The Method Validation Step Of Biological Dosimetry Accreditation Process

Roy L, Voisin Pa, Guillou A.C., Busset A, Grégoire E, Buard V, Delbos M, Voisin Ph.
Institut de Radioprotection et de Sûreté Nucléaire, LDB, BP17, 92262 Fontenay-aux-Roses Cedex, FRANCE

Abstract : One of the missions of the Laboratory of Biological Dosimetry (LDB) of the Institute for Radiation and Nuclear Safety (IRSN) is to assess the radiological dose after an accidental overexposure suspicion to ionising radiation, by using radio-induced changes of some biological parameters. The "gold standard" is the yield of dicentrics observed in patient's lymphocytes, and this yield is converted in dose using dose effect relationships. This method is complementary to clinical and physical dosimetry, for medical team in charge of the patients.
To obtain a formal recognition of its operational activity, the laboratory decided three years ago, to require an accreditation, by following the recommendations of both 17025 General Requirements for the Competence of Testing and Calibration Laboratories and 19238 Performance criteria for service laboratories performing biological dosimetry by cytogenetics.
Diagnostics, risks analysis were realized to control the whole analysis process leading to documents writing. Purchases, personnel department, vocational trainings were also included in the quality system. Audits were very helpful to improve the quality system.
One specificity of this technique is that it is not normalized therefore apart from quality management aspects, several technical points needed some validations.
An inventory of potentially influent factors was carried out. To estimate their real effect on the yield of dicentrics, a Placket-Burman experimental design was conducted. The effect of seven parameters was tested: the BUdr, PHA and colcemid concentration, the culture duration, the incubator temperature, the blood volume and the medium volume. The chosen values were calculated according to the uncertainties on the way they were measured i.e. pipettes, thermometers, test tubes… None of the factors has a significant impact on the yield of dicentrics. Therefore the uncertainty linked to their use was considered as nil.
The main difficulty of the quality program set up in the laboratory was to convince people working in the laboratory of the interest of such a project and to change their way of work.
The laboratory is almost ready to request for accreditation. The establishment of quality system resulted in some practice changes that were positive for the laboratory and improved the results quality.

1. Introduction

In the case of accidental overexposure to ionizing radiations, it is important to assess as soon and as precisely as possible the dose received by the potentially overexposed people. This may help the medical team to adopt the best medical arrangement. To estimate inter-individual sensitivity to ionizing

radiation biological dosimetry is conducted in supplement to clinical and physical dosimetry (IAEA, 2001).
The dicentric assay (Figure 1) is considered as the most sensitive and specific bio-indicator of dose in case of recent accidental overexposure. The dicentrics are measured on circulating lymphocytes and converted to dose using dose effect calibration curves.

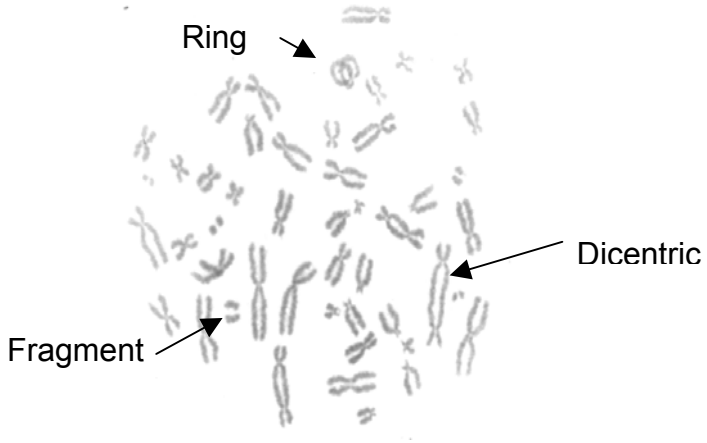

Figure 1: Fragments, dicentrics and rings are observed in a lymphocyte at the metaphase step of the cell cycle, to estimate a dose by biological dosimetry.

