

This article was downloaded by: [EBSCOHost EJS Content Distribution]

On: 11 August 2008

Access details: Access Details: [subscription number 768320842]

Publisher Informa Healthcare

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



International Journal of Radiation Biology

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title-content=t713697337>

A comparison of different cytological stains for biological dosimetry

Thiago Salazar Fernandes ^a; David Lloyd ^b; Ademir Amaral ^a

^a Grupo de Estudos em Radioproteção e Radioecologia (GERAR), Universidade Federal de Pernambuco, Centro de Tecnologia e Geociências/Departamento de Energia Nuclear, Cidade Universitária, Pernambuco, Brazil ^b Health Protection Agency/Radiation Protection Division, Chilton, Oxfordshire, UK

Online Publication Date: 01 January 2008

To cite this Article Fernandes, Thiago Salazar, Lloyd, David and Amaral, Ademir(2008)'A comparison of different cytological stains for biological dosimetry',International Journal of Radiation Biology,84:8,703 — 711

To link to this Article: DOI: 10.1080/09553000802241770

URL: <http://dx.doi.org/10.1080/09553000802241770>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

A comparison of different cytological stains for biological dosimetry

THIAGO SALAZAR FERNANDES¹, DAVID LLOYD², & ADEMIR AMARAL¹

¹Grupo de Estudos em Radioproteção e Radioecologia (GERAR), Universidade Federal de Pernambuco, Centro de Tecnologia e Geociências/Departamento de Energia Nuclear, Cidade Universitária, Pernambuco, Brazil, and ²Health Protection Agency/Radiation Protection Division, Chilton, Didcot, Oxfordshire, UK

(Received 27 November 2007; revised 9 May 2008; accepted 21 May 2008)

Abstract

Purpose: This paper examines the relative accuracy of analysis of unstable chromosomal aberrations (dicentric, rings and fragments) in lymphocyte metaphases using four microscope slide staining options, widely used to assess radiation overdose or to survey occupationally exposed subjects.

Materials and methods: Peripheral blood lymphocytes from a healthy donor were irradiated with 1.5 and 3.0 Gy of X-rays at a dose rate of 0.715 Gy/min. Dicentrics were scored by different cytological stains in order to compare block staining: Giemsa and 4', 6-Diamidino-2'-phenylindole dihydrochloride (DAPI); with techniques that highlight centromeres: C-banding and Centromere Multiplex Fluorescence *in situ* Hybridization (CM-FISH).

Results: At each of the two doses, the values for dicentrics per cell observed with each staining method were compared. In terms of dose estimation, no statistical difference was observed between the evaluated methods (χ^2 p: 0.27 and 0.64, respectively; analysis of variance – ANOVA, $p > 0.99$). Therefore, the evidence of centromeres by C-banding and CM-FISH did not promote an increased discovery of dicentrics. On the other hand, when confirmation of unequivocal identification of dicentrics is needed, C-banding and CM-FISH can be a suitable method to confirm its presence. Economical and social factors must be taken into account in the decision of method as well.

Conclusion: For routine use where several hundreds of cells need to be reliably processed and analyzed daily, processing slides by block staining with Giemsa and DAPI is preferable. However, to assist in resolving the minority of images that are ambiguous, C-banding and CM-FISH provide a better identification of suspected dicentrics.

Keywords: Cytological stains, biological dosimetry, FISH, C-banding, DAPI, Giemsa

Introduction

The estimation of absorbed dose based on bioindicators is an important tool in the investigation of suspected or actual exposure of persons to ionizing radiation. It is particularly valuable in radiological or nuclear emergencies where subjects, such as members of the public, are not wearing conventional physical dosimeters. In such cases, physical dosimetry is not straightforward and the dose evaluation based on bioindicators, so-called biodosimetry, can often provide the necessary information. This information can assist clinicians preparing to treat patients displaying the effects of high dose exposures (International Atomic Energy Agency [IAEA] 2001).

It is also informative for dealing with persons receiving low dose exposures which is likely to be the

majority of people involved in most emergency scenarios. Here, the need is for reliable dosimetric information that can form the basis for counselling persons about risks of later arising stochastic disease. These people generally have exaggerated fears for their risk of radiation-induced cancer (Lloyd 1998, IAEA 2001).

The analysis of unstable chromosome aberrations (dicentric, fragments and rings) in metaphases of cultured peripheral blood lymphocytes is a long established method of biological dosimetry for ionizing radiation (IAEA 2001, Amaral 2002).

The dicentric chromosomes characterized by possessing two centromeres instead of the single one that is borne on normal chromosomes, has been described as the 'gold standard' for biological dosimetry. There is an extensive literature illustrating

how the method can confirm or refute suspected cases of overexposure and, where confirmed, provide an estimate of dose quite independent of physical and mathematical methods (Lloyd 1997, 1998, Lloyd et al. 2000, IAEA 2001).

The radiation dose is derived by comparing the frequency of dicentrics in lymphocyte metaphases from the irradiated subject with an *in vitro* dose response curve produced in the same laboratory with a comparable quality of radiation. Since its beginnings in the mid-1960s, the method has been extensively refined and calibrated so that it now occupies a significant place in the radiological protection programmes of many countries and, in some, has been given special forensic status (Bender 1964, Voisin et al. 2001, 2002).

