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FINAL PUBLISHABLE SUMMARY REPORT

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1 EXECUTIVE SUMMARY

The effective management of an event involving the exposure of numerous people to radioactive material, whether accidental of following malevolent act, requires a mechanism for rapid triage of exposed individuals. In-field quick triage operations require an integrated toolbox with equipment devices adapted for field use, easy to use by first responders without medical or nuclear competences.

The BOOSTER project, gathering seven partners from five different countries, addresses this requirement through a global and unique toolbox including newly developed and adapted measurement tools to quickly assess the radiological situation in the field, to determine by appropriate sensors its consequences, to provide fast and reliable biodosimetric tools to Triage Teams to evaluate the radiological dose received by each victim and the toxicity of particles and products used during the attack in order to speed up allow an efficient categorization and triage of exposed individuals. It also allows a potential further follow-up material for medical staff at medium/long term post-event.

The BOOSTER System architecture was imagined to fit current procedures for radiological crisis management, generally based on the definition of different areas around the scene.

An exclusion area (area directly affected by the event, subject to high levels of radioactivity and contamination, where only duly protected first responders can rescue and evacuate victims) and a controlled area (secured area around the exclusion area) are defined from radioactivity levels measured in these zones. The equipment used in the controlled area allows the cartography of the radiological situation and therefore the real-time assessment of the dose received by irradiation by means of radiological measurements coupled with GPS position of equipment: dose-rate-meters (Colibri & GPS-COM) for real time radiological measurements, rugged PDA for live information from first responders on site, gamma camera (Gampix) for hot spot location, and portable High Purity Germanium detector (FALCON 5000) for identification of radionuclides.

In the Decontamination area, victims are controlled for contamination and a decontamination process is applied if necessary. Several measurement devices are deployed to assess the level and position of external contamination on individuals: bar code wristband for victims' identification and follow-up, detection portal system (MiniSentry) for contamination monitoring, decontamination tent equipment with dedicated measurement probes, sampling equipment to obtain victims' biological samples, creation of a "victim database" for collecting information for triage and medical support.

When affected people arrive to the Support area, a deeper analysis is performed for radiological triage. A complete kit for a first determination of the dose received by internal contamination and irradiation is installed in the Support area: Low-Background Spectroscopy on biological samples and environment samples, portable LIBS analysis of biological samples, retrospective dosimetry using environment samples and SMD resistors from cell phones, biodosimetry using γH2AX quantification. All results, obtained in less than 20 min, are linked with the victim ID and stored in a database processed by Decision Support System for triage instructions and medical care.

Additional analyses off-site (in-lab) can be performed for more precision on the medical impact on the victim: biodosimetry using centrosome quantification, liquid scintillation analysis of biological samples and environment samples, ICP-MS analysis of biological samples, diffraction methods on biological samples for particle granulometry, confirmation of the estimated dose via cytogenetics method. Results, stored in the database, along with in-field results, give additional information (especially toxicity information) for possible medical treatments to be given.

The Command & Control Area is located far away from the incident area, for instance in an existing Crisis Management Center. Two easily deployable tools consisting in rugged laptop with SIMACOP and RODOS applications are available for crisis managers and authorities, to show all information from the Controlled area as well as second level information from the Support area. This information will help the authorities and higher level crisis managers to mitigate the incident effects and manage the available resources in an optimum way.

The BOOSTER equipment was demonstrated at Budapest on May 16, 2013, to present all the techniques developed during BOOSTER project and their final integration.

2 SUMMARY DESCRIPTION OF PROJECT CONTEXT AND OBJECTIVES

Context

From the beginning of the third millennium, Homeland Security applications have been a challenging issue for all societies. 9/11 attacks showed the reality and impact of terrorist threats which forced societies to quickly adapt and develop new technological solutions. Moreover, recent nuclear accidents demonstrated people's fear regarding nuclear risks. A terrorist attack, potentially involving a radiological risk, could have dramatic and long-lasting consequences on the world, from a sanitary and psychological point of view. The effective management of an event involving exposure of a large number of people to radioactive material requires a mechanism for rapid triage.

The BOOSTER project, gathering seven partners from five different countries, addresses this requirement by researching and developing new bio-dosimetric tools to quickly evaluate the level of exposure of potential casualties, determine by appropriate sensors its consequences and allow an efficient triage of exposed individuals. These bio-dosimetric tools will be integrated to a portable toolbox along with a prognostic toolkit based on radiation sensors. The combination of these approaches will allow an effective and fast management of the situation.

Project objectives

The BOOSTER project is a capability project designed to research and develop new measurement tools, to adapt and optimize existing measurement tools to enable a fast evaluation of the dose received by victims in case of radiological attack in order to quickly sort out the victims. More precisely, BOOSTER toolbox must give to the First Responders the required means for assessing the radiological situation in the field, evaluating the level of potential casualties, and determining by appropriate sensors their consequences. BOOSTER must provide to Triage Teams fast and reliable biodosimetric tools to quickly evaluate the radiological dose received by each victim and the toxicity of particles and products used during the attack to speed up the categorization and to perform an efficient triage of exposed people. BOOSTER project will use an integrated approach to define a useful and usable toolbox, combined with a prognostic toolkit to allow effective management of exposed persons. It must also allow a potential further follow-up material for medical staff at medium/long term post-event. Training of civil protection operators and definition of commercial exploitation potentialities must also be planned.

The global objective of the first period was to develop the overall architecture of the system to provide basis for system requirements and data types for integration. This architecture, based on end-users requirements and usage scenario, allowed a first design of the core software. In parallel, all components were developed separately, taking into account the future fusion process.

Objectives of the second period were the technical progress of all technologies with the preparation of their integration in a global toolbox, the definition and preliminary program of the technical and operational scenarios, the preparation of the mid-term review, the search of additional experts and end users to review project scenarios and technical architecture, the delivery of ethical agreement for biological experiments by national and local ethical committees, the dissemination of knowledge through conference and scientific journal papers.

Main objective of the third period was the preparation, organization and execution of the final demonstration exercise requiring the finalization of BOOSTER toolbox, the integration and test of all components in Simacop and Decision Support System modules, the writing of a complete set of documents describing all BOOSTER devices and technologies in different languages and adapted to the use by first responders non specialists in the different techniques, the formation of Hungarian end users and volunteers participating in the exercise, and the approval of the ethical committee for technologies using biological samples. Second objective was the identification and motivation of complementary experts and end users for evaluating BOOSTER documents, exercise scenario and the state of BOOSTER technologies some months before the exercise in order to prepare and adapt the exercise according to experts' comments and finally to evaluate BOOSTER complete and final toolbox during the exercise. Third objective concerned the dissemination, to transmit the scientific and technological knowledge acquired during BOOSTER project to the scientific community through

publications in journals and communications in conferences, to inform the public through the website and the press, and the crisis management teams via specific communication actions. Fourth objective was the definition of the exploitation strategy of BOOSTER toolbox after the project, as some BOOSTER devices can lead to final commercial products after an industrialization step or a patent registration.

2.1 WP100 - Management

Global objectives of WP100 focus on global project management through the organization of communication and collaboration between teams (strategy and financial Steering Committee meetings, technical and organizational meetings, regular phone calls with partners), reporting to the European Commission, monitoring of technical progress and achievements, delivery with a quality control of reports according to the planning defined in the Description Of Work, budget survey, ethical survey. Management also includes particular involvement to solve technical or financial difficulties between partners or work packages, in case of disputes or in phase of preparation and organization of the final demonstration exercise with the integration of all equipment devices.

Management is also in charge of dissemination, to transmit the scientific and technological knowledge acquired during BOOSTER project to the scientific community through publications in journals and communications in conferences, to inform the public and the press through the website (project presentation and news, newsletters, booklet), to inform project experts/end users and the crisis management teams via specific communication actions.

2.2 WP200 – System requirements & design concept

Objective of WP200 is to apply an appropriate methodology to identify the needs and requirements of the different BOOSTER end-users, to define the system architecture design and to propose the possible exploitation strategy.

The main action concerned the identification and motivation of a panel of potential end users and experts to get their needs and requirements in order to propose a definition of the global toolbox architecture and the technical specifications of equipment devices, and to adapt them by iteration according to feasibility criteria and technical experience and competences of the consortium members. The analysis of experts' feedback was also necessary to define the general, organizational and technical scenarios for the demonstration exercise.

Due to the particular innovative aspect of some new developed tools, a particular effort was put on the on-going and future exploitation strategy regarding the industrialization of some prototypes or the deposition of several patents.

2.3 WP300 – Fast evaluation

The objective of WP300 in Booster project is to use and adapt existing sensors together with newly developed ones (e.g. retrospective dosimetric systems) in order to estimate the level of radiation. Information on the radiation level will be gathered with standard visible camera systems (identification, threat evaluation). Information on the estimation of dose absorbed in individuals due to exposure to external radiation is to be gathered by means of retrospective dosimetry.

In the frame of WP300, three different tasks were planned. First task was the development of a mobile and smart gamma camera system. Second was to test other sensors, in particular portable devices developed by Canberra and MTA EK (former IKI). These devices were hand-held systems, portal monitors and other transportable devices such as low-background gamma spectrometers. Third task was to develop retrospective dosimetric methods for posterior dose estimation together with a development of a small, portable fluorimeter.

2.4 WP400 – New bio-dosimetric tools

The objective of WP400 is to develop an integrated system for triage in the first hours post-accident to identify three populations: individuals requiring no further treatment, individuals needing close follow-up and individuals requiring hospitalization using biodosimetric tools. New biodosimetry systems and their integration with other procedures are planned to determine radiation exposure and appropriate response in a combined toolbox that will exploit existing and new technologies and techniques. Using these techniques, the dose absorbed inside the body can be determined within medium-term period (1-2 days) after exposure, applying mainly laboratory-based systems.

In the frame of WP400, the developed and applied methods are mainly based on development of new biomarkers and real biodosimetric tools for dose estimation like γ-H2AX and centrosome quantification techniques. Some other additional methods are also informative and supply more information for better estimation of the inner dose absorbed for further treatments of the victims. These techniques are: low-background gamma-spectrometry, inductively coupled plasma mass spectrometry (ICP-MS), laser-induced breakdown spectroscopy (LIBS) and liquid scintillation counting technique. These are well-applicable systems for analysis of radionuclides in low concentration in biological, environmental and swipe samples. However, the techniques are laboratory based systems and therefore non-applicable in field, furthermore they are more time consuming but also more sensitive than other techniques, hence it is possible to determine also the low dose absorbed by inhalation or ingestion relatively fast. These techniques are relevant in the second phase of triage.

For WP410, besides the development of bio-dosimetric tools, another aim is to quantify radioactive substances and nanoparticles, as well as to determine the toxicity of in-field contamination with radioactive particles and other substances. The risk of contamination by toxic particles must be evaluated by measuring the abundance and nature of different types of particles at the main routes of contamination such as the mouth, nostrils and eyes and the toxicity of the particles. This task involves the development of procedures for sampling and analysis of smears (bucal, nasal mucus, and commissure of the eye), to determine the particle size at the route of entrance into the body to assess the potential level of penetrance. These techniques are useful in the second phase of triage and determination of dose absorbed by victims as bio-dosimetric tools.

Further objective of WP410 is to describe the development of a portable LIBS system for detection of radioactive contamination in the field and patients during the triage and for further analysis of body fluids to determine the inner contamination, as a biodosimetric tool. Laser-Induced Breakdown Spectroscopy (LIBS) is a unique spectroscopic technique with a significant analytical potential and many advantages, e.g. compact, portable systems are available which may also provide adequately high resolution for isotope ratio analysis. In-situ, in-field and remote analysis on samples of any physical phase and form can be performed. Employing the special properties of this versatile technique for in-field analysis, the detection of a nuclear incident or other terror attack can be conveniently carried out.

For WP420 about analysis of γ-H2AX protein, first objective is to demonstrate, on a pig model, the appropriateness of using the hair follicle as measurement vector to assess irradiation of an individual. It is then necessary to develop quick field methods for sampling hair follicles, for blood collection and segmentation of the lymphocytes, for protein extraction compatible with field use, for quick in-field measurement of γH2AX in samples. Further objective is to establish dose-effect curves and a wide correlation for the various methods for measuring H2AX phosphorylation on human fibroblasts and blood lymphocytes at the laboratory level: overall approaches using FACS, foci intensity compared to individual foci scoring under fluorescence microscopy. To validate the global approach, results obtained by laboratory methods will be compared to those obtained with the in-field methods.

WP430 proposes to develop a bio-dosimetric tool based on the finding that centrosome amplification is a dose-dependent response to DNA damage. A recently-described cellular response to DNA damage is the amplification of centrosomes, the principal microtubule organizing centers in animal cells. As these can be detected and quantitated by microscopy, this response offered a potentially new method for detecting radiation exposure. First objective is to generate a suite of monoclonal antibodies that can be used for centrosome quantitation in automated microscopy approaches to biodosimetry. To generate centrosome antigens and monoclonal antibodies, proteins will be expressed in human

tissue culture cells and purified by affinity chromatography; then a series of immunogens that comprises representative centrosomal protein antigens will be prepared by recombinant protein expression in bacteria and will be used for generation and optimization of a battery of commercial monoclonal antibodies. Monoclonals will be tested on primary human cells and centrosome amplification will be analyzed in both cultured cells and ex-vivo human cells. Along with those generations, an automated immunofluorescence microscopy method will be optimized to allow performing a quantification of centrosomes.

For WP440, the goal is to validate technologies by comparison with other techniques, as well as to give a summary of their abilities and functions by comparing their application field, sensitivity, time of response, throughput, complementarity, and readiness level. Finally a program and schedule for using these techniques will be provided.

2.5 WP500 – Software development and integration of components

First objective of WP500 is to integrate all newly developed sensors with existing sensors on a hardware point of view. Sensor platforms including a GPS device must be developed to map the radiological situation, and to provide the availability to send the gathered information to a coordinated network.

The second objective is to develop a software frame to process the monitoring results of the various sensors under development in other work packages, to interpolate results to get a picture of the radiological situation of the area of interest, and to provide an evaluation of the exposure and contamination status of victims, based on the data from deployed sensors and their position in the area.

In addition, a decision supporting tool, taking into account medical, radiological and resources aspects, will be developed for first responders and commandment echelons to support them in carrying out quickly and efficiently the triage, providing guidance in the various steps of the triage.

After hardware development on smart sensors and their integration in BOOSTER toolbox, the interfacing of the many monitoring devices to the software components SIMACOP and DSS must be completed. A prototype of the core software must be developed and tested before and during the final demonstration exercise.

2.6 WP600 – System validation and training

The main objectives of WP6 are the validation of the whole system through a BOOSTER Final exercise, the assessment of the operational efficiency of the toolbox by performing a real field exercise, training for the first responders participating in the exercise and also for the BOOSTER community and potential end users.

During the final exercise to be organized in Hungary, all devices and techniques integrated into the BOOSTER toolbox will be presented and demonstrated in realistic situation based on the scenario analysis performed in WP200. Techniques and methodology will be validated by end users and experts attending to the event. During debriefing, appropriate questionnaires will be used to gather information and feedback from the participants and external observers.

