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Automated High-Throughput Cytogenetic Biodosimetry

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Disclosures

- **All equipment suppliers are commercial vendors.**
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Objective

Develop current “gold standard” chromosome aberration radiation dose assessment method for mass casualty applications

Outline

Section I

Background

Section II

Dicentric assay for clinical triage

Section III

Laboratory automation for sample processing and Laboratory Information Management System (LIMS)

Section IV

Inter-laboratory comparison study

Section I

Background

Diagnostic Biodosimetry Premise

- **Dose to the irradiated person is the primary determinant of the nature, onset, severity, and duration of acute radiation syndrome.**
- **Early diagnostic information on the absorbed dose is essential for effective clinical management.**
- **Medical personnel rely heavily on clinical signs and biological-based assessments of radiation dose.**
- **With appropriate medical and intensive care, the likelihood of survival can be increased significantly.**

Radiation Dose, Clinical Status, and Outcome

Dose Range (Gy)	Prodromal Effects	Manifest Symptoms	Survival Expectancy
0.1 - 1.0	None to Mild (3 h to up to 48 h)	None to slight decrease in blood count	Almost Certain
1.0 - 3.5	Mild to moderate (1 h up to 48 h)	Mild to Severe Bone Marrow Damage	0 to 10% Death
3.5 - 7.5	Severe (1 h up to 48 h)	Pancytopenia, Mild to Moderate GI Damage	10 to 100% Death (within 2 to 6 weeks)
7.5 -10.0	Severe (<1 h up to 48 h)	Combined BM and GI Damage	90 to 100% Death (within 1 to 3 weeks)
>10.0	Severe (minutes to <48 h)	GI, Neurological and Cardiovascular Damage	100% Death (within 2 - 12 weeks)

(Modified from AFRRI 2003, Prasanna *et al.* 2004)

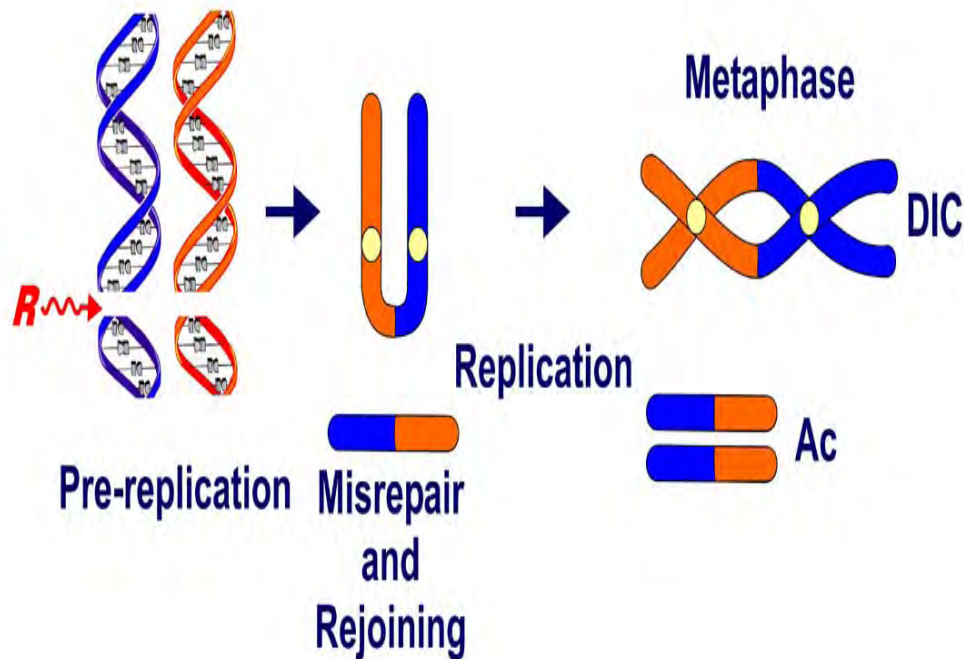
Qualities of an Ideal Biodosimeter

- **Dose-effect relationship**
- **Specific to ionizing radiation**
- **Low inter-individual variation**
- **Early availability of results**
- **Partial-body exposures**
- **Persistency**
- **Fractionated and chronic exposures**
- **Radiation quality effects**
- **Noninvasive or semi-invasive sampling**
- **Automation**

The Dicentric Assay – Effective and Validated Biodosimetry Method

- Validated cytogenetic “gold standard” biodosimetry assay
- Dicentric chromosomes seen in human peripheral blood lymphocytes, are specific to ionizing radiation exposure
- Dose-effect relationship
- Strong correlation between *in vitro* and *in vivo* yield
- Technical manual (IAEA) and international standards (ISO) are available for performing dicentric assay for radiation dose assessment.
- Performed in “reference cytogenetic reach-back biodosimetry laboratory”
- Needs cytogenetic expertise and labor intensive
- Applications in radiation mass casualties will require automation.

