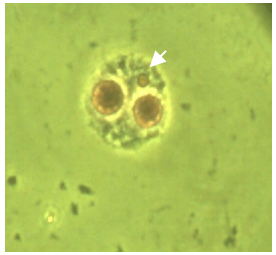
- chromosome aberration (CA), analysis of the first mitosis, 1000 cells



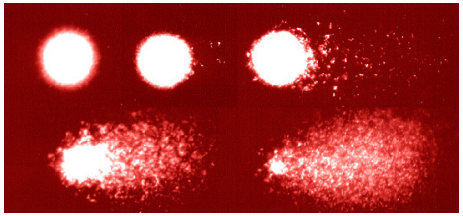
- sister chromatide exchange (SCE), analysis of the second mitosis, 50 cells



- Micronucleus-Test (MN), analysis of binuclear cells,(Cytochalasin B), 2000 cells



- Comet-Assay (CoA) alkaline version, 100 cells



Frame given by the BfS:

- **Analysis/ scoring:**
  in 3 appropriate laboratories
  scoring should be carried out „blind“:
  Assays and scoring of the slides are conducted in different laboratories, the slides are encoded

- **Statistics:** statistician

- **Duration:** 2,5 years

Structure of the project:

ethics commission authorisation
questionaires
recruitment of donors

↓

anamnesis,

blood sampling

↓

stimulation with
Phytohemagglutinine (PHA)
exposure

CA, SCE, MN, Comet
staining of the slides
encoding the slides

↓

scoring in 3 Labs

↓

decoding
statistical analysis
report
publication

Planned realisation of the project:

Donors: male, healthy, non-smokers

- 2-3 donors more per group

  teachers (50-60 years), students (16-17 years)

  donors, who worked for a longer period on the school (highschools in Mainz)

  low exposure, similar environment, open-minded

questionaire

questions about:

Health: acute - latent deseases, allergies, immunisations

utilization of medical benefits:

pharmaceuticals, x-ray examinations, surgical treatments

live style: smoking, drinking, sports,

Nutrition: special diets, weight, body height

Exposure to HF-EMF: use of mobile phone, etc.

→ **groups as homogeneous as possible**

non-smoking, healthy, no acute allergy, no diet, no serious sport, not obese

Planned realisation:

Blood collection: anamese by a medical doctor

1 donor /date and experiment

25 ml blood / donor

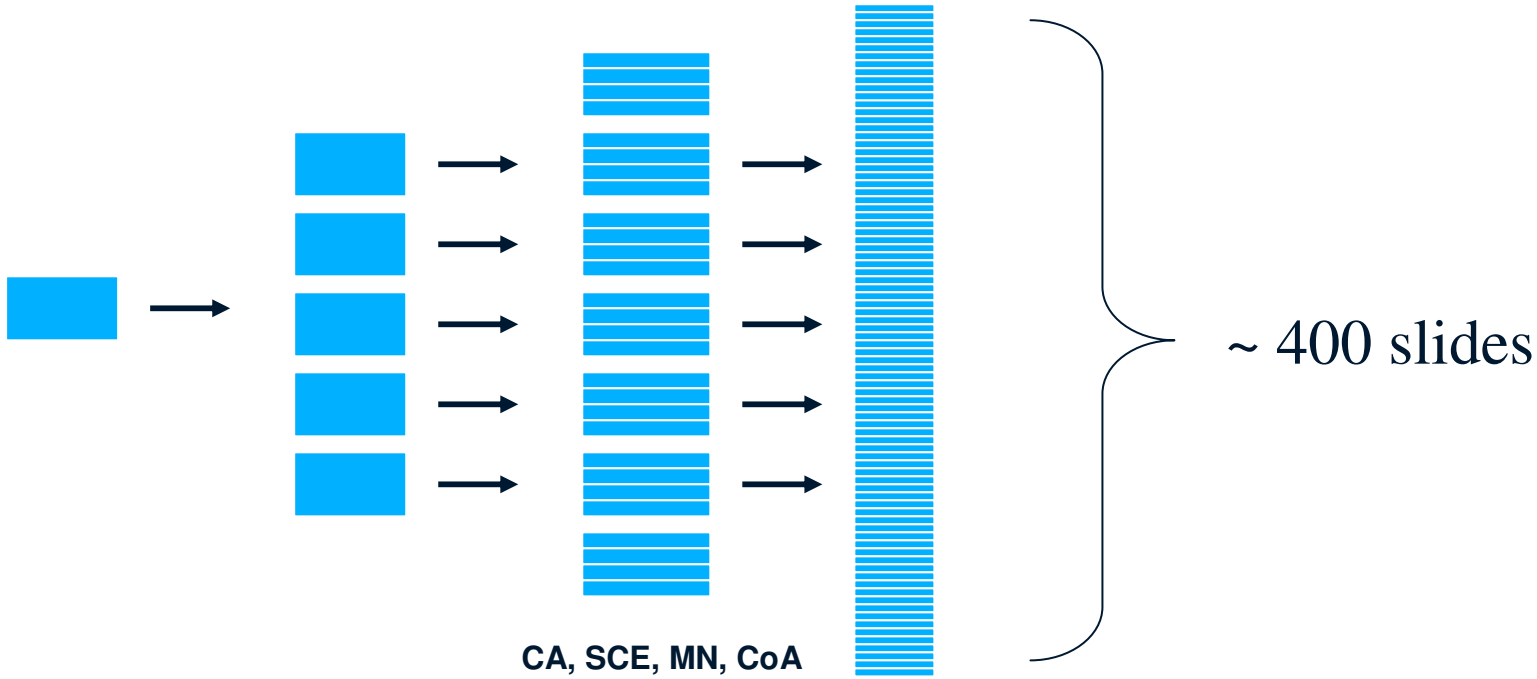
for 4 genotoxicological endpoints

5 treatment groups

3 Labs

2-3 donors / month

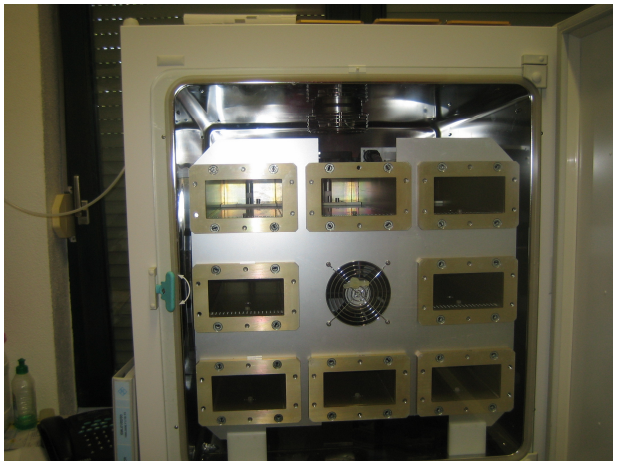
1 donor → doses (0, 0.2, 2,10 W/kg, pos.ctr) → 4 assays → 3 labs



disadvantage:
complex logistics, big wave guide setup

advantage:
blood sampling 1-2 times/ donor, less variation of the results

Exposure:



<u>given:</u> 1800 MHz, GSM-signal, intermittent, 5 min on, 10 min off,

<u>Wave guide setup from ITIS Zurich:</u>
Enables HF-EMF exposure of cells under defined conditions with respect to homogeneity of the HF-EMF, minimum variation of SAR and temperature.
8 wave guides arranged in 4 separately controlled exposure units placed within a CO_2-incubator
2 wave guides /SAR-value

9 x 35 mm dishes can be exposed in 1 wave guide
18 dishes /dose
72 dishes /experiment + positive controls

Details of a smaller version sXc1800 of the wave guide setup are published.

