DICENTRIC TELESCORING AS A TOOL TO INCREASE THE BIOLOGICAL DOSIMETRY RESPONSE CAPABILITY DURING EMERGENCY SITUATION.


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ABSTRACT

Biological Dosimetry is a necessary support for national radiation protection programs and emergency response schemes. The Latin American Biological Dosimetry Network (LBDNet) has standing experience in biological dosimetry network activities with several intercomparison exercises showing almost homogeneous results. One way to increase the network response, overcoming the difficulties associated with blood or slide transport is the scoring of dicentric on a computer monitor using electronically transmitted images i.e. the dicentric telescoring. This procedure removes the difficulties associated with the dispatching and reception of “physical” samples and is probably the most immediately way of share the scoring load among laboratories working in network. Here we present the results of two exercises organised by the LBDNet to test the efficiency of such an approach. During the first exercise, the participant laboratories analysed the same images derived from cells exposed at 0.5 Gy and 3 Gy; In the second exercise an emergency situation was tested, each laboratory was required to score 50 different images in 2 days extracted from 500 downloaded images derived from cells exposed at 0.5 Gy. Then the remaining 450 images had to be scored within a week. The conclusion is that dicentric telescoring seems to be a promising technique for population triage in a large scale accident.

1. INTRODUCTION

Dosimetry evaluation has acquired a new role to guide medical treatment of victims of nuclear or radiological accidents. Dose assessment is performed not only early post-exposure by physical dosimetry calculation (scenario reconstruction) but also from evaluation of serial blood counts and the medical history (timing and severity of prodromal signs and symptoms). A medically significant dose should be subsequently confirmed or discarded by dicentric
assay, the current gold standard for biodosimetry, combined with other physical and biophysical techniques, applying a multidisciplinary approach [1].

The bottleneck in data acquisition during biological dosimetry based on dicentric assay is the need to score dicentrics in a large number of lymphocytes. In addition skilled operators are required, limiting the scoring process to a few people in specialised laboratories around the world. For this reason, dicentric scoring may be critical in a mass casualty event, resulting for malicious or accidental exposure to radiation, when the capability of the local laboratory is exceeded. This latent situation has stimulated biological dosimetry laboratories to develop tools that would help to estimate the dose under such circumstances. Three approaches are currently recommended, the triage scoring, based on a rapid scoring of 50 cells or 30 dicentrics [2-4] the use of dedicated software for metaphase finding [5-7], and the mutual assistance working in networks [8-12]

The Latin American region has standing experience in network activities. The first intercomparison exercise in the region was performed during the 90’s using the dicentric analysis and the micronucleus assay [13] and since then several activities have been performed including the most recently intercomparison exercise involving the 7 countries from the Latin American Biological Dosimetry Network (LBDNet) and 6 European countries [14]. Moreover the ShipEx-1 exercise tested the existing capabilities for safe and expeditious international transport of blood to participating laboratories in 13 countries within the LBDNet and both the IAEA Response Assistance Network and WHO BioDoseNet [15]. However, blood distribution can encounter problems due to national regulations, and slide transportation is not always a speed process. These factors may affect the efficiency of the network in an emergency situation. One way to increase the network response, overcoming the difficulties associated with blood or slide transport is the analysis of electronically transmitted images [8,12,16]. This procedure removes the difficulties associated with the dispatching and reception of “physical” samples and is probably the most immediately way of sharing the analysis among laboratories. Recently a pilot study evaluating the efficiency of Internet scoring based on dicentric frequencies has been published [12]

Here we describe an interlaboratory intercomparison among 10 partners by analysing digitized images shared by internet. The results obtained in two intercomparison exercises in which standardised methods for intercomparisons analysis have been used [17, 18] are presented. The aim of the first exercise (E1) was to test the feasibility of using electronically transmitted images, for the purpose of harmonization of scoring criteria and acceptance or rejection of metaphase images. The aim of the second exercise (E2) was to test the fast response capacity simulating a telescoring triage, and then the exercise followed by a conventional scoring.