A training session will be hold in Hungary before the exercise, to describe and present all BOOSTER devices and techniques used during the exercise to the first responders participating in the final exercise. In addition, a complete course with training material in different European languages will set up in a learning platform for being accessible to all BOOSTER community and potential end users.

3 DESCRIPTION OF MAIN SCIENTIFIC AND TECHNICAL RESULTS/FOREGROUNDS

3.1 WP200 – System requirements & design concept

A **high-level operational scenario** was defined at the end of 2010 and reported in D200.2. It describes the chronology of events and operations following a terrorist attack in a crowded stadium, involving several dirty bombs. This report used knowledge and expertise of partners involved in the project and expert team, in order to get a trustable synopsis for further scenario description.

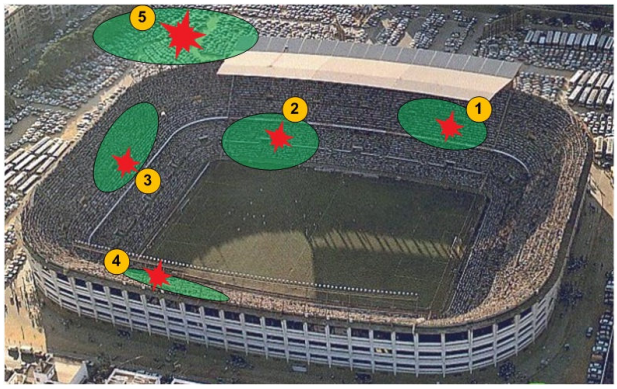

Figure 1: Scenario situation after the attack

The **technical scenario** D200.7 delivered in May 2013, describes the technical configuration to put into practice during the demonstration exercise: necessary zones to be established (red, yellow and green), and technical description of the equipment to be used.

The **trial scenario** D200.8 delivered in May 2013, defines in detail the unfolding of the demonstration exercise with all the procedures and actions described and scheduled minute by minute. BOOSTER demonstration exercise took place on May 16th 2013, at the sport centre at the Csillerberc campus of the Hungarian Academy of Sciences (Budapest, District XII). The different areas including the location of the different elements and devices which participated in the demo are shown in Figure 2.

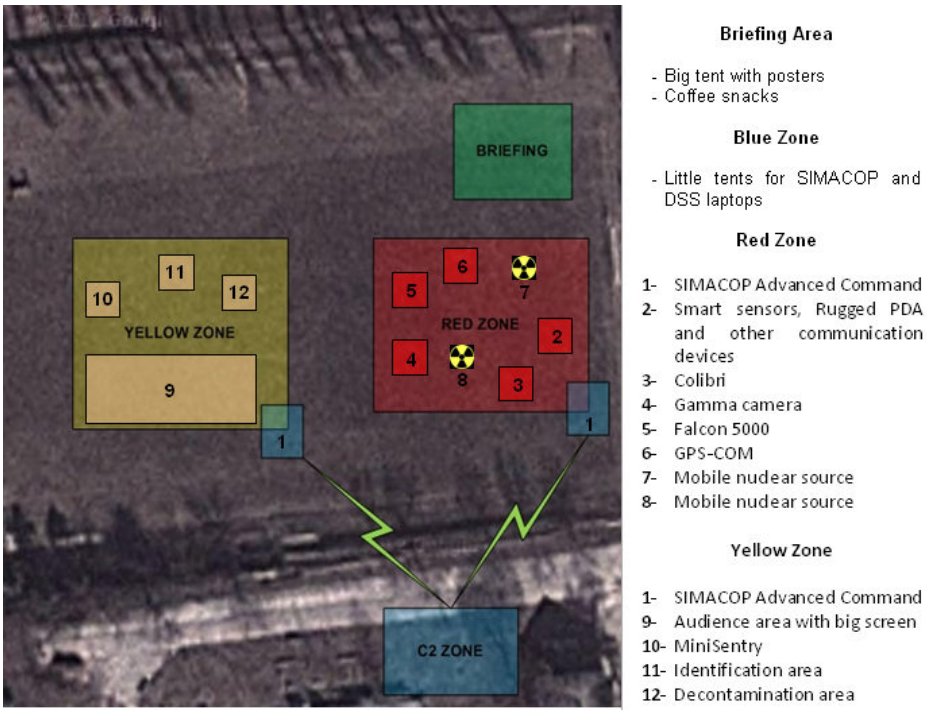

Figure 2: BOOSTER trial areas

User requirement tracking

The large experience and expertise of several project components on bio-dosimetric tool development, gamma camera use and crisis management, helped for preparing a first draft of BOOSTER system user requirements. The lack of related documentation and materials at the beginning of the BOOSTER project made very difficult to find any potential end-user or expert in order to conveniently define user requirements and validate the system that had to be conceived. Besides this evident constraint, HAEA, KIT, IKI and CEA succeeded in recruiting 4 end-users and 2 experts during the first period of the project. The feedback received from the 6 potential end users, who actively participated in defining the final BOOSTER user requirements, was used as additional source for refining the final BOOSTER user requirements, reported in D200.1 in July 2011. Nevertheless, one of the two experts reconsidered his participation one year later arguing multiple overworking issues.

During the mid-term review, reviewers and the commission considered that involvement of external end-users and experts was not large enough and recommended to the project consortium to improve this point. Some additional attempts were carried out by CEA in the year 2012 to contact 14 new persons but only 2 of them definitively accepted participating in the evaluation of the BOOSTER project. From mid-January 2013 and according to the above commission demand, CEA with the help of CANBERRA continued to strive in finding the appropriate panel of end-users and/or experts to include all the different specific competencies present in the project. Up to 43 persons were contacted during this period and 22 of them were very enthusiast with the project scope and showed their availability to participate in the corresponding evaluation process.

Consequently, the whole task of the opinion solicitation from end-users and/or experts required a lot of effort and time, resulting in an extra work of more than 6 PM. In addition, due to experts' overall work overload and agenda restriction, it was impossible to define a common date for all experts to explain the project at a same meeting session. It was thus necessary to present the project to each expert via face-to-face or phone interviews, to answer their doubts or questions during the weeks following the presentations, and finally to insist with them to get their comments.

On the whole, up to 63 end users and experts were contacted during the BOOSTER project. 6 experts participated since the beginning of the project. 16 experts provided their feedback on project scenarios and deliverables in 2013. 12 experts participated in the demonstration exercise.

Deliverable D200.5 describes the process for recruiting end users and experts all along the project life. Deliverable D200.6 focuses on the results obtained from the process for gathering and summarizing the feedbacks (opinions, recommendations, comments or advices) from external end-users and experts on the content of the BOOSTER project. This deliverable also includes a traceability matrix, which has been generated to track and assess the different developments undertaken during BOOSTER in answer to end users' comments.

System architecture design has to provide the specifications and the description of the architecture of the BOOSTER System according to the needs identified by end users. The output is the BOOSTER System architecture which was the main input for design and implementation tasks in WP500. Report D200.3 delivered in July 2011 describes in details the system intended capabilities, appearance, and interactions with users, for software developers. The architecture specification is a guideline and continuing reference point as the developers go on with the developing process. In this document, only the functions from the user's point of view and the links between components are considered.

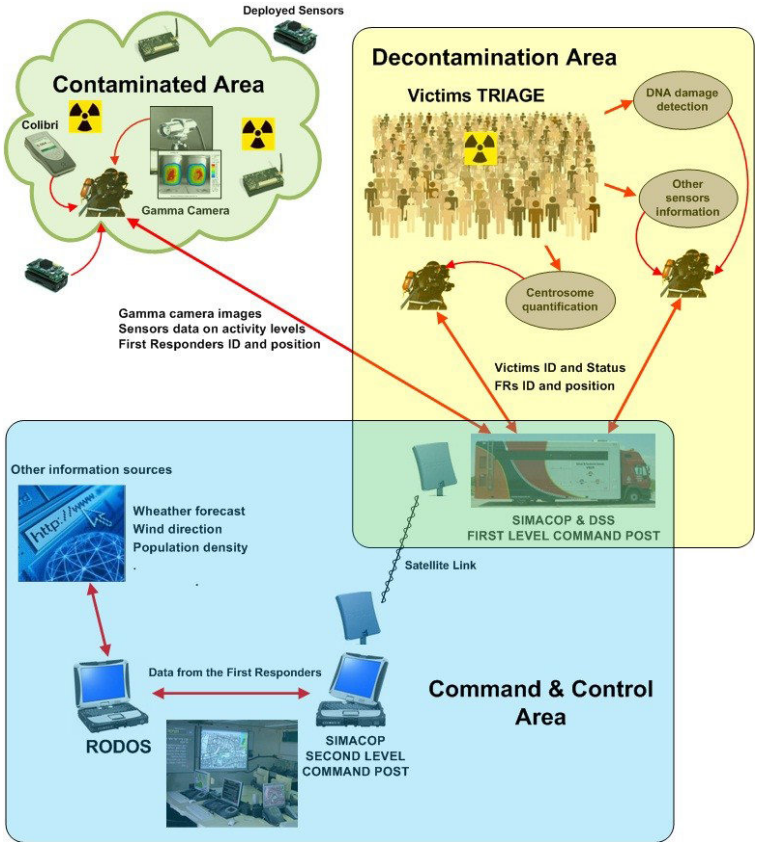

Figure 3: BOOSTER overall architecture

Exploitation strategy was defined at two steps of the project. A preliminary report D200.4 was delivered in August 2011 to develop the potential view of each partner concerning:

- The developments they are carrying out and their fallout on their institutes (e.g. for future researches) or for the field of research and development their institute is involved in;
- The way they plan to disseminate their results (planned publications and conferences);
- The different relations that these developments could open with the other partners and the fallout of their developments in the field of industry/research centers;
- The way developed tools are planned to be exploited out of and after the project.

The final report D200.9 summarizes the main aspects of the on-going and future exploitation strategy concerning the BOOSTER project. It also contains a brief description of all the activities carried out by each partner as well as a qualitative analysis of the results obtained and the means that will be adopted to take profit of the diverse sustainability opportunities created.

A special attention was also made to the non-commercial exploitation of the results, not only in terms of new research activities, start-up and assessment of scientific cooperation inside and outside the BOOSTER Consortium, but also in terms of identification of mid-term scientific and technical challenges for an improvement and an extension of the use of BOOSTER approach in operative conditions for the general frames of nuclear or radioactive emergencies.

CANBERRA, in service for more than four decades, is the worldwide leader in nuclear measurements. The key to our success lies in our ability to identify the right customers and understand their needs, in order to bring them value-adding and innovative solutions. Furthermore, thanks to CANBERRA's long experience in Homeland Security, we can assist the other partners of the BOOSTER project to spread their developed technologies and to overcome much more easily most of the industrial constraints.

Due to the particular innovative aspect of the new developed biodosimetric portable tools, the integration of these tools in mobile vehicles (Figure 4) can be foreseen according to the real necessities of potential stakeholders.

Figure 4: CANBERRA mobile vehicle for in-situ and real-time radiation monitoring.

Although being bulky, the inclusion of laboratory based biodosimetric techniques developed in the BOOSTER project such as centrosome quantification, inductively coupled plasma mass spectrometry (ICP-MS) and liquid scintillation counting (LSC) may be envisaged as well, by means of a turn-key transportable intermodal container, as shown in 5. The latter will be particularly useful in the following situations:

- Detonation of nuclear weapons
- Radioactive or nuclear accidents
- Attacks on nuclear power plants
- Dispersal of radioactive material with or without explosives

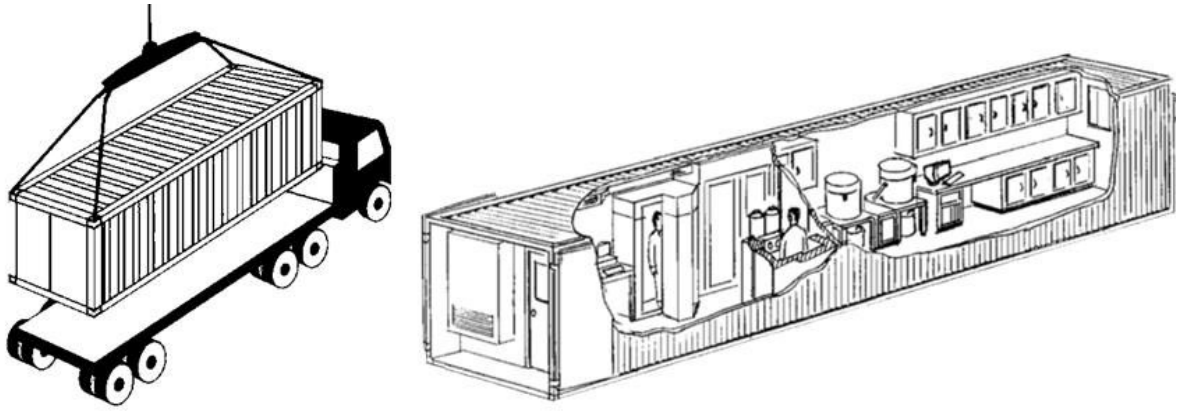

Figure 5: Transportable intermodal container for the whole BOOSTER System.

As conclusion, all the planned objectives for WP200 have been fully met during the BOOSTER project and in some cases they even exceeded their original expectations, namely regarding the whole task of the opinion solicitation from end-users and/or experts. The third period was characterized by a high effort for the recruitment of experts and end-users and the collect of their feed-back, and the definition of the future strategy of exploitation of BOOSTER toolbox. 41 experts and end users were contacted during the third period, according to criteria based on country distribution, competence field, expert/end user, and gender. 16 experts and end-users first reviewed the project documents for the preparation of the exercise and their comments and questions were used to track users' requirements and to adapt the demonstration exercise. Then 12 experts and end-users also reviewed the final BOOSTER toolkit during the demonstration exercise.

The operational, technical and organizational scenarios of the exercise were defined with the definition of several zones in the field, the geographical distribution of the devices in the zones, the strategy of data transmission between the devices and data securing. Finally the exploitation strategy was defined with each partner.

3.2 WP300 – Fast evaluation

3.2.1 WP310 – Miniaturization of gamma-camera

Adaptation of the new generation of gamma camera
GAMPIX gamma camera was adapted by CEA LIST to fit the requirements of a crisis situation deployment by first responders. During period 1, a complete range of coded masks was simulated via MCNPX Monte Carlo simulations, in order to determine a set of coded masks with the best performances (sensitivity level and angular resolution level). This set of selected coded masks was manufactured during the second period, and tested in laboratory at CEA LIST premises.

Coded masks are application-dependent: the configuration of the coded masks (number of holes in the mask, size of these holes, thickness of the mask, etc.) is more or less adapted to a dedicated application. As examples, a thick mask is more adapted to high energies, bigger holes lead to a better sensitivity, whereas smaller holes allow a better spatial resolution. The tests and characterization process, carried out over period 2 and 3, allowed to determine all those characteristics and highlighted a coded mask that gave the best compromise on all features. This coded mask was therefore chosen for being the standard coded mask on the gamma camera.

