Autoridad Regulatoria Nuclear

DEPENDIENTE DE LA PRESIDENCIA DE LA NACIÓN

ARN´s BIOLOGICAL DOSIMETRY LABORATORY

Marina Di Giorgio

Scientific Support Management

Nuclear Regulatory Authority

ARGENTINA

2008

BIOLOGICAL DOSIMETRY LABORATORY

- **OBJECTIVES:**

To ensure the availability of reliable biological dosimeters to evaluate doses in different overexposure scenarios: individual or large scale, when the dosimetry is recent or retrospective, for different radiation qualities and for different distribution of the dose in the body

- **ACTIVITIES:**

Service tasks

Research projects

BIOLOGICAL DOSIMETRY LABORATORY

- **STAFF:**

  María Rosa Taja (Biologist)

  María Belén Vallerga (Advanced student)

  Analía Radl (Biologist)

  Beatriz Roja (laboratory maintenance assistant)

  Marina Di Giorgio (Biologist)- Head of the laboratory

SUMMARY OF CASES EVALUATED:

- The laboratory was established in 1968
- 1968-1986, the lab has just kept the documentation of 10 persons involved in two severe accidents in our country:
  1. Highly inhomogeneous exposure (0.5 Gy - 17 kGy) with a ^{137}Cs industrial radiography source (1968)
  2. Criticality accident, caused by a change of configuration in a research reactor (1 casualty, dose assessment: 43 Gy, 21 Gy by gamma and 22 Gy by neutrons - Neurological Syndrome- 1983)

SUMMARY OF CASES EVALUATED:

- **1986-2007, 191 persons suspected of being overexposed to ionizing radiation were referred to the ARN’s biological dosimetry laboratory**
  1. **2000-2007, 80% of cases arose from industrial uses of radiation (gammagraphy sources)**
  2. **Overall, 37% of dose assessments showed doses above the detection limit of the conventional cytogenetic method**

TECHNIQUES IN USE

Biodosimetry purpose

Standard dosimetry (dicentrics + centric rings)

Cytokinesis Blocked Micronucleus assay

G Banding

FISH technique

- ***Lymphocyte cultures are set up by micromethod applying the FPG technique***

Radiosensitivity purpose

Alkaline single-cell microgel electrophoresis (comet) assay

CALIBRATION CURVES

The Laboratory has developed in-vitro Calibration Curves for different radiation qualities, based on the frequency of unstable and stable chromosome aberrations

TECHNIQUES IN USE

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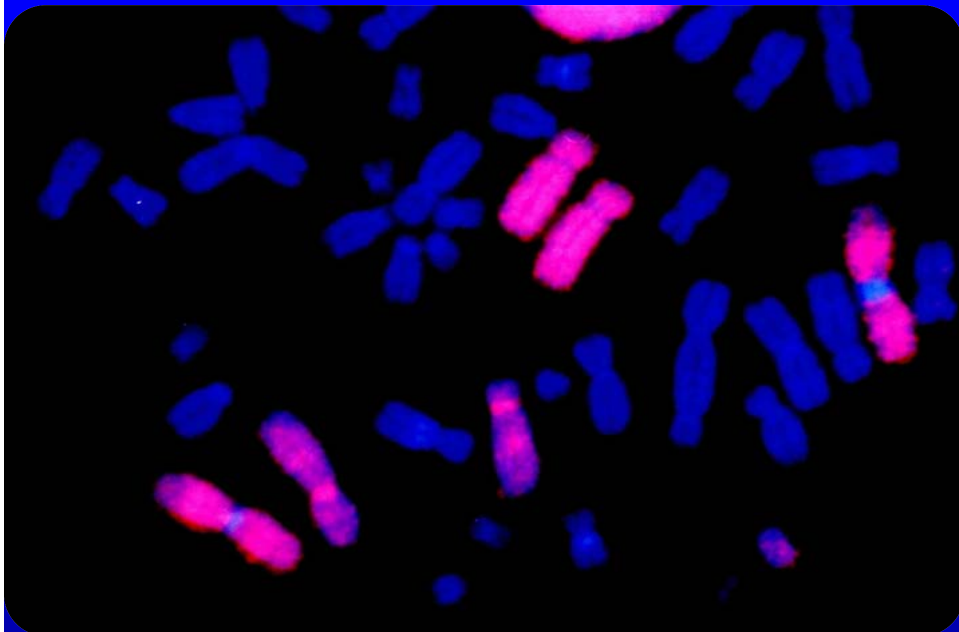