2. MATERIAL AND METHODS

2.1 Study Design

For E1, blood was irradiated at 0.5 and 3 Gy and for each dose the same set of 100 selected images was distributed among participating laboratories. For E2, blood was irradiated at 0.5 Gy and 500 non-selected images were captured to mimic a real emergency scenario where selection would imply reduction in the speed of image uploading and distribution to the cooperating laboratories. These images were split in 10 sets containing different 50
metaphases each. To simulate the situation where only one laboratory receives the blood and asks the members of the network to respond to an accidental situation, a message was sent to all laboratories 48h before sending images. First each laboratory analysed a different set of 50 images, and then to complete the analysis of the 500 images, each laboratory analysed the remaining 9 sets.

In both exercises participant laboratories were requested to send three variables: the number of images scored; the frequency of dicentrics (or dicentric plus ring); and the dose estimated by each laboratory. Rings were registered or considered if necessary for dose estimation according to the calibration curve used in each lab. In E1, 7 participating laboratories were from the Latin American region and 3 from Europe. In E2, 9 participating laboratories were from the Latin American region (including two satellite laboratories) and 1 from Europe. From the 10 laboratories which participated in each exercise, there were 8 that participated in E1 and E2, 7 from the Latin American region and 1 from Europe.

2.2 Sample Irradiation and Blood Culture

For E1, whole blood from one volunteer was exposed at 0.5 Gy and 3 Gy with a dose rate of 0.5 Gy/min with a Caesium-137 source (IBL 637) located at the Institute of Radiation Protection and Nuclear Safety (Fontenay-aux-Roses, France). For E2, whole blood from another volunteer was exposed at 0.5 Gy of X-rays (250kV) at the University of Tuscia (Viterbo, Italy) at a dose rate of 0.3 Gy/min. For both exercises after radiation exposure, the blood was left 2 hours at 37°C. All blood samples were treated according to the standard protocol[1]

2.4 Scanning Systems

For both exercises metaphases obtained were located with a microscope Axioplan 2 Imaging (Zeiss, Oberkochen, Germany) coupled with a camera (Jai, Copenhagen, Denmark) and a motorized scanning stage (Marzhauser, Wetzlar, Germany) linked to a 2-axis stepping motor. The metaphase positions were identified automatically by the Metafer 4 software (version 3.5.101; MetaSystems, Baden-Württemberg, Germany) with a 10x objective (Zeiss). For E1 the metaphase images were automatically acquired with a 63x objective (Zeiss, software Autocapt) and exported to jpg files. Before sending them a selection was done in order to exclude metaphases with a chromosome number clearly higher or lower than 46, and metaphases in their second or further cell division. For E2, the images were manually acquired from the metaphases located by the metaphase finder. In E2 metaphases were not selected before sending them.

2.5 Communication, Image Availability and Scoring

For image transmission and communication among laboratories a Google group was created, and in each exercise the laboratory in charge of cell culture was responsible to upload different sets of images to the other members of the network.

The same criterion used for manual scoring[1] was applied to score the images. Dicentrics (or dicentrics plus ring) with their accompanying fragments were recorded in well spread complete metaphases.
2.6 Statistical Analysis

Each laboratory estimated the dose using its own calibration curve established by conventional scoring of metaphases using a transmitted light microscope. The associated uncertainties were calculated according to the IAEA procedures [1]. The data were then analysed according to both ISO 5725 [17] and ISO 13528:2005 [19]. The application of these standards to the particular case of biological dosimetry has been already presented [14], and nowadays it is recommended their use for intercomparison exercises in biological dosimetry [1]. The z score used allows to classify participant’s results as satisfactory (z < |2|) questionable (|2| < z < |3|) and unsatisfactory (z > |3|) [14,17,18].