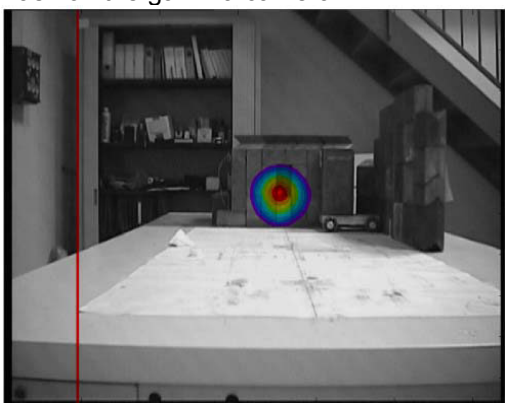

Figure 6: Image obtained with the gamma camera at CEA LIST premises (source of ^{241}Am at 1m)

The chosen mask is a MURA mask of rank 7, with a thickness of 4 mm. Its rank (which determines the number of holes and their diameter) allows a good sensitivity despite a perfectible resolution. The angular resolution was considered as a secondary interest for BOOSTER application, since the main information required is a quick location of sources and hotspots. In parallel, the thickness of 4 mm was a good compromise between the best configuration for high energies (such as ^{60}Co) and the loss of sensitivity on the periphery of the sensor (due to autocolimation effect of high thickness) which degrades the imaging capabilities for extended sources.

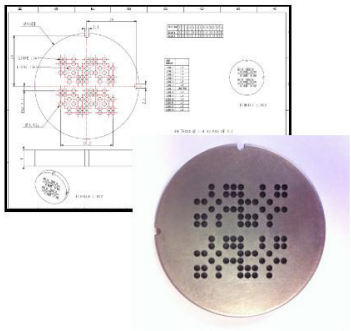

Figure 7: Mechanical realization of coded mask of rank 7

Extended presentation of the adaptation results are presented in deliverable D300.1 delivered in September 2013.

Characterization of the adapted gamma camera for radionuclides of interest
A comprehensive test of the gamma camera was realized during the whole project timeframe, in CEA premises. These tests allowed validating every modification that occurred on the system, from acquisition parameters level to hardware modification of the prototype. This iterative approach aimed at minimizing the risks of failure of developments, with a validation of each step of research.

Tests were therefore realized over the range of operation of the gamma camera (from ^{241}Am to ^{60}Co). They took advantage of the fully equipped imaging laboratory for a characterization of imaging capabilities.

A first measurement campaign at KIT Calibration Laboratory, carried out at the end of 2011, led to the definition of a new calibration plan for the second measurement campaign. Complete results from the first measurement campaign are presented in deliverable D300.1 delivered in September 2013.

A second measurement campaign, carried out in October 2012, allowed:

- The study of the linearity of response of the gamma camera, in terms of dose rate, was tested on the ^{137}Cs irradiator from very low dose rate (some nGy/h) to very high (some Gy/h). This type of characterization requires a range of dose rates wide enough to cover several aspects of the envisaged uses of the gamma camera, and therefore leads to a different type of requirements for its performances.

  For very high dose rates, the ability of the gamma camera to be tested is linked to the efficient detection of high level of threat: hot spots resulting from the dirty bomb. The matter is to detect those threats in the minimum time and at a very long distance. The precision of the response (in particular, with regard to the dose rate estimation) is not so critical.

  For very low dose rate, the response of the gamma camera is important for detecting traces of contamination on people – a sort of scanning – that goes out of the red zone, triggering the contamination portal. The most important is not the sensitivity (as it is a second level of control, it is possible to take more time for detecting the isotopes), but a very precise information on the location of the contamination and a good estimation of the dose rate of the contamination spot.

  Therefore, the test on the 137Cs irradiator gave the necessary information for testing the performances of the gamma camera in its two envisaged uses.

- The study of the energy response of the gamma camera was tested on the X-Ray irradiator. This test allowed to reach the lowest detectable energy of the gamma camera, and to calibrate the different “threshold parameters” for extending the imaging range in terms of energy as much as possible. This second study allowed giving precious information for further development of the gamma camera in the frame of the addition of spectrometric information to the image.

A complete presentation of the results of this second measurement campaign is included in deliverable D300.3 delivered in October 2013.

Finally, additional measurements were carried out at IKI Budapest on sources of interest for Homeland Security applications. Dirty bombs are the major radiological threat addressed in the context of BOOSTER project. These bombs are made of radioactive material of various nature and origins. It therefore appears appropriate to test the gamma camera GAMPIX on the largest possible number of radionuclides. We relied on the American National Standard Performance Requirements for Spectroscopic Personal Radiation Detectors (SPRDs) for Homeland Security, which classifies radionuclides into four categories. The Center for Energy Research has other radionuclides listed as special nuclear materials (SNM), naturally occurring radioactive materials (NORM) and industrial radionuclides. It was not possible to test medical radionuclides but these are rarely used in dirty bombs because of their short half-life.

The tests carried out at the Center for Energy Research of the Hungarian Academy of Sciences, Budapest, had not the aim of determining the performances (in terms of sensitivity of resolution) of the gamma camera, but its ability to image typical radioisotopes liable to be used in the context of a dirty bomb. The results of this measurement campaign allow validating the use of the gamma camera on most of these isotopes, with a good sensitivity with regard to the activity of isotopes imaged. Indeed,

the activity of isotopes that were used for these tests do not represent any threat (too low activity) and therefore, in the case of a real attack with a dirty bomb, the gamma camera would react far faster to detect dangerous area (activity at a level that can easily be considered as threatening).

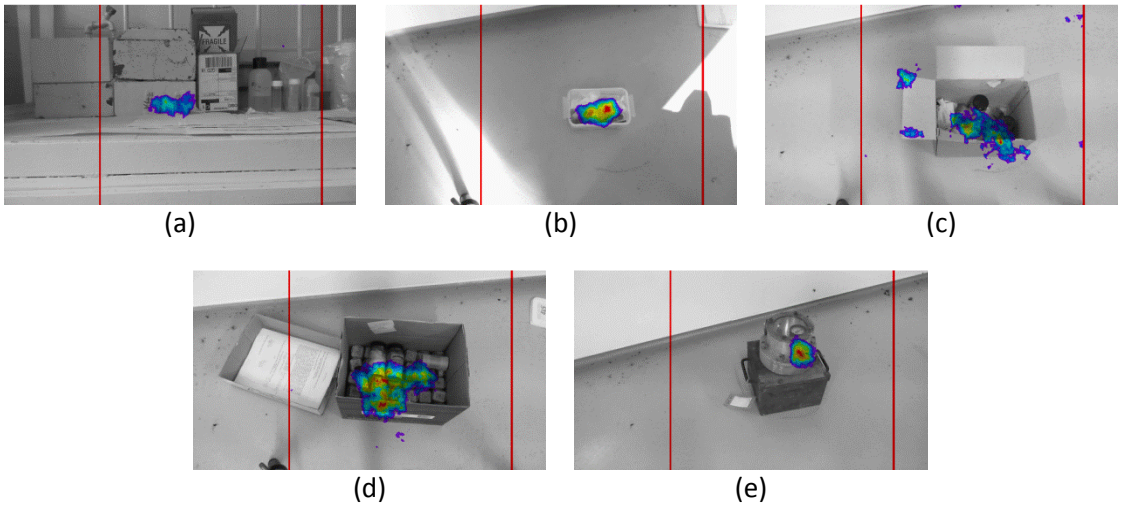

Figure 8: (a) Uranium enriched to 95% (3850s acquisition) , (b) Uranium enriched to 20 % (acquisitions in mask and anti- mask positions of 1600s each), (c) Uranyl nitrate (acquisition in mask/anti- mask positions 2x1800s), (d) Uranyl acetate (acquisition mask/anti- mask 2x1400s), (e) Plutonium 239 (acquisition of 940s)

The results of IKI measurement campaign are presented in deliverable D300.1 delivered in September 2013.

3.2.2 WP320 – Assessment of other sensors

Retrospective dosimetry & Digital fluorimeter development

The main objective of retrospective dosimetry is to assess or, if possible, to determine posteriorly the absorbed dose of a sample after a radioactive incident. On the one hand, some materials available in the environment (such as sodium-chloride, dolomite or calcite) have useful luminescence properties and that is the reason why these can be used for retrospective dosimetry. On the other hand, the subservient luminescence properties of surface-mount devices (SMD) have also been previously proved suitable for retrospective dosimetry purposes.

Thermo-luminescence (TL) and optically stimulated luminescence (OSL) are the most important analytical methods used in retrospective dosimetry. Therefore, retrospective dosimetry is planned to be applied by using naturally occurring minerals, ceramics, semiconductor devices in order to estimate the dose received by the casualties.

Figure 9: Measuring head of the portable TL/OSL reader

The aim of the task was the selection and characterization of the most suitable retrospective dosimeter materials using both TL and OSL techniques: sand (quartz), lime-stone, dolomite and rock-salt (table-salt). Components of mobile phones can serve as emergency personal dosimeters (SMD resistors).

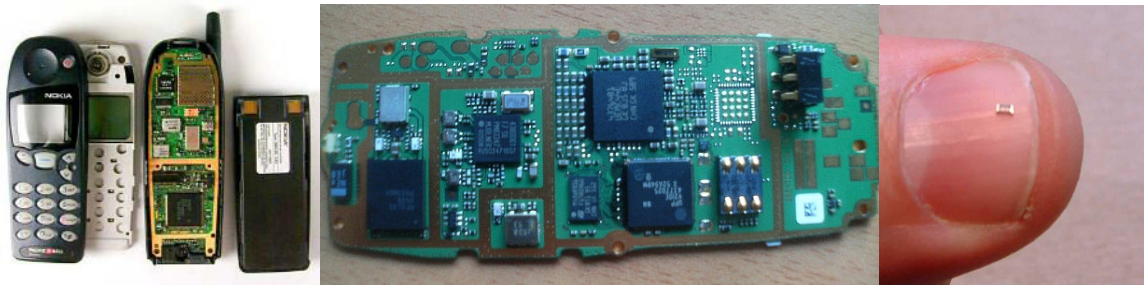

Figure 10: SMS resistors of a mobile phone

Other planned task was the development of a portable and digital fluorimetric reader for in-field analysis of absorbed dose determination. The validation of the method developed will be done by irradiating retrospective dosimetric materials to known doses, in a reference dosimetry laboratory. The measurement of the response will be carried out and comparison of the results achieved from the analysis with reference doses performed in the laboratory will allow a characterization of method applicability.

Based on the data that can be found in the literature, one can say that most of the optically stimulated luminescence (OSL) measurements are carried out with the Risoe TL-OSL Reader worldwide. This is a multifunctional commercially available laboratory device that is suitable for doing series of OSL and thermo-luminesce (TL) measurements on a lot of samples. During the measuring cycles, the heat treatment and the irradiation of the samples are also possible without touching them. The whole procedure, the steps of the measurement and the collection and processing of the data are controlled by a computer.

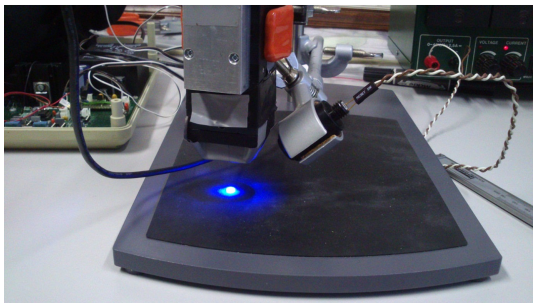

Figure 9: Portable digital fluorimeter for in-field measurements

The Risoe Reader is equipped with two or three illumination units of different wavelengths depending on the configuration ordered. This versatile features of the Risoe TL-OSL reader makes possible its application in numerous fields of dosimetry and other applications like geological and archeological dating, control of food irradiation and of course retrospective dosimetry. Also this reader was applied for the first OSL measurements carried out on different components of modern electronic devices. Our first results relative to the OSL properties of the surface mounted (SM) resistors were achieved using a Risoe Reader too.

Reports D300.5 about retrospective dosimetry method and D300.6 about the construction of a portable fluorimeter were delivered in March and April 2013, respectively.

Development and test of low-background gamma spectrometers

A transportable iron chamber and lead container were designed, constructed and tested at IKI during 2010 and 2011 for low-background gamma spectrometric measurements to obtain a quick identification of radioactive gamma isotopes after a terrorist attack.

These instruments are planned for the measurements of contaminated samples originating from persons (biological samples, as saliva, blood and urine) or from the environment (swipe, soil and plant samples) and for determination of the elemental and isotopic composition of radioactive materials/samples. Planar and coaxial type HpGe crystal detectors were used in the systems in lower and higher energy range.

The total weight of the measurement set-up is about 100-110 kg or 200 – 240 kg, respectively and the required place is about 500 x 500 mm. Therefore these measurement systems can be transported by a van, caravan, jeep or pick-up truck and are useful for quick in-field measurements to the first characterisation of the victims.

The transportable systems developed for in-field measurements were further tested using different type of radioactive materials and sample types.

Figure 10 : Low background portable gamma spectrometer

Test of Canberra systems

To fix the perimeter of the exclusion and controlled area, as well as to perform a continuous control on the radiological situation, two digital dosimeters were proposed by CANBERRA: Colibri and Ultraradiac. Colibri has a large color-touch screen to display measurement results for up to eight CSP-CANBERRA Smart Probes at the same user location. It is integrated into the SIMACOP system for a parallel on-line data transfer with the responder/command emergency post.

Figure 11. Colibri

Canberra also updated the GPS-COM accessory of UltraRadiac dosimeter in order to communicate wirelessly via radio-frequency technology with the decision making team. It will operate using a supervisor unit, which is the main communication link between the GPS-COM, and a series of repeaters. With a minimum setup of one supervisor and one repeater, communication is guaranteed up to 1 km distance.

Figure 12. GPS-COM

CANBERRA has also set-up a contamination monitoring portal that will be located at exit points of the exclusion and controlled area. Therefore, when each person passes through this portal, the first responders will be able to classify him as being contaminated or not. After that, further measurements will be performed using other CANBERRA alpha and beta probes. Once the victims are evacuated from the exclusion and controlled area, they will be subjected to a first medical check-up to be sure that they are fully safe or not. As a next step of development, CANBERRA is being focused on the communication with the supervisory software.

Figure 13: MiniSentry

Preliminary report D300.2 about assessment of other sensors was delivered in August 2012, and final report D300.4 was delivered in June 2013.

3.3 WP400 – New bio-dosimetric tools

3.3.1 WP410a – Characterization of the exposure – Quantification of radioactive substances

Quantification of radioactive substances by gamma spectrometry

Low-background gamma-spectrometry can be used to quantify the radioactive substances incorporated by a person immediately after an accident. It can be carried out by analysis of body fluids, such as blood and urine. By knowing the activity of the radionuclides in the body and the approximate time of the incorporation, the total dose received by the person can be estimated.

Development of methods for the analysis of biological samples (calibration technique) was carried out. Calibration curves were determined using uranium-containing solutions with different concentrations as model biological samples. The important analytical parameters (detection limits, repeatability, and accuracy) of the technique were determined. Analysis time is also an important parameter, therefore it was also optimized. Some of the results obtained can be seen in the following table.

Isotope	U-235		U-238	
Sample type	Blood	urine	blood	Urine
LOD in 1200sec	5 µg	40 µg	6 mg	15 mg
LOQ in 1200sec	15 µg	120 µg	20 mg	45 mg
LOQ in 5000sec	8 µg	40 µg	6 mg	15 mg
LOD long time measuring	2 µg	3 µg	0.5 mg	1.5 mg

Table 1: Detection limits (LOD) and quantification limits (LOQ) obtained with the transportable high resolution gamma spectrometric devices

This work is described in report D400.10 delivered in December 2012.