2. Quality management context
One of the missions of the Laboratory of Biological Dosimetry (LDB) of the Institute for Radiation and Nuclear Safety (IRSN) is to assess the radiological dose after an accidental overexposure suspicion to ionising radiation, by using cytogenetic endpoints. Several reasons have encouraged the laboratory to follow an accreditation process for this operational activity:

- *The LDB is the only laboratory in France to perform this activity. Without any other reference the results could be criticized. Furthermore the results could have a direct impact on the exposed victim. Indeed if an overexposure is confirmed for an occupational worker by the cytogenetic analyses, the patient may have to stop working in exposed area, possibly resulting in a financial and psychological impact.*
- *This technique has a medico legal value. Therefore if some pathology appears a long period after a suspected radiation overexposure, the patient may require a reclassification of his disease to a professional one.*

The quality management was developed to meet two standards:

- *The ISO/CEI 17025 :2005 General Requirements for the Competence of Testing and Calibration Laboratories. The interest of this standard is to describe both technical measures and general quality measures to set up.*
- *The ISO 19238 :2004 Radioprotection – Performance criteria for service laboratories performing biological dosimetry by*

cytogenetics. This new standards describes the technical measures to set up specifically to perform biological dosimetry.

In the accreditation goal, the work performed in the laboratory was held to match the two standards and can be divided in two parts: quality system management and technical validations.

3. The quality management of the system

Figure 2 describes the general organisation of the quality system management set up in the LDB, whose major aspects are detailed in table 1.

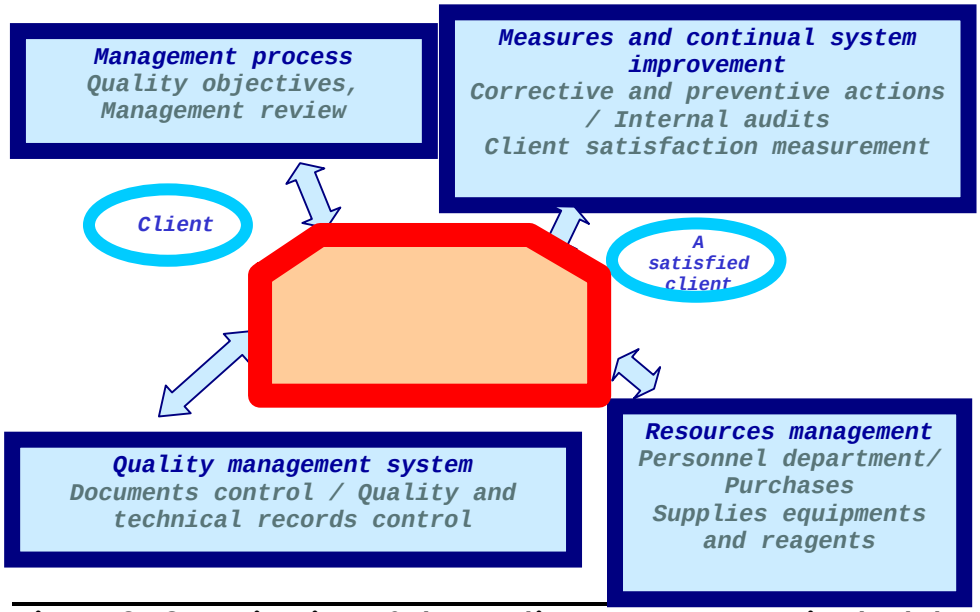

Figure 2: Organisation of the quality system set up in the laboratory to satisfy both ISO 17025 and ISO 19238 standards.

Table 1: Description of the major aspects of Q & A system to meet the 17025 and 19238 standards

Quality process	*Description of the method set up*
Management Process	• *the system is reviewed every year* ➢ *Analyse of all the documents* ➢ *Quality objective check* • *Each staff member has his own responsibility* ➢ *The head of the lab fixes the objectives and participates to the set up* ➢ *One technician is in charge of the quality system* ➢ *One scientist is in charge of the material*