Dicentric Chromosome: A Specific Radiation Biomarker

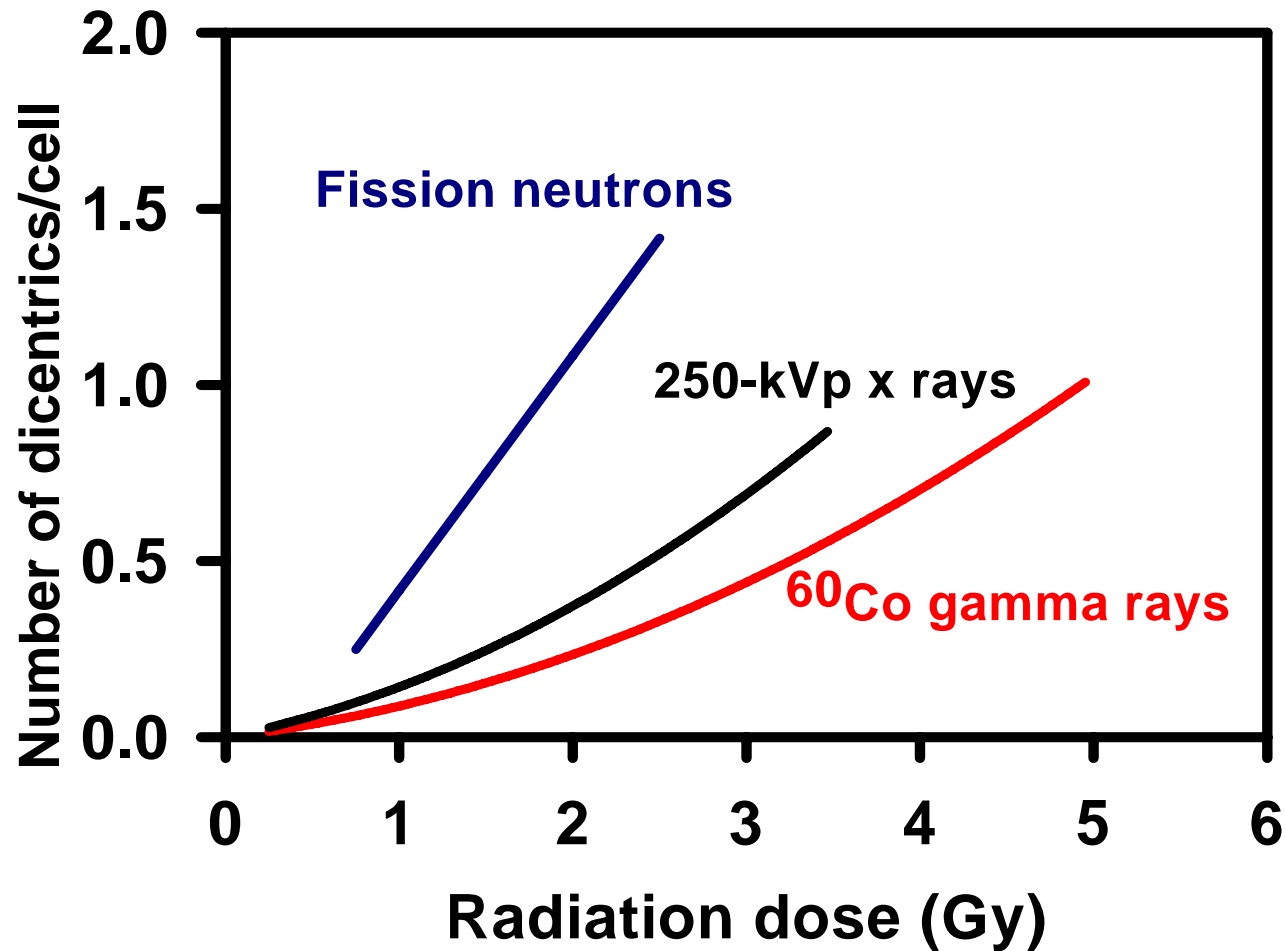


**Mechanism of dicentric
induction**



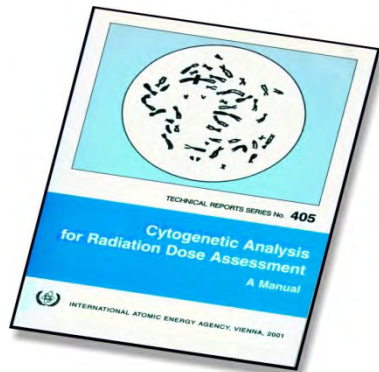
**Metaphase spread
with a dicentric
chromosome**

AFFRI's Dose-Response Calibration Curves



Prasanna et al. (2002)

Dicentric Assay: Current Status



IAEA revised manual



International Standardization Organization

- Revised IAEA manual published in 2001.
- ISO standard for accrediting cytogenetic biodosimetry laboratories (ISO TC85/SC 2, 19238, 2003) is available.
- “Radiation Protection — Performance criteria for rapid cytogenetic assessment of individual exposures in radiological or nuclear mass casualties - I. General principles and application to the dicentric assay, (ISO TC85/SC 2, WG-18, 21243) soon be ready for ratification.

Conclusions: Section I

- **Dicentric assay is currently the “gold-standard” dose assessment method.**
- **Technical manual and standards are available for performing dicentric assay for radiation dose assessment.**
- **Applications in radiation mass casualties will require further assay development.**

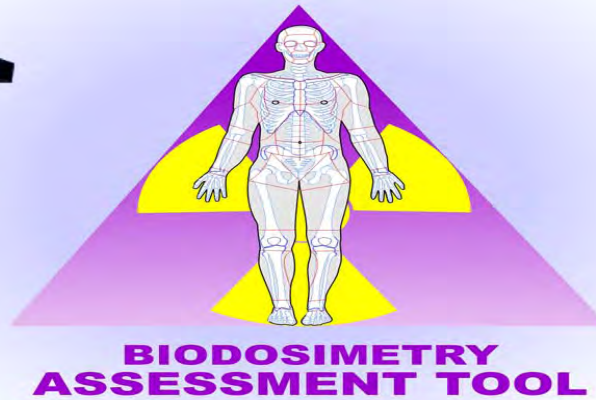
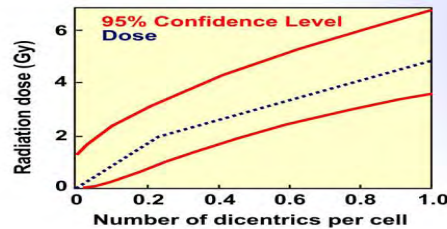
Section II

Dicentric Assay for Confirmation of Clinical Triage

Guidelines for Confirmation of Clinical Triage by Biodosimetry

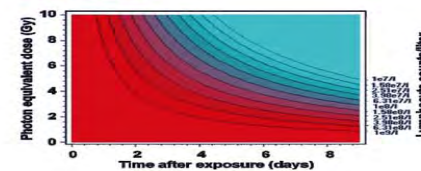
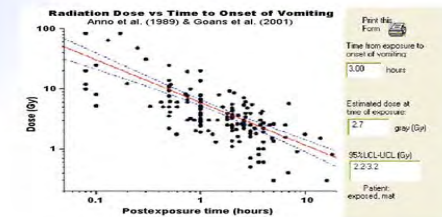
- **Time of emesis is less than 2 hours after exposure**
- **An approximate 50% decline in peripheral blood lymphocyte counts over 12 hours**
- **Geographical location-based physical dosimetry indicate above 3-Gy dose**
- **Multi-parameter clinical symptoms indicate imminent acute radiation syndrome**

Dose Assessment: Cytogenetic Biodosimetry Strategy



B. Prodromal Syndromes

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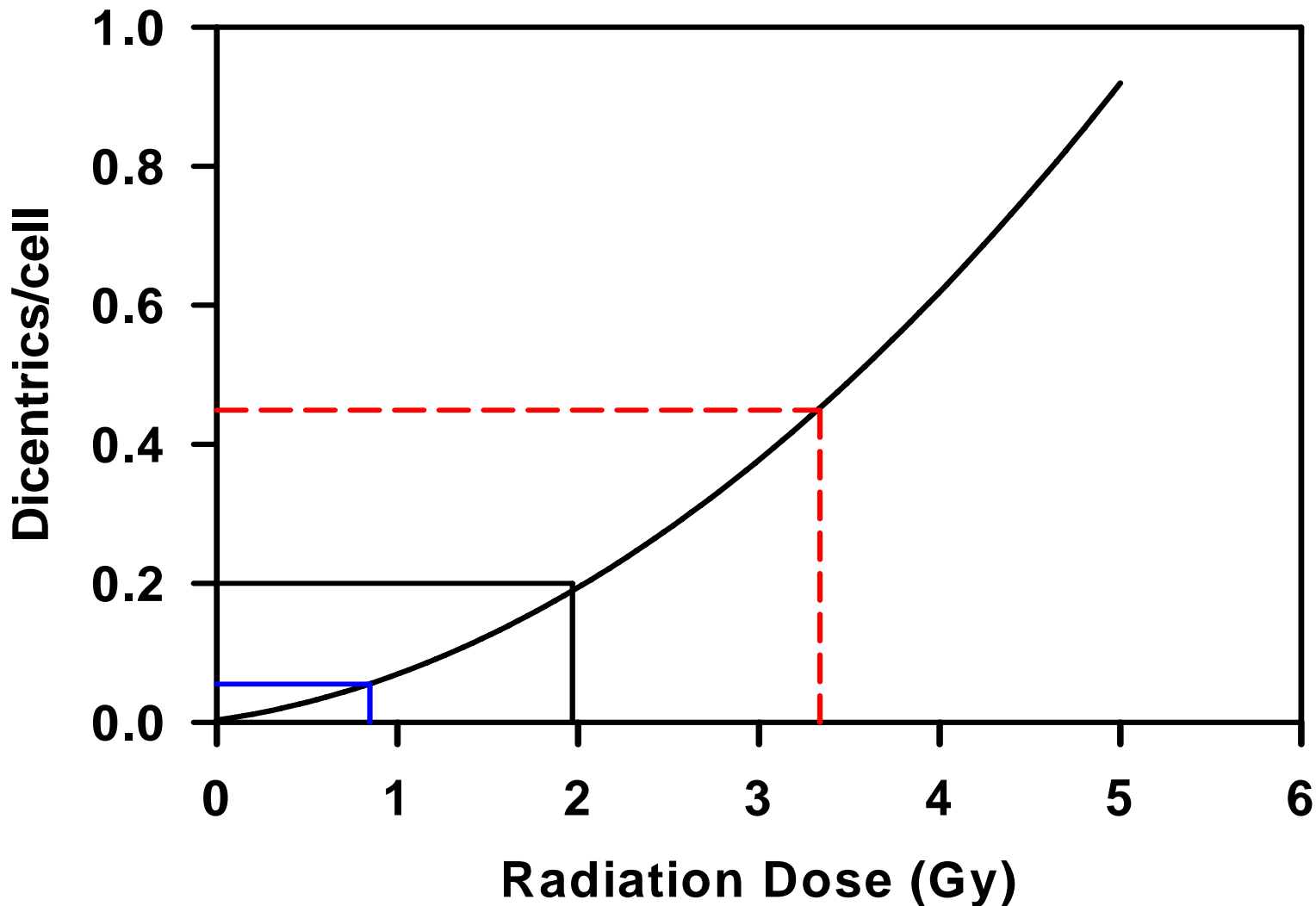