Quantification of radioactive substances by liquid scintillation

The liquid scintillation technique is used for analysis of alpha- and beta-activity in urine samples within a few hours. This laboratory-based system can perform precise determination of the dose for the in-field triage.

Due to high quenching, the activity in blood samples could barely be determined by the LSC measurement technique. In addition, it is necessary to have a complex and time-consuming sample preparation for blood as sample matrix. The determination of the activity in saliva mainly provides the information about the contamination of the oropharyngeal cavities. The difficulty here consists in the determination of the actually incorporated activity to estimate a dose. Therefore, the technique will be used only for urine analysis, which is the most informative and sensitive.

The analytical method has been adapted for analysis of some essential radionuclides and their isotopes (^{208}Po, ^{238}Pu, ^{241}Am, ^{235}U, ^{238}U, ^{229}Th) in urine. The analytical method contains a sample preparation step (preconcentration, filtration and preparation of the residue), an instrumental analysis and calculation method. Dose estimation and determination of dose ranges were carried out using mathematical modelling and calculations. Validation of the technique was carried out by the use of radioactive standards and, in the case of ^{241}Am, by gamma spectrometric control measurement. This method has low detection limits and is rapid and simple enough for rapid sample analysis and absorbed dose estimation after the in-field triage. Using this technique, it is possible to determine an activity level which corresponds to a dose of 20 – 200 mSv.

This work is described in report D400.10 delivered in December 2012.

Quantification of radioactive substances by ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) is used for analysis of radionuclides incorporated in body fluids. Laboratory-based ICP-MS analysis is capable to perform precise determination of the dose after the in-field triage. The analytical protocols which have been developed for environmental and swipe samples were re-optimized for overcoming potential matrix-related interference during the analysis. The adapted and modified analytical methods are capable of the analysis of some essential radionuclides and their isotopes in biological samples (blood and urine).

The methodology has very low detection limits (fg, pg) and is rapid and simple enough for rapid sample analysis and absorbed dose estimation after the in-field triage. Using this technique, it is possible to determine an activity level which corresponds to a dose of 20 – 200 mSv and even lower.

Laser Ablation ICP-MS method has been developed for analysis of hair samples and saliva by IKI. However, analysis of human hair samples using LA-ICP-MS technique was not found to be sufficiently informative for determination of absorbed dose, since the infiltration of the radionuclides into the hair and hair growth are too slow for this analysis. Saliva is not informative enough for dose estimation and the calculation of the whole absorbed dose is very difficult with saliva. Thus these sample types were eliminated.

LA-ICP-MS and LIBS methods were developed for direct analysis of blood and urine samples. The advantage of this method is that only one drop of blood or urine (after drying) can be enough for determination of absorbed dose. This work is described in report D400.10 delivered in December 2012.

3.3.2 WP410b - Characterization of the exposure – Quantification of nanoparticles

Sampling and sample preparation

Particle analysis and assessment of cutaneous particle contamination are possible by surface cutaneous impressions. Particle samples are taken with swabs and analyzed by scanning electron microscope, laser ablation (LA) ICP-MS and also LIBS techniques. Contamination by ingestion and radioactive particle inhalation can be detected from the level of radioactive particles present in the saliva and nasal mucus of an exposed individual. After sampling, the swab tip and the biological sample (nasal mucus and saliva) are dissolved in an aqueous or organic solution using ultrasonic bath and spread on a slide.

The methodology for analysis of radioactive particles on a slide using laser ablation ICP-MS technique was developed using test particles containing U and Pu. For localization and identification of the alpha-emitting particles, nuclear track detectors were used and a mathematical algorithm was developed. The method was validated using reference materials and analysis of the particles in bulk samples by LA-ICP-MS technique. This work is described in report D400.6 delivered in July 2011.

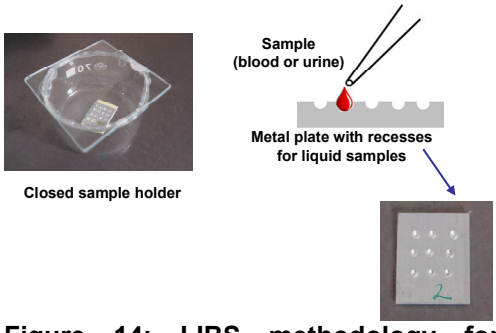

Figure 14: LIBS methodology for biological liquid samples

This work is described in report D400.2 delivered in December 2012.

Development of an analytical method for particle analysis using human body samples and determination of exact concentrations in the samples technique was performed by LIBS. Calibration method for biological samples was developed and many types of sample holder techniques were tested. Standard cesium test solutions and real biological samples (human body fluids) spiked with cesium were used for test and validation of the method.

In the case of LIBS analysis, a 'dribbled-and-dried-sample' technique is used for determination of radionuclides in biological samples. The applied sample volume (2 µl) is so small that the method can be considered as particle analysis. Therewith, it is very fast and relatively simple. This method may represent a supplementary technique to the LA-ICP-MS analysis of samples originating from the swab after dissolution.

Diffraction methods and particle analysis

LIBS analysis can be used for the analysis of radionuclides at low concentrations in natural body fluids (saliva, nasal mucus, and tears at the commissure of the eyes). This technique enables to estimate the abundance of radioactive particles in a body. Moreover, the level of contamination by particles, as well as toxicity of particles, can be taken into account in risk assessment.

The risk of contamination by toxic particles can be evaluated by measuring the abundance and nature of different types of particles at the main routes of contamination such as the mouth, nostrils and eyes, as well as the toxicity of these particles. Indeed, to develop the test, we need to determine which diffraction technologies could be implemented on the field. After various tests, we have chosen to focus on a compact FACS device and specifically on its diffraction functions. We have thus modified the original functions of diffraction of a FACS GUAVA HT8 which was calibrated to be able to analyze the diffraction of particulates in suspension.

The flow cytometry (FACS) can be used to estimate the abundance and granulometry of nanoparticles, as a function of the modification of SSC (Side Scatter) corresponding to the diffraction signal in the FACS technology. The shift of the SSC signal for measuring Nano Diamond (NDs) interactions is sensitive, since the signal remains significant for a nano-diamond solution of < 1 µg/ml. We developed a spatial vector analysis to estimate and segregate the different types of particles present in a complex mixture of particles. Tests for suspensions of nanoparticles of different size concentration, produced in water or in various dilutions of body fluids (saliva and tears), demonstrate that we can differentiate nanoparticles of 20, 50 and 100 nm with very high precision and rapidity.

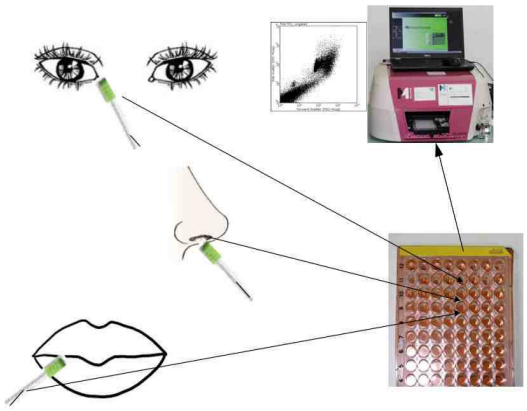

Figure 15: Test of diffraction

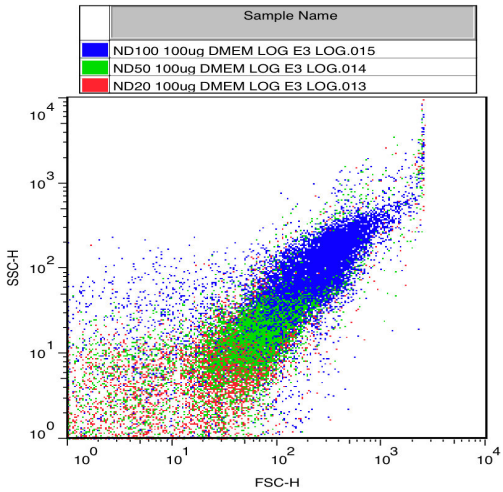

Figure 16: FACS analysis of a mixture of nano-diamonds of 100/20/50 nm

For risk assessment, the toxicity of particles was evaluated through the same FACS technology, with a rapid method using a Caki-1 cell model to evaluate the toxicity of collected particles. This model allows assessing the toxicity of a particle in less than 2 hours.

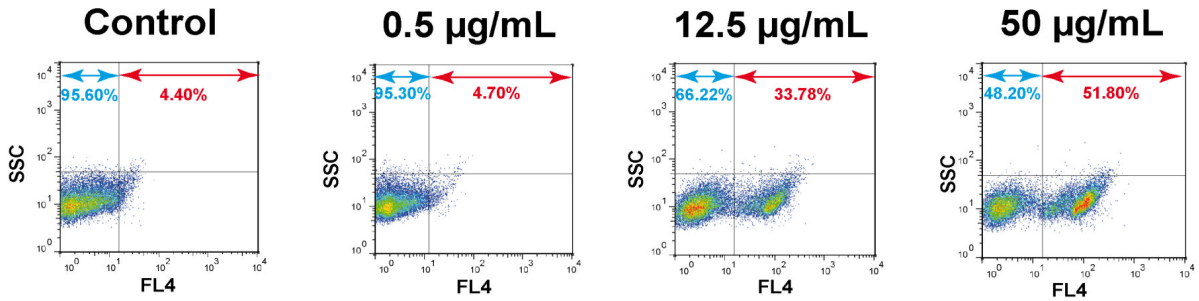

Figure 17: FACS cell mortality induced: living cells in blue, dead cells in red

This work was reported in deliverables D400.12 delivered in April 2013 and D400.13 delivered in October 2013.

LIBS analysis (calibration and miniaturization)

Preliminary information about the contamination of a sample originating from the environment or a victim can be obtained within seconds with LIB technique. Different sample preparation and calibration techniques were developed and tested for analysis of human body fluids to optimize exact concentration assessments.

Biological samples must be analyzed in a closed system to avoid the presence of hazardous materials. Since biological samples cannot be measured directly, different 'dribbled-and-dried' techniques were developed and tested: Fast drying of the sample before the analysis in a closed sample holder.

For testing, certified biological reference materials (urine and blood serum) were spiked with different radionuclides and heavy metals. For liquid measurements 'lab-on-chip' (capillary) techniques and closed sample holders were tested ('sandwich technique'). The method was validated by comparison of results with ICP-MS measurements. Report D400.2 was delivered in December 2012.

Figure 18: LIBS equipment

Development and miniaturization of a portable low volume LIBS system started in 2011. The laser is specific and strong enough for in-field measurement of liquid samples (direct analysis of human body fluids). The laser head has a special portable construction for in-field analysis.

The LIBS system was tested using standard liquid solutions and spiked biological samples. Tests concerned the accuracy of materials found in samples and the repeatability of analyses. An optical detector with the highest resolution physically possible was adapted to obtain a fit-for-purpose instrument: small, portable, useful for in-field analysis, high resolution in spite of its small volume to carry out extended analysis for isotope ratios.

New software was also developed for detection of radioactive contamination sources and surface detection, with a large range of functions. The software is applicable for qualitative and quantitative analysis of elements. LIBSCAN25+ developed system was found well-applicable for analysis of uranium-containing materials and determination of uranium isotope composition, in laboratory and also in-field environment. Report D400.9 was delivered in July 2013.

3.3.3 WP420 – Biodosimetry using the detection of DNA damage response

A major consequence of ionizing radiation is the generation of DNA double-strand breaks (DSBs). DSBs induce a rapid and coordinated series of cellular responses, one of which is the phosphorylation of histone H2AX in large chromatin regions surrounding the break. The quantitation of γH2AX by immunofluorescence microscopy allows the determination of the radiation dose received by the cells. The goal of the test is to provide, in less than 20 minutes, a fast biological measurement of genomic damage caused by ionizing radiation on different parts of the body of an individual exposed, and an estimate of the dose received.

Biological sampling

Figure 19: Bar code wristband and bar code reader for fast identification of victims and follow-up of their personal results

Before going through the contamination control checkpoint, each victim or involved person will be tagged with a bar code wristband with a fixed ID. All samples taken from a specified person (may it be biological sample or personal belongings taken for further analysis) will also be tagged with this same bar code for fast identification and association of results. This bar code system will allow an efficient follow-up of victims' status in real time by sanitary staff and staff responsible for triage.

The biodosimetry test is designed to be performed in less than 20 minutes. Some steps require incubation times of several minutes, so two to three subjects will be tested at a time. As a consequence, the test station can handle 6-9 people per hour.

Although the entire kit should be portable, it is important to note that its implementation should be performed on individuals with no external contamination, implying that people are already decontaminated. The test should be conducted in an area protected from any irradiation or contamination, for example, positive pressure inflatable tents set up in a protected area.

Blood sampling

The sampling toolkit for quick blood test lymphocytes consists of:

- An alcohol heparin sterile compress, to sterilize the fingertip and reduce blood clotting;
- Haemolancea to pick the fingertip and to collect two drops of blood (120 to 150 µl);
- A compress of disinfectant, and calcium chloride applied to the fingertip to stop the bleeding;
- Microtubes containing magnetic beads coated with antibodies against red blood cells.

The test will provide information on potential internal radiation (local or general) of the individual by determining the rate of genomic DNA damage in lymphocytes. The operator of this examination is not necessarily a medical staff, thus we focused on disposable sampling equipment for diabetics "Haemolance". To perform the analysis, we need to collect twice 50 µl of blood. The entire blood collection process is performed in 10 s.

As only extracts free of red blood cells give γH2AX signals proportional to the irradiation rate, red blood cells are removed by adding 50µl of magnetic particles coated with antibodies against blood markers A, B, Rhesus, etc. in the tubes of the 96-well plates which will receive the blood. A clot of red cells forms in the tubes after the addition of the magnetic particles coated with antibodies. The clot can be removed with a magnet. The lymphocytes sampling operation is completed in 2 minutes.

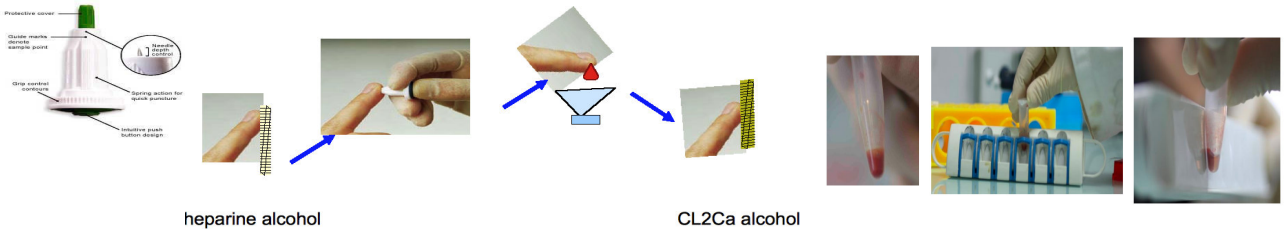

Figure 20: Sampling process for blood

Hair follicle sampling

Sample collection remains the limiting step to carry out a rapid test on a large number of individuals. For the rapid removal of hair follicles, we first developed a method using patches. However, the hair follicles were consistently outside the patch, forcing the operator to manually fold them to introduce them into the extraction solution, this step demanded 10 s.