	➢ *One technician is in charge of purchases* ➢ *One scientist is in charge of documents management*
Continuous improvement system	• *Establishment of a complaints book:* ➢ *Direct benefit for the lab* ➢ *Had to be filled in regularly* • *Audit by external consultants to have an original point of view* ➢ *To evaluate the system periodically* ➢ *To show the way to progress*
Quality management system	• *A quality assurance manual* • *Traceability* ➢ *Of products* ➢ *Of the biological material* • *Device maintenance* ➢ *e.g. microscopes, centrifuges, pipettes,* ➢ *According to the critical points of the technique* • *Maintenance of the laboratory (property, sterility…)*
Resources management	• *General requirement through IRSN quality process* ➢ *Recruiting* ➢ *Formation* ➢ *Purchases: for some products* • *Material management* ➢ *Identification of influent products* ➢ *Use of ready to use products* ➢ *Control of the experimentation temperature*

4. The technical validation

To ensure the quality of the technical process, several actions were undertaken including writing protocols, performing intercomparisons, checking personnel qualification but also measuring the uncertainties on the technical process. The different parameters which uncertainties have to be measured are related to the dicentrics assay detailed in figure 3.

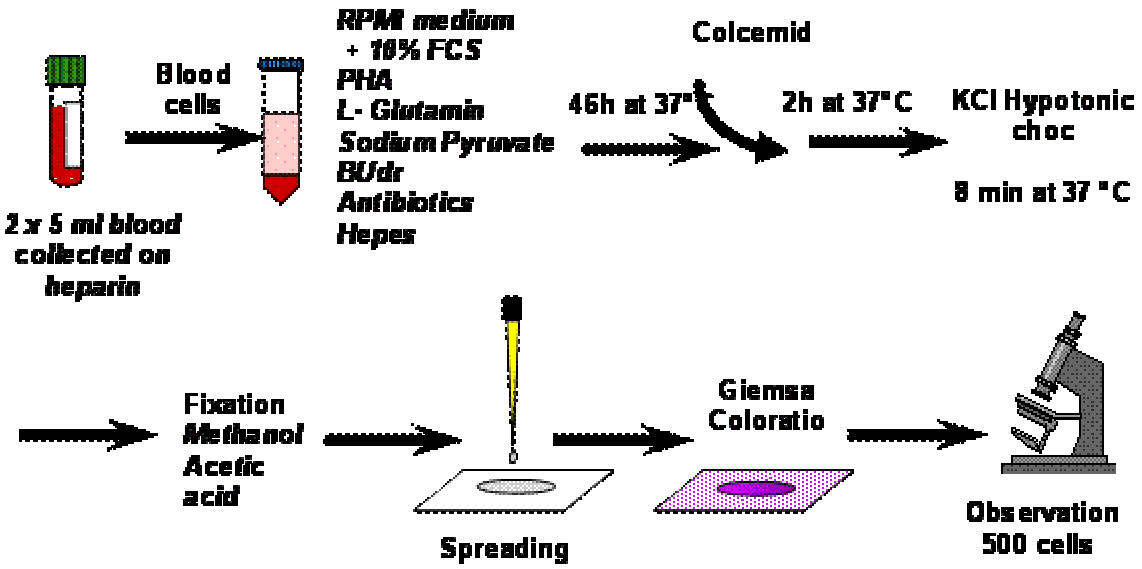

Figure 3: General description of the different steps of the dicentrics assay

They include the following items:

- *Lymphocyte culture and arrest*
- *Spreading onto slides*
- *Scoring, either manually of using image analysis systems*
- *Converting yield of dicentrics to dose using image dose effect calibration curves.*

Some parameters were still well described but others needed some more validations.

Three parameters affect the uncertainty on the dose effect calibration curve (figure 4). The first one is the number of dicentrics which is described by the Poisson law. The second one is the curve fitting which is described in details in a publication (Papworth, 1975). The third one is the uncertainty on the dose which is given by the physical dosimetry. We combined all these uncertainties to have the global uncertainty on the dose effect curve.
In Figure 4 are the parameters which affect the yield of dicentrics. Uncertainties on the number of dicentrics and cells scored were calculated according to the Poisson law (Doggett et al. 1983). Image analysis systems are checked to know and limit their influence. Operators are qualified regularly to avoid any deviance in the dicentrics identification. The last point that could affect the yield of dicentrics is the products used for the culture blood samples.