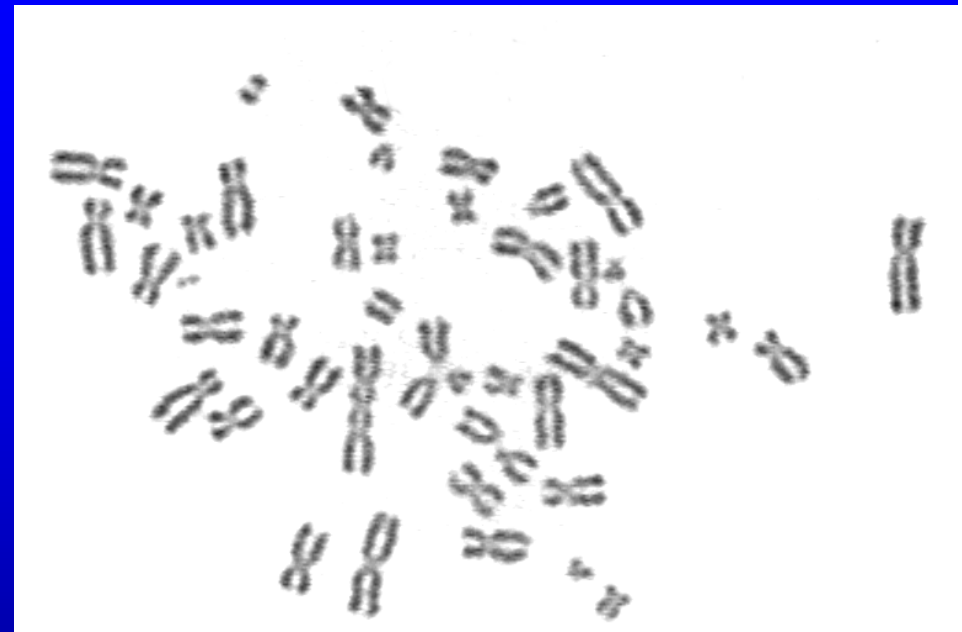

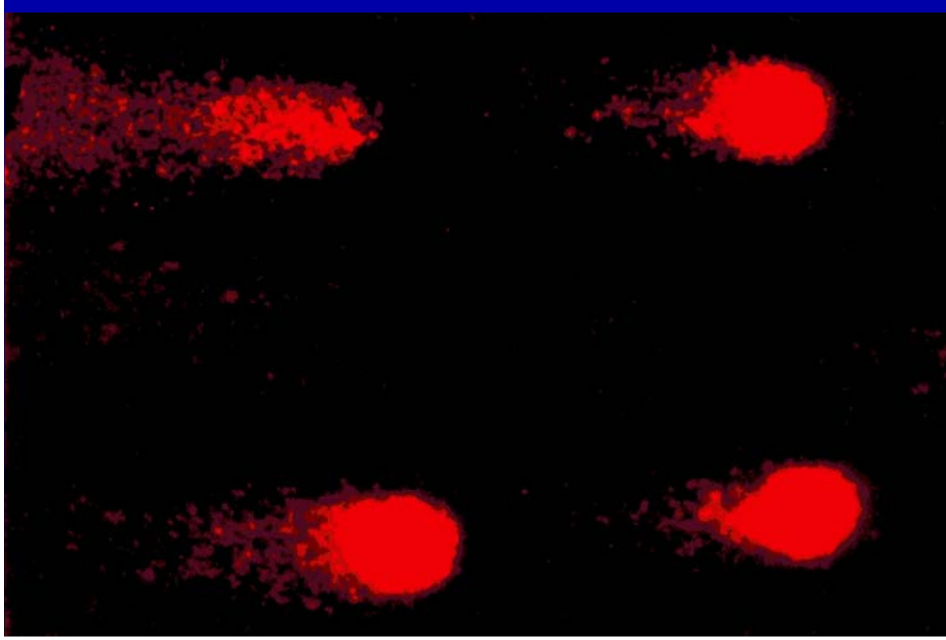

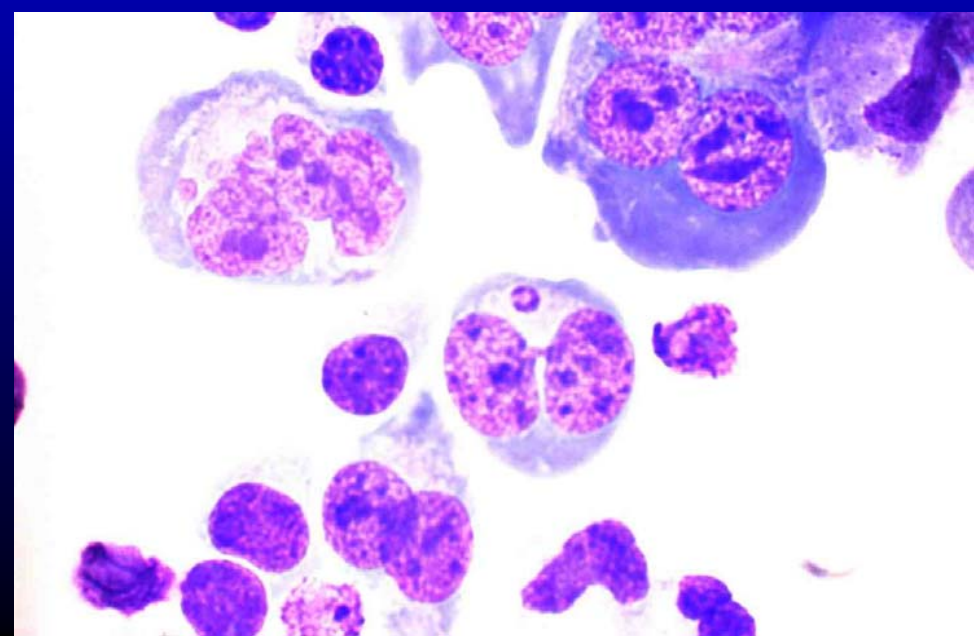

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CALIBRATION CURVES

Unstable chromosome aberrations

Standard dosimetry ➲ <u>dicentrics and centric rings</u>

$$y = c + \alpha D + \beta D^2$$

❖ 20.2 MeV ^4He:

$\alpha = (32.8 \pm 2.9)\, 10^{-2}\ Gy^{-1}$

$\beta = (2.9 \pm 1.6)\, 10^{-2}\ Gy^{-2}$

High energy photons

❖ 6 MeV

$\alpha = (2.79 \pm 1.20)\, 10^{-2}\ Gy^{-1}$

$\beta = (7.00 \pm 1.01)\, 10^{-2}\ Gy^{-2}$

❖ 15 MeV

$\alpha = (3.67 \pm 1.20)\, 10^{-2}\ Gy^{-1}$

$\beta = (6.08 \pm 0.79)\, 10^{-2}\ Gy^{-2}$

❖ γ ^{60}Co:

$\alpha = (1.90 \pm 0.60)\, 10^{-2}\ Gy^{-1}$

$\beta = (7.50 \pm 0.29)\, 10^{-2}\ Gy^{-2}$

CALIBRATION CURVES

Unstable chromosome aberrations

Biodosimetry using micronucleus (MN) frequency in binucleated cells

$$y = c + \alpha D + \beta D^2$$

- γ ^{60}Co (pool) :

$c = (1.73 \pm 0.05)\ 10^{-2}$

$\alpha = (2.72 \pm 0.27)\ 10^{-2}\ Gy^{-1}$

$\beta = (2.81 \pm 0.14)\ 10^{-2}\ Gy^{-2}$

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Influence of age, sex and smoking habit on the spontaneous and radiation induced MN frequency using Multiple Linear Regression and Poisson Regression Models

- **Significant positive correlation (R^2 = 0.59) of spontaneous MN frequency with :**
  - **age (t = 5.0 ; P < 0.0001)**
  - **smoking habit (t = 3.15 ; P < 0.004)**
- **Significant positive correlation (R^2 = 0.96) of radiation induced MN frequency with :**
  - **age (t = 2.43 ; P < 0.02)**
  - **smoking habit (t = 3.63 ; P < 0.0001)**

Main confounding factor ⩘ Smoking habit

Spontaneous and radiation induced MN frequencies did not correlate significantly with sex; a wider dispersion was observed in female data

Smokers and Non Smokers MN - Calibration Curves

❖ **Smokers**

$c = (2.31 \pm 0.25)\ 10^{-2}$

$\alpha = (2.07 \pm 1.27)\ 10^{-2}\ Gy^{-1}$

$\beta = (2.89 \pm 0.86)\ 10^{-2}\ Gy^{-2}$

❖ **Non smokers**

$c = (1.25 \pm 0.084)\ 10^{-2}$

$\alpha = (3.02 \pm 0.75)\ 10^{-2}\ Gy^{-1}$

$\beta = (2.92 \pm 0.50)\ 10^{-2}\ Gy^{-2}$

✓ **Each fitted calibration curve, for smoking and non smoking donors, fell within the 95% confidence curves of the other, with the exception of the spontaneous frequency values of both calibration curves**

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CALIBRATION CURVES

Stable Chromosome Aberrations

translocations + inversions identified by G-Banding

- γ ^{60}Co :

$c = (0.89 \pm 0.60)\ 10^{-2}$

$\alpha = (3.20 \pm 0.90)\ 10^{-2}\ Gy^{-1}$

$\beta = (6.22 \pm 1.32)\ 10^{-2}\ Gy^{-2}$

$$y = c + \alpha D + \beta D^2$$

translocations identified by FISH

- γ ^{60}Co :

$c = (0.37 \pm 0.27)\ 10^{-2}$

$\alpha = (2.26 \pm 1.54)\ 10^{-2}\ Gy^{-1}$

$\beta = (4.23 \pm 0.79)\ 10^{-2}\ Gy^{-2}$

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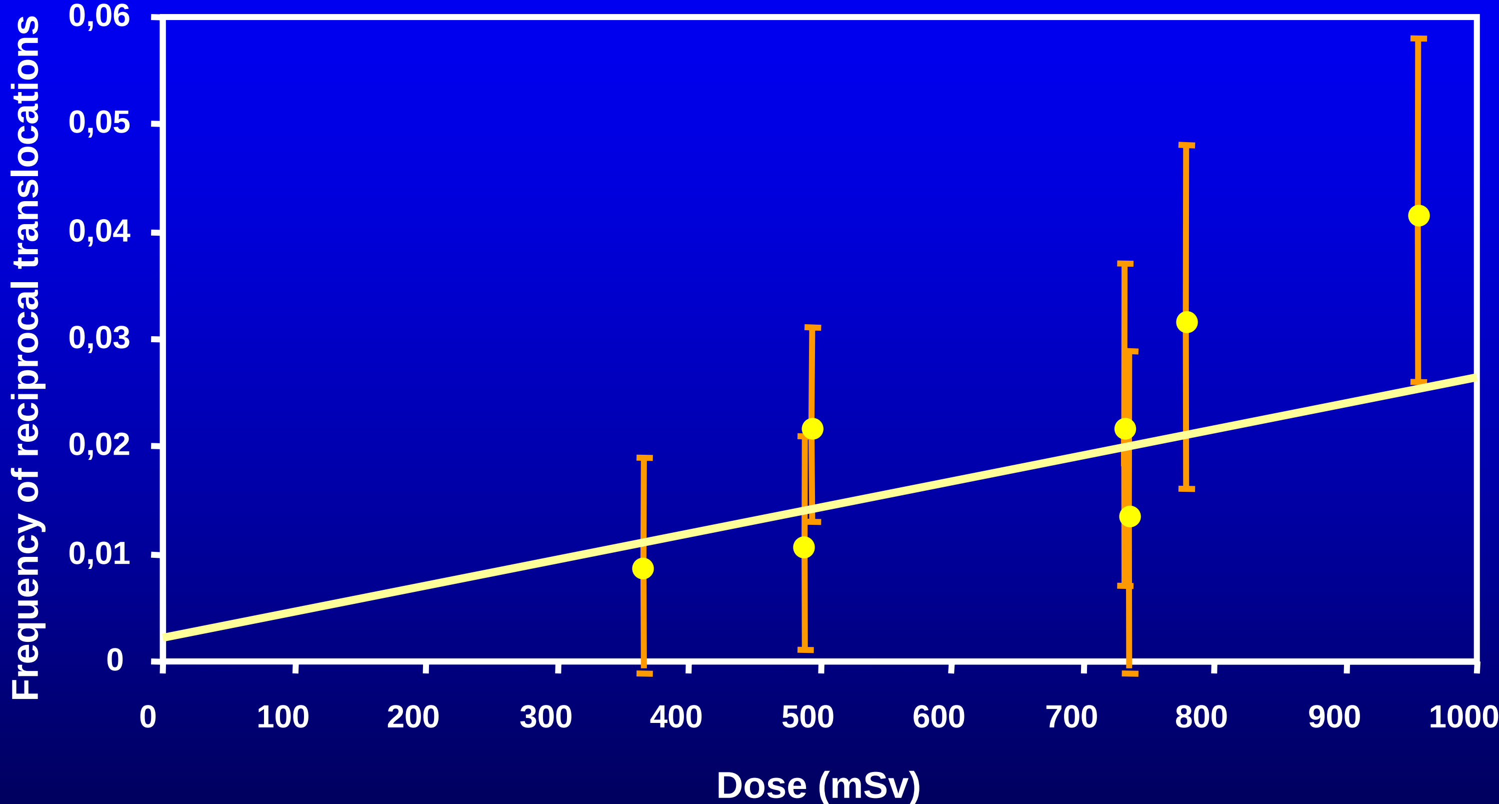
Stable Chromosome Aberrations