Exposure:

<u>Incubation:</u> 37 °C, 5% CO_2

in RPMI-Medium + FBS + Pen/Strep + Amphotericin B
-PHA

The calculated energy impact of HF-EMF on DNA is to low to generate DNA strand breaks directly.
Indirect effects of mechanisms like

repair, replication, mitotic spindle formation
condensation of chromosomes, redox-status etc. can not be ruled out.

exposure should happen during the active phases of cell cycle:

G1-phase, S-phase, G2-phase, (mitosis)

– to cover many potential mechanisms

exposure – time course (flow):

begin of exposure: 20 h after PHA-addition cells enter G1

SCE:

0 h ⟶ **20 h** ⟶ **48 h** ⟶ **72 h** ⟶ **74 h**

+ PHA + BrdU exposure + Colcemid

CA:

0 h ⟶ **20 h** ⟶ **48 h** ⟶ **50 h**

+ PHA exposure + Colcemid

MN:

+ Cytochalasin B

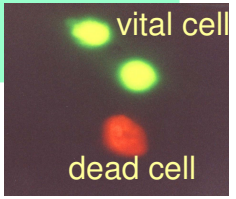
0 h ⟶ **20 h** → **48 h** **52 h**

+ PHA exposure

CoA:

0 h ⟶ **20 h** → **48 h**

+ PHA exposure determination of vitality



INCOS: Research Group for Molecular Mechanisms of Environmental Gentoxicity (AMMUG/ INCOS GmbH)

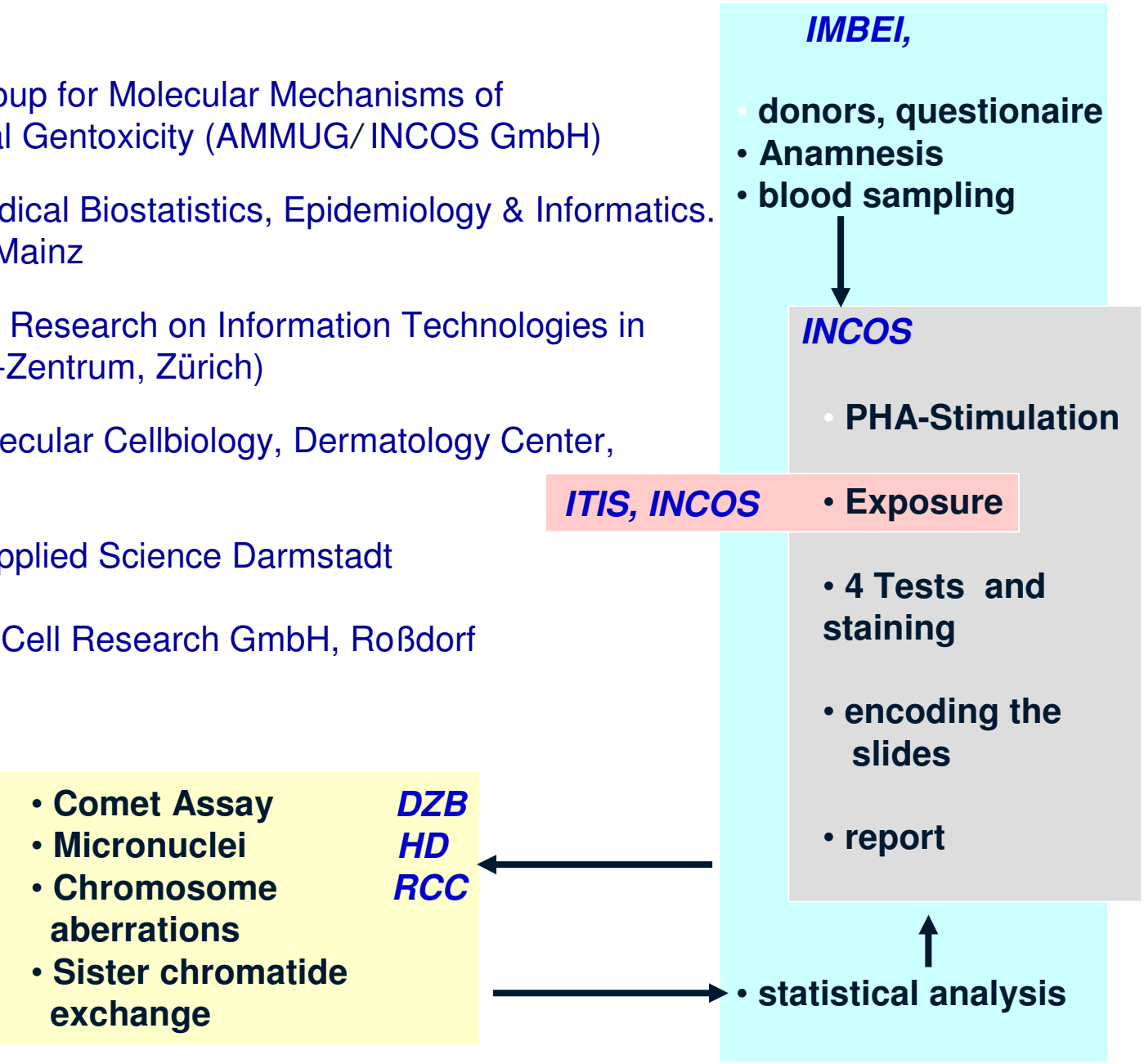
IMBEI: Institute of Medical Biostatistics, Epidemiology & Informatics. University of Mainz

ITIS: Foundation for Research on Information Technologies in Society, (ETH-Zentrum, Zürich)

DZB: Division of Molecular Cellbiology, Dermatology Center, Buxtehude

HD: University of Applied Science Darmstadt

RCC: RCC Cytotest Cell Research GmbH, Roßdorf



Pretest:

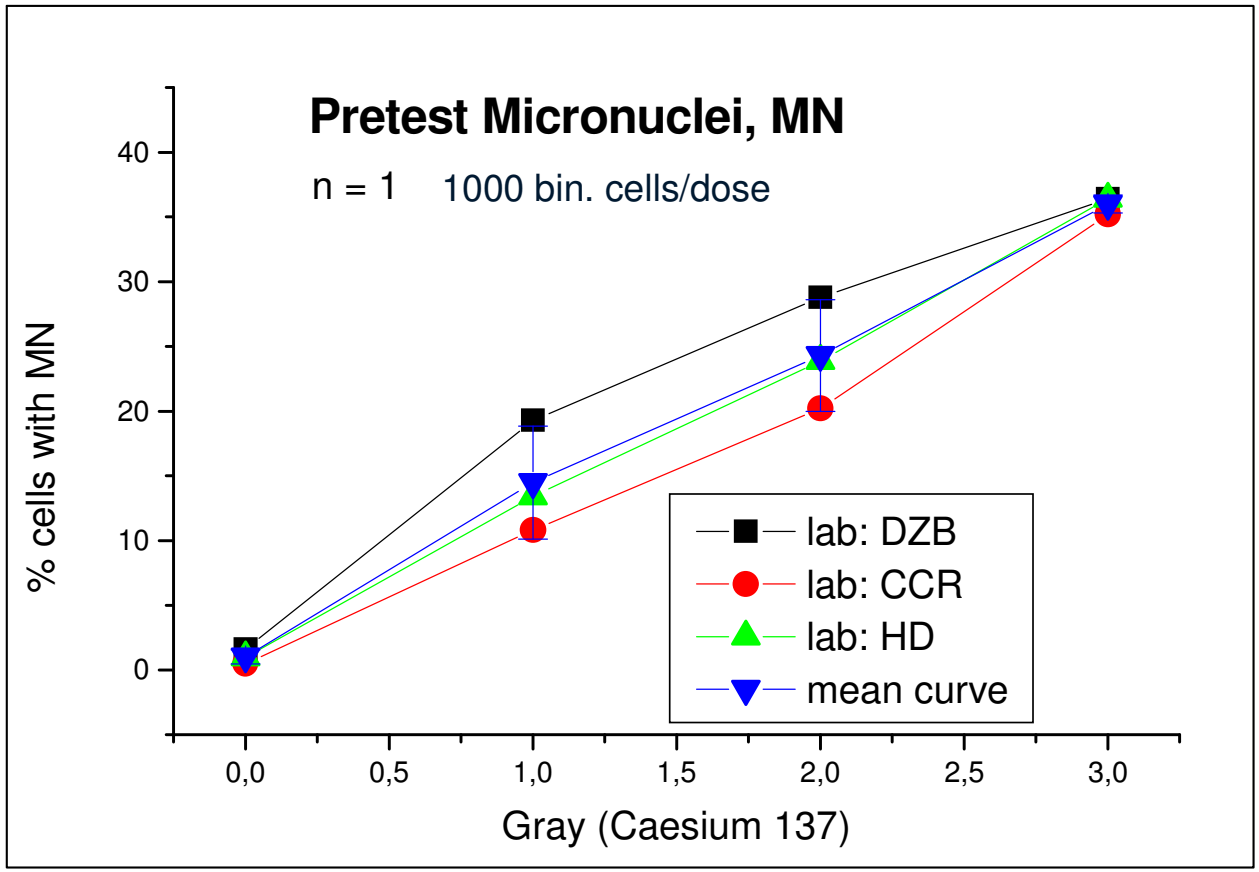
to assure that the 3 labs obtain similar results.

to prove, if all parameters for scoring are well-defined and harmonized.

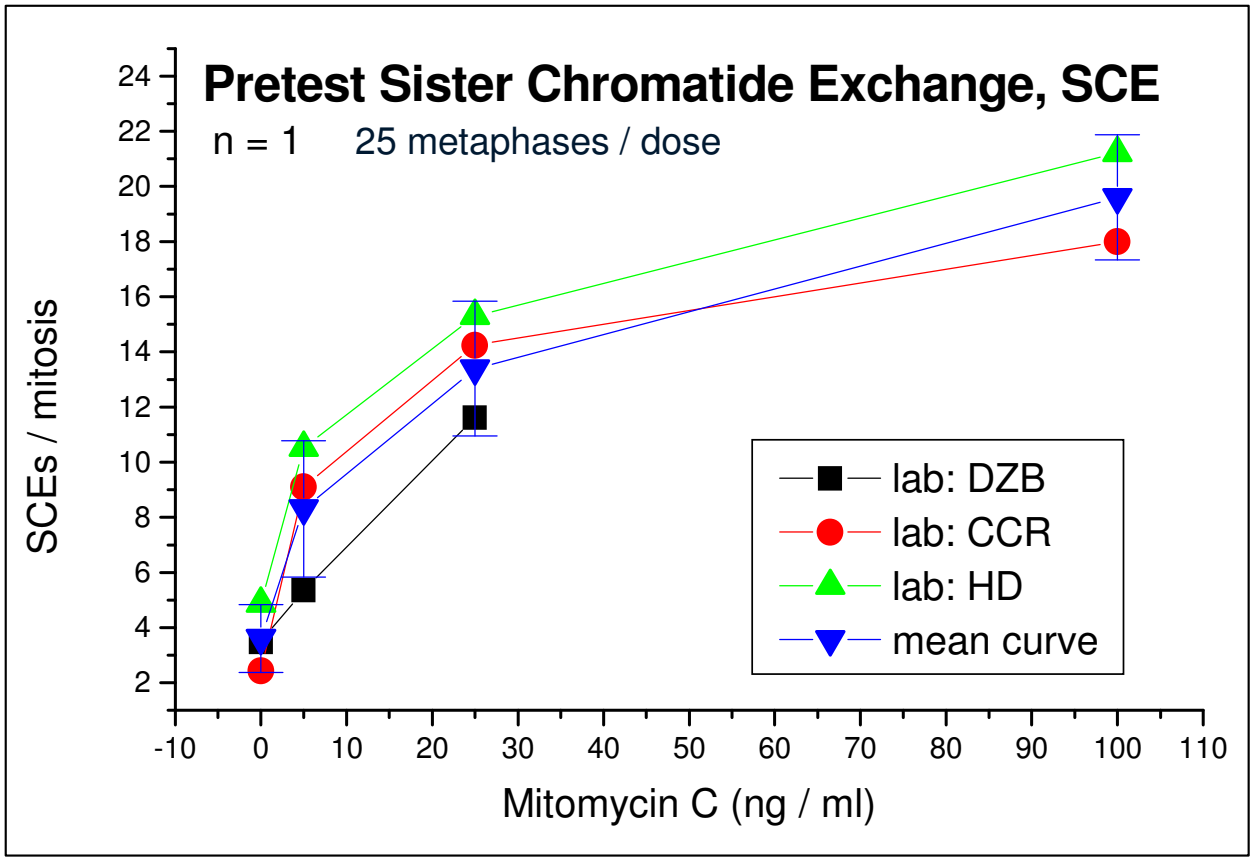
to assure that the prepared slides have an acceptable quality.

to practice the logistics.