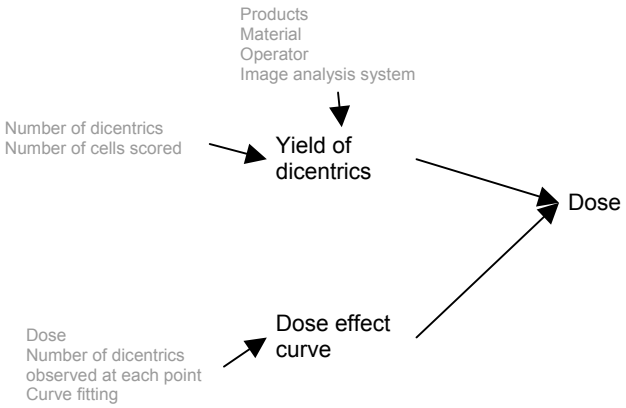

Figure 4: Visualization of the different parameters on which the method validation was performed.

5. The experimental design

Each step of the dicentrics assay was analyzed to identify the factors that might have an impact on dicentrics yields. All these factors are summarized in table 2. The suspected ones were included in the Placket-Burman experimental design conducted to estimate the impact of those factors.

Once the parameters to be tested were chosen, it was necessary to determine the testing values range. Briefly each source of uncertainty related to the way the products were measured were taken into account before combining them. The enlarged uncertainty was calculated assuming a weighting out factor of 2. Table 3 gives the values tested in the experimental design.

Table 2 : List of factors that were analyzed to evaluate their impact on the yield of dicentrics

FACTEURS	*INFORMATIONS*	*Potential impact*
Medium : RPMI	*Culture medium for the division of lymphocytes*	*Yes*
Foetal Calf Serum	*Lymphocytes cannot divide without serum*	*No*
Penicillin-Streptomycin	*The antibiotics avoid bacteria proliferation but don't interfere with lymphocyte division*	*No*
Sodium Pyruvate	*This sugar is only a complement to the one present in the native medium*	*No*
L-Glutamin	*This amino acid acts as fuel and is a complement to the sugar present in the medium.*	*No*
Hepes	*Control the pH of the medium. An alteration of the pH is visible by eye when the medium changes of color.*	*No*
Bromodeoxyuridin (BUdr)	*This is a thymidin analogous; it allows the scoring of dicentrics in first division metaphases only. It has an impact on the cell cycle duration. The number of metaphases*	*Yes*

	can vary according to its concentration.	
Phytohemagglutinin (PHA)	*This bean byproduct is required to have T cells division*	*Yes*
Colcemid	*Required to block cells in the metaphase step of the cell cycle*	*Yes*
Hypotonic choc (KCl)	*Required for cell membrane lyses, is bought ready to use*	*No*
Acetic acid - Methanol	*Required to fix the cells and to have chromosome spread on the slide. It has no effect on the number of metaphases neither on the number of dicentrics.*	*No*
Blood	*The number of lymphocytes in the blood sample can affect the quality of the culture*	*Yes*
Culture duration	*Can have an impact on the number of metaphases*	*Yes*
KCl incubation duration	*Variations are shown without effect in the literature, therefore its impact was not taken into account*	*No*
Incubator temperature	*From IAEA (2001) it should be of 37 ± .0.5°C but difficult to control*	*Yes*

Table 3: Ranges of the parameters tested in the experimental design set up.

Parameters	*value 1 (low level)*	*Usual value*	*value 2 (high level)*
BUdr	*94 mg*	*100 mg*	*106 mg*
PHA	*146 µl*	*150 µl*	*154 µl*
Culture duration	*46 h*	*48 h*	*50 h*
Colcemid concentration	*48 µl*	*50 µl*	*52 µl*
Temperature	*36°C*	*37°C*	*38°C*
Blood volume	*0.4 ml*	*0.5 ml*	*0.6 ml*
Medium volume	*4.75 ml*	*5 ml*	*5.25 ml*

The different values presented in table 3 were experimentally combined in 12 lymphocytes cultures. Six samples were cultivated according to normal values (Voisin et al., 2001). First, the mitotic index was calculated as the ratio of the number of mitosis divided by the number of lymphocyte nuclei. Second, dicentrics were scored in 500 cells.
The operator effect was tested on the six experiments performed according to the standard protocol used in the lab. No operator effect was measured on these points.

