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INTERLABORATORY COMPARISON OF DICENTRIC CHROMOSOME ASSAY USING ELECTRONICALLY TRANSMITTED IMAGES

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The bottleneck in data acquisition during biological dosimetry based on a dicentric assay is the need to score dicentrics in a large number of lymphocytes. One way to increase the capacity of a given laboratory is to use the ability of skilled operators from other laboratories. This can be done using image analysis systems and distributing images all around the world. Two exercises were conducted to test the efficiency of such an approach involving 10 laboratories. During the first exercise (E1), the participant laboratories analysed the same images derived from cells exposed to 0.5 and 3 Gy; 100 images were sent to all participants for both doses. Whatever the dose, only about half of the cells were complete with well-spread metaphases suitable for analysis. A coefficient of variation (CV) on the standard deviation of ~15 % was obtained for both doses. The trueness was better for 3 Gy (0.6 %) than for 0.5 Gy (37.8 %). The number of estimated doses classified as satisfactory according to the *z*-score was 3 at 0.5 Gy and 8 at 3 Gy for 10 dose estimations. In the second exercise, an emergency situation was tested, each laboratory was required to score a different set of 50 images in 2 d extracted from 500 downloaded images derived from cells exposed to 0.5 Gy. Then the remaining 450 images had to be scored within a week. Using 50 different images, the CV on the estimated doses (79.2 %) was not as good as in E1, probably associated to a lower number of cells analysed (50 vs. 100) or from the fact that laboratories analysed a different set of images. The trueness for the dose was better after scoring 500 cells (22.5 %) than after 50 cells (26.8 %). For the 10 dose estimations, the number of doses classified as satisfactory according to the *z*-score was 9, for both 50 and 500 cells. Overall, the results obtained support the feasibility of networking using electronically transmitted images. However, before its implementation some issues should be elucidated, such as the number and resolution of the images to be sent, and the harmonisation of the scoring criteria. Additionally, a global website able to be used for the different regional networks, like Share Points, will be desirable to facilitate worldwide communication.

INTRODUCTION

Victims of nuclear or radiological accidents require prompt diagnosis and treatment, which include an assessment of medical status as well as radiation exposure and/or radioactive contamination conditions. Dosimetry evaluation has acquired a new role to guide medical treatment. Dose assessment is performed not only early post-exposure by physical dosimetry calculation (scenario reconstruction) but also from the 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 a dicentric assay, the current gold standard for biodosimetry, combined with other physical and biophysical techniques, thus applying a multidisciplinary approach[1].

The major disadvantage of a chromosome aberration assay is that it is time consuming, particularly during the scoring process. 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 from 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 with several intercomparison exercises showing almost homogeneous results. The first intercomparison exercise in the region was performed during the 1990's using dicentric analysis and a micronucleus assay[13], and since then several activities have been performed including the most recent intercomparison exercise involving the seven countries from the Latin American Biological Dosimetry Network (LBDNet) and six European countries[14]. Moreover, the ShipEx-1 exercise tested the existing capabilities for the 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 speedy 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 despatching and reception of 'physical' samples and is probably the most immediate 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].

The objective of this paper is to describe an inter-laboratory intercomparison among 10 partners by analysing digitised images shared by the Internet. The rationale of telescoring is the capacity allowing for almost instantaneous transmission of metaphase images acquired in a laboratory from the country in which the event has occurred to other laboratories, permitting the sharing of the scoring of several hundred (thousands) of victims. Here, the results obtained in two intercomparison exercises are presented, in which standardised methods for intercomparison analysis have been used[17, 18]. The aim of the first exercise (E1) was to test the feasibility of using electronically transmitted images from blood irradiated at two different doses, for the purpose of harmonisation of scoring criteria and acceptance or rejection of metaphase images. The aim of the second exercise (E2) was to test the fast response capacity of simulating a telescoring triage, and then the exercise followed by conventional scoring.

MATERIALS AND METHODS

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 a reduction in the speed of image uploading and distribution to the cooperating laboratories. These images were split in 10 sets containing a different set of 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 48 h before the sending of 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, seven participating laboratories were from the Latin American region

and three from Europe. In E2, nine participating laboratories were from the Latin American region (including two satellite laboratories[11]) and one from Europe. From the 10 laboratories that participated in each exercise, there were 8 that participated in E1 and E2, 7 from the Latin American region and 1 from Europe.