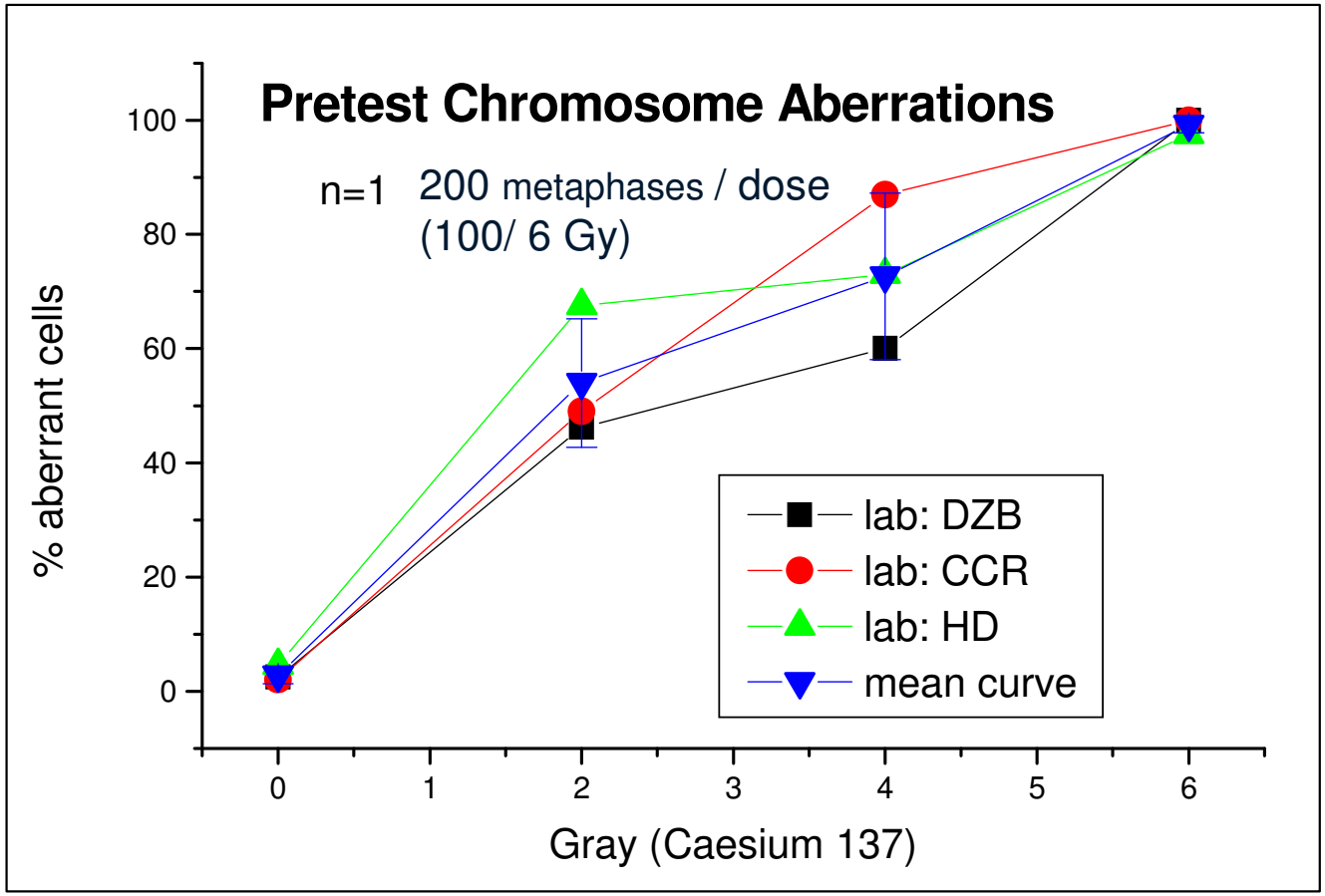
Pretest Micronuclei-Assay



Pretest Sister Chromatide Exchange

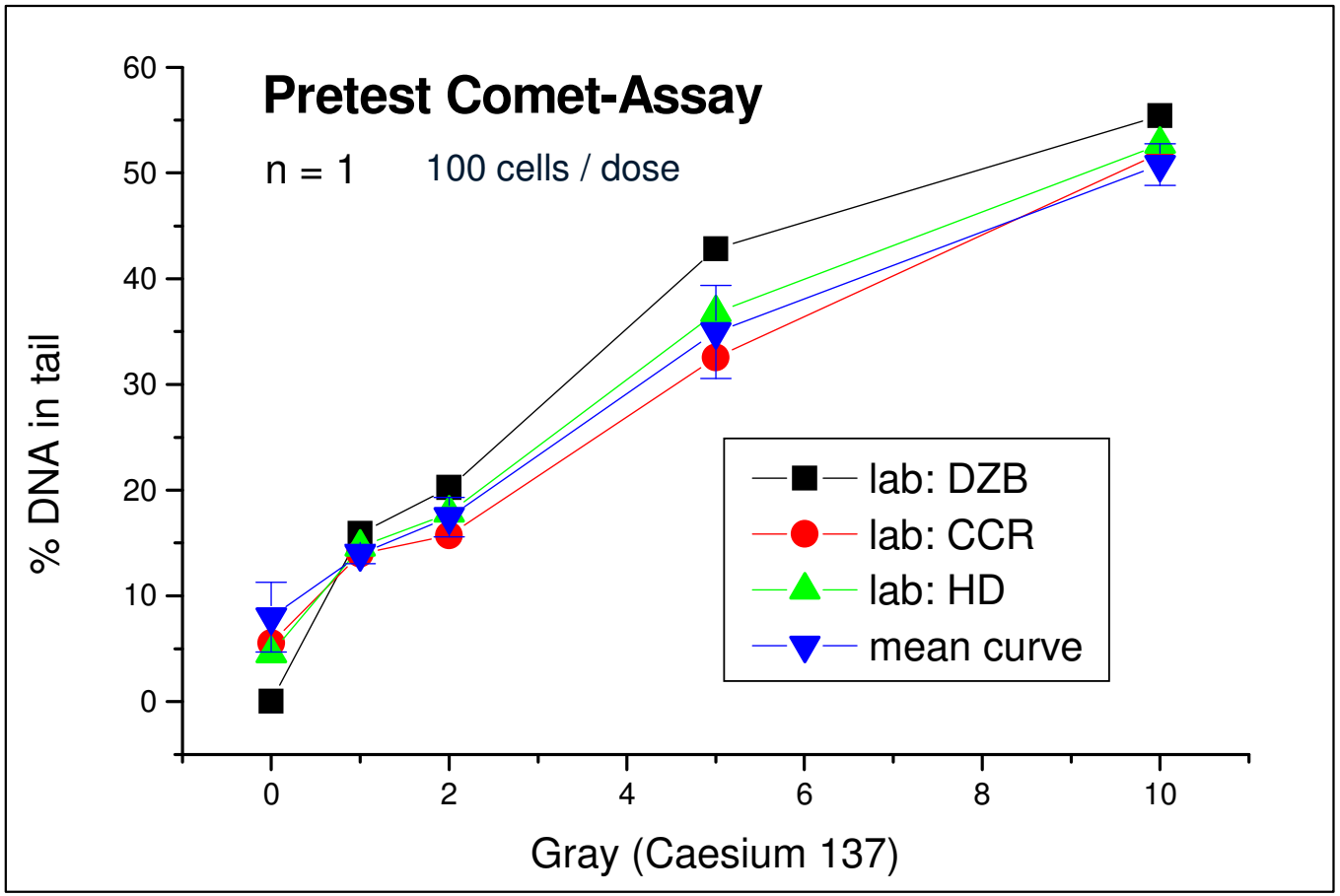


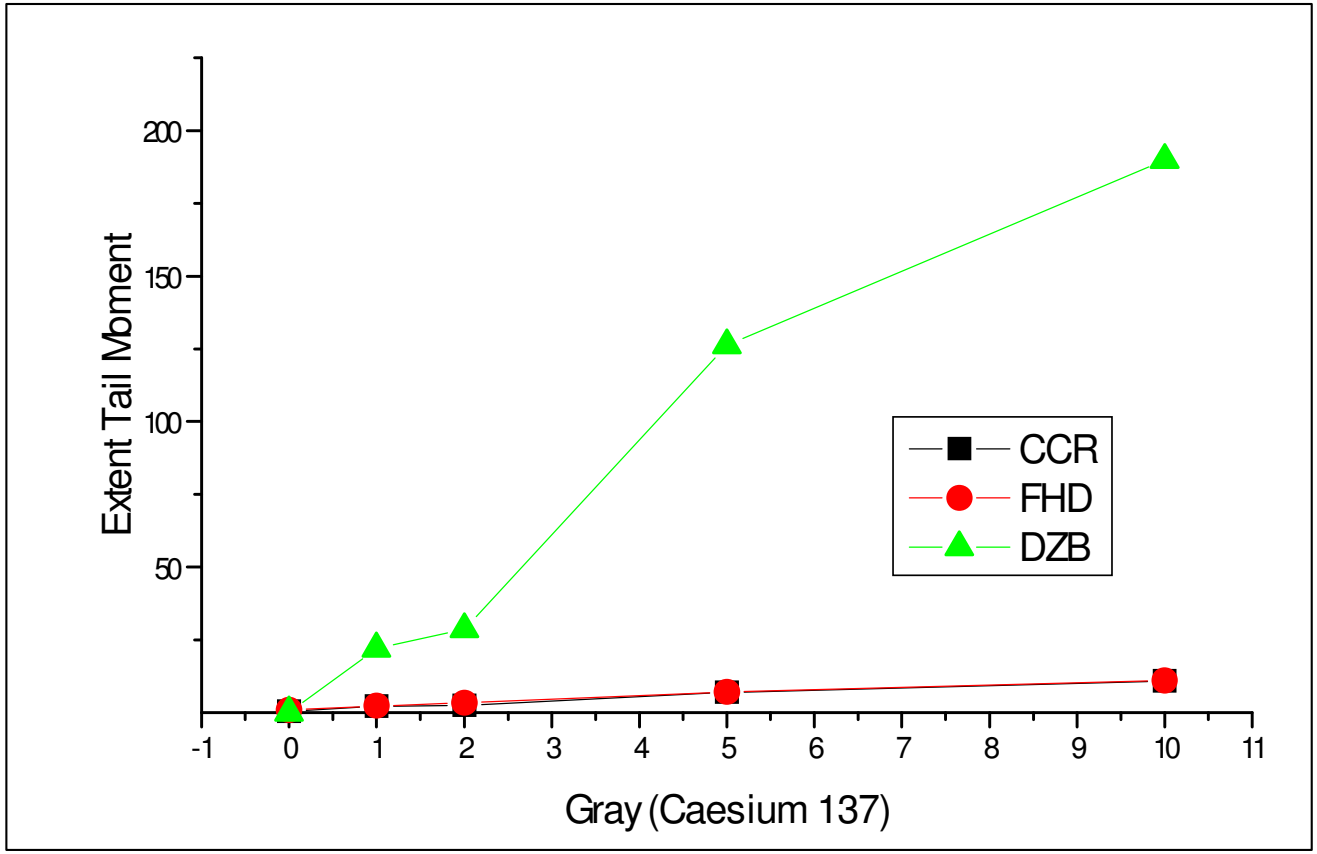
Pretest Chromosome Aberration



Pretest Comet-Assay

2 different image analizing systems:





All Parameters that represent absolutely measured values (tail length) depend on the Imaging system which is used. Camera, lenses of the microscope etc. affect the sensitivity of the system and also the measured values. DZB used a lens with a higher magnification.

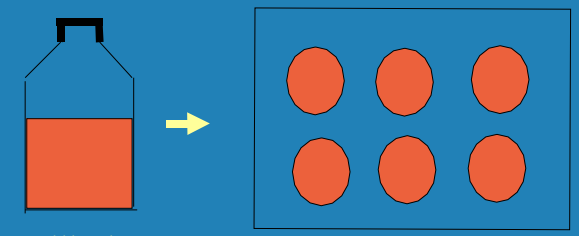
Proceeding of the study:

Most of the problems with the wave guide setup are solved. Exposure is possible.

The blood of 2 donors has been exposed and we will take blood of 2-3 donors per month.