The most commonly used stain for aberration scoring and for obtaining the calibration curves is Giemsa; either as a block stain or in the differential 'harlequin' (fluorescence plus Giemsa – FPG) mode to distinguish the cell's *in vitro* cycling history. Giemsa stain requires simple bright field microscopy. Other block stains may be used such as DAPI (4', 6-Diamidino-2'-phenylindole dihydrochloride) or PI (propidium iodide) combined with UV (ultraviolet) fluorescence microscopy.

Block staining requires the distinction of centromeres as constrictions of the chromosomes, or possibly by a different intensity of stain uptake compared with the chromosome arms. This can lead to interpretational problems when, for example, chromosomes bear secondary constrictions or are acrocentric with satellite association and perhaps with widely separated arms beginning to enter anaphase. Also a dicentric with two juxtapositioned centromeres may be difficult to distinguish from a simple monocentric chromosome. This is why it is often remarked that the assay requires experienced skilled microscopists (Kanda & Hayata 1996, Roy et al. 1996).

Intuitively, staining methods that permit preferential staining of the critical centromeric regions such as C-banding should enhance the discrimination of centromeres and it may therefore be possible to identify dicentrics more positively, eliminating some of the scorer error. However, some options may cause concomitant swelling of chromosomes which distort the optical resolution of the chromosome structure (Prosser 1975, Fernandes et al. 2006).

This paper describes the use of two block stains, Giemsa and 4', 6-Diamidino-2'-phenylindole dihydrochloride (DAPI), and two centromere highlighting methods, C-banding and Centromeric Multiplex Fluorescence *in situ* Hybridization (CM-FISH) on replicate slides, for the visualization of dicentric chromosomes for biological dosimetry, pointing out the advantages and limitations of each method, and

compares the resultant estimates of absorbed doses with known doses given to *in vitro* irradiated human lymphocytes.

Materials and methods

Irradiation and lymphocyte cultures

Heparinized peripheral blood samples were obtained from a healthy male non-smoker donor and exposed to 1.5 and 3.0 Gy of 250 kVp X-rays, at a dose rate of 0.715 Gy/min and 37°C. The radiation beam filtration and exposure geometry were the same as that employed in the same laboratory for constructing its *in vitro* calibration curve for biological dosimetry (Lloyd & Edwards 1983).

The irradiated blood samples were kept at 37°C for 2 h to allow biological repair process, and then placed into culture (Gumrich et al. 1986). The culturing, fixation and slide making followed a standard procedure as described in an IAEA (2001) Manual. In brief, the culture medium used was Eagle's Minimum Essential Medium (MEM) with Earle's Salts (Gibco, USA) supplemented with penicillin (10,000 units/ml), streptomycin (10,000 µg/ml), heparin (2,000 units/ml), L-glutamin (200 mM), 5-bromodeoxyuridine (20 µM), 20% foetal bovine serum and phytohaemagglutinin (reconstituted in 10 ml of sterile analytical grade water).

Each replicate culture comprised 0.3 ml of irradiated blood added to 4 ml of culture medium. They were incubated at 37°C for 48 h with Colcemid (25 µg/ml in 0.85% saline) added at 45 h. The cells were then harvested and fixed by the routine hypotonic potassium chloride/methanol: acetic acid method. Replicate fixed cultures at each radiation dose were pooled into a common stock and two drops of pelleted fixed cell suspension placed on each slide and allowed to dry. All replicate slides used in the study were prepared together in the same conditions of room temperature and humidity.

Fluorescence plus Giemsa (FPG)

This method followed the protocol given in IAEA (2001). In brief, bromodeoxyuridine (BrdU) was included in the cultures in order to permit fluorescence plus Giemsa (FPG) staining. This thymine analogue is taken up preferentially into replicating DNA. When one chromatid is bifilarly and the other one unifilarly substituted, FPG staining produces a 'harlequin' effect in the metaphase chromosome of cells which are in their second or later post-substitution division. Aberrations are normally scored in guaranteed first division metaphases, i.e., those where the chromosomes stain uniformly.

DAPI (4', 6-Diamidine-2'-phenylindole dihydrochloride)

Air-dried slides were mounted with 27 μ l of 400 ng/ml of DAPI in Vectashield antifade mountant that consist of 500 μ l Vectashield plus 2 μ l of DAPI stock 100 μ g/ml.

CM-FISH (Centromeric Multiplex FISH with DAPI counterstained)

This method was adapted from two published protocols: Finnon et al. (1995) and Hone et al. (2005). In essence, a human pan-centromere probe (Cambio, UK) was hybridized onto the metaphases. The fluorochrome Texas Red was then added by the immunological avidin/biotinylated anti-avidin procedure. The metaphases were then counterstained with DAPI.

C-banding

This technique was adapted from two published protocols: Prosser (1975) and Fernandes et al. (2006). Basically, three-day-old slides were placed in hydrochloric acid 0.2 N at room temperature for 30 min and then washed three times in distilled water. Next, they were incubated in barium hydroxide 5% at 60°C for 1 min, washed for 2 min each in 0.2 N HCl and distilled water, then 2 \times SSC solution at 60°C for 45 min and finally in distilled water. Air-dried slides were stained with a solution of Giemsa 2% in phosphate buffer pH 6.8 for 10 min. The stain intensity was checked and if insufficient the slides were re-immersed in Giemsa for a further 5–10 min.