Comparison between reciprocal translocation frequencies of Atucha I NPP workers and the expected frequencies applying the linear coefficient of in vitro FISH calibration curve

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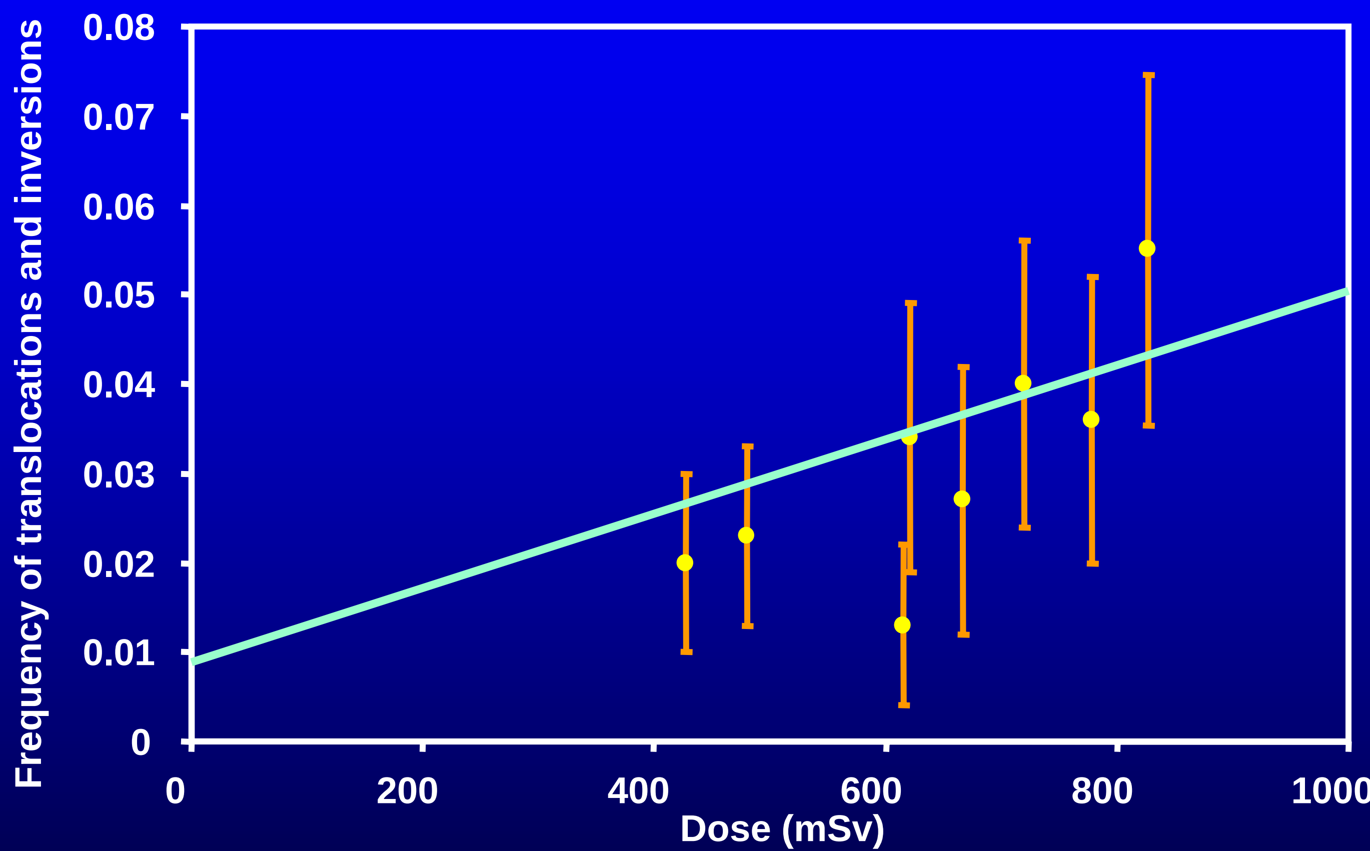
Stable Chromosome Aberrations



Comparison between stable chromosome aberration frequencies of Atucha I NPP workers and G-Banding calibration curve

INTERCOMPARISONS EXERCISES

- **Intercomparison in Cytogenetic Dosimetry Among Five Laboratories from Latin America. Mutation Research 327, 33-39, (1995)**
- **Interlaboratory Comparison in Cytogenetic Dosimetry among Four Latin America Countries. V Regional Congress on Radiation Protection and Safety – Regional IRPA Congress (2001), Brazil**
- **HUman MicroNucleus Project: International Database Comparison for Results with the Cytokinesis-Block Micronucleus Assay in Human Lymphocytes: I. Effect of Laboratory Protocol, Scoring Criteria and Host Factors on the Frequency of Micronuclei. Environmental and Molecular Mutagenesis 37:31-45 (2001)**

INTERCOMPARISONS EXERCISES

- International biological dosimetry intercomparison, at the SILENE experimental reactor (Valduc, France), simulating different criticality scenarios organized by IRSN (2002)
- Intra and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes. Results of an international slide-scoring excercise by the HUMN project. Mutation Research 534, 45-64 (2003)
- Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project. Mutation Research 543, 155-166 (2003)

BIOLOGICAL DOSIMETRY AFTER CRITICALITY ACCIDENTS INTERCOMPARISON EXERCISE IN THE SILENE REACTOR – FRANCE – ARN (2002-2004)

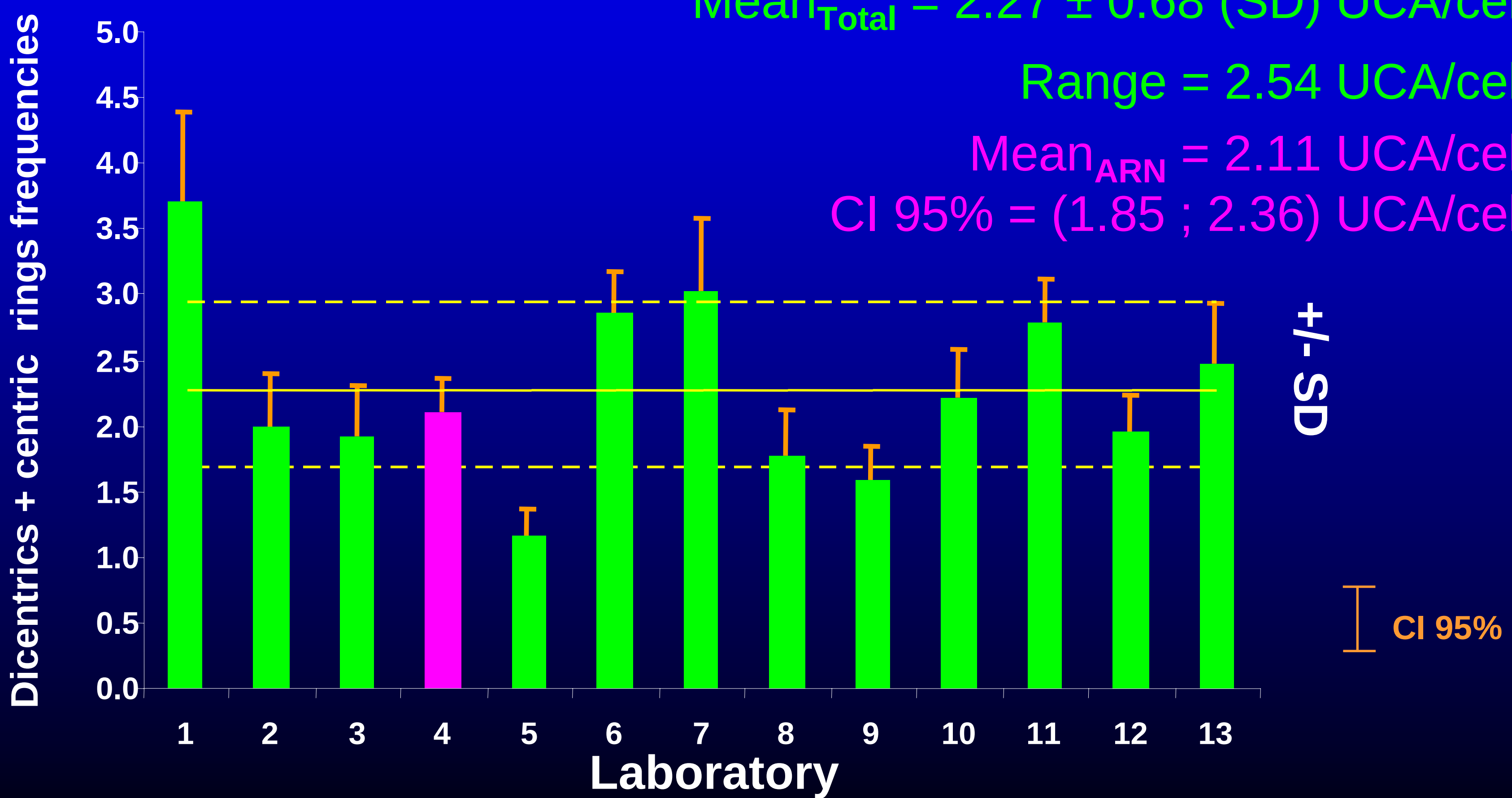
Dicentrics + centric rings frequencies, bare source (4 Gy - γ/n = 1.2)