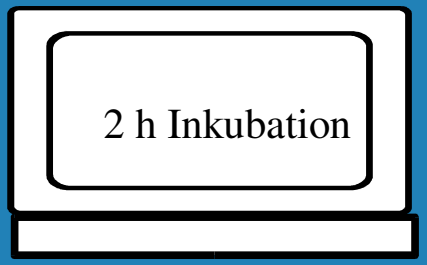
End of the project: ~ August 2008

Cometassay

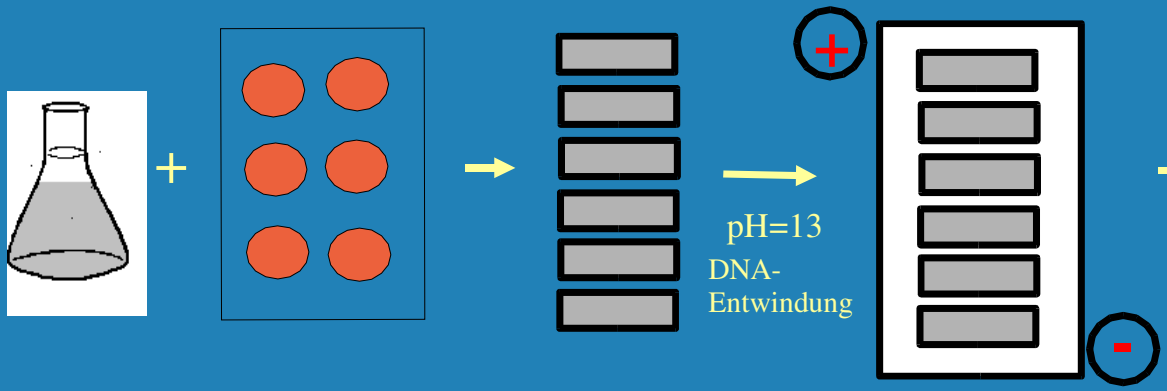


Zellkultur

+ Testgut

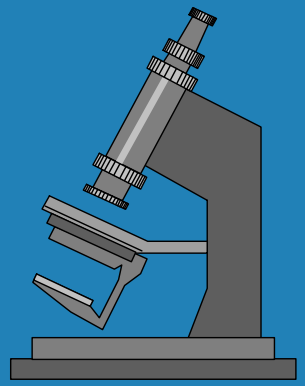


Brutschrank, 37°C, CO_2

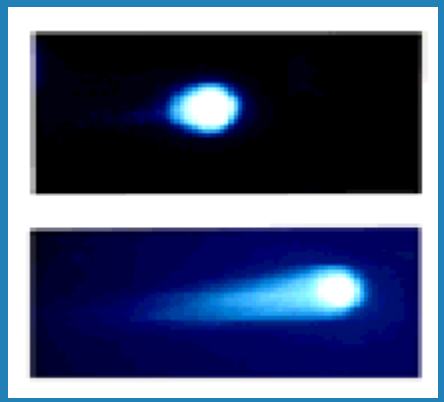


pH=13
DNA-Entwindung

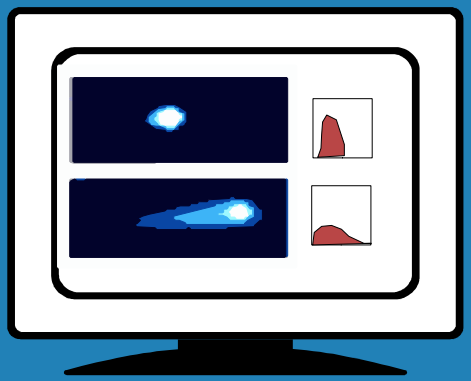
Neutralisation
Fixierung mit Ethanol
Färbung: Fluorochrom