Aberration scoring

The microscopy was carried out by one technician on coded slides. Strict scoring criteria were employed based on those recommended in IAEA (2001). Metaphases were required to be complete, i.e., to contain 46 centromeres. Thus from the examination of all chromosomes in the spread acentric fragments were either classed as excess acentrics or associated with dicentrics or centric rings. It was verified from the FPG material that the frequency of second cycle metaphases was acceptably low (<10%) in the material from both radiation doses (1.5 and 3.0 Gy). The M2 cells were ignored during scoring with FPG. Chromosome Y was not misidentified as a fragment because of the clear demarcation of its heterochromatic region by both C-banding and CM-FISH. The Giemsa and C-banding assays were carried out with a bright field microscope and the DAPI and CM-FISH assays

with a UV fluorescence microscope. With the latter, a DAPI filter was used for both assays to observe the DAPI signal alone and for CM-FISH a triple pass filter also allowed the blue DAPI and red centromeres to be observed together. The images were captured in the magnification of 100 \times using the MetaSystem Karyotyping and FISH Imaging Isis software (Germany). For each staining method, 600 metaphases were scored from the blood samples irradiated to 1.5 Gy and 160 metaphases for 3.0 Gy.

Dose estimation

Doses were estimated from each experimental point for comparison with the given doses (1.5 or 3.0 Gy). For this, the dicentric frequencies observed at the two doses and by the four staining methods were referred to a linear quadratic dose response curve ($Y = 0.001 + 0.04D + 0.06D^2$), not including standard deviations, previously produced in the same laboratory using the same X-ray source and geometry, the same lymphocyte culture method and stained by FPG.

The u-test and ratio of variance to mean were calculated for each method in order to verify that the dicentric frequencies followed a Poisson distribution. Chi squared testing was used to check for homogeneity of the data at each dose. Analysis of variance (ANOVA) was carried out in order to test for differences between the methods at each dose.

Results

Figure 1 illustrates how the addition of centromere highlighting by CM-FISH (A and B) and in a different metaphase by C-banding (C and D) can enhance the identification of a dicentric, particularly where, as in these examples, the two centromeres are close together (Meyne et al. 1989, Finnon et al. 1995, Schmid et al. 1995, Terzoudi & Pantelias 2006). The juxtaposed centromeres are almost indistinguishable, but when the same metaphase was analyzed with the two centromeres highlighted or as dark bands a dicentric chromosome could be seen.

The analysis of such highlighted spots on the same chromosome, or two dark bands in the case of C-banding, should not only aid scoring by eye using block staining methods, such as Giemsa and DAPI, but also improve detection when using computer assisted image analysis systems.

Chromosomes, particularly those in the B group, can sometimes contain a secondary constriction or a band between sister chromatids giving the appearance of a second centromere. Figure 2 shows two chromosomes, one in (A) in a metaphase stained by DAPI and another in (C) stained with Giemsa,