The results of the mitotic index and dicentrics yields are presented in figure 5 and figure 6, respectively.

From the statistical analysis of the results presented in figure 5, five parameters have an impact on the mitotic index: BUdr concentration, medium volume, blood volume, duration of the culture and incubator temperature. Therefore to have a good cell culture quality those parameters need to be controlled.
Further was measured the impact of those parameters on the yield of dicentrics. The statistical analysis of the results doesn't point out any significant effect.

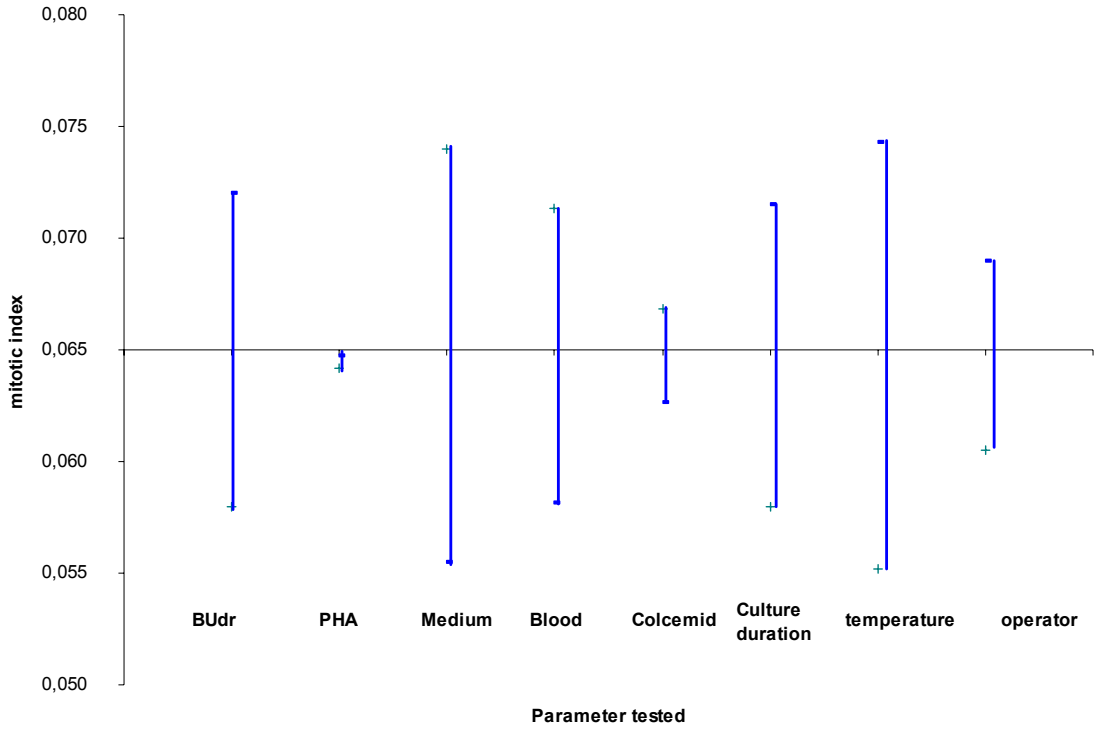

Figure 5: Result of the mitotic index measurement for the different parameters tested. The horizontal line represents the mean of all the data. Vertical lines represent the gap between the mitotic index obtained with value 1 and value 2.

