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MULTIBIODOSE - Final publishable summary report of the project

Executive Summary

In the event of a large scale radiological emergency biological dosimetry is an essential tool that can identify those exposed individuals who should receive immediate medical treatment. A number of biodosimetric tools are available, but they have to be adapted and tested for a large-scale emergency scenario. The aim of MULTIBIODOSE was to analyse a variety of biodosimetric tools and adapt them to different mass casualty scenarios. The assays were chosen because they complement each other with respect to sensitivity, specificity to radiation and the exposure scenario as well as speed of performance. The project involved key European players with extensive experience in biological dosimetry and was completed on time in April 2013.

Within MULTIBIODOSE we tested and validated the following dosimetric assays for their suitability as tools to triage exposed people in case of a large-scale radiological emergency:

1. Manual and automated dicentric assay
2. Automated micronucleus assay
3. Gamma-H2AX assay
4. Skin speckle assay (SSA)
5. Serum protein assay (SPA)
6. Electron paramagnetic resonance (EPR)
7. Optically stimulated luminescence (OSL)

The assays 4 (SSA) and 5 (SPA) were excluded from the battery of tools because they were found unsuitable for triage due to: (1) a long latency period between exposure and signal manifestation (SSA) and (2): large individual variability (SPA).

The other assays were standardised in the participating laboratories and training was provided. They were then tested for their ability to identify a person exposed to a dose higher than 1 Gy of gamma radiation. A statistic software was developed that allows collating the results from all assays. It can be found on the web page of the project: www.multibiodose.eu/software.

Inter-laboratory exercises were carried out to ensure that the all participating laboratories assessed the dose correctly. Blood samples and mobile phone elements were irradiated and shipped to participating laboratories. Three triage categories were aimed at: 0-1 Gy (low dose); 1-2 Gy (median dose) and 2-4 Gy (high dose). The results show that, on the whole, the methods and assays are very good at identifying doses within the correct triage category. Categorisation was without exception most successful based on dose estimates alone, i.e. ignoring the standard errors associated with the estimates. This was somewhat unexpected, as it was initially hypothesised that it would be necessary to use the upper standard error or

confidence limit to ensure that some doses weren't missed. However, this result strongly demonstrates that the assays actually do what they are supposed to be doing, and that the dose estimates provided are indeed a good indication of the actual exposure dose.

A guidance document for radiation emergency responders was developed with information about the possibilities and limitations of the triage tools and about the capacity of the participating laboratories to carry out biodosimetric triage in a mass casualty emergency. The guidance document can be downloaded from the project web page.

In summary, the Multibiodose consortium successfully tested, adapted, and validated five biodosimetry assays for their use in triage biodosimetry in a mass casualty situation. In an emergency situation a MULTIBIODOSE partner laboratory in the affected country should act as the "core" or "administrative" laboratory that will be in charge of the decision which assays to use and how to involve other laboratories. The laboratory will give advice to the health and radiation protection authorities about collection of samples and it will collect the results from other laboratories. In the end, this laboratory will provide the health and radiation protection authorities with dosimetric and radiological triage categorisation results to support medical and public health decisions.

A summary description of project context and objectives

In the event of a large scale radiological emergency, the triage of patients according to their degree of exposure forms an important initial step. Although clinical signs and symptoms of a serious exposure are used for that purpose, there are a number of reasons against their exclusive use as the basis for medical decision making: clinical symptoms are dynamic, show a strong degree of individual variability and are not specific to radiation. A good example is vomiting, which can be psychogenic, whereas its absence does not exclude significant overexposure. Biological dosimetry is an essential tool in the management of a radiological mass casualty, which can provide timely assessment of radiation exposure to the general population and enables the identification of those exposed people and specifically who should receive medical treatment.