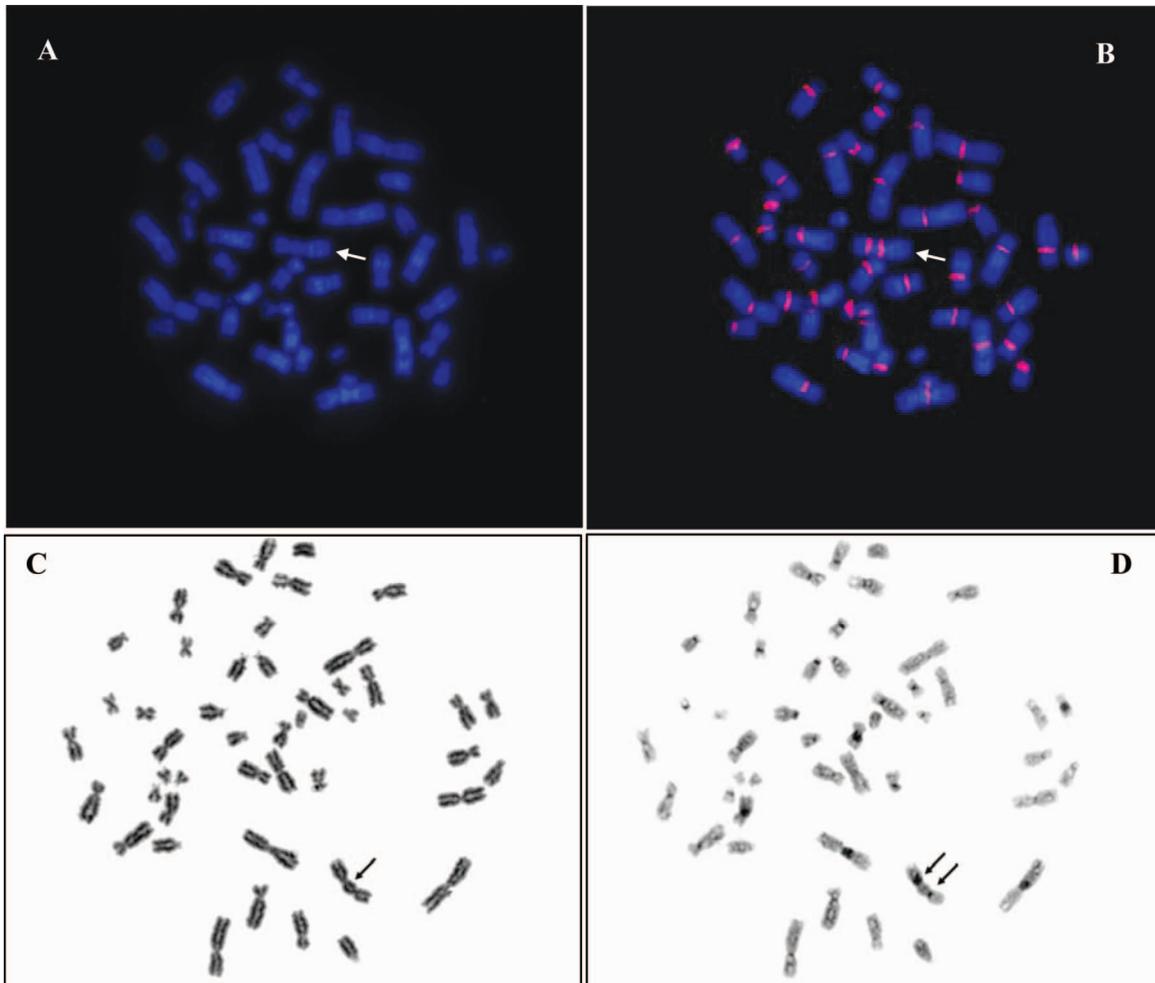


Figure 1. Confirmation of dicentrics by techniques that highlight the centromeres on optical microscope (magnification $100\times$). (A) A metaphase containing a possible dicentric (arrow) observed by DAPI staining and (B) its confirmation by CM-FISH. (C) Other metaphase containing a suspected dicentric (arrow) analyzed by Giemsa staining and (D) its confirmation by C-banding.

which seem to have two centromeres. When examined with CM-FISH (B) and C-banding (D), respectively, they transpired to be monocentric.

Figure 3 illustrates another situation where two chromosomes are linearly aligned and touching end-on and giving the appearance of a dicentric in a metaphase analysed by DAPI (A) or very close to each other in other observed by Giemsa (C). Here, however, their resolution as two separate objects is easier in the DAPI stained and Giemsa preparation without centromere highlighting (B) or in the case of C-banding (D), where it can be observed a satellite association between two acrocentric chromosomes giving to it the appearance of a dicentric.

In this example, if analysis had proceeded straight to the highlighted image there would have been an increased chance of a false-positive dicentric having been recorded. The same would also apply to cases of overlapping chromosomes and this experience reinforces the recommendation that block staining methods and centromeric highlighting should be

applied together, the latter as a confirmatory test of the first. The same conclusion was reached by Prosser (1975) and elsewhere by Fernandes et al. (2006) using only C-banding and Giemsa staining.

At each of the two doses, the values for dicentrics per cell observed with each staining method were compared. The results of scoring unstable chromosome aberrations (dicentrics, centric rings and acentric fragments), and the doses estimated using dicentric frequencies for each method, are presented in Tables I and II. For better comparison of these results, the dicentric frequencies are also presented in Figure 4. The errors presented are based on the scoring statistics alone and the relatively minor contribution from the curve is ignored. This is the so-called 'simplified approach C' in the IAEA Manual (IAEA 2001).

Discussion

The results in both Tables I and II show that there is an overall good agreement between the estimated