We improved the fast collection kit by developing a retractable piston covered with a patch of glue. The piston is applied to the sampling area, the hairs adhere, and when the piston is quickly removed, the hairs are pulled out with their bulb which remains outside the patch. The piston is then retracted, which folds the bulb on the patch in one step. Samples can be directly transferred to the extraction buffer in a 96 well plate. At a rate of 5 s per sample, it is possible to collect 96 samples in 8 mn.

Sometimes due to the low angle applied to the hair pulling, the quantity of samples can greatly vary from one individual to another or from one sampling area to another. This makes the normalization of samples difficult. A new sampling strip was used. The operation of collection and transfer is made in 6 s, 1 s more than that of the last toolkit. However, the amount of material removed is more abundant regardless of the individual or the body sampling area.

Finally a new glue strip distributor enabling patching 30 body locations in 60 s by a single operator was developed. The final sampling toolkit for hair follicles consists of a gun laying strip allowing the user to pull the hairs on the strip in 2 s in order to transfer them to the extraction buffer in a 96-well plate. The sampling with the glue strip distributor was demonstrated at the exercise in May 2013. This work was reported in report D400.5 delivered in July 2011.

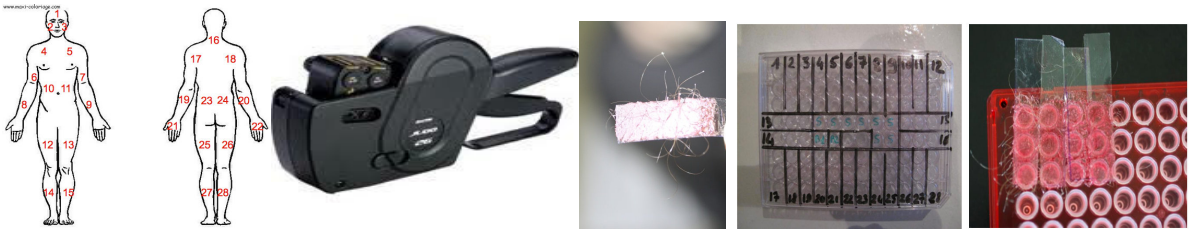

Figure 21: Final hair sampling method for hairs

Sampling preparation

We have developed a protein extraction protocol in 15 s for managing 96 samples of hair bulb or blood at the same time. This protocol was parallelized for an extraction directly in the 96-well plate. Once the sample has been collected and extracted, it is analysed directly in a 96-well plate, where the bottom of each well is functionalised with an antibody against γH2AX. Two strategies were used to validate the γH2AX strip assays in 96-well plates. This work was reported in report D400.3 delivered in July 2011.

Rapid γ-H2AX level assessment with fluorescence and LIBS techniques

The isolation of antigen-antibody complexes are performed directly using the strip as a functionalized support with the antibodies, in the 96-well plate. We tested two different types of measurement for assessing the level of γH2AX.

Fluorescence or chemi-luminescence, using scanner technologies, allows the evaluation of the compatibility of every step of the strip assays with a portable kit. Experiments on pig hair follicles and human blood demonstrated that the analysis of the fluorescent γH2AX strip assays gives a good linear dose response curve (R> 0.98). However, the need to either mark the sample or use two different antibodies, one to capture the sample, and the other to measure it increases the utilisation time of the kit. This work was reported in report D400.1 delivered in July 2011.

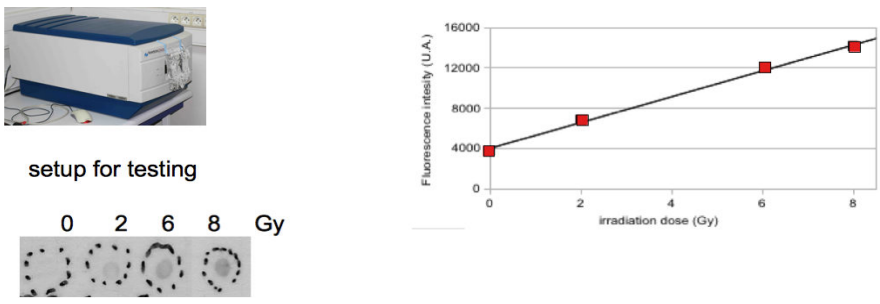

Figure 22: γH2AX strip assays using fluorescence

We adapted a LIBS scanner for measuring the level of γH2AX via the direct measure of phosphorus. First calibration curves of proteins with known levels of phosphorylation provided an LOD of 10^7 protein molecules. We developed mapping software to establish the titration curve of phosphorus for a strip. A first prototype of LIBS scanner and mapping software were developed for in-field use. Results of the γH2Ax strip assays were comparable to those obtained by FACS analysis of the same test samples analyzed using the same γH2Ax antibody. Furthermore we demonstrated that the LOD of 10^7 proteins molecules allows the detection of γH2Ax under in-field experimental conditions. This work was described in report D400.4 delivered in July 2011.

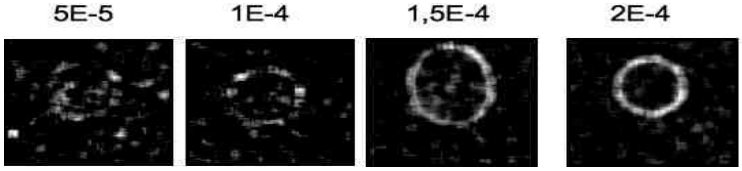

Figure 23: Image analysis of phosphor on protein spot on strip

As we noted that the quality of commercially available anti-γH2Ax antibodies was not consistent from one lot to another and was leading to variation of the results between lots, we fully developed a new antibody using a CEA-patented process, which provides extremely stable and reproducible antibodies irrespective of the lot.

We also developed an image analysis method compatible with the mapping obtained by LIBS or by fluorescence. A new Booster-SurView allows the direct and automatic measurement of signals from each spot and deduces the dose received at each corresponding body position.

To use the LIBS as a means of detection in the field, it is essential the device is compact, sturdy and portable. Following research and testing, we have not yet found a device that fully meets the required specifications. We have, therefore, developed the concept for mobile LIBS equipment which completely meets the required specifications. We are in the process of patenting the principle and are developing the first proof of concept device.

These results are presented in report D400.12 delivered in April 2013.

Rapid analysis of γ-H2AX levels on blood cells

Foci scoring in lymphocytes are not as easy as in fibroblasts because of less defined nucleus area. Thus, we reproduced the global measurement approach already done on fibroblasts, on human lymphocytes in order to validate our observations in a second cell type. We compared global measurements of total fluorescence from phosphorylated H2AX using flow cytometry and microscopy at 10X magnification (plus foci scoring for fibroblasts).

Based on the same immunohistochemistry technique, CEA developed a technique for the quantification of the total intensity of the foci of γH2AX in interphase nuclei. The overall measurement of the fluorescence intensity of the foci was performed by optical microscopy using a 10X objective. The originality of this technique consists of the detection of a large number of cells with precision while taking into account the morphology of the cell. Compared to the classical microscopy-based quantification of the fluorescent signal in cytogenetics with 63X objective, it addresses the heterogeneity of the intensity of different cells, avoids saturation problems in the capture of a fluorescent signals and highly improves the speed and sensitivity (2 mn for 10 000 cells with the 10X objective versus 1 hour for less than 500 cells with the 63X objective).

We also improved the FACS analysis of fluorescent γH2AX signal in the same cohort of donors (15 000 cells per minute). We showed a H2AX signal increase with the dose and a signal decrease with the repair time. We also observed that the γ-H2AX signal is not negligible in non-irradiated cells (intrinsic fluorescence of lymphocytes). Measurements present a large inter-individual variability that increases with the dose received, and tends to drop off with the repair time. Inter-individual variability does not seem to be correlated to the donors' relative radiosensitivity estimated by cytogenetic approaches. This result makes relevant a dose estimation a few hours after radiation exposure.

Thus, four hours post exposure, we measured a linear dose dependence, in 0.5 to 6 Gy:
D = (Y+3.96) / 7.35 for 10x microscopy, and D=(Y+0.58) / 2.77 for FACS quantification, where Y is the intensity measured for γ-H2AX signal and D is the dose received.

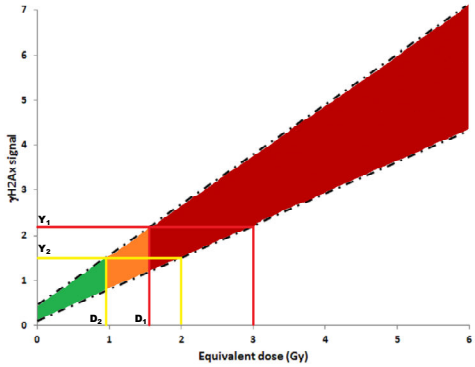

Figure 24: Proposed triage categories

We used the laboratory dose-effect calibration curve to propose triage categories: doses of 2 Gy and 3 Gy were described as minimal values for moderate symptoms (variable care) and severe symptoms (urgent care) respectively. Triage categories are designed to help the medical team and tend to overestimate the medical intervention needed if the victim possesses a radioresistant phenotype. However, it takes into account the overreaction if victims belong to the non-negligible group of moderate radiosensitivity.
Final results for the comparison of several laboratory techniques for H2AX quantification of lymphocytes and dermal fibroblasts were presented in D400.15.

Biological validation

Two important steps of this project are:

- To compare the methods to be used to perform biodosimetry in the Booster kit, with a reference method consisting of fluorescence-activated cell sorting (FACS) while assessing the relevance of the global measure of abundance of the DSB marker, γH2AX, as a biodosimetry tool.
- To establish calibration curves between the radiation dose received and the abundance of the DSB marker γH2AX

The first model used to establish the dose response curves consisted of fibroblast cells. One of the tasks of the CEA-DSV-LRO group was to evaluate the impact of inter-individual radio-sensitivity. The global quantification of γH2AX in cells was assessed by fluorescence-activated cell sorting (Facs) after X-ray irradiation and various repair delays. The first results reported in report D400.1 (July 2011) and in report D400.12 (April 2013), suggest that individual radio-sensitivity which exists among individuals does not have a significant impact on the data obtained by global quantification when analysis is performed between 4 and 6 hours post exposure.

The last year was dedicated to confirm the results by increasing the number of samples analyzed to obtain better statistics. This allowed us to pool all the data to obtain a dose-dependent curve from measurements realized 4 hours after irradiation. The curve was established for human fibroblasts (19 donors) and lymphocytes (11 donors) after integration of a weighting index that reflects the proportion of each group of radio-sensitivity in the general population to be as close as possible to the population that could be found in a stadium. Statistical analyses of the data enabled the definition of three “dose-range” boxes that are significantly distinguishable.

We also validated our global approach for γH2AX quantification by performing intercomparative experiments with the well-established foci-scoring method and with fluorescence quantification by microscopy at 10X magnification. High correlations between the three methods were observed for both cell types, especially for fibroblasts. These results validate the global quantification of γH2AX for radiological triage purposes. Detailed experiments and data are reported in D400.15 and a publication is in preparation.

3.3.4 WP430 – Biodosimetry using centrosome quantification

Quantification of centrosomes as a novel biodosimetric marker

Cells normally have one or two centrosome (a structure within cells), but after irradiation, the number of centrosomes increases proportionally to the amount of radiation received. We performed preliminary centrosome quantitation experiments using automated immunofluorescence (IF) microscopy analysis of human Jurkat (acute T-cell leukaemia) and GM07521 (EBV-transformed B cell line, apparently normal) cells. This work was described in report D400.8 delivered in July 2011.

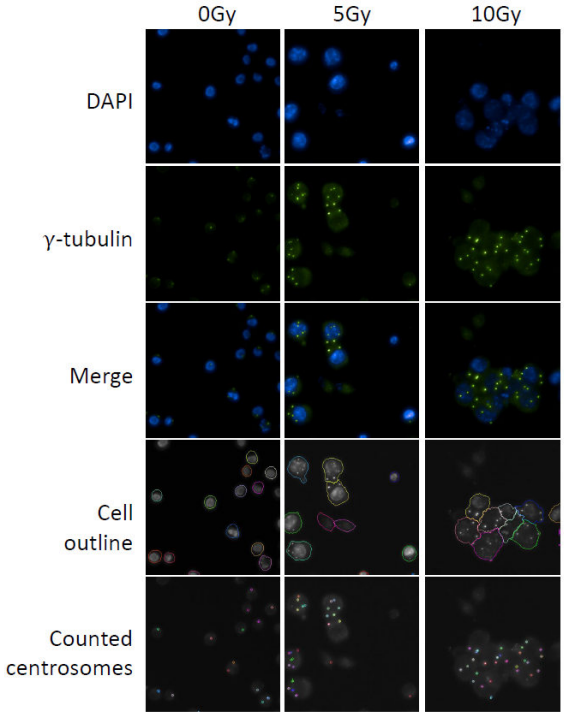

Figure 25: Counting of amplified centrosomes

These analyses were performed over time after irradiation with single-dose treatments of 1-5 Gy from a ^{137}Cs source. While there remained a clear dose-response relationship in terms of centrosome amplification after irradiation, these analyses revealed relatively little difference at earlier timepoints (8h) post-irradiation. Furthermore, Jurkat cells showed different levels of centrosome amplification to the B-cells; this may be related to different impacts of cell death or may reflect different centrosome amplification capabilities. Streamlining of the analysis per cell line/ baseline is therefore necessary for the adaptation of centrosome amplification as a biodosimetric marker.

An analysis of centrosome amplification in volunteer-derived samples showed a good reactivity of our monoclonal antibodies on primary peripheral blood mononuclear cells (PBMCs). Cells were harvested from volunteers and then irradiated; they were quantitated by automated microscopy at 24h and 48h after irradiation.

We observed little centrosome amplification in PBMCs that had been irradiated ex vivo. Mitogen stimulation potentiated centrosome amplification, although the radiation-dependency of any such amplification was not clear. A preliminary analysis was performed on donor-derived PBMCs in parallel with the γ-H2AX analysis by CEA, to allow the direct comparison of the methodologies. However, the sample quality was not optimal and no clear results could be obtained; these experiments indicated the need for fresh sample material.

The choice of cell type for analysis will be important for developing centrosome counts as a biodosimetry technique. Preliminary microscopy experiments were performed on cells from buccal swabs and hair follicles, but robust detection of centrosome signals was not achieved within the Booster period. This work is described in report D400.14 delivered in March 2013.

Preparation of centrosome antigens and monoclonal antibody generation

The development of centrosome quantitation technology involves three principal initial steps:

- Generation of monoclonals to centrosome-localising proteins;
- Analysis of monoclonal antibody reactivities and functionalities;
- Testing of monoclonal antibodies on primary cells and on irradiated cells and validation of centrosome analysis as a biodosimetric approach.

First task sought to generate monoclonal antibodies specific for centrosomal antigens and capable of detecting centrosomes in human cells in indirect immunofluorescence microscopy experiments. This was achieved by recombinant bacterial expression and immunogen purification by affinity chromatography, followed by hybridoma preparation by commercial antibody production companies.