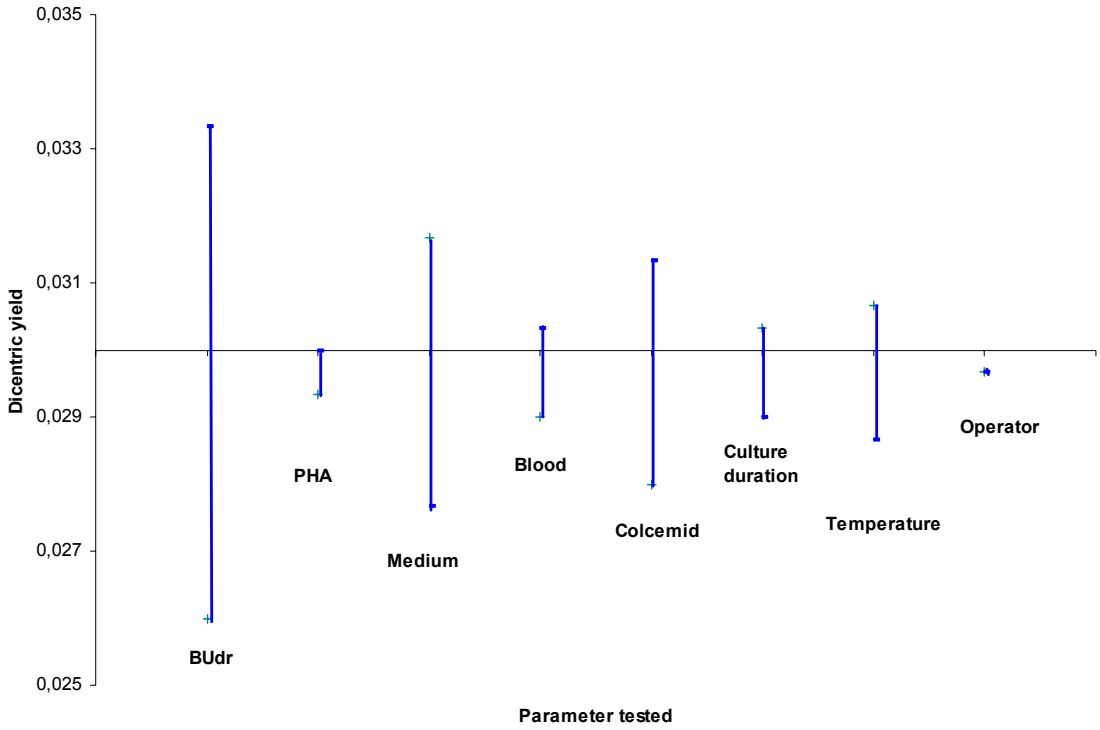

Figure 6 : Results of the yield of dicentrics measurement for the different parameters tested. The horizontal line represents the mean of all the data. Vertical lines represent the gap between the yield of dicentrics measured for value 1 and value 2.

6. Conclusion

While practiced in many countries, the biological dosimetry by cytogenetics is not completely standardized so far, so that it is required specific validation steps. All the uncertainties of the different steps of the assay have been evaluated in this study.

Such a quality management process needs time as habits must be changed. In fact, new process needs to be well integrated to be correctly applied. Then it is essential that each staff member shall be associated to each standardization step.

While this process has required a lot of work from the team, we have noticed an improvement for many steps, such as identification of reagents, products and samples. However, implementing such a quality management system usually produced many documents which needed to be filled in regularly. It is required not to set up a too heavy system in order to keep flexibility enough.

According to last internal audits, we have done most of the job. Then we expect to be accredited by the French accreditation organism by the end of 2006.

7. References

DOGGETT, N. A. and MCKENZIE, W. H. (1983) An analysis of the distribution and dose response of chromosome aberrations in human lymphocytes

after in vitro exposure to 137 cesium gamma radiation. Radiat. Environ. Biophys., 22, 33-51.

INTERNATIONAL ATOMIC ENERGY AGENCY (2001) Cytogenetic analysis for radiation dose assessment: a manual, 405 Vienna, IAEA.

PAPWORTH, D. (1975) Curve fitting by maximum likelihood. Appendix to paper by JRK Savage: Radiation-induced chromosomal aberrations in plant Tradescantia: Dose response curves. Radiation Botanics, 15, 127-131.

VOISIN, P., BENDERITTER, M., CLARAZ, M., CHAMBRETTE, V., SOROKINE-DURM, I., DELBOS, M., DURAND, V., LEROY, A. & PAILLOLE, N. (2001) The cytogenetic dosimetry of recent accidental overexposure. Cellular and Molecular Biology, 47, 557-564.