

FINAL REPORT (Technical Part)

(Summary)

EU funded Project “EQADeBa - Establishment of Quality Assurances for Detection of Highly Pathogenic Bacteria of Potential Bioterrorism Risk“

(EAHC Agreement n°: 2007 204)

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1 Executive summary

1.1 Project Description

EQADeBa is an EU funded project (EAHC n° 2007 204) on the topic of “Establishment of Quality Assurances for Detection of Highly Pathogenic Bacteria of Potential Bioterrorism Risk“, coordinated by the Robert Koch-Institut (RKI), Germany, and carried out in cooperation with, originally, 23 partners from 21 European countries. The project started in May 2008 and runs for 36 months. A cost neutral prolongation of 3 months was agreed until 31 July 2011. This project was initiated in response to results of a tender by DG SANCO on an External Quality Assurance Exercise (EQAE) in 2006 revealing the need for further improvement of the diagnostic reliability concerning bacteria of potential bioterrorism risk.

The objective of the EQADeBa project is to provide a suitable infrastructure and to design, organize, and manage practical EQAEs for bacteria of potential bioterrorism risk causing anthrax, tularemia, plague, glanders, melioidosis, brucellosis, and Q-fever. The project will directly support the early warning and rapid response capacity to ensure an effective reaction to bioterrorist attacks as well as to natural outbreaks. In this context, a supportive European high threat pathogen laboratory network capable of identifying and characterising potential bioterrorism agents was established.

A repository of relevant reference samples was established and can be further extended. These bacteria were and will be provided by the project participants. They were authenticated by all available microbiological, molecular and immunological approaches. The reference material

was used by the project participants for quality assurance exercises as well as for validation of new detection methods and instruments. Furthermore, it will be offered for participants in relevant activities in the future.

Three rounds of External Quality Assurance Exercises using samples of different complexity were applied in order to estimate the current laboratories' capabilities including all aspects of biosafety and biosecurity. The coordinating institution of the project (RKI) has organised the EQAEs including preparation, characterisation, quality assurance, delivery of samples and analysis of results. The planning of the exercises, discussion of the results and development of recommendations were carried out in cooperation with all participants at the general meetings. The final aim was to develop a "Gold standard" for the detection and identification of high threat bacteria. To ensure biosafety and biosecurity while handling high threat biological samples, a questionnaire was developed to evaluate the current stage of adequate regulations and their implementation and to be used for further improvements.

Almost all partners took part in training provided by other partners. The training aimed to improve the diagnostic capabilities, to learn more about the partners' institutes and their work and to foster a reliable laboratory network between the EU Member States. The efficacy of training of personnel at network partners and corresponding improvements were validated by a third round of EQAE.

Thus, the project has substantially optimized the preparedness of individual appointed laboratories in EU Member States to respond to any potential bioterrorism threat or event. The established network of European laboratories has highly facilitated the infrastructure to create capabilities, critical mass and skills necessary for the detection of high threat bacterial pathogens.

1.2 Summary of results

1.2.1 Coordination, Dissemination, Evaluation (WP 1-3)

The partnership, training and exchange of material were regulated by confirmed Consortium Agreements for both associate and collaborative partners. Six meetings of all participants (General Assembly) in Berlin, Vienna, Rome, Salisbury, Stockholm and Brussels and three meetings of the Advisory Board have been performed (in Berlin, Rome and Stockholm). Furthermore, three challenging proficiency tests / EQAEs have been completed comprising in every case, 30 samples for each laboratory.

During the six project meetings, data were analysed and discussed and next steps planned. The cooperative atmosphere of the meetings encouraged most of the partners to present their results and openly discuss the advantages of their approaches but also the need for improvement. At quite an early stage the idea came up to combine meetings with topical workshops in order to foster the exchange of scientific knowledge among the partners. Thus, four workshops have been carried out with the focus on particular "dangerous pathogens and Leptospirosis", "antibiotic susceptibility", "advanced rapid diagnostic assays", and "surveillance of highly pathogenic bacteria". External experts invited in addition to professionals among the project partners contributed to the high quality of these workshops. The project website as well as presentations and/or publications were used as ways of dissemination. An evaluation plan was set up. The Advisory Board also served as Steering Committee. Critical issues have not been identified for the project performance.