Prasanna *et al.* 2004

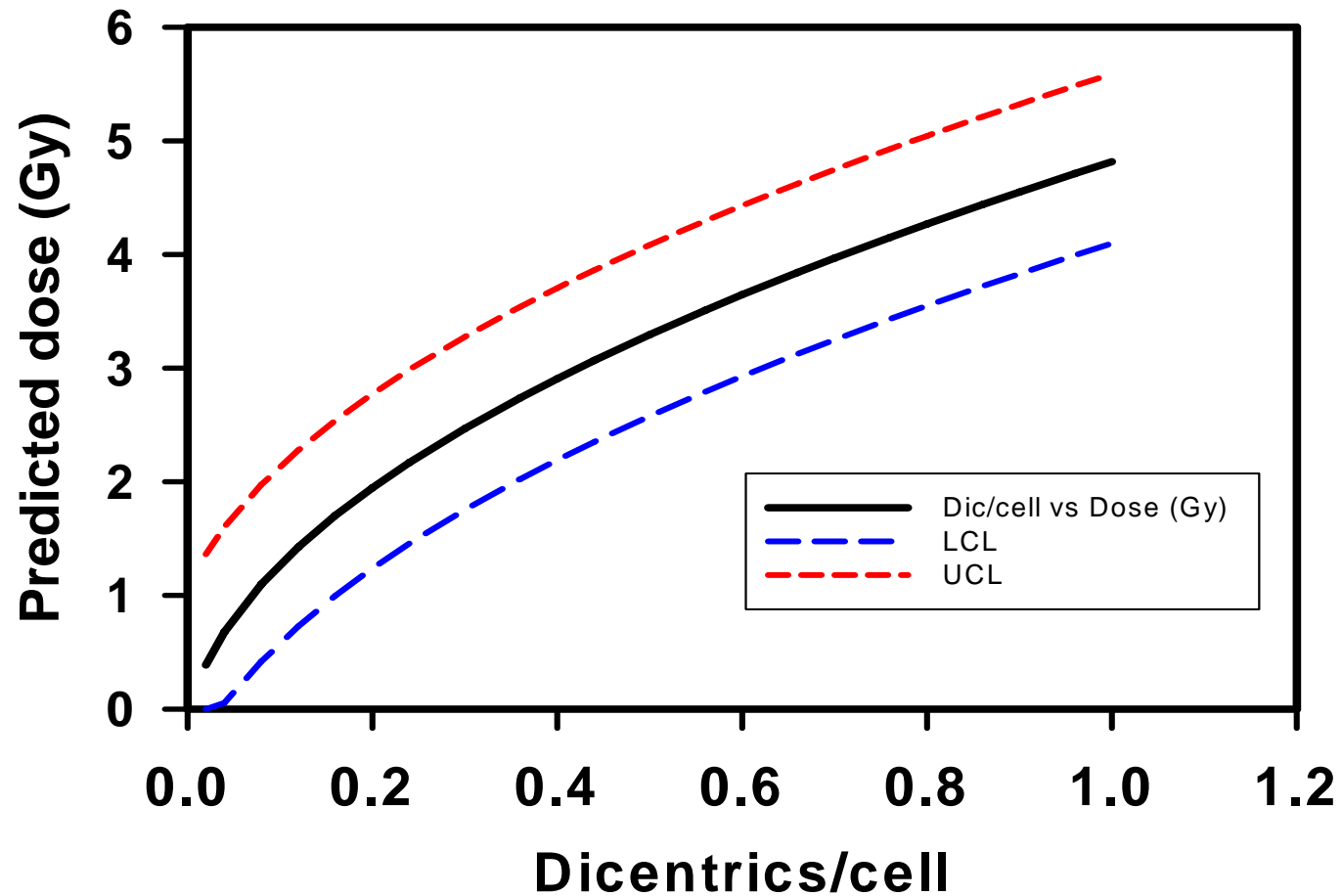
Emergency Triage Analysis for Mass Casualties

- **Triage for management of the Acute Radiation Syndrome (ARS) will require estimation of whole-body dose in the ranges of >4 Gy, 2-4 Gy and <2 Gy**
- **Scoring 20-50 metaphases per patient allows stratification of patients in to dose categories**
- **Current activities of ISO focus on developing performance criteria for assessment of individual exposures in radiological or nuclear mass casualties**

Triage Dose Prediction Model: Confirm "Significant" Exposure (20 cells)



Dose Prediction by Analyzing 50 Cells



Cytogenetic Dose Assessment in Mass Casualties: Challenges and Solutions

- **Labor-intensive and time-consuming**
 - **Automate**
 - **Sample processing**
 - **Chromosome-aberration analysis**
- **Needs expertise**
 - **Establish inter-laboratory networks**
 - **Train individuals**
 - **Perform tele-analysis via Virtual Private Network (VPN)**
 - **Automate analysis**