Sample irradiation

For E1, whole blood from one volunteer was exposed to 0.5 and 3 Gy with a dose rate of 0.5 Gy min^{-1} with a ^{137}Cs 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 to 0.5 Gy of X-rays (250 kV) at the University of Tuscia (Viterbo, Italy) at a dose rate of 0.3 Gy min^{-1}. For both exercises after radiation exposure, the blood was left standing 2 h at 37°C.

Blood culture

All blood samples were treated according to the standard protocol[1]. Briefly, 0.5 ml of whole blood was cultured with 5 ml of RPMI 1640 supplemented with 10 % of foetal calf serum, 3 % Phytohemagglutinin, 2 mM of L-glutamine, 1 mM of sodium pyruvate, 10 mM of Hepes, 50 U of penicillin G and 50 μg ml^{-1} of streptomycin (all reactives from Invitrogen, Paisley, UK), and 50 μg ml^{-1} of 5-bromodeoxyuridin (BrdU) (Sigma-Aldrich, St Louis, MO, USA). Two hours before harvesting Colcemid (0.1 μg ml^{-1}, Invitrogen) was added. A hypotonic shock was done at 37°C during 9 min (0.075 M, Biowest, Nuaillé, France). This step was followed by three washing steps using a fixative (methanol: acetic acid; 3:1). Cells were spread onto slides and stained using the FPG procedure[1].

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 motorised scanning stage (Marzhauser, Wetzlar, Germany) linked to a two-axis stepping motor. The metaphase positions were identified automatically by the Metafer 4 software (version 3.5.101; MetaSystems, Baden-Württemberg, Germany) with a 10× objective (Zeiss). For E1, the metaphase images were automatically acquired with a 63× 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 stage of 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.

Communication and image availability

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 for uploading different sets of images to the other members of the network.

Scoring

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.

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[18]. The application of these standards to the particular case of biological dosimetry has already been presented[14], and nowadays their use for intercomparison exercises in biological dosimetry is being recommended[1]. In brief, the global coefficient of variation (CV) and the trueness were calculated. The CV or 'relative variability' was used to compare the reproducibility of both frequency and dose assessments. CV was defined as the ratio S_R/x^* and expressed as a percentage, where S_R is the robust standard deviation and x^* is the robust average. The CV measures the precision of the exercise (the closeness of agreement between independent test results obtained under stipulated conditions); the quantitative expression of the precision is the standard deviation. The trueness was calculated as (x^* − reference value)/x^* and represents the closeness of agreement between the robust average value obtained from the laboratory results and the accepted reference value (the administrated physical dose). In addition, for each laboratory a z-score of the dose is presented in order to evaluate its performance. The z-score allows one to classify participant's results as satisfactory ($z < |2|$), questionable ($|2| < z < |3|$) and unsatisfactory ($z > |3|$)[14, 17, 18].

RESULTS

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 (Tables 1–4). Large variations were observed between laboratories and for the same set of images a ratio of 4–6 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.

Dose estimation and laboratories performance for exercise 1

In the E1 exercise, after irradiation at 0.5 Gy, the observed frequencies of dicentrics per cell (or dicentrics plus rings) mainly ranged from 0.05 to 0.08, and only one laboratory (L2) did not observe any dicentric (Table 1). The same laboratory was the one identifying a fewer number of cells as scorable (35 among 100). The estimated doses ranged from 0 to 0.97 Gy. When the 95 % confidence limits were considered, only two of the reported doses did not include 0.5 Gy (L2 and L3). According to the *z*-score, three results were classified as satisfactory, three as questionable and four as unsatisfactory (Table 1). The global CV 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 irradiation at 3 Gy 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 to 3.45 Gy. In two cases, the 95 % confidence intervals of the estimated doses did not

Table 1. Exercise 1. Results obtained from the analysis of 100 identical metaphase images from a peripheral blood sample exposed to 0.5 Gy.

Lab code	Number of scorers	Number of scored cells	Number of aberrations (Dic.) or (Dic.+ring)	Frequency (± Poisson error)	Dose, Gy (CI 95 %)	*z*-value (dose)
L1	1	58	3+0	0.05 ± 0.03	0.74 (0.25–1.38)	1.72
L2	1	35	0+0	—	—	−3.59
L3[a]	14	48	3+0	0.06 ± 0.04	0.87 (0.62–1.08)	2.69
L4[a]	3	49	4+0	0.08 ± 0.04	0.92 (0.54–1.19)	3.00
L5	1	50	3+0	0.06 ± 0.03	0.80 (0.00–1.24)	2.14
L6	1	45	3	0.07 ± 0.04	0.84 (0.34–1.49)	2.43
L7[a]	2	44	2	0.05 ± 0.03	0.71 (0.00–1.09)	1.51
L8	1	61	3	0.05 ± 0.03	0.67 (0.20–1.33)	1.22
L9[a]	4	46	3	0.07 ± 0.03	0.97 (0.34–1.79)	3.38
L10	1	57	4+0	0.07 ± 0.03	0.92 (0.42–1.55)	3.04

[a]When several operators have done the scoring, the number of cells and the number of dic.+rings presented are the mean of all scorers.