1.2.2 Repository (WP 4)

To perform quality assurance exercises and to allow the laboratories to self-evaluate newly established methods and instruments, a repository of reference samples, comprising all target bacteria, has been established in the course of the project. The bacteria of the repository, provided by the partners, were characterised by means of all available microbiological and molecular approaches. All quality-controlled repository strains, stored at -80°C , as well as the quantified DNA and inactivated material out of these strains, stored at -20°C , are part of the reference material. At the end of the project, the repository comprises 161 strains. A detailed list is given in Annex 1 (Repository Content) describing the repository of highly pathogenic bacteria at the RKI established in the framework of the EQADeBa project.

Some partners ordered living (native) bacteria or DNA out of the repository. The number of strains, inactivated material or DNA ordered by the partners was at maximum 15 for the duration of the project, which was agreed at the meeting in Vienna/Austria. To guarantee a long-term availability of this source of reference material of highly pathogenic microorganisms a continuous maintenance and expansion is essential.

1.2.3 EQAEs (WP 5)

For transport and handling of pathogenic material, a questionnaire on biosafety and biosecurity (cf. Annex 2 - Questionnaire on biosafety and biosecurity for handling of highly pathogenic agents under BSL 3 conditions) was developed and was the basis for the exchange of pathogenic bacteria among project partners. The sample preparation was conducted by the RKI. While the first exercise included DNA and inactivated bacteria only the last two exercises contained both, living and inactivated bacteria, as the two components of the exercise. The detection range of high threat bacteria included *Bacillus anthracis*, *Francisella tularensis* ssp. *tularensis* and ssp. *holarctica*, *Yersinia pestis*, classified under category A agents, according to the Centers for Disease Control and Prevention (CDC). Category B strains involved were *Burkholderia mallei*, *Burkholderia pseudomallei*, *Brucella melitensis*, *Brucella abortus* and *Coxiella burnetii*. To ensure the high quality of the samples, the provider (RKI) controlled the sterility or viability of the bacteria, the purity of material and strains, cross-contamination of the samples by adequate real-time PCR and additional PCRs for further characterization of the strains. The exercises were designed with an increasing level of difficulty. The aim was to assess, maintain and/or improve the partner institutes' skills and capabilities to detect highly pathogenic bacteria. The exercises were agreed by all participants, and the set of provided samples were appropriately composed to fulfil the aims of the exercises.

1.2.4 Analyses of results of EQAEs (WP6)

All samples arrived at the partners' laboratories without complications. The speed of diagnostics during the three exercises could considerably be improved. A supplementary task was to test isolated bacteria for susceptibility to various groups of antibiotics. Another supplementary issue was the estimation of the bacterial load of the inactivated samples containing various matrices. The participants were asked to describe the applied methods and to provide results in a final protocol, cf. Annex 3 (Summary Results 1st EQAE).

The most challenging samples consisted of living bacteria (EQAE 2 and 3) containing mixed cultures. A clear improvement of correct positive and correct negative results could be observed over the course of the project. In the case of inactivated samples in different

concentrations and within complex matrices, contamination posed the biggest problems during the 1st and 2nd exercise. This could also be improved during the third exercise. Reduced bacterial load within the inactivated samples of the 2nd (see Annex 4 - Summarized results of the 2nd EQAE) and 3rd exercise (Annex 5 - Summary Qualitative Results EQAE3) increased the risk for not obtaining correct results. Reasons for this were a low sensitivity of applied methods, besides cross contamination, specificity of the applied methods, and mixing up of samples. The improvement of laboratories' performances was achieved by solving the problems listed following the previous EQAE. The major impact, however, has been given by personal troubleshooting according to partners' results, followed by exchange of best practice and approaches among the partner institutes and by subsequently run training wherever reasonable.

Three partners participated in their new constructed BSL-3 containment for the first time (one partner within the 2nd exercise and two partners in the 3rd exercise), one BSL-3 laboratory is under construction and all participants extended also their detection spectrum to the entire range of all target bacteria during the exercises.

