



Robert Koch-Institut

Centre for Biological Security 2

**Establishment of Quality Assurances for  
Detection of Highly Pathogenic Bacteria  
of Potential Bioterrorism Risk**

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**Luxembourg**

**30 Sept - 01 October 2009**

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Agency for  
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# Robert Koch-Institut Berlin, Germany

ROBERT KOCH INSTITUT



RKI is the central federal institution responsible for disease control and prevention in Germany.

## Berlin



### Staff

820 employees,  
incl. 330 scientists

(graduate students and trainees incl.).  
Approx. 320 are on limited-term contracts.

Nordufer 20

Seestr. 10

(General-Pape-Str. 62-66)



## Wernigerode



# Centre for Biological Security (ZBS)

**October 2001: The German government decided to establish the Centre for Biological Security at the RKI**  
**Currently: More than 120 employees**

**IBBS**      *Federal Information Centre for Biological Security*

**ZBS 1**      *Highly Pathogenic Virus*

**ZBS 2**      *Highly Pathogenic Microbial Pathogens*

**ZBS 3**      *Microbial Toxins*

**ZBS 4**      *Imaging Techniques; Rapid Morphology-Based  
Diagnostics of Infectious Organisms*

**ZBS 5**      *BSL 4 Laboratory*

# Highly Pathogenic Bacteria

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## Bacteria like causative agents of anthrax, plague, and tularemia

- are often zoonotic pathogens,
  - require BSL3 laboratory containment,
  - are mainly diagnosed by In-house-assays,
  - occur naturally and causes epidemics,
  - could be suspected for deliberate release.
- **Almost no reference materials and quality assurance exercises for diagnostics!**

# Measures for Quality Assurance

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## Internal and external QA of laboratory diagnostics

- Wet labs, proficiency tests, ring trials
- Appropriate reference material
- Improvement, training
- **Question: Who takes the responsibility?**

**Situation of laboratory preparedness to diagnose agents of potential bioterrorism risk across the European Union is not well known.**

# Previous Initiative

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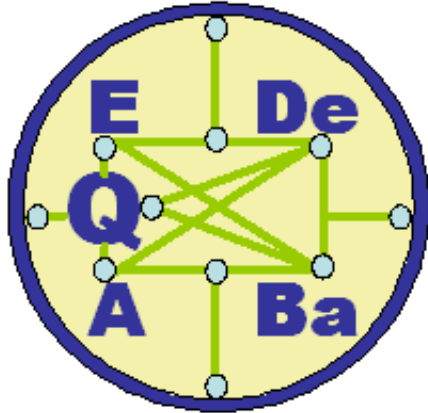
**Tender DG SANCO No. 2005/C3/04-SI2.419417**

**„Setting up quality assurance schemes for diagnosis of very high threat pathogens“**

- **Robert Koch-Institut, Wernigerode, 2006**
- **13 countries (14 labs)**
  
- **Outcome: Strong requirements for improvement**

# Establishment of Quality Assurances for Detection of Highly Pathogenic Bacteria of Potential Bioterrorism Risk

Acronym: **EQADeBa**  
(Agreement No 2007 204/EAHC 2007)



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\* Centre for Biological Security (ZBS 2)

# Financial Administration (ZV2-DRM1)

- **Granted since May 2008; Duration: 2008-2011,**
- **Financial volume: 2 million EUR**

# EQADeBa Participants

## Participants:

25 labs from  
22 European countries

## External participants

- CDC BRRAT LAB  
Atlanta, USA
- Public Health Agency of Canada  
National Microbiology Laboratory  
Winnipeg, Canada