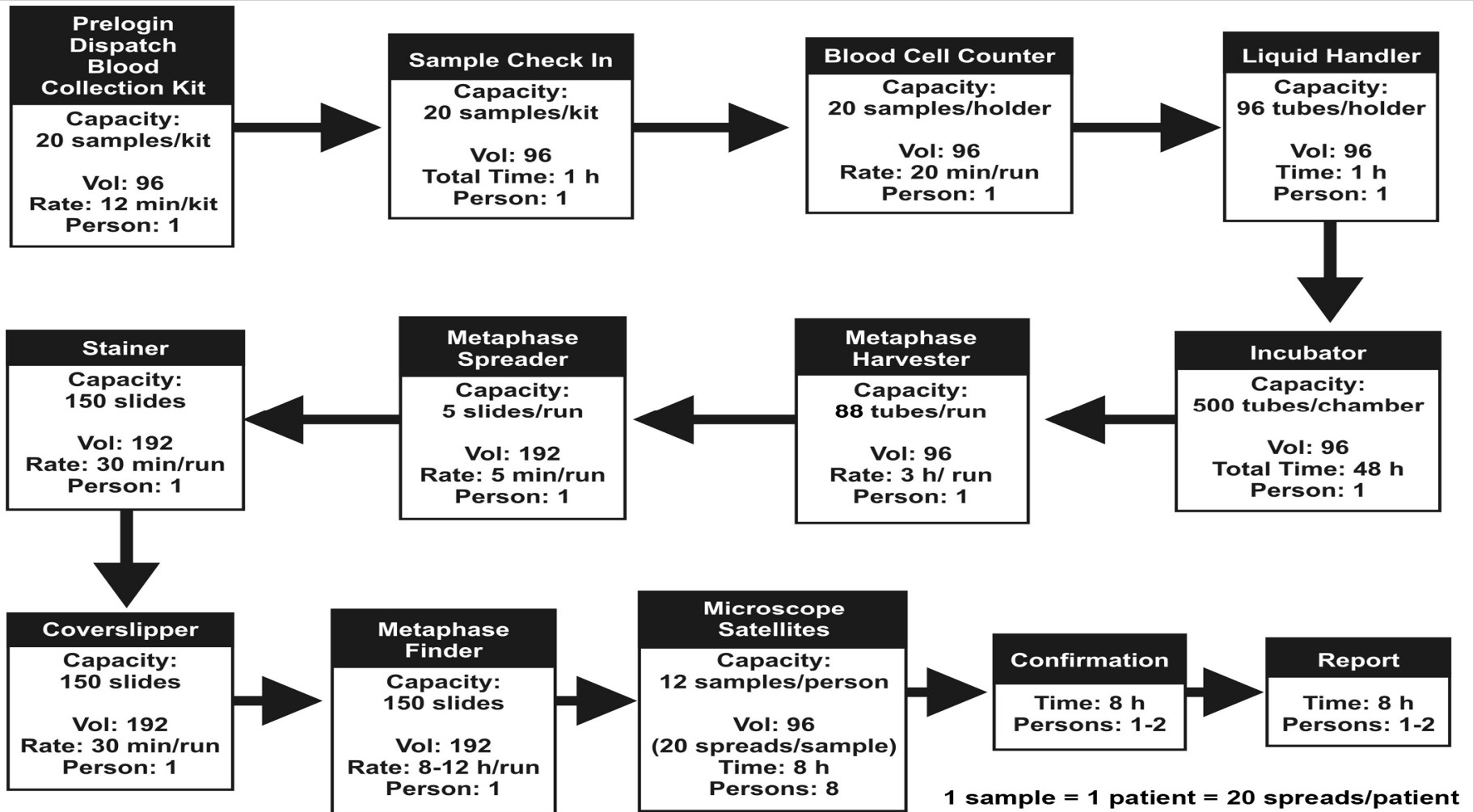
Conclusions: Section II

- **Triage for management of the Acute Radiation Syndrome (ARS) will require estimation of whole-body dose in the ranges of >4 Gy, 2-4 Gy and <2Gy.**
- **Scoring 20-50 metaphases per patient allows stratification of patients in to dose categories.**
- **Current activities of ISO focus on developing performance criteria for assessment of individual exposures in radiological or nuclear mass casualties.**
- **Dicentric assay can be used for confirming clinical triage in radiation mass casualties**

Section III

Laboratory Automation and Laboratory Information Management System

Sample Processing: Assay Overview and Industrial Analysis

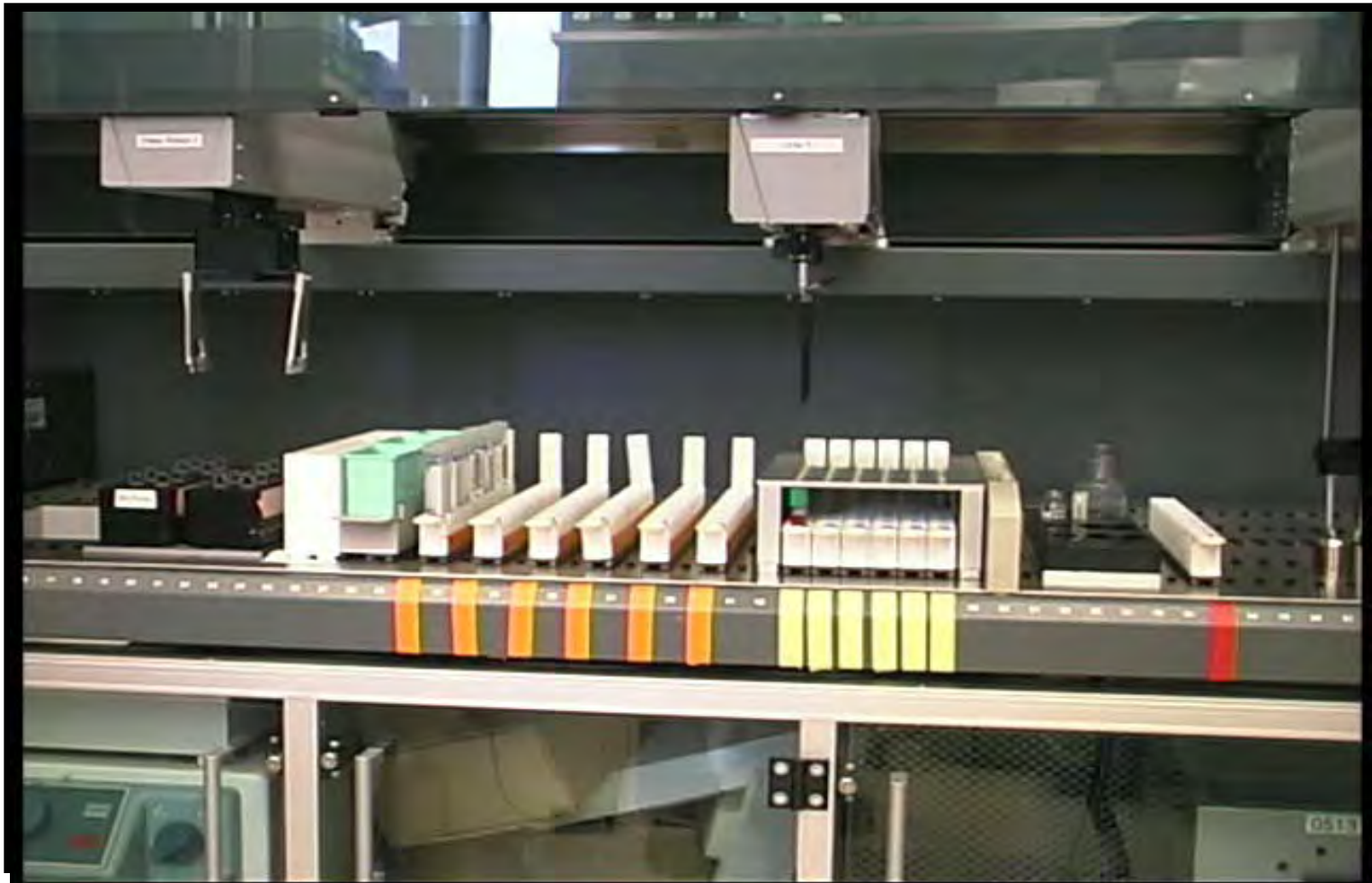


Martin et al. 2007

Automation Challenges

- **System Integration**
 - Liquid handling
- **Protocol Optimization**
 - Metaphase harvester
- **Equipment Customization**
 - Metaphase finder
- **Data management and Sample Tracking**
 - LIMS (Sample Master®) → CytoTrak™