3. RESULTS

3.1 Number of images acceptable for dicentric identification

Whatever the exercise and the dose, on average half of the images were selected by the operators as acceptable for dicentric identification as can be seen in the tables below. Large variations were observed between laboratories and for the same set of images a ratio of 4 to 6 in the number of analysable images could be found. This variability was lower in E1. The reasons for image rejection were incomplete metaphases, overlapping chromosomes, unfocused images, or chromosomes with bad shape.

3.2 Dose estimation and laboratories performance for exercise 1.

In the E1 exercise, after 0.5 Gy only one laboratory (L2) did not observe any dicentric (Table 1).

<table>
<thead>
<tr>
<th>Lab code</th>
<th>Number of scorers</th>
<th>Number of scored cells</th>
<th>Number of aberrations (Dic) or (Dic. + ring)</th>
<th>Frequency (± Poisson error)</th>
<th>Dose (Gy) [CI 95%]</th>
<th>z - value (Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>1</td>
<td>58</td>
<td>3 + 0</td>
<td>0.05 ± 0.03</td>
<td>0.74 [0.25-1.38]</td>
<td>1.72</td>
</tr>
<tr>
<td>L2</td>
<td>1</td>
<td>35</td>
<td>0 + 0</td>
<td>-</td>
<td>-</td>
<td>-3.59</td>
</tr>
<tr>
<td>L3*</td>
<td>14</td>
<td>48</td>
<td>3 + 0</td>
<td>0.06 ± 0.04</td>
<td>0.87 [0.62-1.08]</td>
<td>2.69</td>
</tr>
<tr>
<td>L4*</td>
<td>3</td>
<td>49</td>
<td>4 + 0</td>
<td>0.08 ± 0.04</td>
<td>0.92 [0.54-1.19]</td>
<td>3.00</td>
</tr>
<tr>
<td>L5</td>
<td>1</td>
<td>50</td>
<td>3 + 0</td>
<td>0.06 ± 0.03</td>
<td>0.80 [0.00-1.24]</td>
<td>2.14</td>
</tr>
<tr>
<td>L6</td>
<td>1</td>
<td>45</td>
<td>3</td>
<td>0.07 ± 0.04</td>
<td>0.84 [0.34-1.49]</td>
<td>2.43</td>
</tr>
<tr>
<td>L7*</td>
<td>2</td>
<td>44</td>
<td>2</td>
<td>0.05 ± 0.03</td>
<td>0.71 [0.00-1.09]</td>
<td>1.51</td>
</tr>
<tr>
<td>L8</td>
<td>1</td>
<td>61</td>
<td>3</td>
<td>0.05 ± 0.03</td>
<td>0.67 [0.20-1.33]</td>
<td>1.22</td>
</tr>
<tr>
<td>L9*</td>
<td>4</td>
<td>46</td>
<td>3</td>
<td>0.07 ± 0.03</td>
<td>0.97 [0.34-1.79]</td>
<td>3.38</td>
</tr>
<tr>
<td>L10</td>
<td>1</td>
<td>57</td>
<td>4 + 0</td>
<td>0.07 ± 0.03</td>
<td>0.92 [0.42-1.55]</td>
<td>3.04</td>
</tr>
</tbody>
</table>
The same laboratory was the one identifying the fewer number of cells as scorable (35 among 100). The estimated doses ranged from 0 to 0.97 Gy. According to the z score, 3 results were classified as satisfactory, 3 as questionable and 4 as unsatisfactory (Table 1).

The global coefficient of variation on the standard deviation on dose was 17.3% and the trueness on dose 37.8%. In the same exercise, E1, the results obtained after 3 Gy irradiation are indicated in Table 2. Observed frequencies of dicentrics per cell ranged from 0.32 to 0.72, and the estimated doses ranged from 1.92 and 3.45 Gy. The z score values were satisfactory for 7 reported doses and questionable for two laboratories. After 3 Gy irradiation the coefficient of variation was 14.8% and the trueness on dose 0.6%.