$Mean_{Total}$ = 2.27 ± 0.68 (SD) UCA/cell

Range = 2.54 UCA/cell

$Mean_{ARN}$ = 2.11 UCA/cell

CI 95% = (1.85 ; 2.36) UCA/cell



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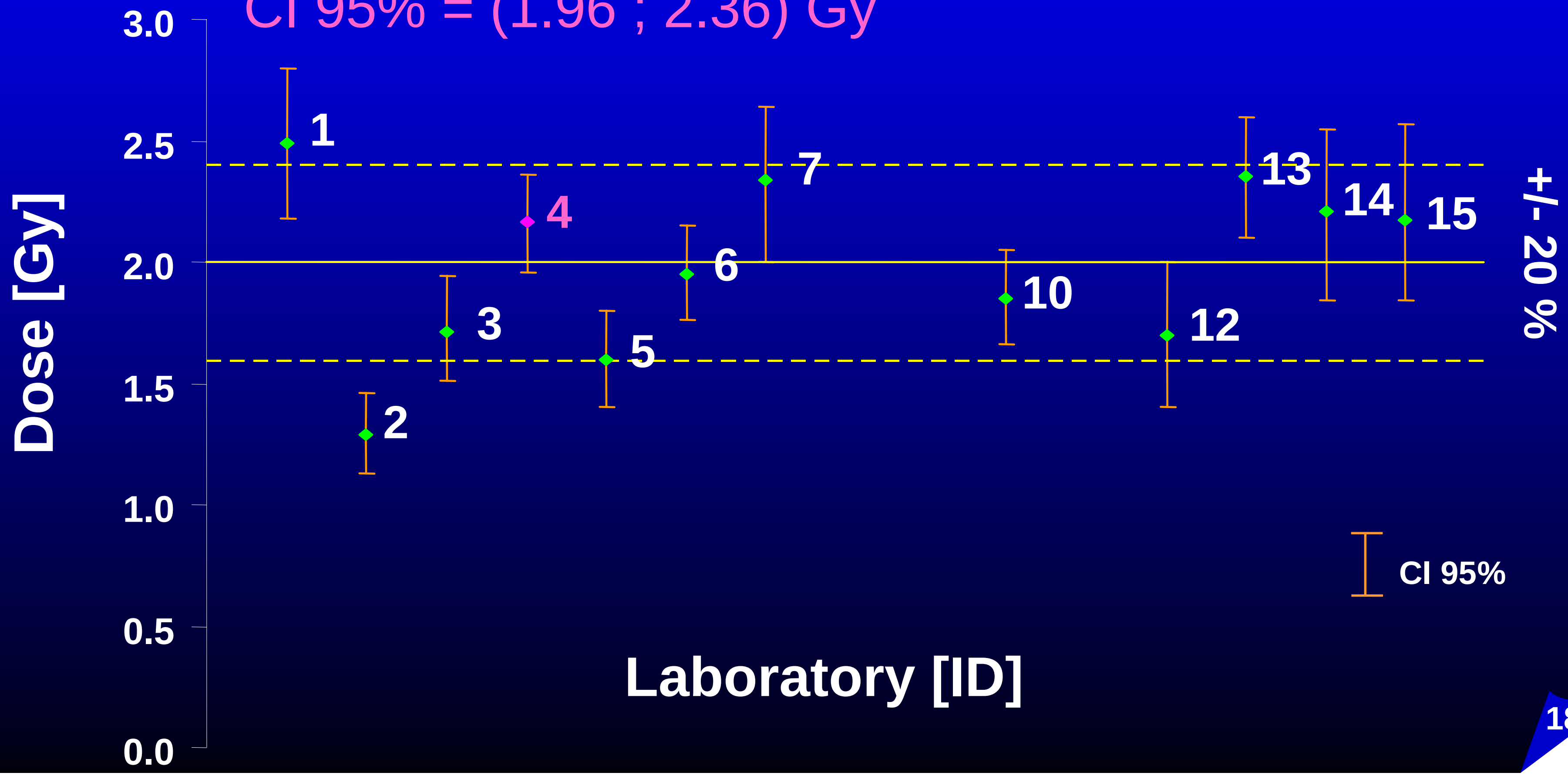
INTERCOMPARISON EXERCISE IN THE SILENE REACTOR

Dose Estimate: γ Pure ^{60}Co source - 2 Gy

D_{ARN} = 2.17 Gy
CI 95% = (1.96 ; 2.36) Gy



INTERLABORATORY ASSAY PROGRAM 2007-2008

- **Frame: IAEA Regional Project - RLA/9/054 Strengthening the National System for Preparedness and Response to Nuclear and Radiological Emergencies**

- **Organizer : BDL of Argentina**

- **Collaboration: Regional Reference Center for Dosimetry of the National Atomic Energy Commission**

PARTICIPANT LABORATORIES
Latin American network

- **Argentina – Nuclear Regulatory Authority (ARN)**
- **Argentina, La Plata – Universidad Nacional de La Plata – CIGEBA**
- **Brazil – Instituto de Radioprotección y Dosimetría (IRD)**
- **Chile – Comisión Chilena de Energía Nuclear (CCHEN)**
- **Cuba – Centro de Protección e Higiene de las Radiaciones (CPHR)**
- **Mexico – Instituto Nacional de Investigaciones Nucleares (ININ)**
- **Peru– Instituto Peruano de Energía Nuclear (IPEN)**
- **Uruguay – Instituto de Investigaciones Biológicas Clemente Estable**

PARTICIPANT LABORATORIES
European network

- **Finland – Radiation and Nuclear Safety Authority (STUK)**
- **France– Institut de Radioprotection et de Sureté Nucléaire (IRSN)**
- **Germany – Bundesamt für Strahlenschutz (BfS)**
- **Spain – Universidad Autónoma de Barcelona – Fac. Biociencias**
- **Turkey - Cekmece Nuclear Research and Training Center**
- **United Kingdom – Health Protection Agency (HPA)**