Auswertung



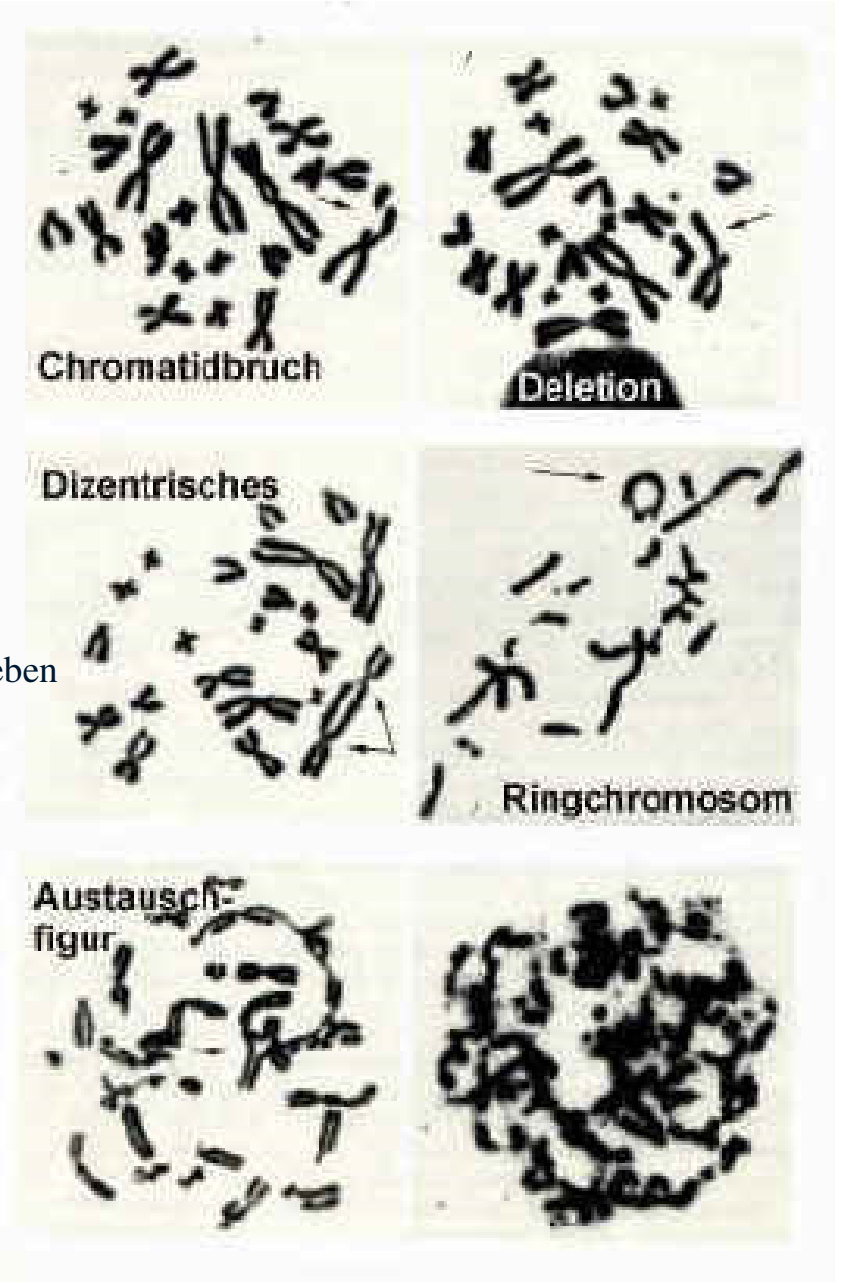
Mikroskopisches Bild

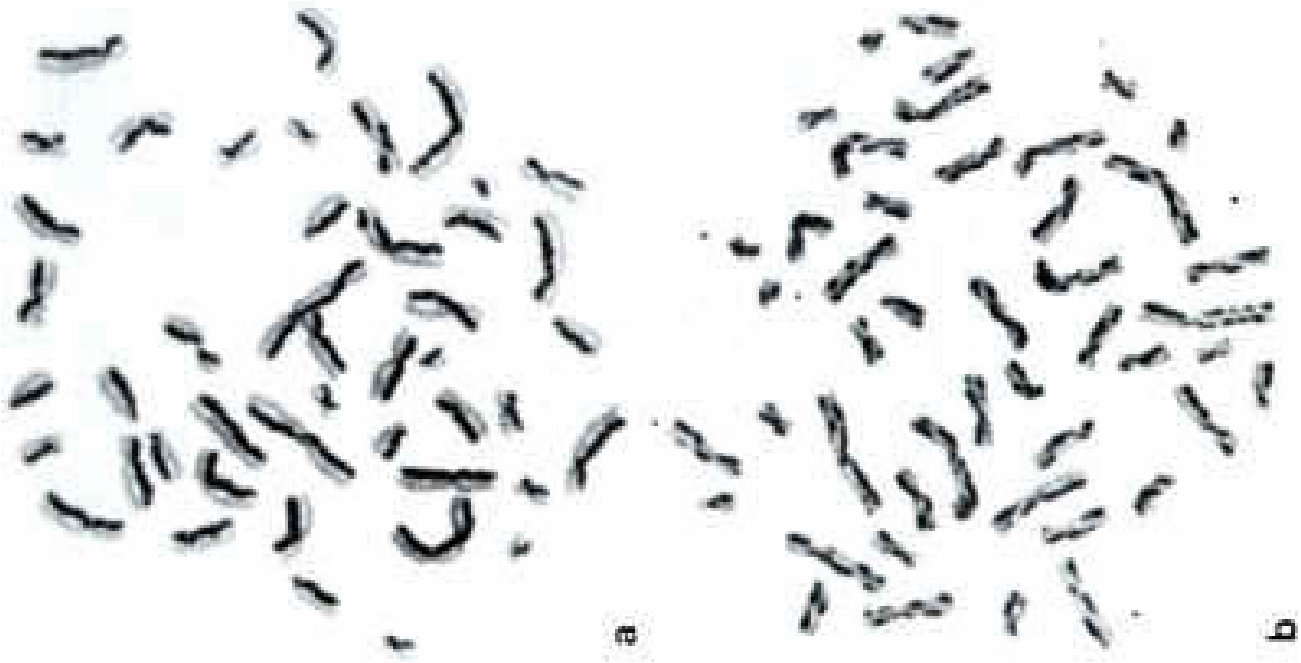


Bildanalyse

Chromosomenpräparation

- Nach Beendigung der Kulturzeit Zellen resuspendieren und in 10ml Zentrifugenröhrchen überführen
- Zentrifugieren (10 min, 150x g)
- Überstand bis auf ca. 1 cm absaugen
- Pellet resuspendieren und tropfenweise 9 ml hypotoner Lösung (37°C) dazugeben, zwischendurch immer wieder resuspendieren, bessere Spreitung
- Zentrifugieren (10 min, 150x g)
- Überstand bis auf ca. 1 cm absaugen
- Pellet resuspendieren
- Vortexen und tropfenweise 9 ml Fixativ (-20°C) dazugeben
- Zentrifugieren (10 min, 150x g)
- Überstand bis auf ca. 1 cm absaugen
- 9 ml Fixativ dazugeben und resuspendieren
- Zentrifugieren (10 min, 150x g)
- Überstand bis auf ca. 1 cm absaugen
- Objektträger anfeuchten (Aqua dest., es muss ein Wasserfilm entstehen)
- mit einer Eppendorfpipette (gelbe Spitze) einen Tropfen Zellsuspension auftragen und vorsichtig das verdrängte Wasser vom Rand abwischen
- 24 h lufttrocknen lassen
- Giemsa Färbung (10 min)
- 2 x mit a.d. spülen