A number of biodosimetric tools are currently in use or potentially available, but all of these must be adapted and tested for a large-scale emergency scenario. These methods differ in their specificity and sensitivity to radiation, the stability of signal and speed of performance. A large scale radiological emergency can take different forms. Based on the emergency scenario, different biodosimetric tools should be applied so that the dose information can be made available with optimal speed and precision. The aims of this multi-disciplinary collaborative project were:

1. To analyse a variety of biodosimetric tools and adapt them to different mass casualty scenarios. The following biodosimetric tools were validated: the dicentric assay, the micronucleus assay, the gamma-H2AX assay, the skin speckle assay (SSA), the blood serum protein assay (SPA) and electron paramagnetic resonance/optically stimulated luminescence (EPR/OSL) dosimetry in components of portable electronic devices.
2. To validate and then embed the techniques in a number of European laboratories. The project involved the key European players with extensive experience in biological dosimetry. The staff of the participating laboratories were trained to perform dosimetric triage in a timely and reliable manner and a programme of on-going training (including other relevant laboratories) was established in collaboration with the EU-funded network RENEB.
3. To develop an operational guide and disseminate it among emergency preparedness and radiation protection organisations. This activity will make the stakeholders aware of the existence of the operational biodosimetric network and will inform them about the possibilities and costs of applying the biodosimetric triage tools in a mass casualty situation.
4. To develop an algorithm, based on the TMT-handbook, that will be helpful in deciding which tools should be applied for optimal triage and are best related to the type/circumstances of the emergency.
5. Automation and commercialisation was pursued and a software package was developed for integrated statistical analysis of data from each of the assays.

The assays were chosen because they complement each other with respect to sensitivity, specificity to radiation and the exposure scenario as well as speed of performance. Some of the assays were well established as biodosimetric tools and only needed to be adapted to a mass casualty scenario, while other assays needed validation. This approach guaranteed that the final important deliverable of this project is the establishment of a biodosimetric network that is fully functional and ready to respond in case of a mass casualty situation. Thus, the project strengthens the European security capabilities by achieving tangible technical and operational results.

THE OVERAL DESCRIPTION OF THE PROJECT RESULTS

THE BIODOSIMETRIC TOOLS AND THEIR APPLICABILITY FOR TRIAGE

The following dosimetric assays were tested and validated for their suitability as tools to triage exposed individuals in case of a large-scale radiological emergency:

- Manual and automated dicentric (Dic) assay
- Automated micronucleus (MN) assay
- Gamma-H2AX assay
- Skin speckle assay (SSA)
- Serum protein assay (SPA)
- Electron paramagnetic resonance (EPR)
- Optically stimulated luminescence (OSL)

In the following, the tools are shortly presented and their applicability for biodosimetric triage described as resulting from the work done during the project. The tools were tested for their ability to identify a person exposed to a dose higher than 1 Gy of gamma radiation.

Manual and automated dicentric assay (Dic)

The dicentric assay is based on assessing the frequency of dicentric chromosomes in peripheral blood lymphocytes (PBL) of an exposed person. The dicentric chromosome is specific for ionising radiation and the spontaneous frequency is very low in the healthy population (Romm et al, 2009). It is internationally standardised (ISO 19238 and ISO 21243) and regarded as the gold standard of biological dosimetry (Blakely et al, 2005). The assay requires ca. 5 ml venous blood and subsequent in vitro culturing of PBL for a 48 h period for visualisation of chromosomes. Dicentrics can be scored manually under the microscope or automatically/semi-automatically with the help of an image analysis system coupled to a microscope equipped with a motorised stage. The MULTIBIODOSE team relied on the image analysis system distributed by MetaSystems, Germany (Schunck et al, 2004). Following this approach the absorbed doses can be assessed up to few months after exposure (IAEA 2011).

It was found that manual scoring of 50 cells per donor or automatic scoring of 150 cells per donor is sufficient to identify a person exposed to a dose higher than 1 Gy. Partial body exposure covering 50% of the cells and protracted exposure (irradiation time of 16 hours) were also tested and found to be detectable (Romm et al, 2012, Romm et al, 2013).

Automated micronucleus assay (MN)

The micronucleus assay is based on assessing the frequency of micronuclei in PBL of the exposed person. Micronuclei (MN) are not specific for ionising radiation and the spontaneous frequency is much higher than that of dicentrics. However, radiation is a very potent inducer of MN, so a high frequency of MN strongly indicates radiation exposure. A large number of cells can be scored within a time shorter than that required for scoring dicentrics. International standardisation is in progress (ISO 17099). This assay also requires ca. 5 ml venous blood and subsequent in vitro culturing of PBL for ca. 72 hours. The big advantage of MN is that they can be scored automatically with high speed using an image analysis system coupled to a microscope equipped with a motorised stage. The MULTIBIODOSE team relied on the image analysis system distributed by MetaSystems, Germany (Schunck et al. 2004). The absorbed dose can be assessed up to few months after exposure (IAEA 2011).