DI: 0.75 Gy

Reporting results – Reproducibility

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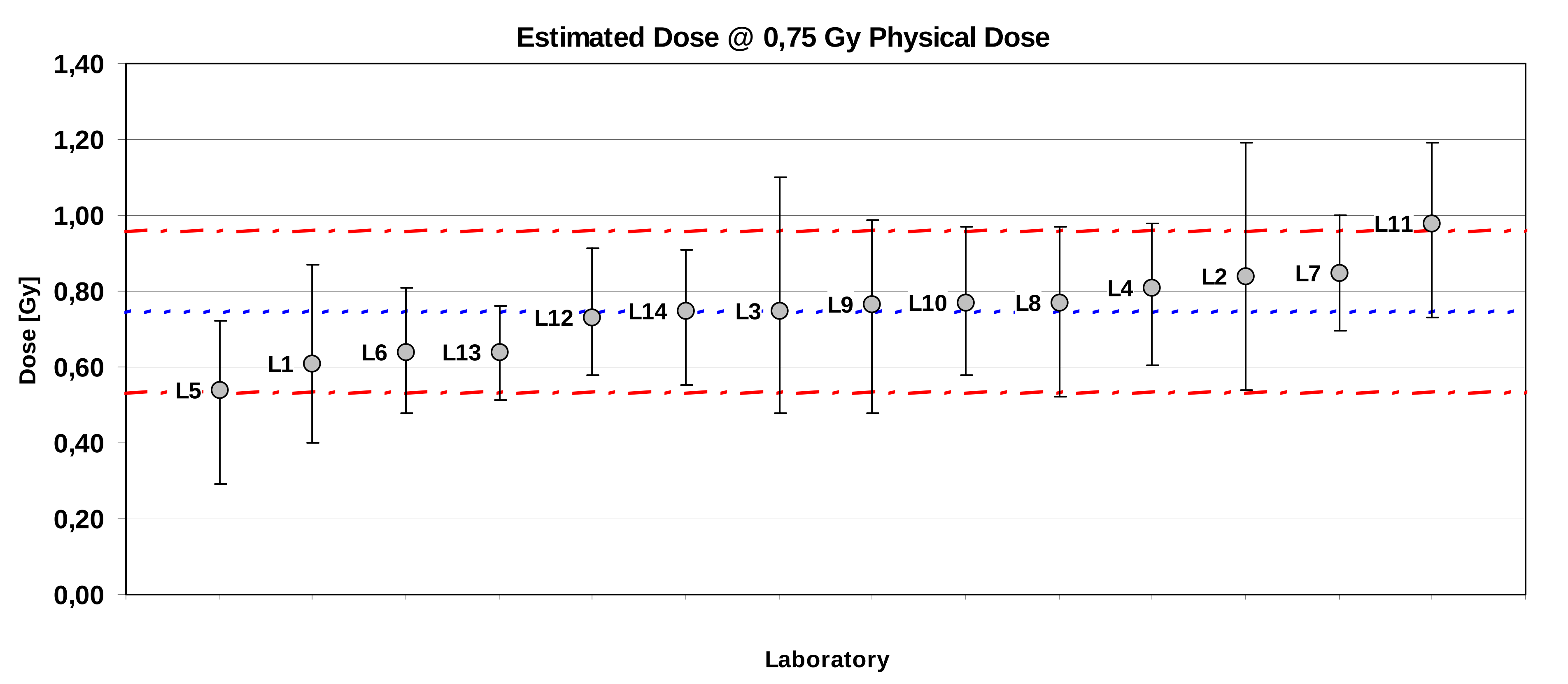

•Dose values determined by the laboratories with their expanded uncertainties; x* ; x*+1.96s* and x*-1.96s* **Precision**

•x*[Gy]=0.744±0.109; Physical dose[Gy]= 0.75 ±0.023 **Truness**

Accuracy purpose is met

**Robust values of the average and standard deviation of the data were applied*

DI: 0.75 Gy

Reporting results – Reproducibility

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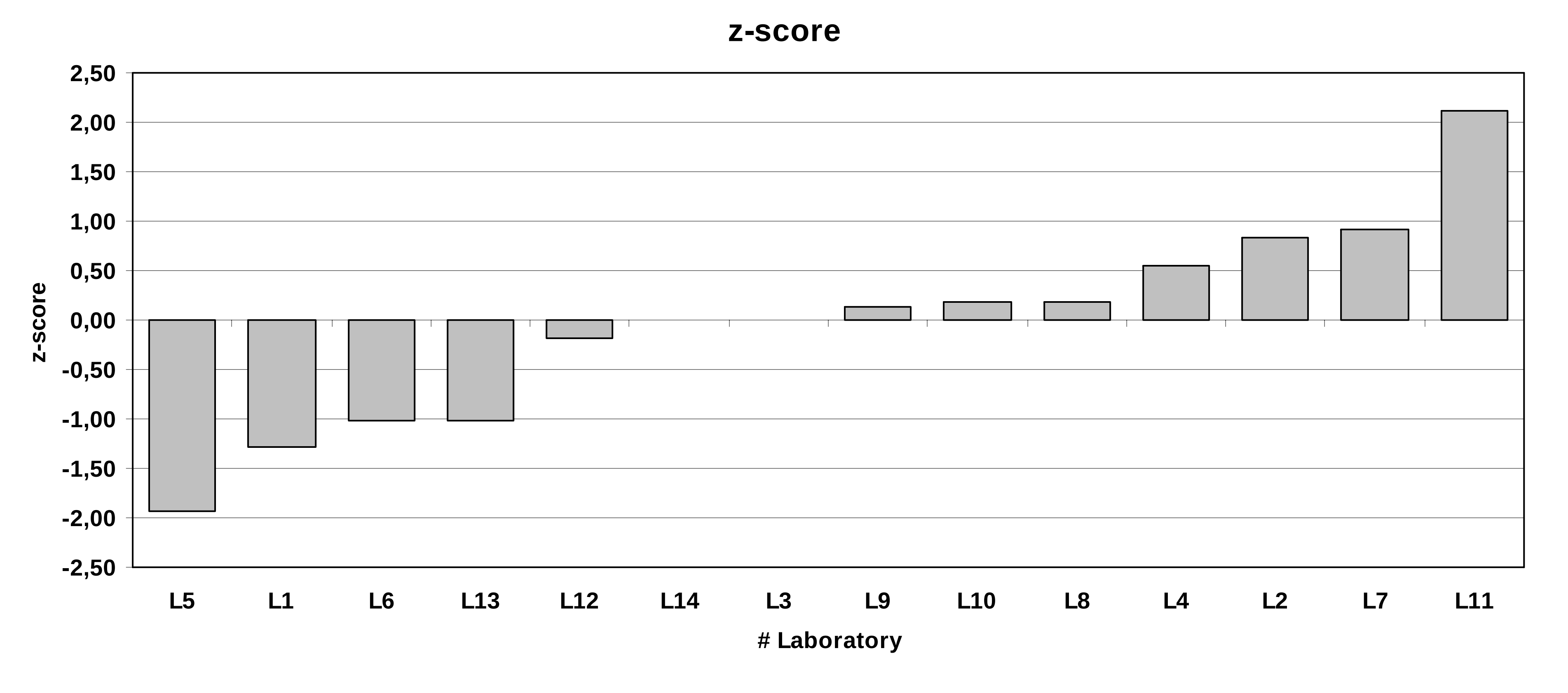

- If ABS(z-score) is above 2.0 or below 3.0, the result is questionable - "warning signal“
- If z-score is above 3.0 or below -3.0, the result is unsatisfactory - "action signal"

DII: 2.50 Gy

Reporting results – Reproducibility

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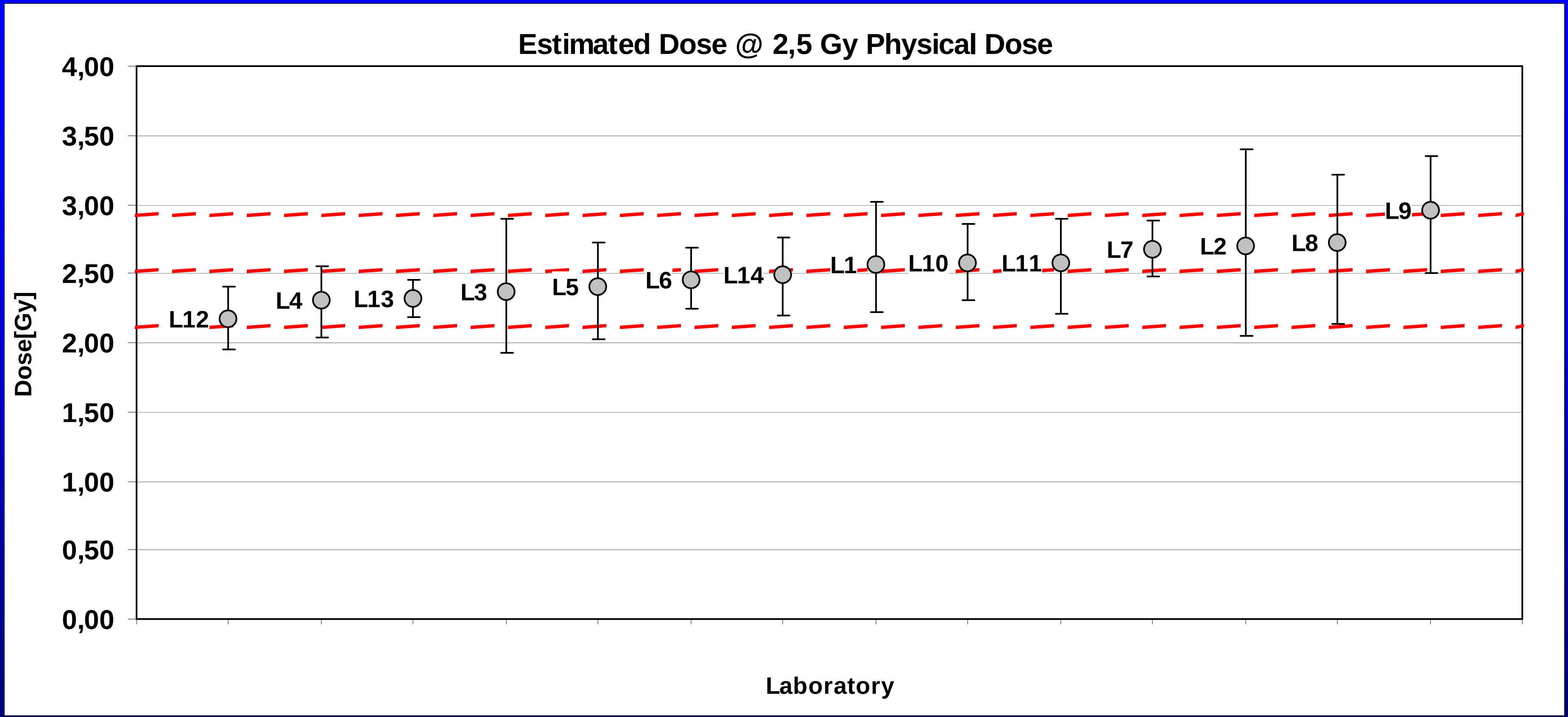