The ultimate aim was to identify best practices in order to develop a "Gold Standard" for the detection and identification of high threat bacteria, yet, not implying that the various successfully applied methods must necessarily be identical. The objective is rather to provide and recommend the best practices identified to maintain or improve the high quality standard of the laboratories. Mutual training was provided to learn from each other and find partners in cases of real scenarios. The "Gold Standard" developed was based on best practices at pre-analytical, analytical and post-analytical stages of diagnoses.

1.2.5 Training (WP 7)

6 partners offered 1- to 2-weeks training courses and 17 partners took the opportunity to participate (cf. Annex 6 - Training Programme List). Biosafety and biosecurity were tackled as well as detection methods for highly pathogenic bacteria (HPB). This exchange of experiences was one major aspect for the improvement of the performances shown during the EQAEs. The most important outcome of these courses was that all partners became more familiar with one another, stabilising this European laboratory network and establishing contacts in case of emergencies. The operational capacities of the network should be further developed in future activities and should be provided to the EC. To evaluate the quality of the training courses by trainees and trainers, accompanying sheets were recommended. Several training programmes were collected and made accessible through the website.

Training offers were mainly based on the abilities of the providers. In the future, trainees will have the opportunity to select training more specifically tailored to their individual needs.

1.2.6 Conclusions and Recommendations

All in all, the following summary, including recommendations, may be given:

- Proficiency tests for diagnostics of highly pathogenic bacteria are recommended as a continuous process.
- Most EU Member States do probably not carry out proficiency tests for diagnostics of highly pathogenic bacteria at national level although this would be strongly required to ensure the necessary quality of diagnostics [15,16]. Moreover, new diagnostic techniques, including rapid assays and MALDI, will continuously be developed and

will, of course, raise new questions regarding validation and implementation. In the long run, it is, therefore, highly recommendable to implement proficiency tests as performed within the EQADeBa project. This would, however, need further support through the European Commission for such activities at European level.

- The degree of laboratory preparedness for the detection of highly pathogenic bacteria varies at (national and) international level.
- All project partners underlined the usefulness of the project and could improve their diagnostic capabilities and/or evaluate their high standard.
- The training courses offered significant benefits to trainees and trainers.
- The project has collected experiences on biosafety, biosecurity, and transportation issues throughout Europe. During the three exercises, no problems occurred in terms of transportation due to an intensive preparation of shipment with the carrier World Courier considering all relevant national and international regulations.
- The project is linked with GHSAG and WHO initiatives. The ECDC has been kept updated and will be involved in the future projects.
- The questionnaire on biosafety and biosecurity is offered to the EU for further development and implementation as a standardised document and recommendation for safe and secure exchange of pathogenic material between European Member States and EFTA as well as other countries.
- Concluding, the participating Member and EFTA/EEA States turned out to be relatively well prepared to detect highly pathogenic bacteria although there is potential for further improvement.
- A repository for reference material of highly pathogenic microorganisms has been set up and should be maintained on a long-term basis.
- A network of laboratories responsible for the diagnostic of highly pathogenic bacteria is required on the long run as these bacteria also occur with often unknown and underestimated prevalence. A first step to set up this network has been achieved in this project.
- The network could also be linked with other networks, e.g. on viruses and toxins.
- European Reference Laboratories which organise and perform quality assurance exercises should be appointed and provide appropriate reference materials for validation of diagnostic methods and instruments.
- For the future, there is a need for comparable evaluation of existing in-house and commercial assays as well as instruments for the detection of selected agents.
- Common recommendations for the testing of antibiotic susceptibility for highly pathogenic bacteria should be developed for European countries.

It should be considered that all activities described above are not only important from the perspective of intentional release of these highly pathogenic microorganisms as these bacteria also occur naturally [18,19,20,21,22] in the environment. This in turn requires diagnostic methods to be supplied to the laboratories [23]. The European scope is the most appropriate framework to evaluate, improve and sustain these diagnostics to have a broader platform to develop and exchange knowledge, methods and reference material on these often “neglected” but potentially very dangerous diseases. Therefore, the final recommendations will comprise the desired support through the European Commission to ensure a long-term perspective for this network of laboratories dedicated to the diagnostic of highly pathogenic bacteria.