It was found that automatic scoring of 1000 cells per donor with the MN assay is sufficient to identify a person exposed to a dose higher than 1 Gy with a high precision. Protracted (irradiation time of 16 hours) whole body, and partial body exposure were also tested and found to be detectable.

Gamma-H2AX assay

The gamma-H2AX assay is based on analysing the formation of DNA repair protein clusters – called gamma-H2AX “foci” - in peripheral blood lymphocytes of an exposed person (Rothkamm and Loebrich 2003, Rothkamm and Horn 2009). Similar to micronuclei, the foci are not specific to but

indicative of ionising radiation exposure and the spontaneous frequency is quite low. The analysis can be done manually or automatically using a microscope or automatically using a flow cytometer or fluidic device (Pope et al, 2011). The advantages of the assay are its high sensitivity (if used within a few hours post exposure), that it can be used with only a drop of blood (finger prick) and that it does not require culturing of lymphocytes as in the case of the dicentric and MN assays and therefore provides results within a few hours. However, the absorbed dose (whole and partial body) can only be assessed up to a few days after exposure (Horn et al. 2011, Lassmann et al, 2010). It was found that automatic scoring of 50 cells per donor with the gamma-H2AX assay is sufficient to identify a person exposed to a dose higher than 1 Gy. Protracted (irradiation time of 16 hours) whole body exposure was detectable, but doses could not be accurately estimated (Rothkamm et al, 2013). Partial body exposure was also tested and found to be detectable only using manual, but not automated analysis (Horn et al, 2011; Rothkamm et al, 2013).

Skin speckle assay (SSA)

The skin speckle assay is based on the ability to detect radiation-induced speckle patterns in the skin. The assay is specific to radiation-induced skin damage and its unique advantage is the possibility to detect a dose to a small area of the skin in a totally non-invasive and very fast way. However, the results obtained in this project suggest that at least one month must pass between radiation exposure and analysis before a radiation-induced skin speckle pattern is detectable. For this reason this assay was found not suitable for a timely triage of people exposed in a large-scale radiation emergency.

Serum protein assay (SPA)

The change of concentration of selected proteins in serum following localised exposure of skin to radiation was tested in samples collected from patients treated by external beam radiotherapy as a tool for identifying partial-body exposure. Although promising results were obtained in earlier mouse experiments, the changes in protein concentration in patients showed very strong individual variability that makes the assay unsuitable for use in emergency situations, when the individual levels of proteins before radiation exposure are not known.

Electron paramagnetic resonance (EPR)

EPR is a spectroscopic technique for studying radiation-induced radicals in biological or artificial materials (IAEA 2002). Ionising radiation induces radicals in glass displays of portable electronic devices such as smart phones. Consequently, these can be used as individual dosimeters, but must be removed and destroyed for measurement. The advantage of EPR is its high radiation specificity, good signal linearity in the high dose range (>1 Gy) and long signal stability (several months). Its detection threshold is 1 Gy. Analysis must be carried out in a laboratory equipped with an EPR spectrometer.

Optically stimulated luminescence (OSL)

OSL assesses the dose of ionising radiation by measuring light emitted from irradiated objects following optical stimulation. Electronic elements used in mobile phones have luminescent properties and can be used as individual dosimeters, but require removal and destruction of the phone's electronic circuitry board. The advantage of OSL is its very high specificity and sensitivity to radiation (from several mGy to several Gy). There is a signal loss of 50% in the first 10 days after irradiation and fading correction must be applied. Analysis must be carried out in a laboratory equipped with an OSL reader.