System Integration for “Walk-Away” Automated Blood Processing



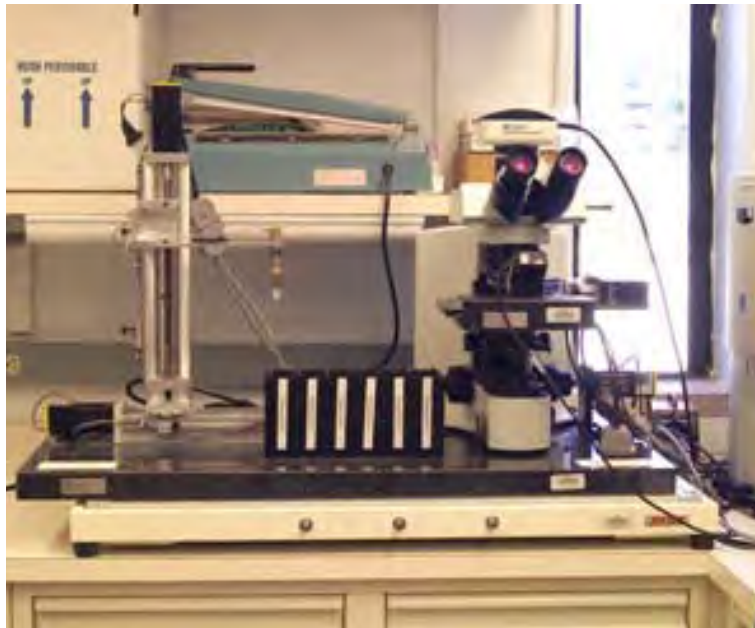
Protocol Optimization: Metaphase Harvesting



Metaphase harvester

- **Equipment is originally designed to harvest metaphase spreads from cell cultures.**
- **Manual protocols are automated.**
- **Standard Operating Procedures (SOP) are developed for harvesting metaphase spreads from peripheral blood and isolated lymphocytes.**

Equipment Customization: Bar-code Integration in Metaphase Finder and Satellite-Scoring Stations



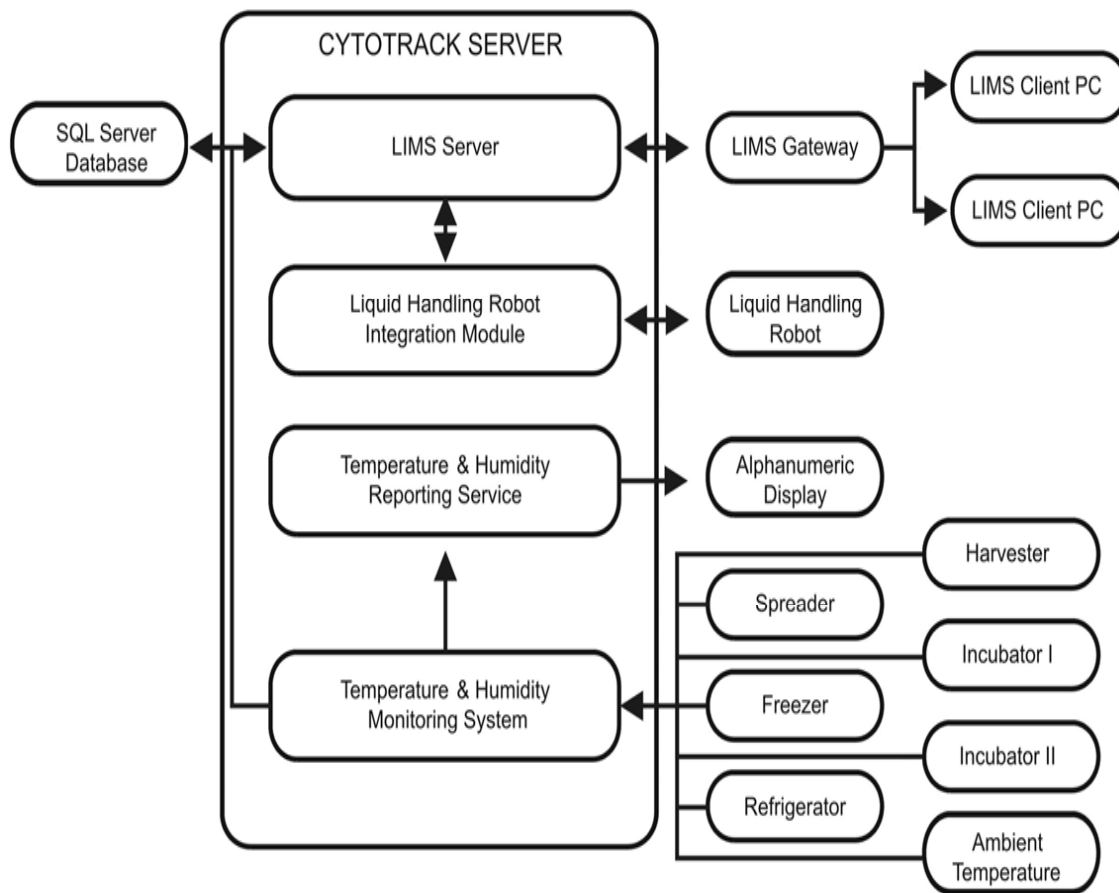
High-throughput automated metaphase finder and bar-coded slide

- Customized to scan barcodes on slides as it automatically locates chromosome spreads.
- Software customizations allow barcode information to be associated with data and image files.
- Allows case file data storage on a centralized server.
- Customized network of satellite chromosome-aberration analysis workstations provides direct access to case files on the centralized server, eliminating data transcription errors.

LIMS (SampleMaster Pro®)

- **Quality Control and Quality Assurance**
- **Sample tracking**
- **Data entry**
- **Sample scheduling and stability**
- **Electronic data transfer**
- **Chemical inventory**
- **Resource management**
- **Sample conditions**
- **Test set up**
- **Security**
- **Auditing**
- **Configuration**
- **Reporting**
- **Instrument integration**