- Dose values determined by the laboratories with their expanded uncertainties; x* ; x*+1.96s* and x*-1.96s*

Precision

- x*[Gy]=2.515±0.204; Physical dose[Gy]= 2.50±0.075

Truness

Accuracy purpose is met

**Robust values of the average and standard deviation of the data were applied*

DII: 2.50 Gy

Reporting results – Reproducibility

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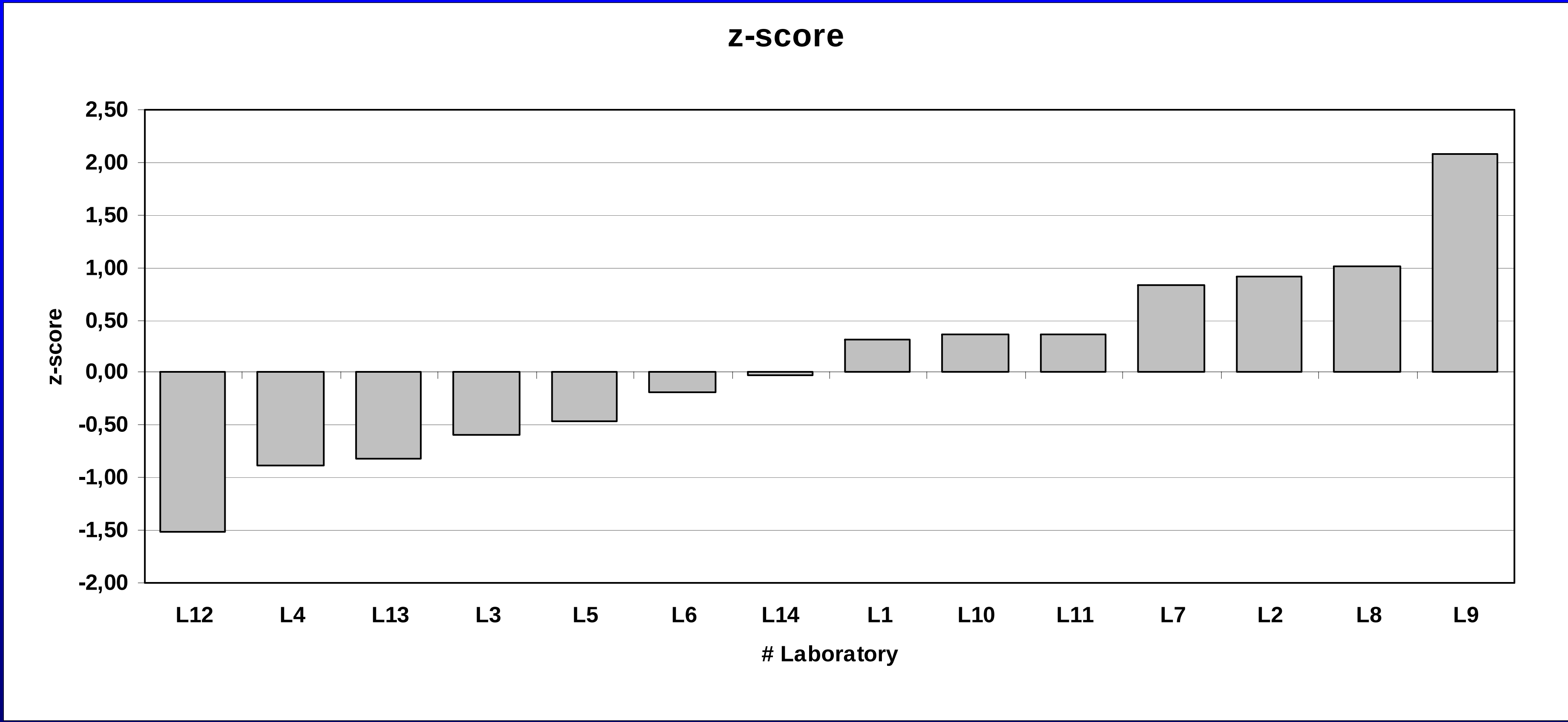

- **If ABS(z-score) is above 2.0 or below 3.0, the result is questionable - "warning signal“**
- **If z-score is above 3.0 or below -3.0, the result is unsatisfactory - "action signal"**

Reporting results

- **To discuss the final report during the next meetings to be held in Argentina (10/2008):**

1. **IRPA-12**
2. **Latin American Biological Dosimetry Network workshop**

- **To implement corrective actions, if necessary, to reinforce the response capability in accidental situations requiring the activation of mutual assistance mechanisms**

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RESEARCH PROJECTS

Automation to increase laboratory sample throughput:

- To validate the use of an automated metaphase finder and capture system to help triage radiation-exposed individuals during an emergency

Biodosimetry for high dose estimation:

- To implement drug-induced prematurely condensed chromosome assay (okadaic acid, PCC-ring in Giemsa preparations)

ISO-WG18

- To support ISO's efforts to draft a new standard on cytogenetic triage for assessment of mass casualties

Relative Biological Effectiveness Studies

- To determine the EBR of 20 MeV α particles through in vitro irradiations in track segment mode.
- To compare the distribution of DNA damage induced by different radiation qualities.

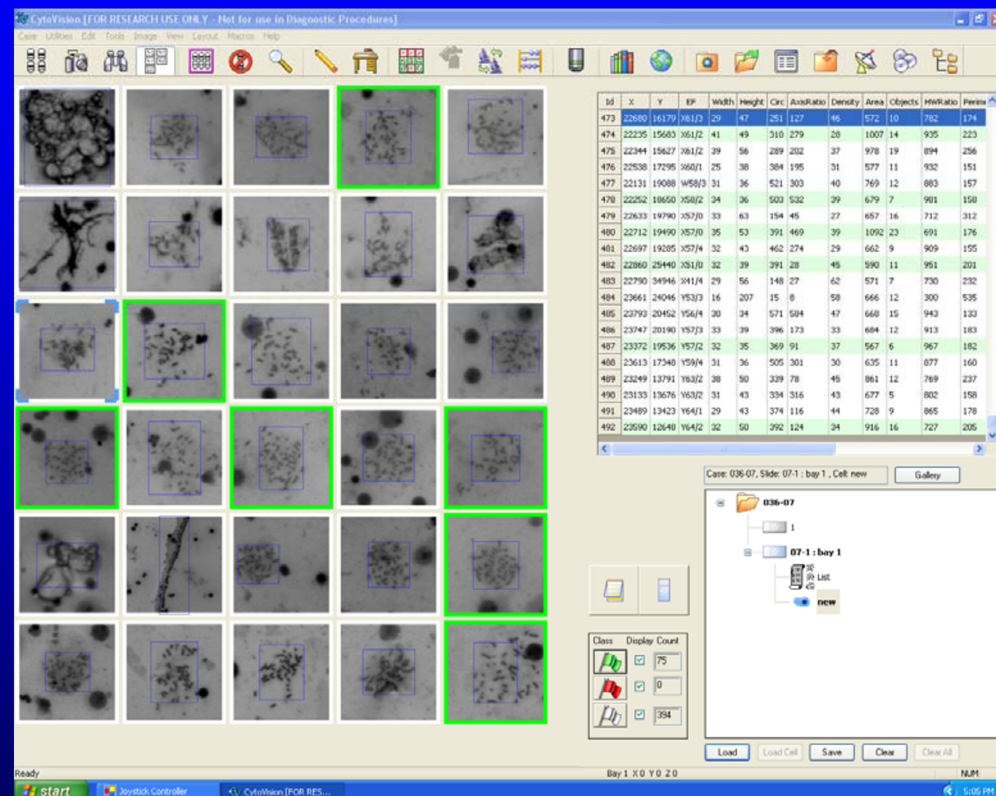
Triage – ARGENTINA
Laboratory capacity and detection limits