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

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

[a]When several operators have done the scoring, the number of cells and the number of dic.+rings presented are the mean of all scorers.

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

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

s, satellite laboratory.

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

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

s, satellite laboratory.

include the 3 Gy dose (L5 and L6). The *z*-score values were satisfactory for seven reported doses and questionable for two laboratories. After irradiation at 3 Gy, the CV was 14.8 % and the trueness on dose 0.6 %.

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, and in two cases the 95 % confidence limits on the estimated dose did not include 0.5 Gy (L2 and L6). 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 CV 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. For the estimated doses, in three cases the associated 95 % confidence interval did not include 0.5 Gy (L5–L7). The *z*-score identified only one dose as questionable. The CV on dose was 30.3 % and the trueness 22.5 %.

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 h and 500 images in a week. These laboratories coincidentally were those with more scorers involved in the exercise.

DISCUSSION

In cases of radiation exposure due to accidents or acts of terrorism, the dose has to be provided as fast as possible to guide patient treatment. Biological dosimetry provides an 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 despatch of blood samples or slides. A way out for these obstacles is to score electronically transmitted images. Telescoring removes difficulties associated with the despatching and reception of 'physical' samples and is probably the most immediate way to share capabilities among laboratories working in the 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, the feasibility of estimating a dose based on telescoring has been tested, each laboratory using its own dose-effect curve.

For telescoring, it is necessary to standardise 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 website 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 the 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 work 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 CV obtained in E1 compared with E2. The better quality can be due to the fact that images in E1 were selected prior to their uploading, whereas images in E2 were not. Another source of variability could be attributed to the heterogeneity of the images, according to their file size, in E2 (manual capture) compared with the relative homogeneity of the images in E1 (automated capture). For E1, the image file sizes varied from 100 to120 kb, whereas for E2 they varied from 21 to 201 kb. This variability would have an impact on the results of the E2 exercise mainly for the purpose of triage when different sets of 50 images were assigned to the distinct laboratories, showing the biggest dispersion (CV: 79.2 % for 50 images compared with 30.3 % for 500 images). Such heterogeneity would limit the comparability of the results for the purpose of triage. When the same 500 images were analysed, the impact of heterogeneity diminished because the same set of images were analysed by all laboratories.

At present, two of the three laboratories within the LBDNet that possess automated scanning, metaphase finding and capturing systems had recently acquired devices for capturing high-magnification images. To strengthen the efficiency of the network, it would be desirable to increase the presence of automated microscopes with the possibility of automatically capturing 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 the execution of exercises E1 and E2, a discussion on the conflicting 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 the purpose of mutual cooperation.

The other associated cause that led 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 in 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 with image analysis, as it was possible to adjust the focus and to localise isolated chromosomes[19].

The aim of the E2 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, taking 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 CV was lower for the estimated doses than for the reported frequencies (data not shown). This agrees with the idea that scoring differences are minimised when each laboratory uses its own dose-effect curve[9, 14]. In the exercise done here, the dose-effect curves were 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 of using such calibration curves in dose estimation by telescoring.

The *z*-test values and the global CV 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 CV on the dose while the trueness remained stable. The impact of the low number of accepted cells was higher at the lowest dose; in E1, after irradiaton at 0.5 Gy there were only 3 *z*-scores considered as satisfactory, whereas after that at 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, CV 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].

Overall, the results obtained here 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) the determination of an appropriate resolution in capturing and uploading images. Additionally, a global website able to be used for the different regional networks, like Share Points, will be desirable to facilitate worldwide communication.

Finally, as there is a growing need for proficiency testing for other conformity assessment activities such as preparedness for networking assistance, intercomparison exercises within and among networks are considered necessary. These exercises will contribute to the enlargement of the net capacities and enhancement of the efficiency in emergency biomedical response. For such a purpose, exercises should follow the technical and management requirements of the ISO 17043 standard[20] to allow comparisons among different exercises, guarantee transparency of the procedures and the results.

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