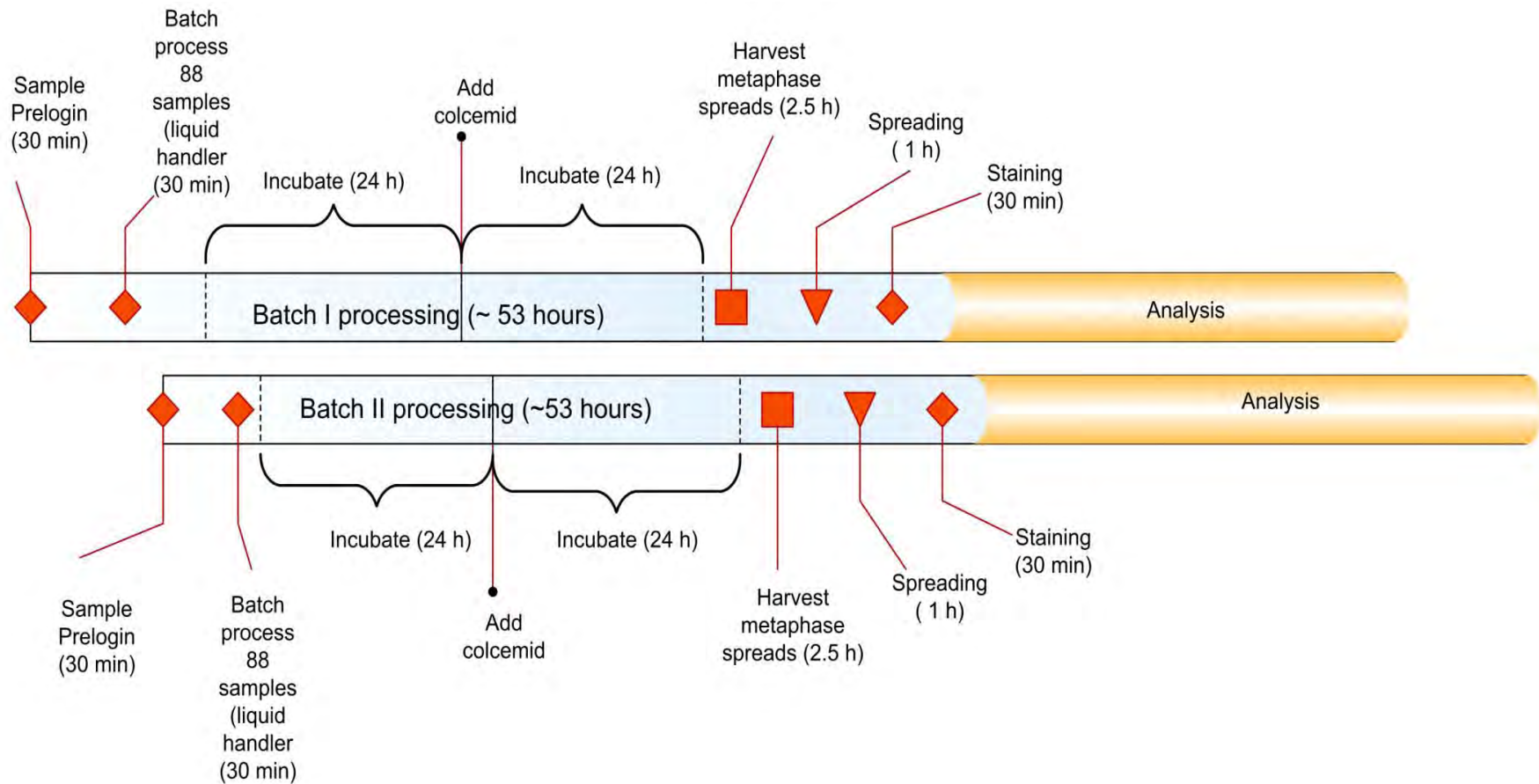
The CytoTrack™ System for Sample Tracking and Data Management: Overview



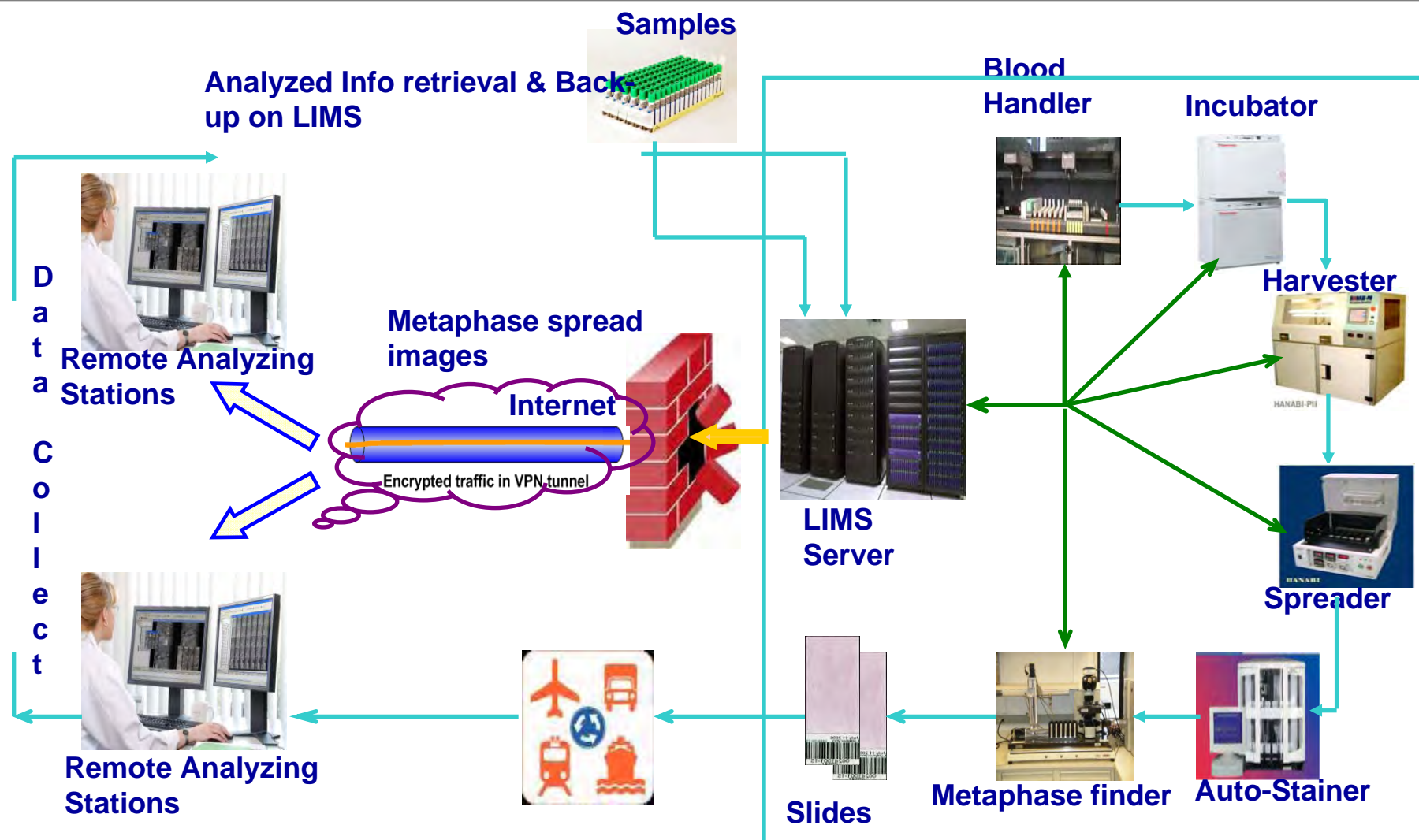
CytoTrack component and data flow

- **LIMS Server and SQL relational database represent the heart.**
- **Individual equipments are integrated via customized interfaces and data translators.**
- **Data translation and processing modules running on centralized server communicate between equipments by importing, recording, and exploring data automatically.**
- **The server also monitors the status of each equipment, reagent inventories, staff training, and accreditation and environmental conditions.**