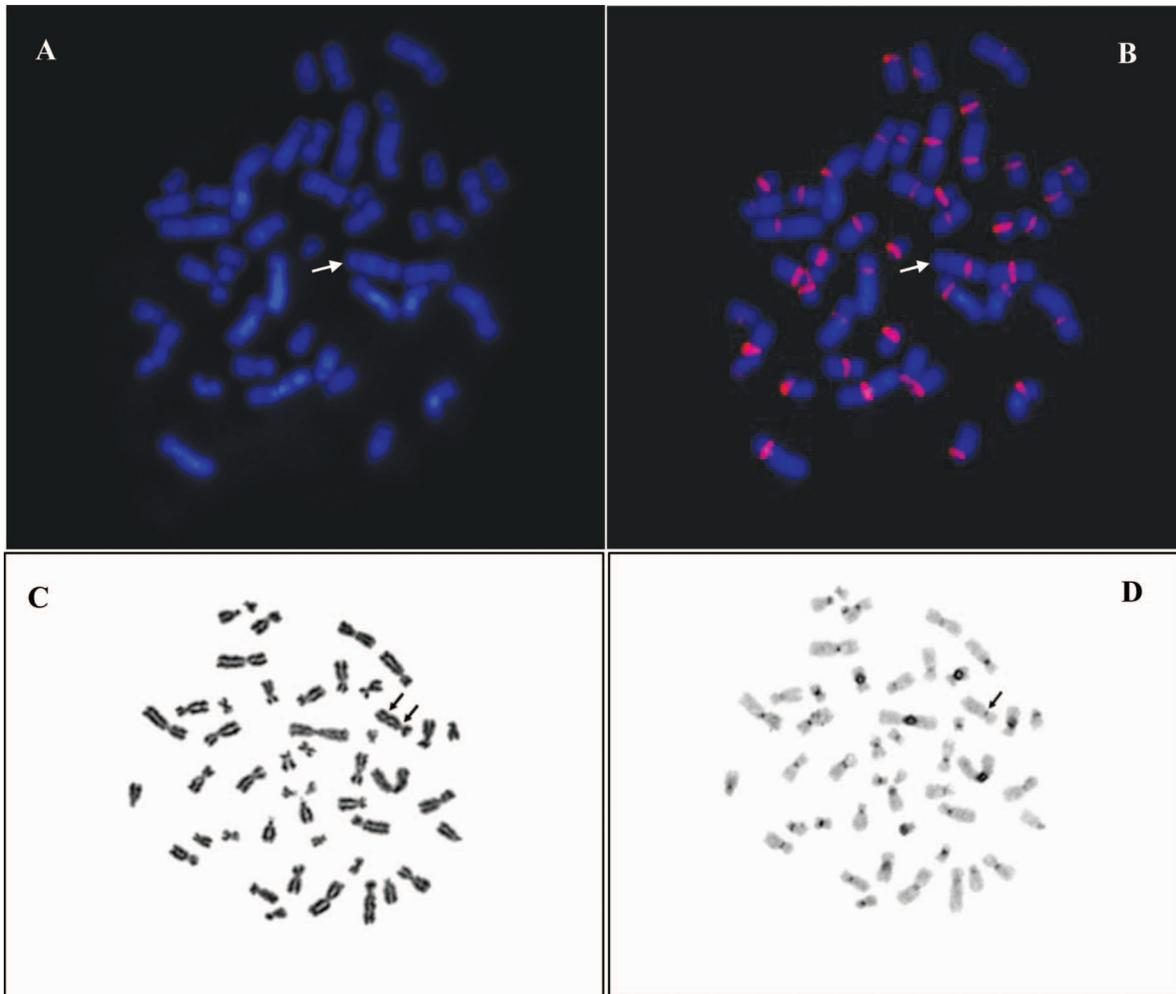


Figure 2. Confirmation of monocentric chromosomes on optical microscope (magnification $100\times$). (A) A metaphase containing a possible dicentric (arrow) observed by DAPI and (B) its confirmation as monocentric with CM-FISH. (C) Other metaphase containing a suspected dicentric (arrow) analysed by Giemsa and (D) its confirmation as monocentric with C-banding.

absorbed doses using each method and the actual ones given to the blood samples. The actual dose is within the 95% confidence limits ($2 \times \text{SE}$) of the estimated values for all staining methods. The u-tests and ratios of variance to mean indicate that, in most cases, the dicentric distribution was Poissonian as expected for X-rays (Edwards et al. 1979).

There is some indication of under-dispersion with the C-banding method at both 1.5 and 3 Gy. This might indicate that the C-banding method is less efficient at detecting cells with more than one dicentric than the other methods. This can be explained by the fact that with barium hydroxide treatment the quality of chromosome staining becomes inferior and some metaphases are disrupted or are with dirty of barium crystals on the slides, most of them impeding the total visualization of metaphases.

Other additional explanation is that C-banding stains heavily centromeres of 1, 9, 16 and entire Y

chromosome, thus, some dicentrics involving the others chromosomes can be mis-scored. Furthermore, scorer variation can interfere in the scoring of more than one dicentric per cell by C-banding, for subjective reasons. Nevertheless, estimated dose results are close to the estimates using the other three methods indicating that C-banding is reliable method for biological dosimetry.

The method which yielded a dose estimate closest to 1.5 Gy (Table I) was CM-FISH. It was a small improvement over DAPI alone and Giemsa and this is perhaps not surprising as the specific highlighting allows recognition of a fraction of less obvious centromeres. CM-FISH produced a dose estimate essentially the same as obtained with C-banding, but also the contribution of false-positive dicentrics must be considered here for the higher yield of dicentrics scored by these methods.

However this trend is not so obvious at 3.0 Gy (Table II) where Giemsa staining resulted in the