Nine candidate proteins were selected as targets for the development of monoclonal antibodies, on the basis of their different defined centrosomal localizations, to ensure that the reagents being generated would have a good range of specificities for centrosome visualization by immunofluorescence microscopy. Hybridomas were generated that expressed immunoglobulins that hybridised with centrin2, centrobin, CEP250/ C-NAP1, kizuna, ODF2/cenexin and Cep290. These reagents were then expanded and stored cryogenically.

This work was described in report D400.7 delivered in July 2011.

Validation of centrosome reactivity of candidate monoclonal antibodies in cultured cells

Various assays (ELISA, immunoblot and indirect immunofluorescence microscopy) were used to confirm the reactivity of the new monoclonals in human T- and B-cell lines. IF microscopy experiments used recognition of γ-tubulin as a centrosomal control marker. The antibodies to centrobin, CEP250/ C-NAP1, kizuna, ODF2/cenexin and Cep290 were all suitable for further use, giving good reactivity in both immunoblot and immunofluorescence experiments.

The next step will be to produce a large quantity of hybridoma to allow increasing the amount of antibody available per antigen and to standardize the methodology used with these antibodies as we develop them for use in centrosome quantitation.

This work was reported in report D400.11 delivered in August 2012.

Implementation of combined biodosimetric toolbox

The developed bio-dosimetry Booster kit is already functional. We developed all the tools required for in-field use for versions using fluorescent labeling. In particular, we developed the portable mini-scanner used during the demonstration test in May 2013 at Budapest. The kit can be used to quantify irradiation down to 0.5 gray on any part of the body and up to 6 hours after irradiation.

In addition, we developed an approach that does not require fluorescent labeling of samples through a direct quantification of phosphorylation of H2AX. This version of the kit, which requires less reacting agents because the fluorescent or chemi-luminescent labels are removed, requires a miniaturization of the LIBS system necessary for dosing the phosphorus. We showed that the direct quantification of the phosphorylation H2AX by LIBS scanning gave comparable results to that obtained by fluorescence.

The results obtained with the bio-dosimetry Booster Kit on hair follicles are comparable to those obtained with this same kit for lymphocytes, with a detection limit of 0,5 Gy. As FACS measurements were not available on the hair follicles, we compared FACS measurements between fibroblasts and lymphocytes: both showed a detection limit of 0,5 Gy for FACS. This approach validates the hair follicles, as a support for the evaluation of the irradiation of individuals. It also positions the Booster kit as a sensitive field technique as well as a laboratory technique in comparison with FACS technique (requiring a stationary in- laboratory equipment), with an equivalent detection limit of 0,5 Gy.

However, other methods for individual irradiation dose evaluation exist, with detection limits lower than 0.5 Gy, such as cytogenetic method or the method of foci. Nevertheless these methods require stationary laboratory equipment, and the time to implement is incompatible with an immediate field method.

Cells normally have one or two centrosome (a structure within cells), but after irradiation, the number of centrosomes increases proportionally to the amount of radiation received. In order to detect centrosomes in cells by recombination, monoclonal antibodies able to recognize centrosomes were generated. When these monoclonal antibodies were tested on cultured blood cells, or on blood cells from informed volunteers, they labelled centrosomes and enabled to detect them by fluorescence microscopy. An automated counting by microscopy was developed to count the centrosomes in cells labelled with our new monoclonals, to assess how much radiation the cells had received.

The monoclonal antibodies were tested on primary cells and on irradiated cells. They demonstrated good reactivity on peripheral blood mononuclear cells (PBMCs) from volunteers, with automated microscopy for centrosomes being demonstrated as feasible. However, ex vivo irradiated and cultured PBMCs did not show robust centrosome amplification, unless stimulated with mitogens that might confound such analysis. Preliminary analysis of additional cell types that could serve as targets for triage analysis, such as hair follicles and buccal cells, did not indicate clearly which cells would be best for determining centrosomal responses in irradiated individuals.

This work was described in report D400.15.

3.3.5 WP440 – Validation of the tools

Validation of the tools

The validity of BOOSTER method for assessment of γH2AX level in irradiated human cells (fibroblasts and lymphocytes) was validated through parallel analysis with different techniques, for both cell types irradiated within a dose range from 0.5 to 6 Gy and analysed 4 h after exposure. A good correlation of the measurements was obtained for both global methods (FACS and microscopy 10X magnification quantification of fluorescence) against the usual approach of foci scoring (fluorescence microscopy at 63X magnification). Furthermore we demonstrated that the individual variability in biological response to irradiation could be neglected when analysis is performed several hours after the exposure (4-6 hours) when DNA repair was already initiated. Thus a calibration curve applicable to the general population, as can be encountered in a public place, can be generated and used for radiological triage purposes.

CEA study proves that FACS analysis of γH2AX levels can be used as a reference for validation of the device based on global measurement of γH2AX from total protein extracts as conceived and developed for the BOOSTER biodosimetry kit. Using the antibody strip assay and signal quantification by LIBS, a calibration curve was realized for human lymphocytes in a range of doses that greatly fits with the FACS reference curve (D400.15).

In addition, to get rid of the variations that could exist during the detection process, it was decided to include several standard samples with known quantities of γH2AX in each antibody detection strip for the in-field assessment of radiation exposure. Thus, each detection strip, corresponding to one single person will have its own calibration curve.

For Inductively coupled plasma mass spectrometry (ICP-MS), different methods were developed for analysis of biological samples:

- Chemical sample preparation (digestion, extraction, chromatographic separation and evaporation) for analysis of the liquid samples;
- Analysis of solid materials (one droplet of blood or urine dried onto a glass surface);
- Analysis of small particles collected from nasal mucus and saliva using a swab (particles are collected by soaking and shaking the swab into an aqueous solution containing organic solvent, and dropping and drying the solution on a sticky membrane).

These preparation methods were compared and resulted highly accurate. The typical uncertainties for this laboratory method are included in 0.5 to 2%.

Liquid scintillation counting (LSC) was validated using samples of known composition. With the preparation protocol developed during BOOSTER project, this laboratory method is quite accurate with typical uncertainties between 1 and 3%.

Laser-Induced Breakdown Spectroscopy (LIBS) is well adapted for in-field measurements of solid samples. A special dropping and drying technique was developed as a fast and easy sample preparation of liquid biological matrices (blood, urine or particles originating from the mouth). The method was validated using biological matrices spiked with an analyte (e.g. cesium) in known amounts, and the precision was found in a typical range of 15-20% uncertainty.

Portable low-background gamma-spectrometry equipment was developed during BOOSTER project. The method was validated using samples of know composition and it was found that the precision of the technique was typically of 20-30% uncertainty.

The measurement of particle granulometry in solution was performed by diffraction using a modified FACS technology, and in-laboratory Dynamic Light Scattering. It was possible to differentiate particles of 20, 50 and 100 nm with diffraction. An assessment of particle toxicity could be performed by exposing caki-1 cells to different concentrations of PS-NH2 nanobeads of 50 nm and assessing the cell mortality/viability by FACS; this assessment is still at research state.

For centrosome amplification assessment, primary peripheral blood mononuclear cells (PBMCs) were harvested from consented volunteers, placed in cell culture and irradiated at 5 Gy. Cells were then

stained with antibodies to γ-H2AX and demonstrated a robust DNA damage response, as determined by γ-H2AX focus formation. Centrosomes in untreated and irradiated PBMCs were visualised by immunofluorescence microscopy of the indicated centrosomal components (pericentrin,centrobin or Cep250. But quantitation of centrosome numbers in PBMCs at 24h and 48h post-irradiation did not indicate any robust increase in centrosome number.

It is possible that PBM Cells, as a quiescent population, might not activate the cell cycle regulators that direct centrosome duplication sufficiently to allow a detectable radiation response. An alternative possibility is that the culture conditions necessitated by the ex vivo approach here might not be suitable to support centrosome over-duplication.

A cell treatment with mitogenic lectins, known for their impact on centrosome overduplication in PBMCs, at the same time as cells were irradiated, showed a small increase in centrosome numbers at 48h post-treatment, but did not appear to lead to any increase after irradiation.

However, PBMCs irradiated at 5 Gy and then cultured with mitogenic lectins, showed centrosome amplification, so that PBMCs are capable of mounting a response similar to that seen in vitro.

With the idea that the centrosome amplification might not happen in quiescent cell populations, as typified by PBMC, we explored additional cell types that could serve as targets for triage analysis. Preliminary experiments with buccal cells suggested that centrosome visualization will be possible in this cell type.

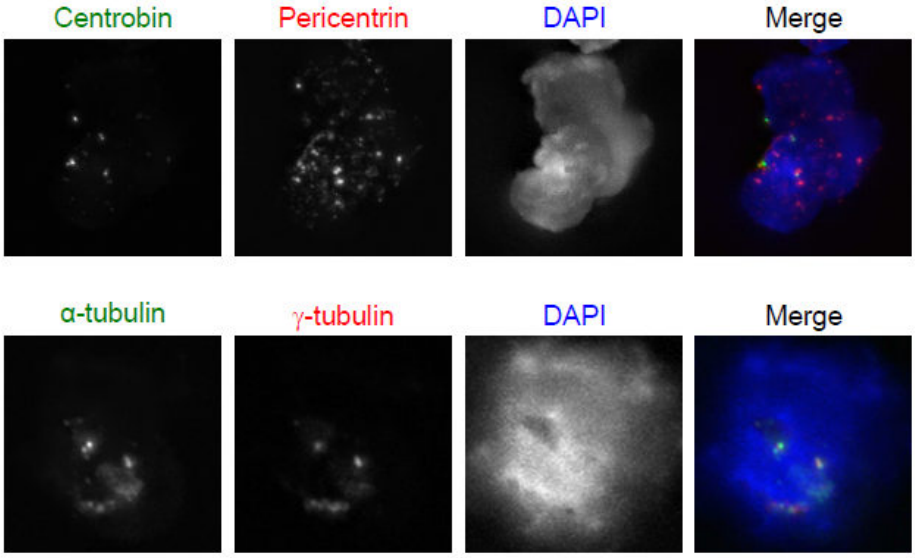

Figure 26: Detection of centrosomes in irradiated buccal cells

All validation work is described in report D400.15. Report 400.15 also reports the procedures and protocols set up for ethical purposes in the development of the BOOSTER prototype.

3.4 WP500 – Software development and integration of components

Activities in WP500 aimed at the development of hardware and software components related to a toolbox that can be used to support the triage in case of a radiological emergency. The system designed is a first prototype and has demonstrated that monitoring equipment of different types can be coupled together with a Command and Control system via the SIMACOP framework. The coupling of the Command and Control system with a decision support component (DSS) was also realized and demonstrated.

In the first phase of the triage process, the different BOOSTER measurement components will analyze the radiological situations and provide a picture of the contamination. This is handled by the SIMACOP system. The SIMACOP system and DSS will exchange relevant information for characterizing the incident area, and help the first responders deployed on the field and starting the first phase of the victims' triage. The different kinds of information exchanged by SIMACOP system and DSS are shown in the figure 27.

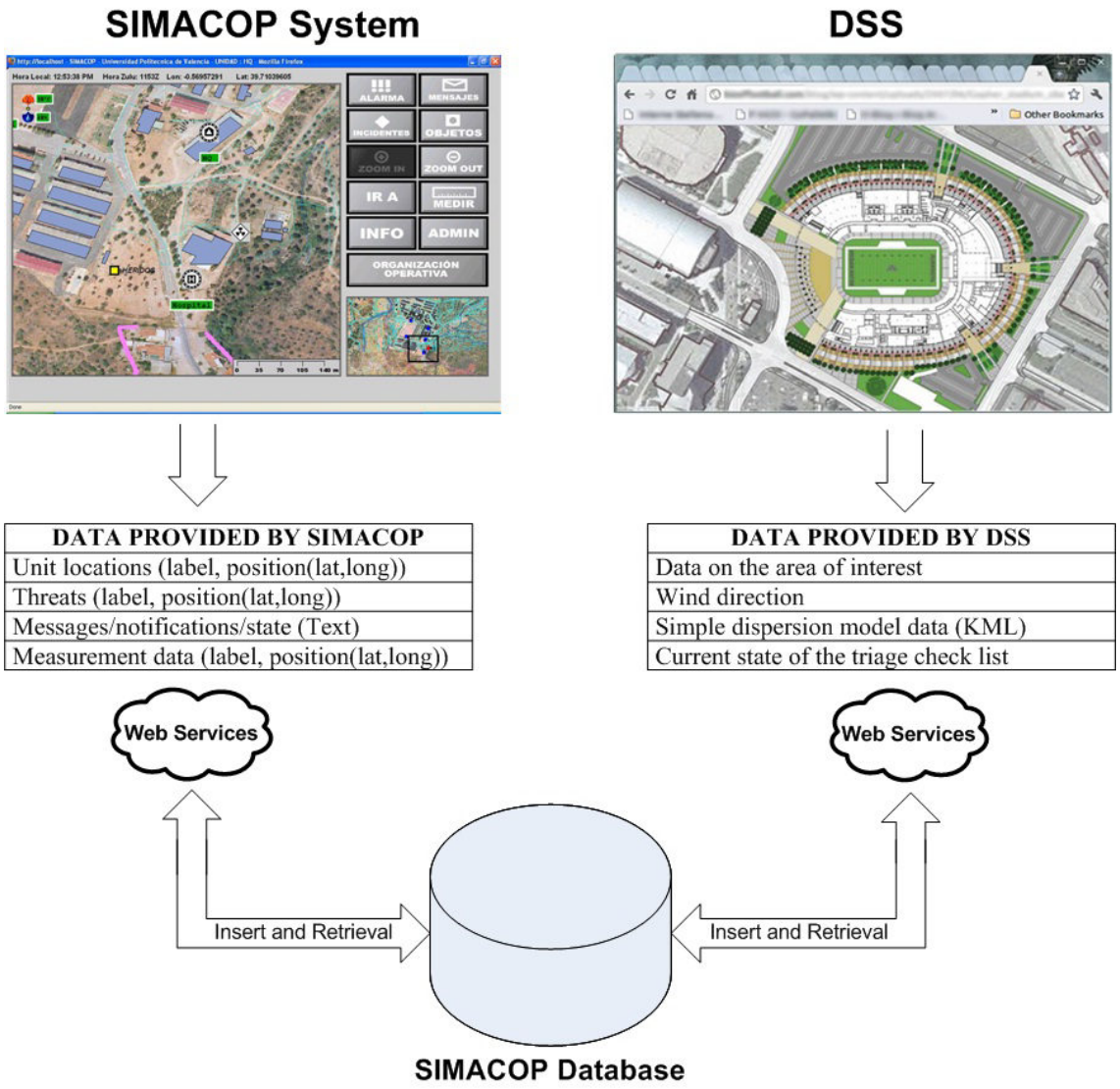

Figure 27: SIMACOP and DSS integration

With the information taken from the field, the BOOSTER system will delimitate the red zone (radiological dangerous area) and its potential evolution (specially based on weather conditions). In addition, this red zone delimitation will serve for taking an assessment of the potential victims' location during the incident which can give an idea on the dose received and type of contamination. Each victim potentially affected will indicate his/her approximate location on the red zone map at the beginning of the decontamination process in the yellow zone (secure area) entrance. This initial information will be automatically associated with the ID (wrist with barcode) which will be given to each victim at the yellow zone entrance in order to initiate the triage. The triage process and the different tests performed in the yellow zone to both, the victims and their samples are described in figure 28.