High-Throughput Batch Processing of Samples



Analysis of Samples



Conclusions: Section III

- **Sample throughput for the current gold standard method can be increased significantly by laboratory automation.**
 - **Transitioned manual sample preparation methods to automated walk away platforms for cytogenetic dose assessment.**
 - **Processing of 500 to 1000 samples per week is possible**
- **Quality control and quality assurance can be improved by implementing LIMS.**
- **Automated cytogenetic laboratory can support physical transfer of slides or images for rapid dicentric analysis for dose assessment**

Section IV

Quality Control Essentials

Inter-Laboratory Comparison Study

Collaborators

- **A. Awa (REAC/TS)**
- **M. Jenkins (REAC/TS)**
- **T.C. Kao (USUHS)**
- **G. Livingston (REAC/TS)**
- **U. Oestreicher (BFS, Germany)**
- **T.C. Pellmar (AFRRI)**
- **H. Romm (BFS, Germany)**
- **R. Wilkins (Health Canada)**
- **M. Yoshida (NIRS, Japan)**

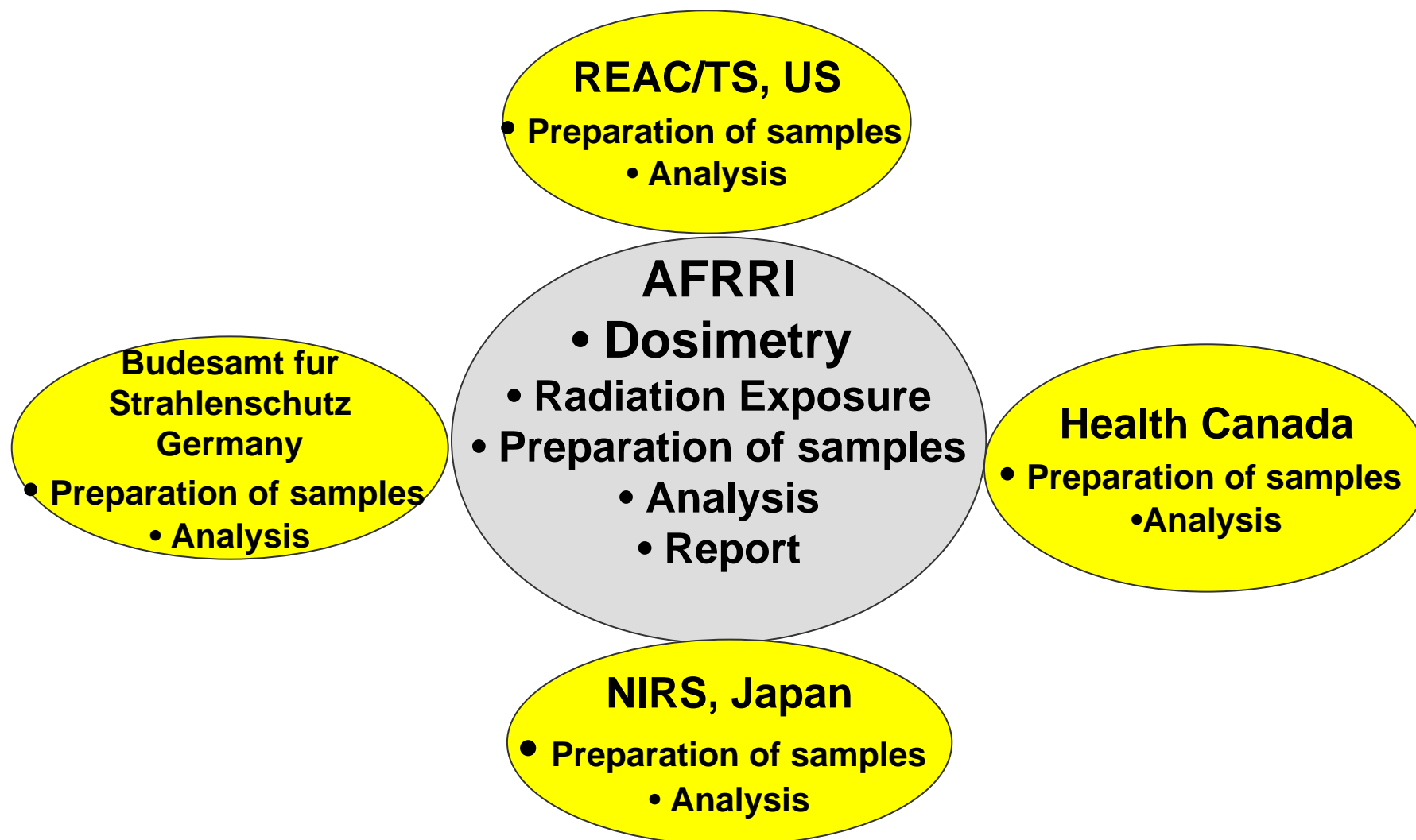
Background and Objective

- Variations in sample preparation methodology
- Analysis of dicentrics by different scorers
- Differences in dose-effect calibration curves

Will influence dose assessment.

- **Objective:** Perform first inter-laboratory comparison after establishment of ISO guidelines

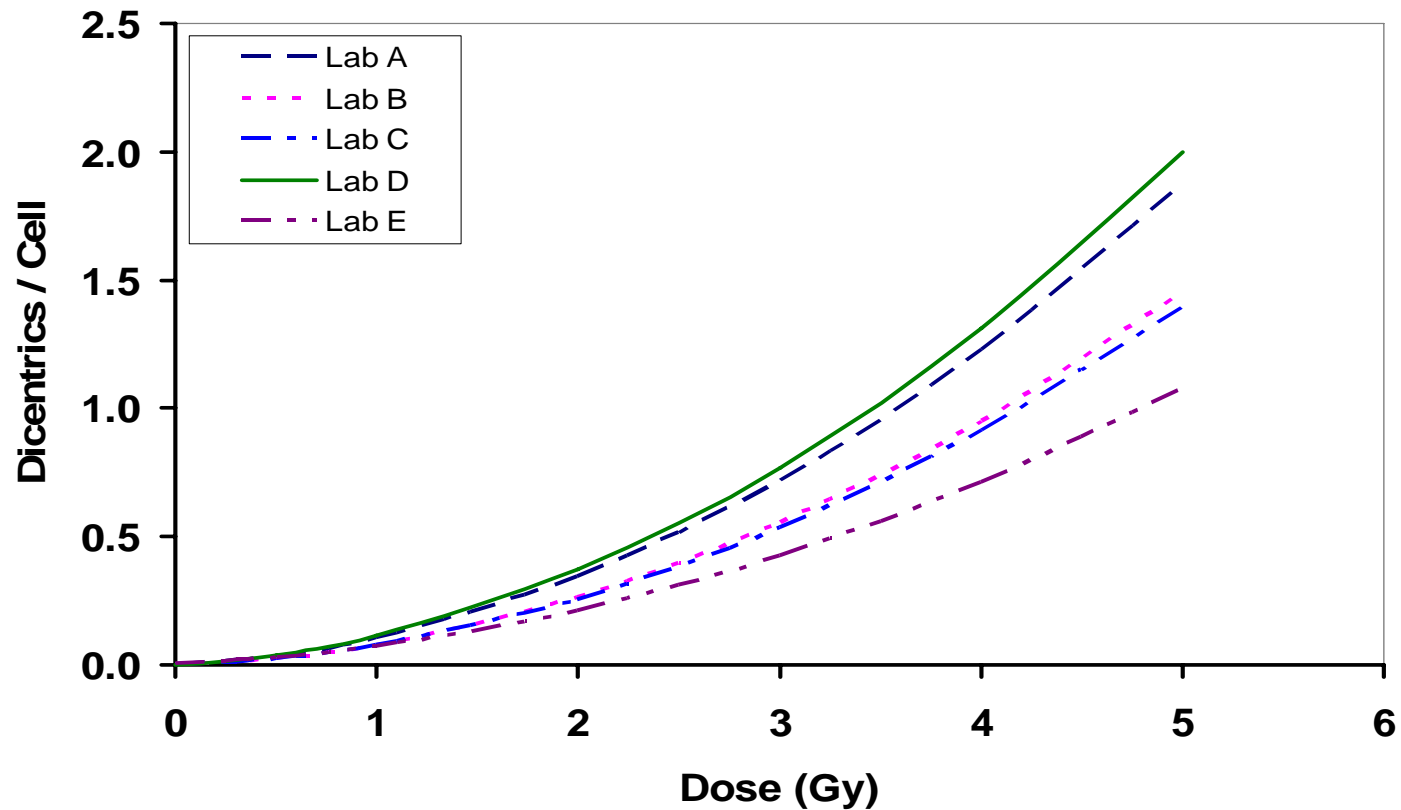
Inter-Laboratory Comparison Study: Laboratories and Roles