Comparison of the performance of the tools through an exercise

In order to validate the tools an exercise was performed where blood samples and elements of mobile phones were irradiated and shipped to project partners for analysis. For the biological assays, blood collected from a total of 8 volunteers was exposed to high dose rate Co-60 gamma irradiation at STUK and UGent. A total of 5 doses/irradiation schemes were included: A control (0 Gy); a medium acute

high-dose-rate dose (1.5 Gy); a high acute high-dose-rate dose (2.75 Gy); a simulated medium partial body exposure (1.5 Gy, mixed 1:2 with 0 Gy blood from the same donor); a simulated high partial body exposure (2.75 Gy, mixed 2:1 with 0 Gy blood from the same donor). A total of 8 samples of blood and/or separated lymphocytes (with a 4 hr timepoint) were then shipped to each participating laboratory. 8 labs participated in the exercise: 6 laboratories carried out the dicentric assay (BfS; BIR; HPA; INCT; IRSN; STUK); 5 laboratories carried out the micronucleus assay (BfS; BIR; HPA; INCT; UGent) and 5 laboratories carried out the gamma-H2AX foci assay (BIR; HPA; IRSN; STUK; UGent). The dicentric assay was run in manual triage mode (at least 50 cells or 30 dicentrics scored) and/or automatic mode (at least 150 captured metaphases scored). The micronucleus assay was run in automated mode with cut-off > 4 MN and without cut-off as well as in semi-automated mode and participants were asked to score at least two slides and two cultures per sample. The foci assay was carried out in manual (≥ 20 cells scored) and/or automatic (200 cells scored) mode, and participants were asked to use a positive control to test the ongoing validity of their calibration curve. For all three assays, standardised scoring sheets were provided which contained the method for calculating standard errors which was defined in deliverable 6.4. Further, each participating laboratory was asked to use their own most appropriate calibration curve. The exercise was run in ‘real time,’ so the return of results was timed, in order to simulate and test a realistic accident response as far as possible. The triage results were based on the labs’ first reported result for each sample, using their own preference for which method (automatic or manual) to apply in triage mode, i.e. to apply first.

For the physical assays, 11 and 13 institutions took part in the EPR and OSL intercomparisons, respectively. During a two-day preparatory meeting, organized at IRSN, the participants were trained on the use of the OSL and EPR protocols and on the various steps of the method, i.e. sample preparation, measurement, signal evaluation and uncertainty assessment. During this meeting, the participants also received the blind dose samples, i.e. mobile phone touchscreens for EPR and mobile phones for the OSL. Irradiations at 3 unknown doses were performed at IRSN in terms of air kerma using a cobalt source. Each of the 3 unknown doses fell within one of the 3 triage dose ranges: 0-1 Gy (low dose), 1-2 Gy (medium dose) and >2 Gy (high dose). For EPR, participants also received a set of calibration samples to determine the calibration curve and a software for the evaluation of the signal intensity. Participants were asked to measure the blind dosed samples in their own laboratories following the MULTIBIDOSE protocol (described in D 5.2). Nine and eleven participants reported the final results for EPR and OSL. The EPR intercomparison was carried out in parallel in two groups of laboratories: in the first group, formed by three participants, samples were taken from a bulk of glass fragments of three smart phones of the same model and stored in the same conditions at one laboratory for the first week, whereas for the second group, made of eight participants, the samples were individually prepared from different smartphones. Shipping and storing conditions were different among laboratories and therefore on average the samples were less uniform than those of the first group (but more similar to a “real” situation). For OSL, the intercomparison was carried out using two different protocols: a “fast mode” protocol, where no preheating is applied so that measurements are faster and a “full mode” protocol, where preheating leads to the isolation of a more stable signal at the expense of longer measurement times. The “fast mode” protocol could thus be suitable for a first triage in a radiological mass casualty, whereas in “full-mode” a more accurate dose assessment should be possible.

As the ‘administrating laboratory,’ i.e. the laboratory in the country in which the simulated radiation emergency occurred, HPA collated the results of each assay from each lab, and entered the data into the MULTIBIDOSE software, in order to assign a triage status to each simulated exposed individual. Once the results were collated, the codes on the samples were broken, in order to ascertain the success of the triage categorisation exercise. In addition to direct comparison of the experimental results with

the irradiation doses, statistical analysis of all the experimental variables was carried out using General Linear Model Analysis of Variance (ANOVA) and post-hoc testing where appropriate. In addition, a number of alternative methods of triage categorisation were tested: categorisation based on upper 95% confidence limit; categorisation based on upper standard error and categorisation based on mean dose value alone, and for either whole body equivalent or actual measured (whole body or partial body doses).