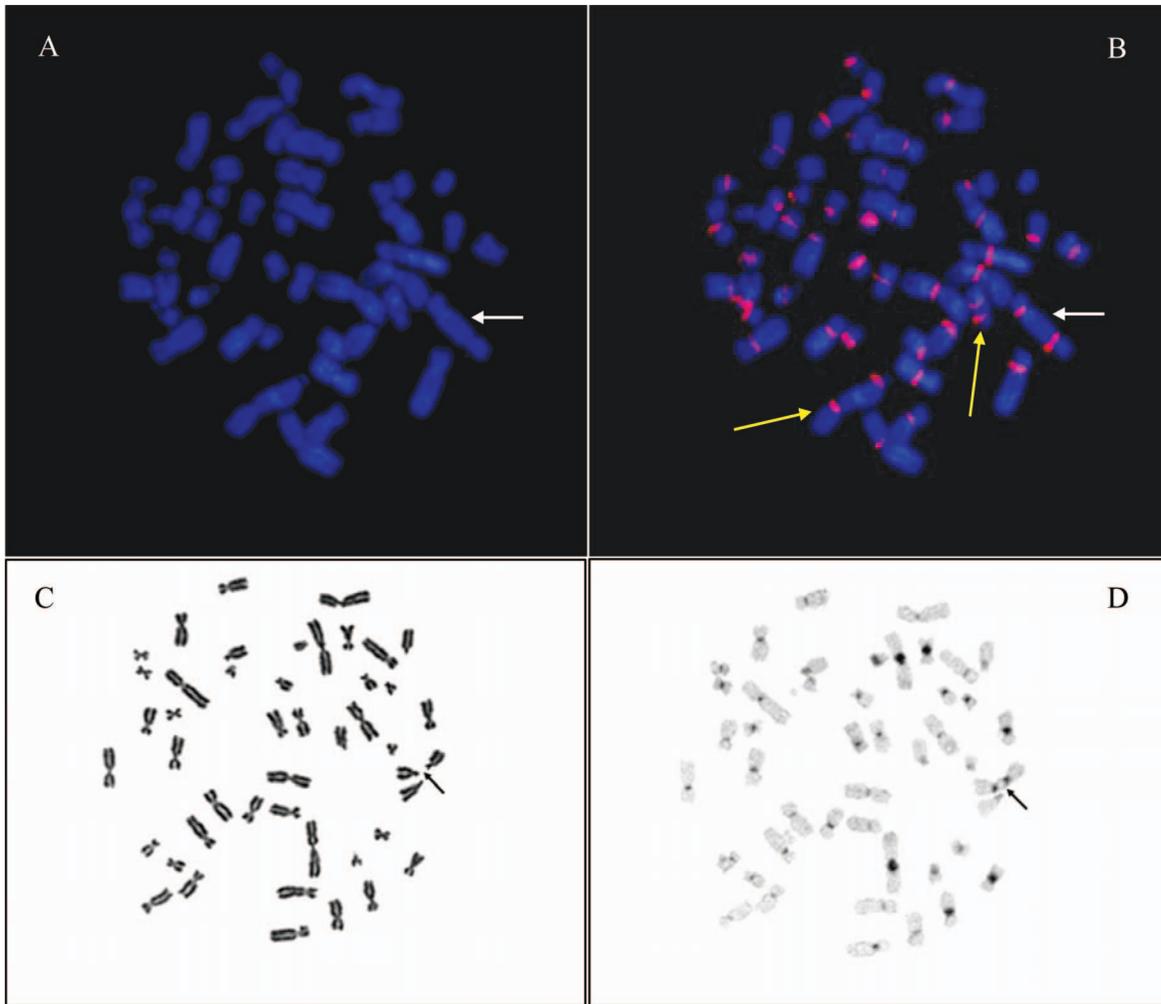


Figure 3. Presence of false-positive dicentrics by block staining methods on optical microscope (magnification 100 \times). (A) A possible dicentric (arrow) observed with DAPI and (B) its confirmation with CM-FISH. The CM-FISH staining also shows two examples of false-positive dicentrics. (C) Other metaphase with two separate acrocentric chromosomes (arrow) observed by Giemsa and (D) a satellite association between them giving the appearance of dicentric (false-positive) after C-banding.

Table I. The aberration yields scored with various staining methods, dicentric distributions and tests for their conformity with Poisson and estimated doses with standard errors and 95% confidence limits from cells irradiated with 1.5 Gy.

Aberrations in 600 cells					Dicentric distribution					Var/mean	u-test	Estimated dose (Gy)
	Method	Dicentrics	Centric rings	Acentric fragments	Dicentric yield	0	1	2	3			
Giemsa	105	4	53	$0.175 \pm 0.016^*$	503	89	8	0	0	0.979 ± 0.058	-0.365	1.40 ± 0.08 (1.24 - 1.57)
DAPI	102	3	62	$0.170 \pm 0.016^*$	507	84	9	0	0	1.010 ± 0.057	0.142	1.37 ± 0.08 (1.22 - 1.55)
C-band	125	7	52	$0.208 \pm 0.017^*$	480	115	5	0	0	0.873 ± 0.058	-2.200	1.55 ± 0.08 (1.40 - 1.73)
FISH	124	5	77	$0.207 \pm 0.017^*$	482	112	6	0	0	0.892 ± 0.058	-1.880	1.54 ± 0.08 (1.39 - 1.72)

*The errors on the dicentric yield indicate the Poisson standard error in the mean of 600 cells.

estimate closest to the true dose. It is interesting to note that all staining methods at 3.0 Gy tended to underestimate the dose but the upper confidence limits nevertheless extend to over 3.0 Gy. Overall, there were no statistical differences between the estimated doses at 1.5 or 3.0 Gy (χ^2 p: 0.27 and 0.64,

respectively; analysis of variance - ANOVA $p > 0.99$).

Cytological staining methods that highlight centromeres such as C-banding and Centromeric-Multiplex FISH technique can be an important tool in cytogenetic dosimetry for identifying more easily

Table II. The aberration yields scored with various staining methods, dicentric distributions and tests for their conformity with Poisson and estimated doses with standard errors and 95% confidence limits from cells irradiated with 3.0 Gy.

Method	Aberrations in 160 cells			Dicentric yield	Dicentric distribution					Var/mean	u-test	Estimated dose (Gy)*
	Dicentrics	Centric rings	Acentric fragments		0	1	2	3	4			
Giemsa	102	9	41	$0.637 \pm 0.038^*$	81	61	14	3	1	0.937 ± 0.112	-0.565	2.94 ± 0.16 (2.63 – 3.27)
DAPI	86	3	39	$0.537 \pm 0.039^*$	88	61	9	1	1	0.887 ± 0.112	-1.020	2.67 ± 0.16 (2.36 – 3.01)
C-band	94	8	18	$0.588 \pm 0.039^*$	74	78	8	0	0	0.586 ± 0.112	-3.710	2.81 ± 0.16 (2.50 – 3.14)
FISH	88	6	21	$0.55 \pm 0.039^*$	90	53	16	1	0	0.887 ± 0.112	-1.010	2.70 ± 0.16 (2.40 – 3.04)

*The errors on the dicentric yield indicate the Poisson standard error in the mean of 160 cells.