Phase I Studies

- **Construct dose-response calibration curves (Dose range 0.25 to 5.0 Gy)**
- **Compare calibration curves and determine variability**

Calibration Curves

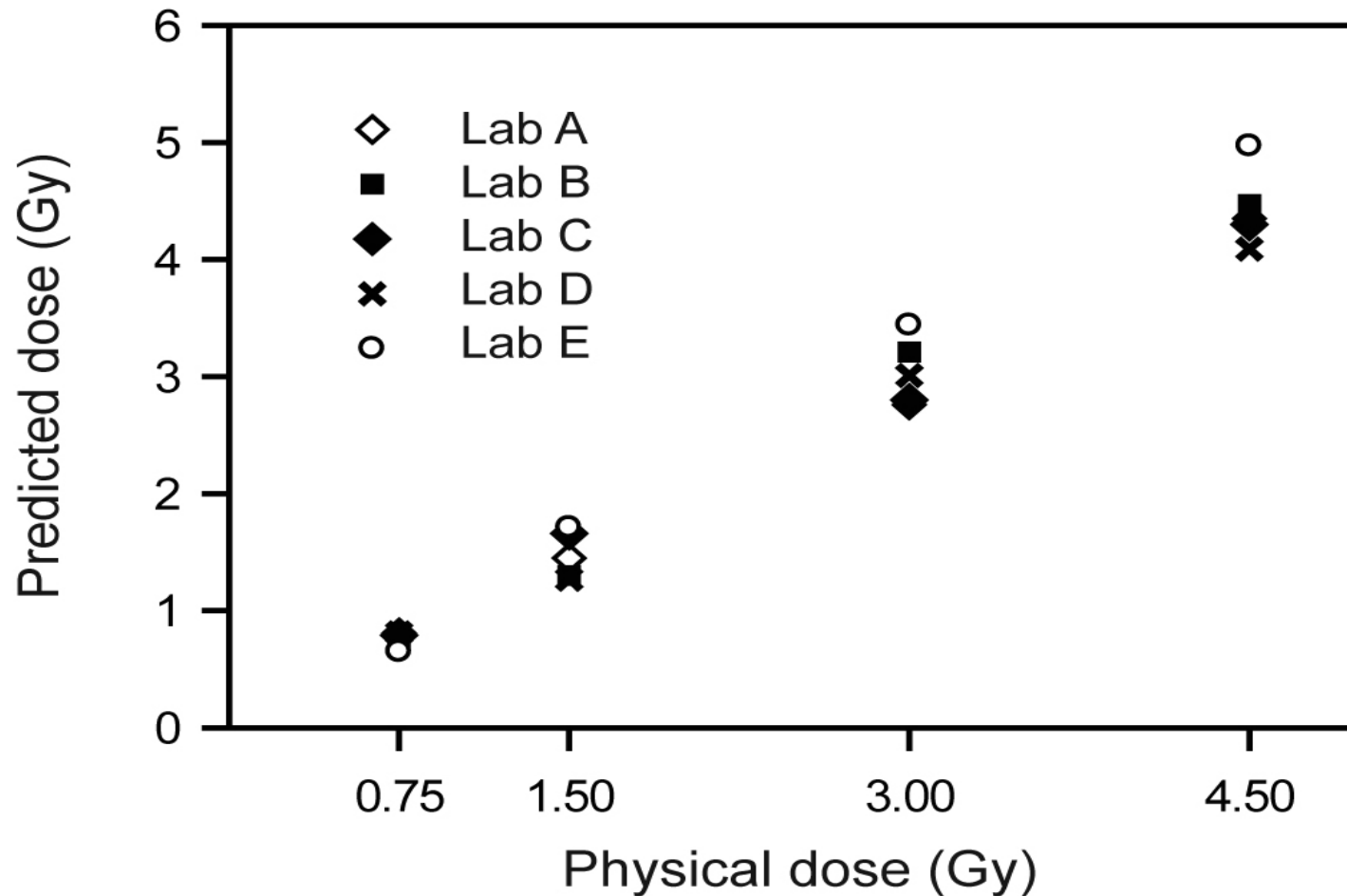


Wilkins *et al.* Rad. Res. 169: 551-560 (2008)

Phase II Studies

- **Dose blinded study**
- **Irradiate samples with unknown doses**
- **Estimate radiation dose and determine accuracy**

Distribution of Estimated Biological Doses for Laboratories A - E.



Wilkins *et al.* Rad. Res. 169: 551-560 (2008)

Estimated Biological Doses in All Labs

Physical Dose (Gy)	Estimated Biological Dose (Gy) and (SD) in various laboratories					Mean (SD)	Percent Error in Estimated Dose
	A	B	C	D	E		
0.75	0.81	0.72	0.79	0.81	0.65	0.76±0.13	1.33
1.5	1.45	1.30	1.66	1.27	1.71	1.48 ±0.17	-1.33
3.0	2.76	3.21	2.80	3.01	3.44	3.04 ±0.31	1.33
4.5	4.35	4.47	4.30	4.09	4.97	4.44 ±0.45	-1.33

Conclusions: Section IV

- **Dose-effect relationships for calibration curves between laboratories are similar but co-efficients of the calibration curves differ.**
- **More importantly, estimated radiation doses from dose blinded samples by a comparison with each laboratory's calibration curve were quite accurate in all laboratories at all doses.**
- **Developing a mathematical dose prediction algorithm for confirmation of clinical triage.**

Summary

- **Further developed and validated the “gold-standard” biodosimetry method for radiation mass casualty applications.**
- **Automation, system integration, protocol optimization, equipment customization, and data management solutions are critical for dicentric assay’s application in mass casualties.**
- **The laboratory is ready for advanced “stress” testing of automated systems and CytoTrack (LIMS) using real samples from a simulated mass casualty event.**
- **Degree of variability on dose effect calibration curves and assessed dose for the dose blinded samples between laboratories was determined.**
- **Mathematical algorithms for triage dose assessment is developed using inter-laboratory comparison study data, but needs testing.**
- **High throughput cytogenetics can be valuable for triage dose prediction in mass casualties.**

Acknowledgment

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