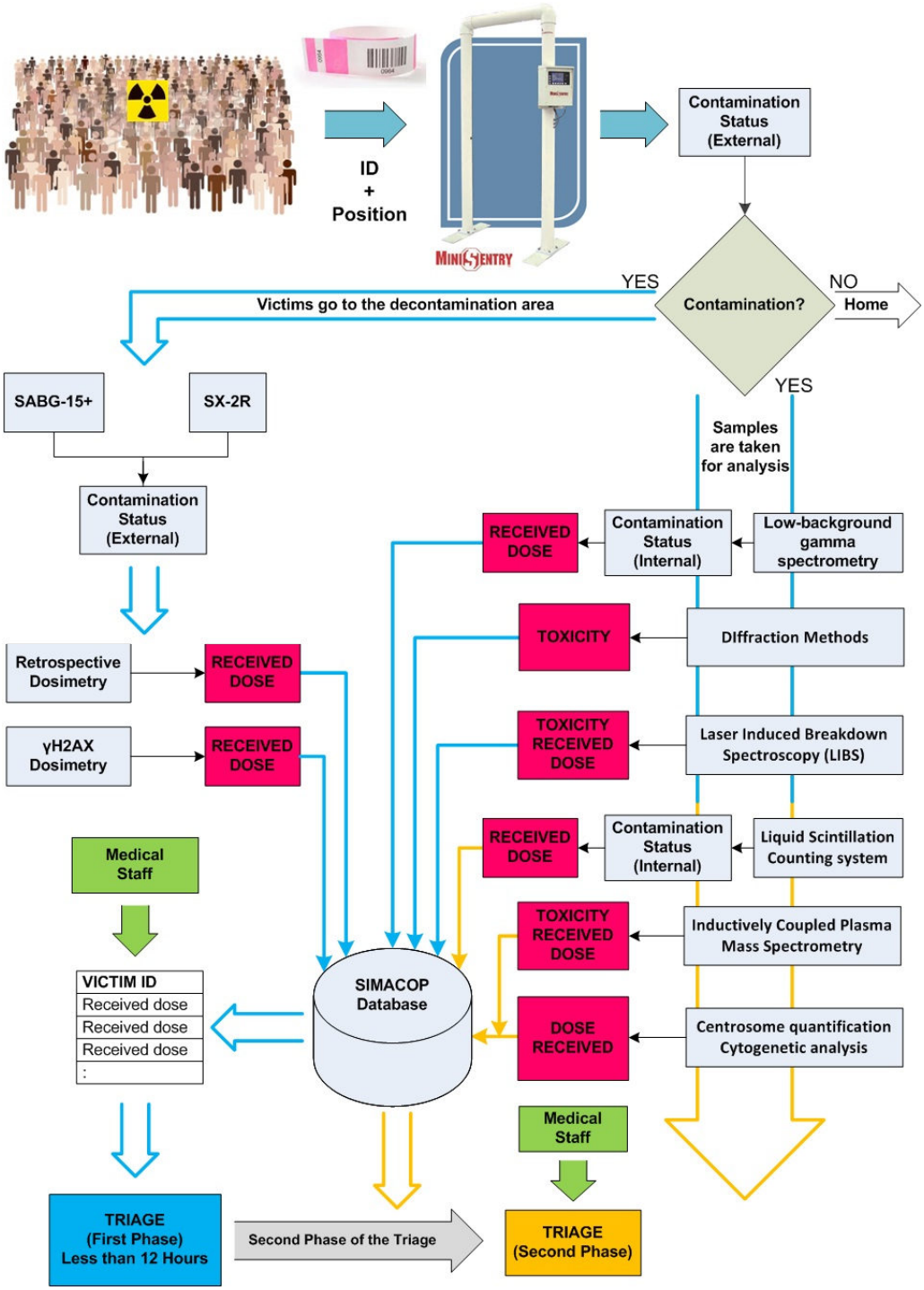

Figure 18: Test performed through BOOSTER and triage process

All this information can be visualized in the SIMACOP system. Figure 29 shows the adapted system to the training case in Hungary.

Figure 29: Main screen of the SIMACOP system

Together with the customized version, a user guide has been developed and provided to the consortium. This user guide describes the system and various modes of operation. The key task of the DSS in this process is to support the definition of the red zone, and the triage of the victims including the trauma triage. Any real-time information is taken from SIMACOP for the application in the DSS. This comprises in particular information about the victim, dose rate and dose measurements.

The DSS is subdivided into two main sections supporting

- Determination of the contaminated area via monitoring information and simple dispersion calculations (Map – Tab)
- Determination of trauma and radiological triage (Triage – Tab)

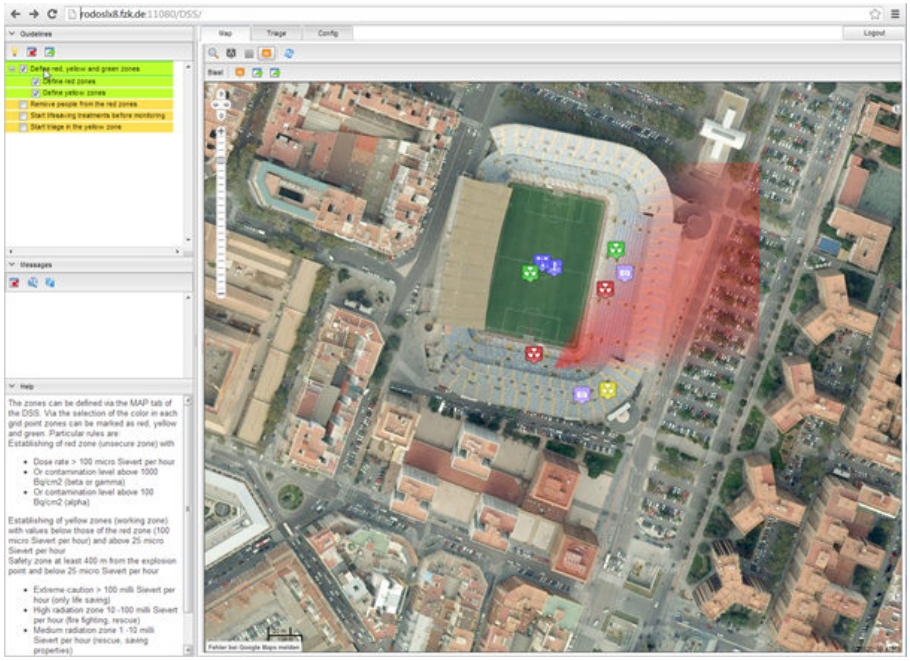

Figure 30: Initial screen of the DSS

The DSS also contains material supporting the individual tasks via Help files. As the trauma triage has to be carried out first, the DSS also provides interactive facilities to mark the location of the wounds on a victim sheet for an adult or child for the non-radiological aspects. The radiological triage is performed by using information collected within SIMACOP or data that can be entered into the DSS from the user. This data is related to monitoring systems such as ICP-MS Methods or Liquid Scintillators. Entering a nuclide concentration value, the DSS calculates the resulting internal dose to the victim. In addition, the DSS estimates roughly the external dose. Depending on the information available the DSS indicates a radiological triage status.

Besides the system development and integration of monitoring components, the UltraRadiacs, which are personal electronic dosimeters integrated within their dedicated GPS-COM devices became part of the BOOSTER core system.

With a simple and flexible graphical user interface (GUI), GPS-COM allows to rapidly visualize data and to localize them using the freely available Open Streetmap. In this way, GPS-COM is fully integrated in the BOOSTER system what has been demonstrated during the May exercise in Budapest.

Figure 31: GPS-COM device and user interface

Figure 32: GAMMA camera and hot spot

A further component of the core BOOSTER system is the Gamma camera which has been made portable and improved with specific codes maps that allows a better detection of hot spots.

In addition to a coded mask development, the gamma camera was equipped with a GPS, in order to get the exact position of the camera for positioning the information on the map of the SIMACOP software.

With these components, the BOOSTER toolbox was demonstrated in an exercise in May 2013 in Hungary. The whole system has been set-up and exercised in a way similar to a real radiological event. This resulted in defining areas for the red zone (explosion area and first responders), yellow zone (monitoring and screening) and blue zone with support for the triage. The various components or the BOOSTER toolbox have been distributed and interconnected via the SIMACOP system.

Figure 33: Set-up of Exercise

This work is described in the successive reports D500.1 about system design, D500.2, D500.3 and D500.4 about the successive versions of the BOOSTER kit prototype.

3.5 WP600 – System validation and training

The technical evaluation of BOOSTER components was performed all along the project for each technique and tests are described in the reports relating to the different techniques. Integration tests with SIMACOP and DSS were performed for each technique (except diffraction) from February to April 2013, as a preliminary step to the demonstration exercise.

Final demonstration for operational evaluation

During several months, UPV with the whole consortium planned step by step the location, preparation and execution of the BOOSTER final exercise. This work is described in report D600.1 delivered in March 2013.

Finally in the final exercise took place on May 16, 2013 in Budapest, the BOOSTER system validation was performed as follows:

After the explosion of a “simulated dirty bomb”, several victims have been affected and the first responders enter in the red zone with the BOOSTER main sensors in order to use them for the following actions:

- To test both, the tactical communications on the field and the first responders’ situation awareness through the Rugged PDA and the MESH communications devices.
- To obtain information on humidity, temperature and wind speed from the red zone through the smart sensors deployed on the field.
- To delimitate the red area and the potential victims’ external contamination through the use of both Colibri and GPS COM devices.
- To obtain gamma images from the field through the portable gamma camera in order to identify potential nuclear sources or contaminated objects.
- To obtain the nuclide form from the field through the FALCON 5000 in order to identify the kind of nuclide spread during the attack.

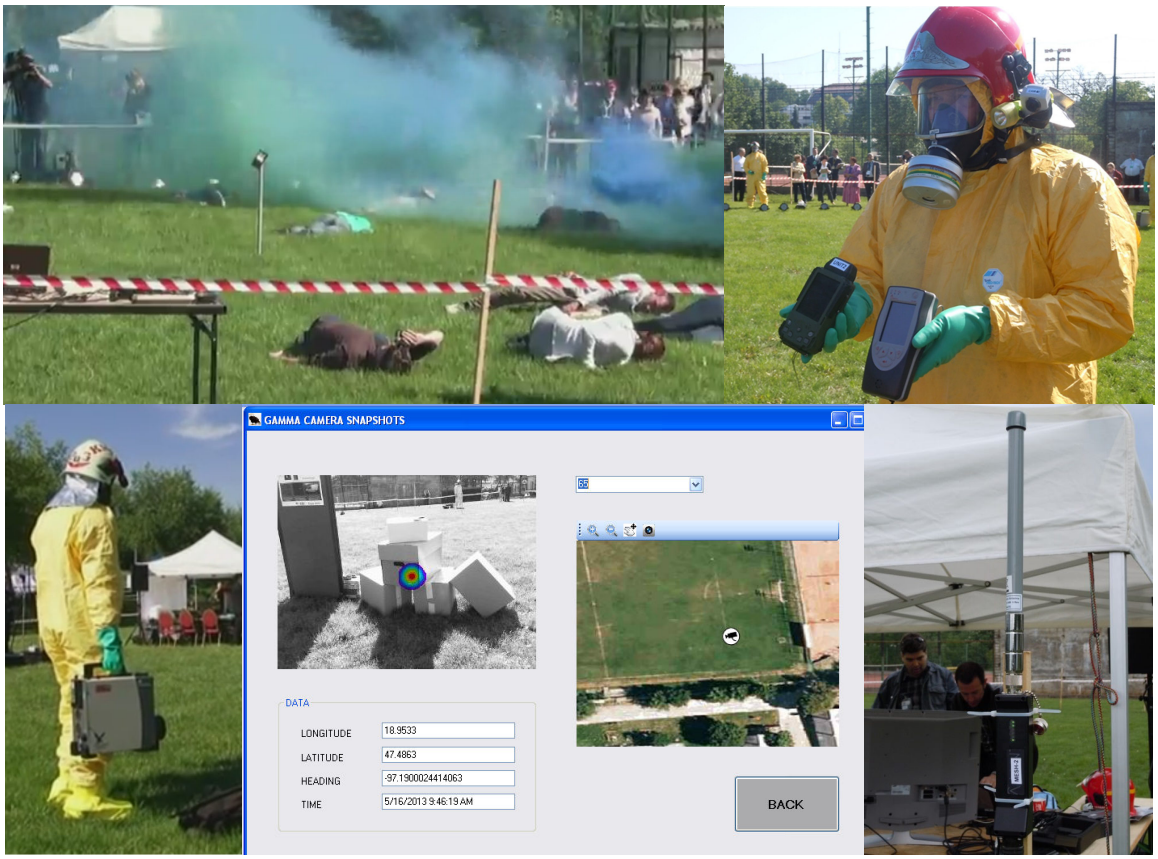

Figure 34: Overview of the red area

Once the data from the field were gathered and the injured people evacuated, the rest of the potential victims went to the yellow area in order to perform the triage of these victims. The triage was performed following a decontamination and analysis process in which the main BOOSTER techniques and probes were used. This process was the following.

All victims were identified with a barcode in a bracelet and passed through the Minisentry portal in order to determine whether external contamination was existing. The second step was a complete decontamination protocol through the use of the following devices: SABG-15+ and SX-2R. After that several samples of saliva, blood and hair were taken from each victim for further analysis.

Figure 35: Contamination tests and victims' identification

The third step for performing the triage was to analyze the samples taken from each victim with the following techniques and probes developed under BOOSTER project:

- Laser Induced Breakdown Spectroscopy (LIBS)
- Low-background gamma spectrometry
- Biological dosimetry with detection of γH2AX
- Retrospective dosimetry
- Centrosome quantification
- Liquid Scintillation technique
- ICP-MS Methods

Figure 36: Overview of the yellow area

With the results of these probes, the dose received by each victim was estimated and the medical staff performed the triage.

The following BOOSTER probes: Liquid Scintillation Counting (LSC), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Centrosome quantization techniques were presented in the yellow zone even though these tests will take more time than the previous ones and would not be able to be performed on the field. For these reasons, the results of these techniques were available to the medical staff later on for performing a more in depth tracking of the victims in case it was necessary.

The blue zone deployed during the exercise simulates the control room where the crisis managers are facing the crisis. As much information as possible from the hot spot should arrive to this control room in order to give an idea on the situation to the crisis managers.

Video flow measurements performed in the field from sensors and devices and also information on the status of victims have to be available in order to help the crisis managers to take the more accurate decision in each moment of the crisis. The main SIMACOP system command post provided by UPV and the DSS command post provided by KIT were deployed.

The demonstration exercise was attended by numerous participants: European experts, embassies, media, volunteers and Hungarian first responders using BOOSTER devices during the demonstration. The exercise resulted very successfully with numerous positive comments from the experts, the end users and the media. This work is described in report D600.3 delivered in July 2013.

Analysis of questionnaires

A questionnaire for evaluating BOOSTER system was defined in order that experts and representatives of embassies attending the exercise demonstration can evaluate each one of the BOOSTER devices, the global system, the sizing of the system, and its implementation.

A questionnaire was filled in by 16 participants: 11 experts, 1 end-user and 4 representatives of embassies.

Globally the participants were satisfied by BOOSTER system and provided positive comments.