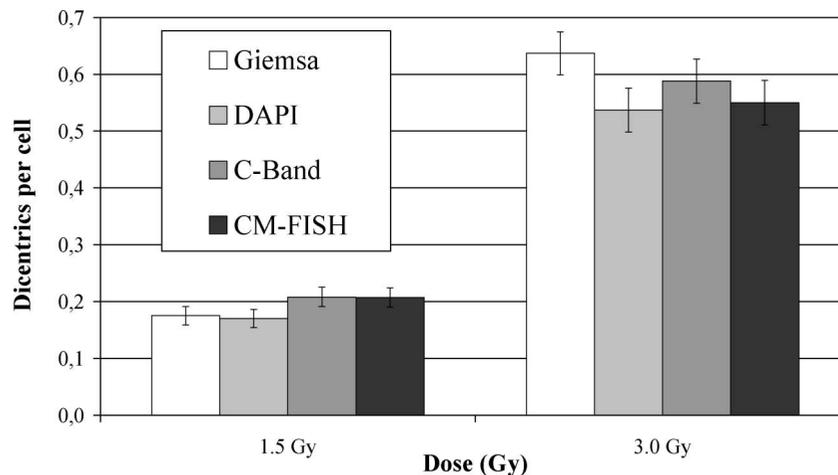


Figure 4. Frequencies \pm SE (standard error) of dicentrics scored at each dose by the four staining methods.

the asymmetrical chromosome aberrations, such as dicentrics (Meyne et al. 1989). On the other hand, this method still has risk of mis-scoring due to loss of resolution associated with fluorescence compared with bright-field staining (Bauchinger et al. 1993, Kanda & Hayata 1996, Roy et al. 1996, Henegariu et al. 2001).

The quality of the chromosome images can be affected by the procedures used for C-banding because the chromosomes may become swollen after barium hydroxide treatment and a proportion of metaphases are disrupted (Prosser 1975). This swelling may make it difficult to resolve individual chromosomes where, for example, acrocentric chromosomes tend to be arranged with satellite association in metaphase spreads (Fernandes et al. 2006).

Table III resumes the ambiguous images and the perceived advantage of combining block staining with centromere highlighting with either fluorescence or bright field microscopy. The possibility in combining DAPI staining with centromeric probes, and the ability to C-band slides previously stained with Giemsa, raises the possibility of re-examining aberrations when identification is uncertain providing

confirmation in cases of doubts. It must be considered here the personal-dependence or scorer variation, in addition to technical factors, which may influence precise aberration identification for subjective reasons.

On the other hand, most of the scorer errors can be solved by using the MetaSystem Karyotyping and FISH Imaging Isis software (Germany), which provides a variety of tools to enhance, edit, annotate, archive, measure, and print the images.

Moreover, supplementary evidence of acentric fragments may draw attention to the presence of dicentrics or provide confirmation in cases of doubt, independently of the staining method.

In addition to scoring accuracy of the various methods there are other factors, such as cost of reagents and overall slide processing time that have to be considered when undertaking biodosimetry investigations. Processing by CM-FISH is not only the most expensive for reagents but also requires about 72 h to perform the method (Finnon et al. 1995, Hone et al. 2005); whilst Giemsa or DAPI staining takes just a few minutes. C-banding is intermediate at about 3 h. Although dicentrics were readily identified in C-banded and CM-FISH

preparations, scoring speeds were no quicker than with normally stained material.

Table IV summarizes these factors. Where the screening of several persons is required conventional block staining (Giemsa) is quite adequate when employing the dicentric analysis in a rapid response triage mode (Lloyd et al. 2000) or where following a serious overexposure biodosimetry is primarily being undertaken to advise clinicians of the magnitude of likely tissue and organ injuries that will need active management. Moreover, initial biological dose estimates need not be very precise when highly irradiated persons have already been identified by the symptoms of the acute radiation syndrome (Lloyd 1997).

However, in situations where confirmation of suspected dicentrics is vital, the ability of re-examine material with C-banding or CM-FISH may be an advantage, especially because every single aberration is important in order to achieve good statistics and each dicentric contributes significantly to the overall estimate of the absorbed dose. Then it is recommended that centromere highlighting techniques such as C-banding and CM-FISH could be used as a confirmatory test for the identification of dicentrics in such cases. On the other hand, costs are relevant for social and economic reasons, depending on the financial resources from the country or laboratory. This is particularly crucial when a great number of samples should be analyzed.

The present research was performed with blood samples from just one donor in order to evaluate

whether the cytological stain method influences per se in the identification and scoring of unstable chromosome aberrations. Despite a degree of variability in individual radiosensitivity, this parameter was not considered here once the experience from biological dosimetry would suggest that such variability would have a little influence on this practical application (IAEA 2001, Hone et al. 2005). In this context, independent of the number of studied subjects, it can be expected that the performance here obtained for each investigated method will be the same as for one donor.

Conclusions

The comparison among Giemsa, C-banding, DAPI and CM-FISH in the analysis of unstable aberrations, especially dicentrics, has shown no statistical difference between the yields of dicentrics or doses estimated using all these methods. However, these centromere highlighting methods (C-banding and CM-FISH) allows a more precise detection of dicentrics and provide confirmation in cases of doubt resolving unequivocal identification of suspected dicentrics. This suggests the use of centromere painting and C-banding as a complementary method in biological dosimetry where each dicentric makes a significant contribution to the overall dose estimate. However, for routine use, standard block staining methods still appear preferable because of its less cost and time for slide preparation.