Overall, the results of the exercise showed good consistency in dose estimates provided between laboratories and dosimetry methods, although some variation has been detected. However, importantly, consistently successful triage categorisation has been demonstrated for the full range of doses and irradiation schemes tested as part of the exercise.

OVERALL CHARACTERISTICS OF THE TOOLS

The assays were characterised not only with respect to their dosimetric performance but also their time performance. Table 10 shows the approximate duration (in days) between the time point of sample arrival at the laboratory and the completion of dose estimations, calculated for different numbers of samples analysed by one or five laboratories. The calculation was made for one person per lab working 8 hours per day. In case of automatic scoring it was assumed that the scoring system works 24h per day.

Table 10. Approximate duration (in days) between the time point of sample arrival to the laboratory and the completion of dose estimation for the purpose of triage.

Biodosimetric tool	Total time in days to analyse samples (excluding time of shipment)					
	1 sample 1 lab	50 samples 1 lab	100 samples 1 lab	100 samples 5 labs	1000 samples 1 lab	1000 samples 5 labs
Dic manual	2.5	6	9	5	65	16
Dic automated	2.5	3	4	3	19	6
MN automated	3.5	4	5	4	20	6
Gamma_H2AX	< 1	1	1	1	3	3
EPR	< 1	1	4	1	40	14
OSL	< 1	1	4	1	40	14

The aim of the table is not to provide precise time estimates, but rather to give a comparative overview of the characteristic of each method. The stated times may change based on the momentary capacity and work load of a laboratory. It is evident that the fastest method is the gamma-H2AX assay, followed by EPR and OSL. It must be remembered that, due to signal loss, gamma-H2AX can only be used up to a few days post exposure. The speed of analysis decreases for EPR and OSL as the number of samples increases. This is due to the fact that there is no possibility to analyse samples in parallel in a laboratory equipped with a single EPR and OSL reader. In contrast, the preparation of samples for the analysis of dicentric chromosomes and micronuclei can be done in parallel and the analysis itself is automated, so many samples can be analysed in a reasonable period of time.

OVERALL CAPACITY OF THE MULTIBIODOSE LABORATORIES

A major aim of MULTIBIODOSE was to set up networking of the partner laboratories so that in case of a large radiological emergency samples can be shared and analysed in parallel, leading to a large

capacity. This overall capacity was assessed and the results are summarised in table 11, expressed as number of samples that can be processed per week and per month.

Table 11. Approximate total capacity of the MULTIBIODOSE partner laboratories expressed as number of samples that can be analysed for the purpose of triage per week and per month.

Tool:	Dic and MN		Gamma- H2AX		EPR and OSL	
Time period:	Week	Month	Week	Month	Week	Month
	1100	4200	2300	9100	4200	17900

The major reason why the capacity for the dicentric and micronucleus assays is lowest lies in the necessity to culture lymphocytes for 48 hours (Dic) and 72 hours (MN). The highest capacity for EPR and OSL results from the possibility to mobilise a large number of competent laboratories that are incorporated in the EURADOS network of retrospective dosimetry. It is important to bear in mind that the values represent approximations and that the numbers can change based on the momentary personal capacity and work load of a laboratory.

CONCLUSIONS

The Multibiodose consortium successfully tested, adapted, and validated five biodosimetry assays for their use in triage biodosimetry in a mass casualty situation. The automation of the assays has also been validated. The application of EPR and OSL assays in portable electronic devices was developed and validated not only among MULTIBIODOSE laboratories but also in 27 EURADOS associated laboratories, some of which are placed outside the EU. The intercomparison exercises and validation of results between the laboratories have made it possible to act in a concerted way in case of a mass casualty accident. As a result of this, both the speed of performing the assays and the throughput of the laboratories have been optimised.

In an emergency situation a MULTIBIODOSE partner laboratory in the affected country (or another national laboratory designated to perform biodosimetry) should act as the “core” or “administrative” laboratory that will be in charge of the decision which assays to use and how to involve other laboratories. The laboratory will give advice to the health and radiation protection authorities about collection of samples and it will collect the results from other laboratories. In the end, this laboratory will provide the health and radiation protection authorities with dosimetric and radiological triage categorisation results to support medical and public health decisions.