Cytogenetic technique	Nº of samples	Nº of cells scored per sample	Delay to estimate a dose *	Minimum dose detected and its confidence interval
dicentrics	1 to 5	500	1 week	0.1 Gy ± 0.1 Gy
dicentrics	50	50	10 days	1 Gy ± 1 Gy
micronuclei	1 to 5	1000	3 – 4 days	0.3 Gy ± 0.3 Gy
micronuclei	50	500	1 week	0.5 Gy ± 0.5 Gy

*Including: lymphocyte culture time, 8 h/working day, 2 people and 1 metaphase finder

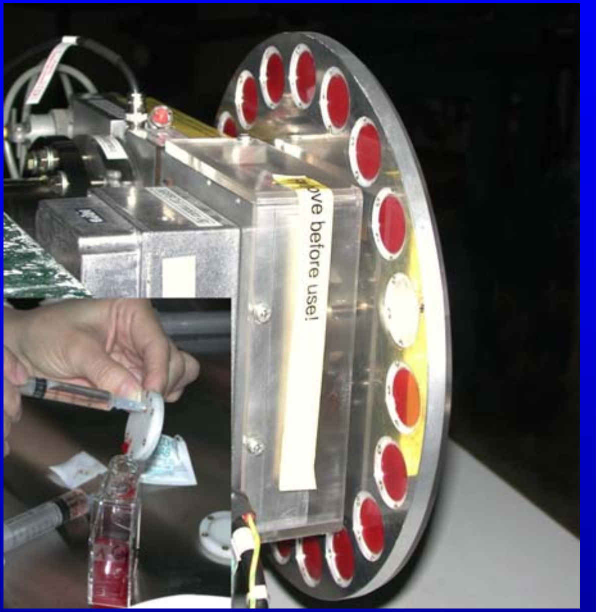
More than 50 samples: International help would be requested

Automated metaphase finder and capture system



The use of this system will allow, aproximately, a 20 % decrease in scoring time.

Relative Biological Effectiveness Studies



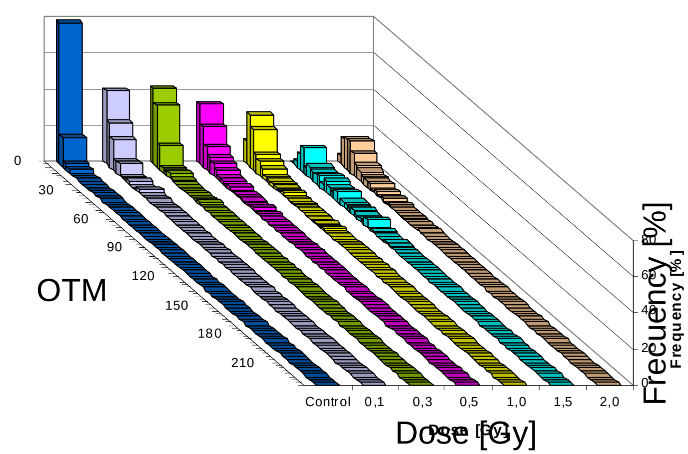
Blood samples were irradiated with 20 MeV α particles in track segment mode.

Dientric and centric ring data were fitted to different models by an iteratively reweighted least square method

Model	α [Gy^{-1}]	β [Gy^{-2}]
$y = \alpha D$	0.373 ± 0.018	-
$y = \alpha D + \beta D^2$	0.328 ± 0.029	0.029 ± 0.016

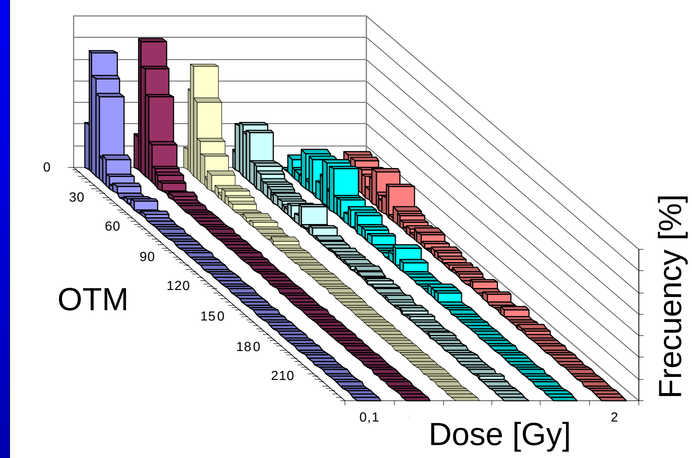
Distribution of DNA damage evaluated through comet assay

Co-60 gamma rays

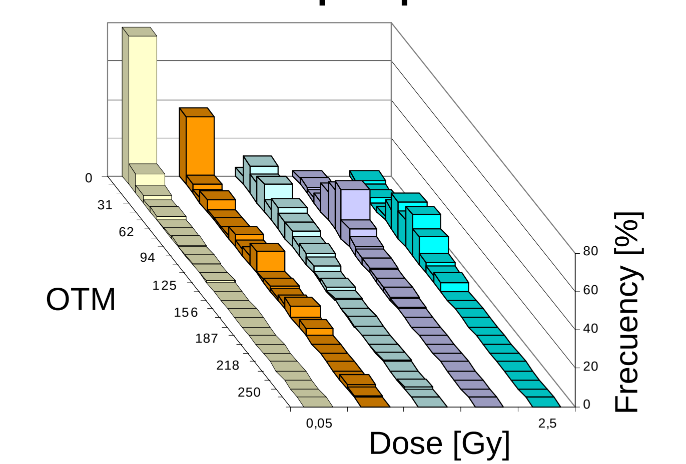


At increasing doses the tail moments are shifted towards higher values with the same asymmetrical distribution.

4 MeV Protons



20 MeV alpha particles



The shift to higher tail moments at increasing mean dose is more pronounced and a broadening in comet distribution is observed.

RESEARCH PROJECTS

Radiosensitivity tests (DNA repair capacity):

- To assess the in-vitro radiosensitivity in peripheral blood lymphocytes of cancer patients and patients with genetic disorders associated with hypersensitivity to ionizing radiation, using comet and MN assays

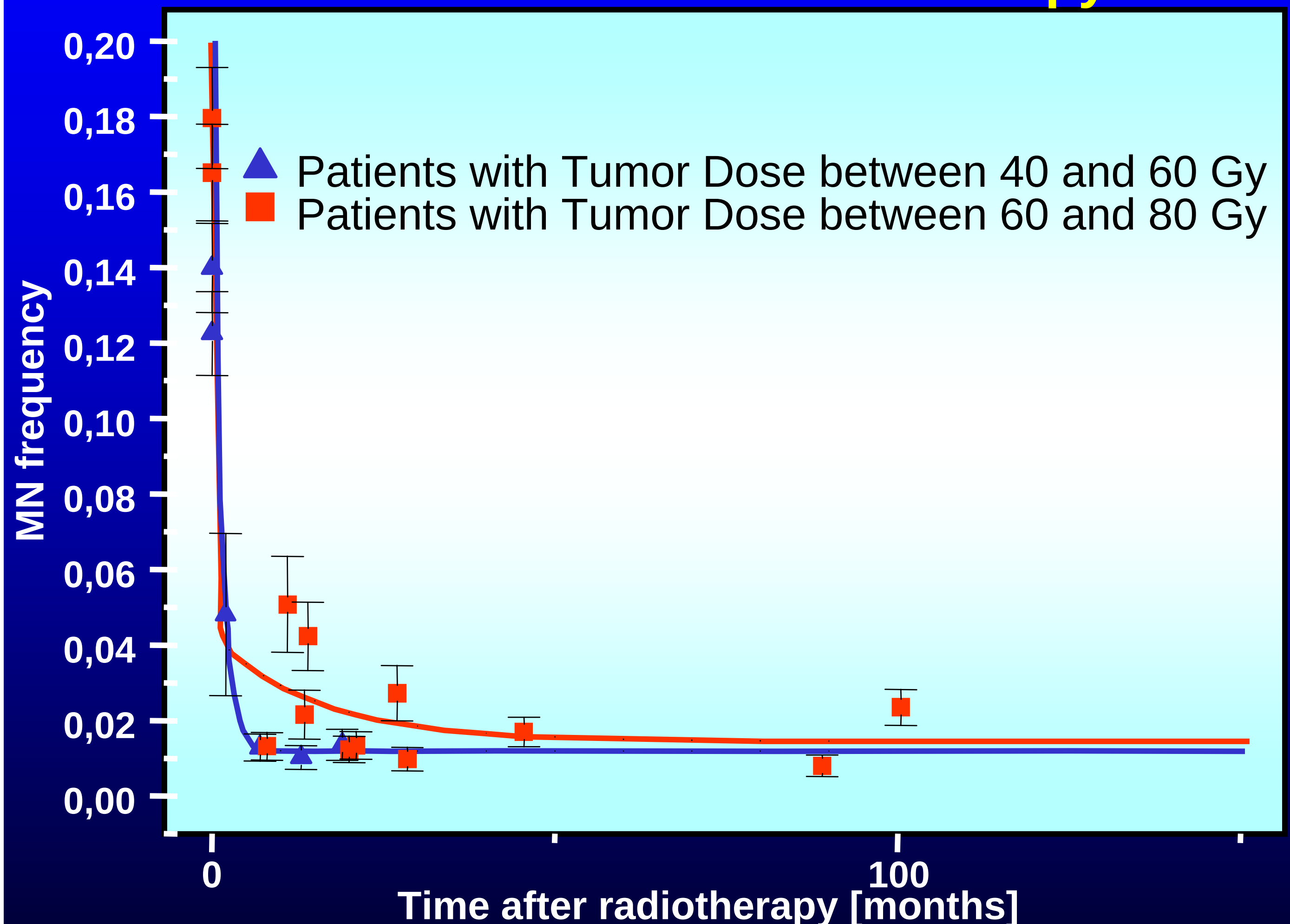
Persistency of radiation-induced damage:

- To determine the persistency of unstable and stable aberrations and the influence of sampling delay on dose estimation

Biological dosimetry of patients treated with open sources:

- To assess, by conventional cytogenetics, MN and FISH techniques, whole body dose or bone marrow dose in patients treated with Iodine-131 for Differentiated Thyroid Carcinoma

MN persistency of 17 patients with head and neck cancer treated with radiotherapy



$$y(t) = a.e^{-(b.t)} + c.e^{-(d.t)}$$

y(t): MN frequency after radiotherapy

a, c: initial MN frequency for the fast and slow components

b, d: rate constants for MN disappearance

Patients receiving higher tumor doses initially showed a faster decrease in MN frequency than those with tumor doses between 40 and 60 Gy.

QUALITY MANAGEMENT SYSTEM

Present status

Institutional Frame

- **Based on processes – Series of ISO 9000:2000 standards**

Scientific Support Management

- **Based on processes – In compliance with the series of ISO 9000:2000 standards and ISO 17025:1999 for laboratories**

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QUALITY MANAGEMENT SYSTEM

Present status

Biological Dosimetry Laboratory

- **Reference standards:**
  - ISO/IEC 17025:2005
  - ISO 19238:2004, Radiation Protection – Performance criteria for service laboratories performing biological dosimetry by cytogenetics.
  - INTERNATIONAL ATOMIC ENERGY AGENCY, Biological Dosimetry: Chromosomal Aberration Analysis for Dose Assessment, Technical Reports Series Nº 260, IAEA, Vienna (1986).
  - INTERNATIONAL ATOMIC ENERGY AGENCY, Cytogenetic Analysis for Radiation Dose Assessment, Technical Reports Series Nº 405, IAEA, Vienna (2001).

- ✓ Quality manual and the system were developed in compliance with ISO/IEC 17025:2005 and 19238:2004 standards
- ✓ Procedure and records are already developed
- ✓ At the moment the system is at the implementation stage
- ✓ Following stage: accreditation →

EMERGENCY RESPONSE SYSTEM

- All ARN-regulated activities involving the use of ionizing radiation must have emergency procedures or plans. Such procedures or plans are a condition within the process of licensing and controlling those activities. The ARN establishes the emergency criteria and evaluates the radiological and nuclear emergency plans and procedures developed by the facilities under its control
- Legal frame:
  - Nuclear Law 24804.
  - Statutory Decree 1390
  - Resolution ARN 25/99
- ARN has SIER/SIEN systems integrated to other organization systems

EMERGENCY RESPONSE SYSTEM

Radiological Emergency Intervention System (SIER)

- To act in the event of radiological emergencies arising in minor facilities and practices or involving the general public
- To act in the event of radiological emergencies in public areas
- To advise public authorities and users

Nuclear Emergency Intervention System (SIEN)

- To act in the event of emergencies resulting from accidents in nuclear power plants, the consequences of which extend to the outside of the facility
- To participate in planning and training stages aimed at intervention in emergencies
- To take actions within the Federal Emergency System (SIFEM)

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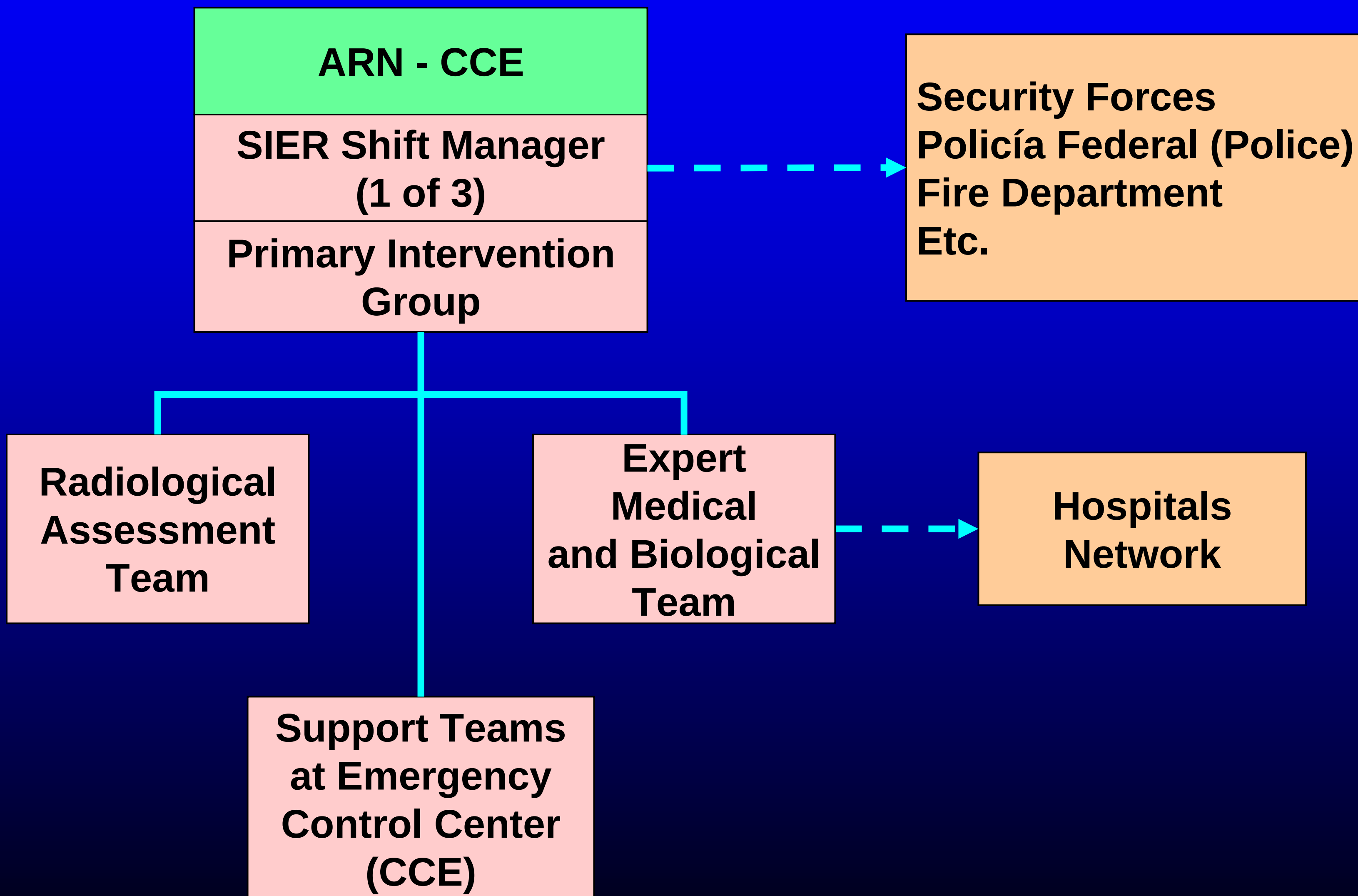
•SIER
Structure and Organization



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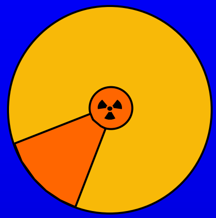
• SIEN - Local Intervention Organization



10 km

ARN

ARN Crisis Committee

ARN Crisis Committee Coordinator

Emergency Control Center

Country (Municipio)

DC

•JOEN Nuclear Energy Operational Manager (ARN)

- Country Manager
- NPP Advisor
- ARN Advisor
- Police
- Etc.

Hospitals Network

Support teams at Emergency Control Center (ARN)

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•SIEN - National and Provincial Response Organization