Acknowledgements

The authors would like to thank the Health Protection Agency (HPA), UK, especially Alan Edwards, Jayne Moquet, Pat Hone and Liz Ainsbury, for the contribution in this research; and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brazil) for supporting this work.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- Amaral A. 2002. Trends in biological dosimetry: An overview. *Brazilian Archives of Biology and Technology* 45:119–124.
- Bauchinger M, Schmid E, Zitzelsberger E, Braselmann H, Nahrstedt U. 1993. Radiation-induced chromosome aberrations analysed by two-colour fluorescence *in situ* hybridization with composite whole chromosome-specific DNA probes and a pancentromeric DNA probe. *International Journal of Radiation Biology* 64:179–184.

Table III. Causes of ambiguity and its resolution by the different methods (Giemsa, DAPI, C-banding and FISH).

Ambiguous images	Interpretation of ambiguous images (is it a dicentric?)	
	By Giemsa or DAPI	By C-banding or CM-FISH
Chromatids overlap	Doubt	Negative
Satellite association	Not seen	False-positive
Secondary constrictions	Doubt	Negative
Possible dicentric	Doubt	Positive or negative

Table IV. A summary of the relative time, cost and accuracy of the four methods.

Method	Parameters		
	Slide processing time	Costs	Accuracy
Giemsa	Few minutes	Low	Good
C-banding	3 hours	Low	High
DAPI	Few minutes	Medium	Good
FISH	72 hours	High	High

- Bender MA. 1964. Chromosome aberrations in irradiated human subjects. *Annual New York Academy of Science* 114: 249–251.
- Edwards AA, Lloyd DC, Purrott, RJ. 1979. Radiation induced chromosome aberrations and the Poisson distribution. *Radiation and Environmental Biophysics* 16:89–100.
- Fernandes TS, Amaral A, Cavalcanti MB, Braga LR, Melo RA. 2006. Unstable chromosome aberrations and micronuclei analyses in the biomonitoring of workers occupationally exposed to ionizing radiation. *International Journal of Low Radiation* 3:299–309.
- Finnon P, Lloyd DC, Edwards AA. 1995. Fluorescence *in situ* hybridization detection of chromosomal aberrations in human lymphocytes: Applicability to biological dosimetry. *International Journal of Radiation Biology* 68:429–435.
- Gumrich K, Virsik-Peuckert, Harder D. 1986. Temperature and the formation of radiation-induced chromosome aberrations. I. The effect of irradiation temperature. *International Journal of Radiation Biology* 49:665–672.
- Henegariu O, Heerema NA, Wright LL, Bray-Ward P, Ward DC, Vance GH. 2001. Improvements in cytogenetic slide preparation: Controlled chromosome spreading, chemical aging and gradual denaturing. *Cytometry* 43:101–109.
- Hone PA, Edwards AA, Lloyd DC, Moquet JE. 2005. The yield of radiation-induced chromosomal aberrations in first division human lymphocytes depends on the culture time. *International Journal of Radiation Biology* 81:523–529.
- International Atomic Energy Agency (IAEA). 2001. Cytogenetic analysis for radiation dose assessment. A manual. Tech Rept. 405. Vienna: IAEA.
- Kanda R, Hayata I. 1996. Comparison of the yields of translocations and dicentric chromosomes measured using conventional Giemsa staining and chromosome painting. *International Journal of Radiation Biology* 69:701–705.
- Lloyd DC, Edwards AA. 1983. Chromosome aberrations in human lymphocytes: Effect of radiation quality, dose and dose rate. *Radiation-Induced Chromosome Damage in Man* 23–49.
- Lloyd DC. 1997. Chromosomal analysis to assess radiation dose. *Stem Cells (Suppl. 2)*.
- Lloyd DC. 1998. New developments in chromosomal analysis for biological dosimetry. *Radiation Protection Dosimetry* 77: 33–36.
- Lloyd DC, Edwards AA, Moquet JE, Guerrero-Carbajal YC. 2000. The role of cytogenetics in early triage of radiation casualties. *Applied Radiation and Isotopes* 52:1107–1112.
- Meyne J, Littlefield LG, Moysis RK. 1989. Labelling of human centromeres using an alphoid DNA consensus sequence: Application to the scoring of chromosome aberrations. *Mutation Research* 226:75–79.
- Prosser JS. 1975. Identification of dicentric chromosomes in irradiated lymphocytes by C-banding. *Mutation Research* 27: 131–133.
- Roy L, Sorokine-Durm I, Voisin P. 1996. Comparison between fluorescence *in situ* hybridization and conventional cytogenetics for dicentric scoring: A first-step validation for the use of FISH in biological dosimetry. *International Journal of Radiation Biology* 70:665–669.
- Schmid E, Braselmann H, Nahrstedt U. 1995. Comparison of γ -ray induced dicentric yields in human lymphocytes measured by conventional analysis and FISH. *Mutation Research* 348: 2934–2938.
- Terzoudi GI, Pantelias GE. 2006. Cytogenetic methods for biodosimetry and risk individualisation after exposure to ionizing radiation. *Radiation Protection Dosimetry* 122:513–520.
- Voisin P, Benderitter M, Claraz V, Chambrette I, Sorokine-Durm M, Delbos V, Durand AL, Paillole N. 2001. The cytogenetic dosimetry of recent accidental overexposure. *Cellular and Molecular Biology* 47:557–565.
- Voisin P, Barquintero F, Blakely B, Lindholm C, Lloyd D, Luccioni C, Miller S, Palitti F, Prasanna PGS, Stephan G, Thierens H, Turai I, Wilkinson D, Wojcik A. 2002. Towards a standardization of biological dosimetry by cytogenetics. *Cellular and Molecular Biology* 48(5):501–504.