Table 2: Exercise 1. Results obtained from the analysis of 100 identical metaphase images from a peripheral blood sample exposed at 3 Gy.

<table>
<thead>
<tr>
<th>Lab code</th>
<th>Number of scorers</th>
<th>Number of scored cells</th>
<th>Number of aberrations (Dic) or (Dic. + ring)</th>
<th>Frequency (± Poisson error)</th>
<th>Dose (Gy) [CI 95%]</th>
<th>z - value (Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>1</td>
<td>42</td>
<td>26 + 0</td>
<td>0.62 ± 0.12</td>
<td>2.97 [2.37-3.64]</td>
<td>-0.06</td>
</tr>
<tr>
<td>L2</td>
<td>1</td>
<td>29</td>
<td>21 + 0</td>
<td>0.72 ± 0.16</td>
<td>3.41 [2.36-4.22]</td>
<td>0.93</td>
</tr>
<tr>
<td>L3*</td>
<td>13</td>
<td>41</td>
<td>26 + 0</td>
<td>0.63 ± 0.03</td>
<td>3.11 [2.72-3.45]</td>
<td>0.25</td>
</tr>
<tr>
<td>L4*</td>
<td>2</td>
<td>37</td>
<td>26 + 0</td>
<td>0.70 ± 0.02</td>
<td>2.96 [2.48-3.37]</td>
<td>-0.10</td>
</tr>
<tr>
<td>L5</td>
<td>1</td>
<td>41</td>
<td>13 + 0</td>
<td>0.32 ± 0.09</td>
<td>2.01 [1.23-2.56]</td>
<td>-2.24</td>
</tr>
<tr>
<td>L6</td>
<td>1</td>
<td>38</td>
<td>12</td>
<td>0.32 ± 0.09</td>
<td>1.92 [1.36-2.57]</td>
<td>-2.43</td>
</tr>
<tr>
<td>L7*</td>
<td>2</td>
<td>36</td>
<td>26</td>
<td>0.72 ± 0.10</td>
<td>3.45 [2.83-3.98]</td>
<td>1.02</td>
</tr>
<tr>
<td>L8</td>
<td>1</td>
<td>50</td>
<td>30</td>
<td>0.60 ± 0.11</td>
<td>3.02 [2.43-3.67]</td>
<td>0.06</td>
</tr>
<tr>
<td>L9*</td>
<td>4</td>
<td>33</td>
<td>21</td>
<td>0.64 ± 0.02</td>
<td>3.23 [2.49-4.07]</td>
<td>0.52</td>
</tr>
<tr>
<td>L10</td>
<td>1</td>
<td>53</td>
<td>31+3</td>
<td>0.64 ± 0.10</td>
<td>3.03 [2.50-3.60]</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*When several operators have done the scoring, the number of cells and the number of dic+ring presented are the mean of all scorers.

3.3 Dose estimation and laboratories performance for exercise 2.

For E2, where a blood sample was irradiated at 0.5 Gy, the first stage was to evaluate 50 images (half of the number of cells scored the E1). From these 50 cells, some scorers recorded only 7 cells and others accepted to score 46 cells (Table 3). The frequency of dicentrics per cell ranged from 0 to 0.27. The corresponding estimated doses ranged from 0 to 1.75 Gy. In this first stage (the analysis of 50 images), the z score indicated only one result as questionable and the other nine results as satisfactory. The coefficient of variation on the standard deviation on dose was 79.2 %, and the trueness 26.8 %.

When the number of images to analyse was increased up to 500 images (Table 4), the number of accepted cells to be scored ranged from 106 to 437. The dicentric frequency ranged from...
0.03 to 0.10. The z score identified only one dose as questionable. The coefficient of variation on dose was 30.3% and the trueness 22.5%.

Table 3: Exercise 2. Results obtained from the analysis of 50 different metaphase images from a peripheral blood sample exposed at 0.5Gy.