The equipment devices considered as most interesting for radiological evaluation were:
1) Colibri
2) DSS and Simacop
3) Gamma-camera and GPS-COM
4) Falcon 5000 and Retrospective dosimetry

The equipment devices considered as most interesting for victims' triage were:
1) MiniSentry, External contamination probes, γH2AX biodosimetry
2) Victims' identification system
3) Centrosome quantification and DSS
4) Biological sampling and Retrospective dosimetry

The participants considered that the technologies for which Booster project enabled a scientific progress, compared to the state of the art, were :
1) Victims' identification system, Retrospective dosimetry and γH2AX biodosimetry
2) Colibri and gamma-camera
3) Rugged PDA, Falcon 5000, LIBS and centrosome quantification

The synthesis of questionnaires is described in report D600.3 delivered in July 2013.

Training courses

Training courses in different languages were written for all technologies of BOOSTER toolbox and were communicated to experts and first responders. The BOOSTER community could access to the BOOSTER learning materials at https://poliformat.upv.es/portal/site/BOOSTER. Courses are presented in report D600.4 delivered in July 2013.

A team of Hungarian first responders participated in the demonstration exercise and received a preliminary dedicated formation about the use of BOOSTER equipment. Demonstration of BOOSTER devices in the red area and the decontamination area was performed by these trained first responders.

The BOOSTER on-line site was widely described in D600.2 delivered in June 2013. In this report all training material produced by the different partners in different European languages were consolidated and exposed. The BOOSTER on-line course gave access to all learning material produced by the consortium.

4 POTENTIAL IMPACTS

From the beginning of the third millennium, Homeland Security applications have been a challenging issue for all societies. 9/11 attacks showed the reality and impact of terrorist threats which forced societies to quickly adapt and develop new technological solutions. Moreover, recent nuclear accidents demonstrated people's fear regarding nuclear risks. A terrorist attack, potentially involving a radiological risk, could have dramatic and long-lasting consequences on the world from a sanitary and psychological point of view, and locally with psychological effects, environmental and economic damages.

The effective management of an event involving exposure of a large number of people to radioactive material requires a mechanism for rapid triage. The BOOSTER project, gathering seven partners from five different countries, addresses this requirement by researching and developing new bio-dosimetric tools to quickly evaluate the level of exposure of potential casualties, determine by appropriate sensors its consequences and allow an efficient triage of exposed individuals. These bio-dosimetric tools will be integrated to a portable toolbox along with a prognostic toolkit based on radiation sensors. The combination of these approaches will allow an effective and fast management of the situation.

Medical impact

First impact of BOOSTER project is medical as BOOSTER toolkit will allow performing a very fast triage by discriminating very quickly persons who need to be checked carefully from people who have no medical risks.

BOOSTER project developed a fast and simple toolkit for taking representative biological samples from victims in a very short time, compatible with the sampling of a crowd. Sampling process includes:

- Some drops of blood taken by sticking fingertip with disposable sampling equipment for diabetics "Haemolance" and a fast preparation process using magnetic particles coated with antibodies against red cells to extract lymphocytes;
- Hair follicles taken with a glue strip distributor enabling patching 30 body locations in 60 s by a single operator, followed by a quick transfer to an extraction buffer in a 96-well plate;
- Saliva, nasal mucus, and tears at the commissure of the eyes to assess the abundance and toxicity of radioactive particles in a body;
- Urine;
- Extraction of some mobile phone components for personal dosimetry;
- Identification of each victim, as well as biological sample or personal belongings, with a bar code wristband with a fixed ID for fast identification and association of results and efficient follow-up of victims' status in real time by sanitary staff and staff responsible for triage.

A kit including 5 in-field techniques for assessing personal dosimetry and biodosimetry was developed and is now functional.

Mini-Sentry is a gamma sensitive portal monitor designed to be quickly set up and operated with very little training or expertise in radiation detection technology. This portable system is used to quickly provide a gamma scan of pedestrians or vehicles for emergency scenarios. It was improved to check also for surface radioactive contamination (mainly due to the presence of X-rays and/or alpha/beta particulates) on head, hands and feet.

BOOSTER project enabled major achievements in bio-dosimetry to assess a posteriori the dose absorbed by a victim. The first portable quick toolkit for global measurement of phosphorylation of H2AX protein in fibroblasts and lymphocytes was developed and validated. Results are provided very quickly (30mn) compared to the laboratory technique (8h), with a comparable precision. A dose-effect calibration curve between 0.5 and 6 Gy was proposed for triage: doses of 2 Gy and 3 Gy were described as minimal values for moderate symptoms (variable care) and severe symptoms (urgent care).

Retrospective dosimetric method was developed. Using small surface mounted devices originated from mobile phones and also environmental materials, like dolomite, doses can be estimated posteriorly. SMD resistors of mobile phones were identified and characterized as good emergency personal dosimeters. The reliability of the dose estimation with SMD via Thermo Luminescence and Optically Stimulated Luminescence was demonstrated in the range 10 mGy – 6 Gy. A portable in-field digital fluorimeter (TL/OSL reader) was developed and used also during the final exercise.

Preliminary information about the contamination of a sample originating from the environment or a victim can be obtained within seconds with LIB technique. Different 'dribbled-and-dried' sample preparation and calibration techniques were developed and tested for analysis of human body fluids to optimize exact concentration assessments. A portable low volume LISB system was developed and miniaturized.

Transportable low-background gamma spectrometer has been also designed and tested. Quantitative methods were developed for absorbed dose determination in victims via analysis of collected biological samples and swipe samples to assess surface contamination on victims.

Complementary laboratory techniques can provide more accurate and sensitive results in a second step for the triage, for refining diagnosis for victims little irradiated or contaminated. The BOOSTER kit includes 4 additional in-laboratory techniques.

Liquid scintillation counting (LSC) is well-applicable for analysis of alpha and beta emitting radionuclides in low concentration in biological samples. A new sample preparation method was developed before the direct analysis of urine samples The method with very low detection limits (mBq) is rapid and simple enough for short sample analysis and absorbed dose estimation after the in-field Triage.

Inductively coupled plasma mass spectrometry (ICP-MS) is a well-applicable technology for analysis of long-lived radionuclides in low concentration in biological, environmental and swipe samples. This technique can detect radionuclides incorporated in body fluids (blood and urine), using a dropping and drying sample preparation. It is more time consuming but also more sensitive than other techniques, hence it is also possible to determine the low dose absorbed by inhalation or ingestion.

In vitro analysis of cultured cancer cells showed that the antibodies generated under Booster were suitable for the detection and automated quantitation of centrosome amplification after irradiation. These antibodies were also capable of detecting the centrosome in primary peripheral blood mononuclear cells (PBMCs) from consented volunteers. However, the irradiation of PBMCs did not lead to the amplification of centrosomes to the same extent as had been seen in vitro. The translation of the Booster centrosome analysis technology to provide a viable in vivo biodosimetry technique that might be comparable to the currently established protocols requires the identification of an appropriate tissue for analysis and readout. Preliminary data suggest that buccal cells may prove suitable.

The risk of contamination by toxic particles can be evaluated by measuring the abundance and nature of different types of particles at the main routes of contamination such as the mouth, nostrils and eyes, as well as the toxicity of these particles. The flow cytometry (FACS) can be used to estimate the abundance and granulometry of nanoparticles, as a function of the modification of SSC (Side Scatter) corresponding to the diffraction signal in the FACS technology. It was possible to differentiate nanoparticles of 20, 50, and 100 nm with very high precision.

Environmental impact

The deployed tools also allow securing the first responders from the radiation exposure as these tools do not only check the affected people but also provide clear information on the existing contamination level which is far beyond that from existing radiation equipment available so far.

The second impact is environmental as the BOOSTER kit enables drawing a contamination map in the exclusion and controlled areas, and limits disseminating contamination in the periphery thanks to the contamination test and decontamination process of people at the exit of the controlled area. This process should avoid contaminating the whole city, like during Goiânia accident, by contaminated people ignoring their state.

Colibri is the smallest versatile radiation monitoring instrument, capable of measuring precisely and quickly background level of dose-rate changes from 10 nSv/h up to several mSv/h, with a saturation limit allowing the quantification of significant dose-rate hazard. Its wireless data collection enables a continuous survey associated with the geographical GPS position of the operator.

GPS-COM system consists of a plug-and-play Supervisor unit, 1 to 100 maximum GPS-COM personal radiation monitoring devices, and repeater units to enhance the communication range between the Supervisor and the control PC up to 1 km. GPS-COM communicates wirelessly via radio-frequency technology with the decision making team. It also allows drawing a contamination map.

Falcon 5000 is a portable Radionuclide Identifier based on a High Purity Germanium (HPGe) detector. It is able to identify special nuclear materials or to determine the basic isotopic content of the sample. By identifying very early the radionuclides involved in the attack or accident, commandment team can adapt preventive/curative treatment for the population and mitigation actions for the environment.

Gamma imaging enables the location of a radioactive source via the superimposition of a visible image and a gamma image. Gamma imaging helps locating radioactive hot spots in a given area while staying on a reasonably high distance of the danger. The identification of hot spots enables to protect first responder team as most of the dose received by irradiation can be avoided when avoiding dangerous areas, but also to identify objects to evacuate in priority or zones to decontaminate in priority.

CANBERRA has also set-up a contamination monitoring portal that will be located at exit points of the exclusion and controlled area. Therefore, when each person passes through this portal, the first responders will be able to classify him as being contaminated or not. After that, further measurements will be performed using other CANBERRA alpha and beta probes. Once the victims are evacuated from the exclusion and controlled area, they will be subjected to a first medical check-up to be sure that they are fully safe or not. In this way, environmental contamination around the controlled zone should be prevented or strongly limited by contaminated people.

To assess the dispersion of radioactive particles in the atmosphere following the explosion, a simplified particle model based on the spreading of a plume was developed in Decision Support System. This model does not require too much computing time as it is bi-dimensional and as the area of interest was limited to several 100 meters. According to the direction and speed of the wind, this model provides a prevision of the global contaminated area or whose contamination is in progress by atmospheric transport of particles.

BOOSTER system designed is a first prototype and has demonstrated that monitoring equipment of different type can be coupled together with a Command and Control system and a Decision and Support system. BOOSTER project was presented during the RODOS congress on February 2014 in order to couple BOOSTER Decision and Support system to the german national wide decision support RODOS system (Real-time On-line DecisiOn Support system for nuclear and radiological emergencies) via simple exchange mechanisms. KIT will explore this path further and develop appropriate interfaces as soon as the end users of the RODOS system decide on spending resources for such an activity.

Social impact

Third impact is social as BOOSTER will significantly improve the mitigation capability of a radiological incident and will participate in ensuring that people do not overreact to the mere presence of radiation without full knowledge of the extent and type of contamination (psychological effects are generally disproportionately greater than the actual physical threat – panic effect).

By deploying quickly an intervention team with specialized material, the crisis managers will give to the media and the population guarantees that the crisis situation is correctly managed and is in a good way to be under control. This should limit the risk of broadcasting totally imaginary and nightmarish information liable to lead the whole population to a global panic situation.

By informing and reassuring non contaminated people present during the accident or terrorist attack about their good health and offering a sanitary survey of victims in the future, it should also limit panic effect by avoiding dishing the dirt to their neighborhood.

Obviously these efforts will need to be associated to psychological assistance for victims that may overreact due to posterior shock effect, and large communication campaigns to clearly explain the situation, the risks and the mitigation measures undertaken to the global population and the victims.

Economic impact

By avoiding a large panic situation based on a popular supposal that a broad area may be contaminated and evacuated, the city will avoid being completely paralyzed for some times and local economy will continue “almost” normally outside the identified contaminated areas.

The early identification of well-defined contaminated areas will enable taking early measures to prevent a global dissemination of contamination. Thus the areas to decontaminate will be reduced and the decontamination cost will be limited too.

A long-term follow-up of the victims should enable the early identification of starting cancers, which should alleviate cancer treatment for victims and also reduce the medical costs.

Economic competitiveness

BOOSTER project enabled the development of several breakthrough technologies in biodosimetry. Two kinds of new biodosimeters were developed to assess a posteriori the radiological dose received by a victim.

The first portable and quick toolkit was developed for global measurement of phosphorylation of H2AX protein in fibroblasts and lymphocytes; BOOSTER technique is much quicker (30mn) than classical laboratory γH2AX technique (8h), and it provides a comparable precision. Thanks to the 3 patents applied for BOOSTER γH2AX technology, new products for biodosimetry should be developed and industrialized in the future.

A proof of concept was provided for centrosome numbering: BOOSTER project proved that centrosome numbers present in chromosomes increased with radiological absorbed dose. Monoclonal antibodies were developed to label centrosomes and enabled an easy detection and automatic counting of centrosomes. The proof of concept for centrosome increase and counting paves the way for new biological and medical applications, in particular in cancer research. This work on centrosomes was the first step before determining which antibodies are of commercial value and discussing licensing with antibody companies.

Retrospective dosimetric method was developed. Using small surface mounted devices originated from mobile phones and also environmental materials, like dolomite, doses can be estimated posteriorly. SMD resistors were identified as good emergency personal dosimeters and characterized. BOOSTER project demonstrated the reliability of the dose estimation with SMD via Thermo Luminescence and Optically Stimulated Luminescence in the range 10 mGy – 6 Gy, and a portable in-field TL/OSL reader, more sensitive than the classical laboratory devices, was built and tested during the final exercise. Further development will permit commercialization.

A new generation of gamma-camera was developed and fully tested during BOOSTER project; it is capable to detect radioactive sources in the field and serves a picture about the source together with the location. The new generation gamma-camera is portable (~1 kg in place of the 15kg of first generation gamma-cameras), easy to use with a USB connection, optimized for a large range of gamma energies, and it provides an improved sensitivity thanks to the use of coded masks. The feasibility prototype was tested and optimized during the project and an industrial product is expected in 2014 after the end of the project.

In term of economic competitiveness, BOOSTER project provided new generation products to the French SME CANBERRA (gamma camera and GPS-COM dose rate meter) and to Spanish spin-off of Polytechnic University of Valencia (SIMACOP integration software). After complementary characterization, calibration and testing, retrospective dosimetry should lead to a portable industrial Thermo Luminescence – Optically Stimulated Luminescence reader.

Scientific impact

BOOSTER also induced a scientific impact as four breakthrough technologies were developed while several technologies were greatly improved.

Main achievement of BOOSTER project was the success of the demonstration exercise in May 2013; it was a technical achievement as all technologies could be integrated in a global toolbox and ran satisfactorily altogether. BOOSTER technologies received positive appreciations from the European experts and Embassy representatives having attended the demonstration. Several devices could be handled by Hungarian first responders who followed BOOSTER training. The event was largely covered by the media with the presence of about 50 journalists from Hungarian televisions, radios and newspapers.

A large dissemination of BOOSTER results was undertaken via the website, a booklet presenting final BOOSTER results and technologies, publications of journal and conference papers, a workshop with partners of MultiBiodose FP7 project, a newsletter of Eurados network, a paper in the French General Nuclear Journal proposing a demonstration of BOOSTER toolkit on demand, the presence of experts at the demonstration exercise and the distribution of Booster training courses to all experts, and several press articles and TV/radio news.

5 CONTACT DETAILS

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