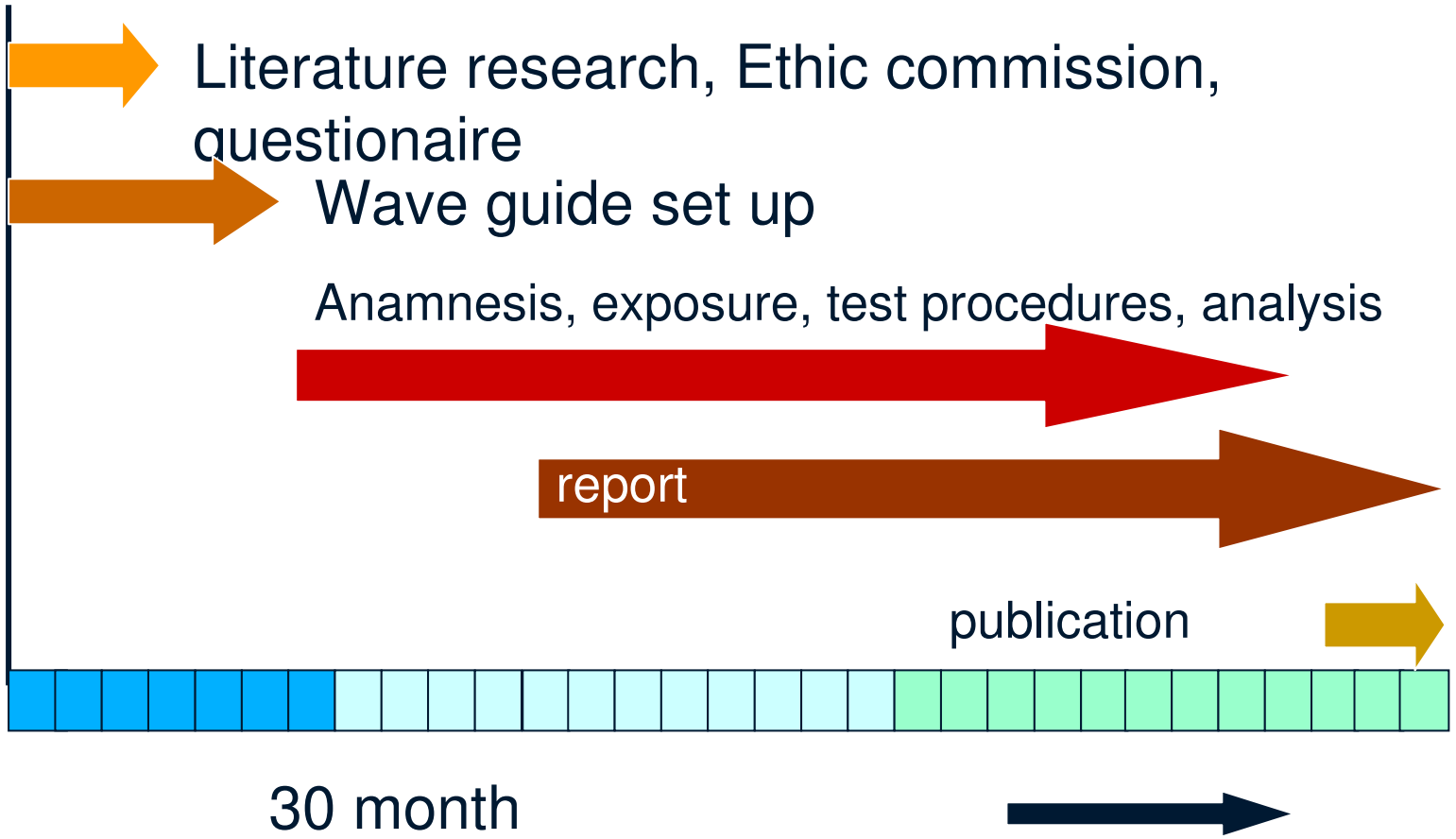
Mean of the 3 labs

treatment group (Gy)	Dic/cell	extra Ac/ cell	rings/ cell	Chromatidtyp/ cell	aberrant cells [%]
0	0,001	0,007	0,003	0,005	2%
2	0,323	0,313	0,062	0,015	55%
4	0,490	0,387	0,082	0,045	73%
6	2,496	1,521	0,419	0,721	99%

treatment group (Gy)	Dic/cell	extra Ac/ cell	rings/ cell	Chromatidtyp / cell	aberrant cells [%]
0	0,001	0,011	0,006	0,009	0,005
2	0,101	0,109	0,103	0,026	0,114
4	0,131	0,034	0,095	0,074	0,150
6	1,071	0,937	0,525	1,238	0,014

class	A	B	C	D	E		
mean of class	2,5	12,5	30	67,5	97,5	summe	
cells	950	25	18	5	2	1000	
tail factor %	2,375	0,3125	0,54	0,3375	0,195	**3,76**	
class	A	B	C	D	E		
mean of class	2,5	12,5	30	67,5	97,5	summe	10 more cells
cells	940	25	18	5	12	1000	in S-phase?
tail factor %	2,35	0,3125	0,54	0,3375	1,17	**4,71**	
class	A	B	C	D	E		
mean of class	2,5	12,5	30	67,5	97,5	summe	
cells	940	35	18	5	2	1000	
tail factor %	2,35	0,4375	0,54	0,3375	0,195	**3,86**	
class	A	B	C	D	E		
mean of class	2,5	12,5	30	67,5	97,5	summe	
cells	940	25	28	5	2	1000	
tail factor %	2,35	0,3125	0,84	0,3375	0,195	**4,035**	
class	A	B	C	D	E		
mean of class	2,5	12,5	30	67,5	97,5	summe	
cells	940	25	18	15	2	1000	
tail factor %	2,35	0,3125	0,54	1,0125	0,195	**4,41**	
class	A	B	C	D	E		
mean of class	2,5	12,5	30	67,5	97,5	summe	
cells	930	35	23	8	4	1000	
tail factor %	2,325	0,4375	0,69	0,54	0,39	**4,3825**	
class	A	B	C	D	E		
mean of class	2,5	12,5	30	67,5	97,5	summe	
cells	920	35	23	8	14	1000	
tail factor %	2,3	0,4375	0,69	0,54	1,365	**5,3325**	

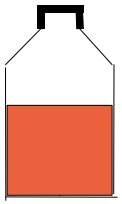
Time course:

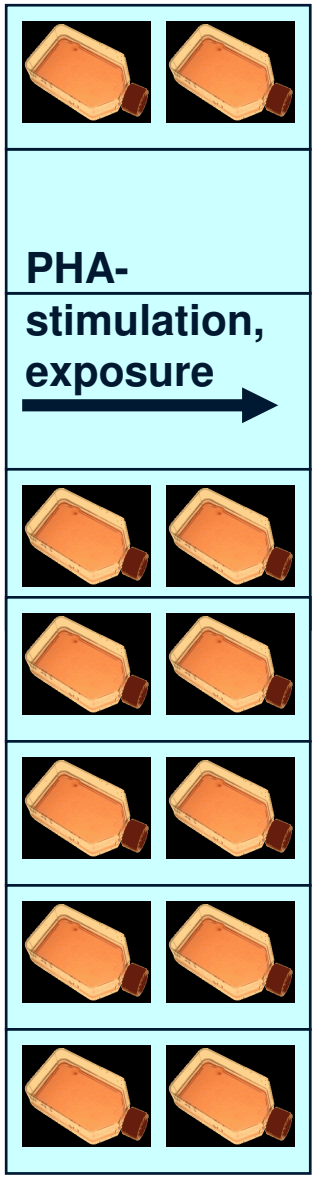

Literature research, Ethic commission, questionaire
Wave guide set up
Anamnesis, exposure, test procedures, analysis
report
publication
30 month

Exposure, test procedures, staining, encodingng, scoring

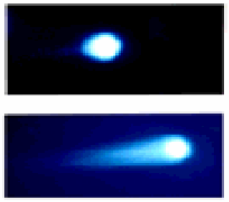
8 wave guides

1 donor: questionaire taking blood



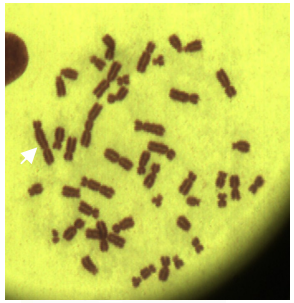


Comet assay



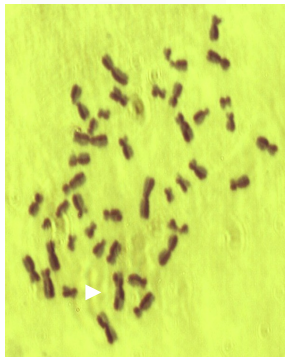
→ lab 1
→ lab 2
→ lab 3

Chromosome-aberration test CA



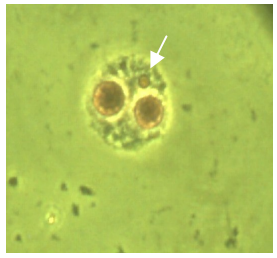
→ lab 1
→ lab 2
→ lab 3

SCE-Test



→ lab 1
→ lab 2
→ lab 3

Micronucleus-test, MN



→ lab 1
→ lab 2
→ lab 3