<table>
<thead>
<tr>
<th>Lab code</th>
<th>Number of scored cells</th>
<th>Number of aberrations (Dic) or (Dic. + ring)</th>
<th>Frequency (± Poisson error)</th>
<th>Dose (Gy) [CI 95%]</th>
<th>z - value (Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>46</td>
<td>2 + 0</td>
<td>0.04 ± 0.03</td>
<td>0.66 [0.15-1.43]</td>
<td>0.30</td>
</tr>
<tr>
<td>L2</td>
<td>7</td>
<td>0 + 0</td>
<td>-</td>
<td>-</td>
<td>-0.93</td>
</tr>
<tr>
<td>L4</td>
<td>23</td>
<td>2 + 0</td>
<td>0.09 ± 0.06</td>
<td>0.85 [0.00-1.47]</td>
<td>0.65</td>
</tr>
<tr>
<td>L4s</td>
<td>27</td>
<td>2 + 0</td>
<td>0.07 ± 0.05</td>
<td>0.76 [0.00-1.33]</td>
<td>0.49</td>
</tr>
<tr>
<td>L5</td>
<td>23</td>
<td>0 + 0</td>
<td>-</td>
<td>-</td>
<td>-0.93</td>
</tr>
<tr>
<td>L5s</td>
<td>33</td>
<td>1 + 0</td>
<td>0.03 ± 0.03</td>
<td>0.52 [0.00-1.00]</td>
<td>0.04</td>
</tr>
<tr>
<td>L6</td>
<td>37</td>
<td>10</td>
<td>0.27 ± 0.09</td>
<td>1.75 [1.22-2.41]</td>
<td>2.31</td>
</tr>
<tr>
<td>L7</td>
<td>11</td>
<td>1</td>
<td>0.09 ± 0.09</td>
<td>1.08 [0.00-2.29]</td>
<td>1.08</td>
</tr>
<tr>
<td>L8</td>
<td>44</td>
<td>1</td>
<td>0.02 ± 0.02</td>
<td>0.39 [0.00-1.19]</td>
<td>-0.20</td>
</tr>
<tr>
<td>L9</td>
<td>26</td>
<td>2</td>
<td>0.08 ± 0.05</td>
<td>1.07 [0.25-2.20]</td>
<td>1.05</td>
</tr>
</tbody>
</table>

*s satellite laboratory

Table 4: Exercise 2. Results obtained from the analysis 500 identical metaphase images from a peripheral blood sample exposed at 0.5Gy.

<table>
<thead>
<tr>
<th>Lab code</th>
<th>Number of scored cells</th>
<th>Number of Aberrations (Dic) or (Dic. + ring)</th>
<th>Frequency (± Poisson error)</th>
<th>Dose (Gy) [CI 95%]</th>
<th>z - value (Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>437</td>
<td>12+2</td>
<td>0.03 ± 0.01</td>
<td>0.50 [0.28-0.75]</td>
<td>0.00</td>
</tr>
<tr>
<td>L2</td>
<td>106</td>
<td>2 + 1</td>
<td>0.03 ± 0.02</td>
<td>0.48 [0.12-0.97]</td>
<td>-0.10</td>
</tr>
<tr>
<td>L4</td>
<td>289</td>
<td>12 + 2</td>
<td>0.05 ± 0.01</td>
<td>0.57 [0.32-0.77]</td>
<td>0.36</td>
</tr>
<tr>
<td>L4s</td>
<td>291</td>
<td>9 + 2</td>
<td>0.04 ± 0.01</td>
<td>0.48 [0.23-0.67]</td>
<td>-0.11</td>
</tr>
<tr>
<td>L5</td>
<td>311</td>
<td>27 + 3</td>
<td>0.10 ± 0.02</td>
<td>0.98 [0.68-1.22]</td>
<td>2.45</td>
</tr>
<tr>
<td>L5s</td>
<td>313</td>
<td>9 + 2</td>
<td>0.04 ± 0.01</td>
<td>0.50 [0.19-0.71]</td>
<td>0.00</td>
</tr>
<tr>
<td>L6</td>
<td>258</td>
<td>19</td>
<td>0.07 ± 0.02</td>
<td>0.80 [0.57-1.04]</td>
<td>1.54</td>
</tr>
<tr>
<td>L7</td>
<td>172</td>
<td>10</td>
<td>0.06 ± 0.02</td>
<td>0.82 [0.51-1.43]</td>
<td>1.66</td>
</tr>
<tr>
<td>L8</td>
<td>402</td>
<td>16</td>
<td>0.04 ± 0.01</td>
<td>0.58 [0.35-0.76]</td>
<td>0.41</td>
</tr>
<tr>
<td>L9</td>
<td>197</td>
<td>9</td>
<td>0.05 ± 0.02</td>
<td>0.78 [0.47-1.15]</td>
<td>1.43</td>
</tr>
</tbody>
</table>