REFERENCES

- Alexander GA et al. BiodosEPR-2006 Meeting: Acute dosimetry consensus committee recommendations on biodosimetry applications in events involving uses of radiation by terrorists and radiation accidents. *Radiation Measurements* (2007) 42:972 – 996.
- Blakely WF, Salter CA, Prasanna PG.. Early-response biological dosimetry - recommended countermeasure enhancements for mass-casualty radiological incidents and terrorism. *Health Phys* (2005) 89:494-504.
- Fattibene P, Wojcik A. (Eds.), Biodosimetric tools for a fast triage of people accidentally exposed to ionising radiation. *Ann Ist Super Sanità* (2009); 45(3).
- Horn, S., Barnard, S. and Rothkamm, K. Gamma-H2AX-based dose estimation for whole and partial body radiation exposure. *PLoS One* (2011), 6, e25113.
- IAEA, Cytogenetic dosimetry: Applications in preparedness for and response to radiation emergencies. International Atomic Energy Agency (2011), Vienna, Austria.
- IAEA, Use of electron paramagnetic resonance dosimetry with tooth enamel for retrospective dose assessment. Report IAEA-TECDOC-1331, International Atomic Energy Agency (2002), Vienna, Austria.
- Lassmann, M., Hanscheid H, Gassen D, Biko J, Meineke V, Reiners C and Scherthan H. In vivo formation of gamma-H2AX and 53BP1 DNA repair foci in blood cells after radioiodine therapy of differentiated thyroid cancer. *J Nuc Med* (2010); 51, 1318-1325.
- Pope, I.A., Barber, P.R., Horn, K., Ainsbury, E., Rothkamm, K. and Vojnovic, B. A portable microfluidic fluorescence spectrometer device for gamma-H2AX-based biological dosimetry. *Radiat Meas* (2011), 46, 907-911.

Romm H, Oestreicher U, Kulka U. Cytogenetic damage analysed by the dicentric assay. In: Biodosimetric tools for a fast triage of people accidentally exposed to ionising radiation. Fattibene P, Wojcik A. (Eds.) *Ann Ist Super Sanità* (2009); 45(3): 251-259

Romm H., Ainsbury E, Bajinskis A, Barnard S, Barquinero J-F, Beinke C, Puig-Casanovas R, Deperas-Kaminska M, Gregoire E, Kulka U, Oestreicher U, Lindholm C, Moquet J, Rothkamm K, Sommer S, Thierens H, Vral A, Vandersickel V, Wojcik A. The dicentric assay in triage mode as a reliable biodosimetric scoring strategy for population triage in large scale radiation accidents. *Proc IRPA* (2012); 13:TS 2c

Romm H, Ainsbury E, Barnard S, Barrios L, Barquinero JF, Beinke C, Deperas M, Gregoire E, Koivistoinen A, Lindholm C, Moquet J, Oestreicher U, Puig R, Rothkamm K, Sommer S, Thierens H, Vandersickel V, Vral A, Wojcik A. Automatic scoring of dicentric chromosomes as a tool in large scale radiation accidents. *Mutation Research* (2013) in press.

Rothkamm, K. and Horn, S. γ -H2AX as protein biomarker for radiation exposure. *Ann Ist Super Sanità* (2009), 45:265-271.

Rothkamm K, Barnard S, Ainsbury EA, Al-hafidh J, Barquinero JF, Lindholm C, Moquet J, Perälä M, Roch-Lefèvre S, Scherthan H, Thierens H, Vral A, Vandersickel V (2013). Manual versus automated gamma-H2AX foci analysis across five European laboratories: can this assay be used for rapid biodosimetry in a large scale radiation accident? *Mutat Res* (2013) , doi: 10.1016/j.mrgentox.2013.04.012.

Rothkamm K and Loebrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc Natl Acad Sci U S A* (2003), 100:5057–5062.

Schunck C et al. New developments in automated cytogenetic imaging: unattended scoring of dicentric chromosomes, micronuclei, single cell gel electrophoresis, and fluorescence signals. *Cytogenet Genome Res* (2004), 104:383-389.