*s satellite laboratory
A point tested during E2 was the ability of each laboratory to respond quickly after being notified. Three laboratories were able to respond on time based on the scoring of 50 images in 72 hours and 500 images in a week. These laboratories coincidentally were those with more scorers involved in the exercise.

4. DISCUSSION

In cases of radiation exposure due to accidents or terrorism actions the dose has to be provided as fast as possible to guide patient treatment. Biological dosimetry provides one important input to obtain this information when physical measures are not available. A disadvantage of the cytogenetic assay is that it is time consuming, particularly during the scoring process. For that reason it is essential to develop tools to help to estimate doses in emergency situations. An important issue is to overcome the difficulties associated with the dispatch of blood samples or slides. A way out for these obstacles is to score electronically transmitted images. Telescoring removes difficulties associated with the dispatching and reception of “physical” samples and is probably the most immediate way to share capabilities among laboratories working in network. The feasibility to score electronically transmitted metaphase images for biological dosimetry purposes has been previously described using a single dose-effect curve as reference [8] and by comparing frequencies of dicentrics [12]. In the present study we have tested the feasibility to estimate a dose based on telescoring, using each laboratory its own dose-effect curve.

For telescoring it is necessary to standardize the process of transmitting images through the internet and to consider all aspects that could affect the method as a whole. The two exercises demonstrated that it was very important to have a web site group for LBDNet laboratories in order to host heavy image files. Although, the capacity of the site, 100 MB, was appropriate for the exercises in case of a real emergency event the site capacity needs to be larger. In this sense a special website, like the pilot website DicentricCount.org is needed [12].

In the present study, two different laboratories captured the images either using automated (E1) or manual methods (E2). At the time of E2 exercise, none of the laboratories within the Latin American network had an auto capture system at high magnification. For this reason just one dose was evaluated, due to the hard working and time involved in the manual capture and the limited capacity of the website. Taking into account the mentioned limitations, it was decided to select a dose of 0.5 Gy as the low dose range showed the biggest dispersions in both, the E1 exercise (CV on dose: 17.3 after 0.5 Gy vs. 14.8% after 3.0 Gy) and the previous intercomparison exercise of the network [14] (CV: 15.6% after 0.75 Gy vs. 8.8% after 2.5 Gy).

Clearly, using automated acquisition the images were captured faster and also exhibited a better quality that resulted in a lower variability in the number of accepted cells to be scored. This could explain the lower coefficient of variation obtained in E1 respect to E2. The better quality can be due to the fact that images in E1 were selected previously to its uploading while images in E2 were not selected. Another source of variability could be assigned to the heterogeneity of the images, according to its file size, in E2 (manual capture) compared to the relative homogeneity of the images in E1 (automated capture). For E1 the image file sizes varied from 100 to 120 kb, while for E2 varied from 21 to 201 kb. This variability would have impact on the results of E2 exercise mainly for the triage purpose when different sets of 50 images where assigned to the distinct laboratories, showing the biggest dispersion (CV: 79.2
% for 50 images respect to 30.3 % for 500 images). Such heterogeneity would limit the comparability of the results for the triage purpose. When the same 500 images where analysed the impact of heterogeneity diminished because the same set of images where analysed by all laboratories.

At present, two of three laboratories within the LBDNet that possess automated scanning, metaphase finding and capturing systems, had recently acquired devices for high-magnification capturing. To strengthen the efficiency of the network, it would be desirable to increase the presence of automated microscopes with the possibility to capture automatically metaphase spreads with low and high magnification. This will enable those laboratories to perform the sending of images inside the net and to intermediate future contacts of LBDNet with other international biodosimetry networks. In addition, the accuracy of the analysis can be improved by a better resolution in capturing and uploading images. In cases with low number of victims involved, a preliminary image selection by the laboratory responsible for generating the images can be decided.

After finishing exercises E1 and E2 a discussion on the conflictive images for its acceptance or rejection was performed, which proved to be a valuable tool to reach consensus. Finally, performing regular intercomparison exercises within the network would allow to reduce variability among the laboratories and to improve Latin American network competence for mutual cooperation purpose.

The other associated cause that leaded to variability was the number of cells scored. Laboratories that scored the lower number of images presented the higher discrepancies in both dicentric frequency and dose results. The possibility of a small decrease on the number of accepted metaphases in telescoring can be balanced by increasing the number of images generated. In the Japanese network exercise the images sent were 470 and 190 for 1 and 5 Gy, and the required images to be analysed were 200 and 50 respectively [8]. It has been demonstrated that when dicentrics were scored directly with the microscope usually fewer cells were rejected compared to image analysis, as it was possible to adjust the focus and to localise isolated chromosomes [19]

The aim of the second exercise was to test the fast response capability of each laboratory of the network. The delay obtained for some laboratories was related to the number of scorers in each one. However, taken into account that it was an exercise, it should be certainly expected that in a real situation daily work will be stopped for a quick response.

For both exercises the coefficient of variation was lower for the estimated doses than for the reported frequencies (data not shown). This agrees with the idea that scoring differences are minimized when each laboratory uses its own dose-effect curve [9,14] In our exercise the dose effect curves where those previously established in each laboratory by conventional microscope analysis. So, the intercomparison only addresses the CV of dicentric scoring, not the experimental conditions of blood culture and metaphase acquisition by different laboratories. The obtained results stress the possibilities to use such calibration curves in dose estimation by telescoring.

The z-test values and the global coefficient of variation and trueness were highly impacted by the number of cells scored. Large variations in the estimated doses are expected whenever they are based on the observation of dicentrics in a small number of cells such as 50 cells. In E2 the increase of the number of cells from 50 to 500, resulted in a division by a factor of 2
of the coefficient of variation on the dose while trueness remained stable. The impact of the low number of accepted cells was higher at the lowest dose; in E1, after 0.5 Gy irradiation there were only 3 z-scores considered as satisfactory whereas after 3 Gy the number of z-scores considered as satisfactory were 8 of 10. This agrees with the previous intercomparison where participating laboratories received a set of slides [14] in which the three parameters analysed (z-score, coefficient of variation and trueness) improved with the number of cells analysed, and were better at the highest dose. Contrasting the two intercomparisons, the one using slides showed better results than the one presented here, because all laboratories reached to score 50, 100 or 500 cells under the microscope. The Japanese network obtained a good agreement between the real doses and the estimated ones transmitting electronically more images than required [8]

4. CONCLUSIONS

The results here obtained support the feasibility of networking using electronically transmitted images. However, in order to improve this methodology, future intercomparisons should consider: a) the transmission of a higher number of images than required, to avoid dose-estimations based on a low number of cells; b) a homogeneity of the sample, to ensure that each participant receives comparable test items; and c) an appropriate resolution in capturing and uploading images should be determined. Additionally, a global website able to be used for the different regional networks, like Share Points, will be desirable to permit a world-wide communication.

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