**MP** Main partner  
16 AP Associated  
Partner  
8 CP Collaborative  
Partner

<b><u>EU Countries</u></b>					
Austria	AP	AGES	Latvia	-	
Belgium	AP	VAR	Lithuania	AP	NPHIC
Bulgaria	AP	NCIPD	Luxembourg	CP	LNS
Cyprus	-		Malta	-	
Czech Rep.	CP	NINBCP	Netherlands	AP	RIVM
Denmark	-		Poland	AP	PZH
Estonia	CP	HPI	Portugal	CP	INS-RJ
Finland	AP	THL	Romania	-	
France	CP	CRSSA	Slovenia	-	
Germany	MP AP CP	RKI FLI IMB	Slovakia	-	
Greece	AP	NKUA	Spain	AP	BIOEF
Hungary	AP	NCEBACT	Sweden	AP	SMI
Ireland	CP	PHL	UK	AP	HPA
Italy	AP AP	ISS IZSBB			
<b><u>EFTA/EEA</u></b>			Switzerland	CP	Spiez Lab
Norway	AP	NIPH			

# Aims and Challenges of the EU Project

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## Overall

- Broad participation
- Analyses of current laboratory capabilities and after their optimisation
- Sustained effect by setting up a network, exchange of information
- Training and visiting of laboratories
- Experience and profit for national attempts to improve diagnostics
- Find partners

## Specific

- Repository: Providing of reference materials (Consortium Agreement)
- Aspects of biosafety and biosecurity
- Transportation, Import/Export controls
- 3 rounds of EQAE for detection of selected high threat bacteria

# Questionnaire on Biosafety and Biosecurity

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## Sources

- World Health Organization (ed.): *Laboratory biosafety manual*. 3rd edition. Geneva 2004.
- Centers for Disease Control and Prevention (ed.): *Biosafety in Microbiological and Biomedical Laboratories*. 5th edition. Washington 2007.
- Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (ed.): *Technische Regeln für Biologische Arbeitsstoffe*. 2006.
- Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the Protection of Workers from Risks related to Exposure to Biological Agents at Work. Official Journal of the European Communities, 2000, L 262/21-45.

# Questionnaire on Biosafety and Biosecurity

## Content

### A. Standard Microbiological and Work Practices

### B. Special Practices

- Handling infectious material
- Decontamination procedures
- Handling of sharps
- Compressed gas suppliers

**About 180 check points  
for self-evaluation**

### C. Safety Equipment (Primary Barriers)

### D. Laboratory Facilities (Secondary Barriers)

- Approval of BSL-3 facility
- Construction of BSL-3 facility
- Electricity
- Biological Safety Cabinets
- Centrifuges
- Emergency situations

### E. Security Assurance

### F. Shipment and transportation of material outside the BSL3 containment

### G. Training and regulatory instructions

### H. Personal precaution for biosafety reasons

### I. Documents

# Study Design of EQAEs

## ➤ Target organisms:

*B. anthracis* veg., *B. anthracis* spores

*Y. pestis*

*F. tularensis* ssp. *holarctica*

*F. tularensis* ssp. *tularensis*

Category A

*B. mallei*

*B. pseudomallei*

*B. melitensis*, *B. abortus*

*C. burnetii*

Category B

Closely related bacteria

**Challenge:**  
**Handling and shipment must be in accordance with regulations for BSL3-organisms**

- 3 proficiency tests with approx. 30 samples for each laboratory
- 1<sup>st</sup> round inactivated samples, 2<sup>nd</sup> and 3<sup>rd</sup> rounds native samples
- including pure culture, clinical and environmental surrogates

# Study Design 1<sup>st</sup> Round of EQAE

Performed in March 2009

## Samples for 23 laboratories:

- 15 DNA samples (10 target pathogens and 5 closely related bacteria)
- 15 samples thermally or chemically inactivated bacteria in 3 different matrices (PBS, river water, tissue)

## Tasks:

- **Correct identification** by molecular genetic and/or immunological and/or other methods
- **Time to identification** (15 DNA samples)
- **Detection limit** (titration of DNA samples)
- Analytical **specificity**
- Additional characterisation or approaches like genotyping

## Methods:

- Microbiological, molecular biological, immunological, others; selected by the individual participants

# Sample Quality

## Samples suitable for different methods

1. Molecular genetic methods
2. Immunological methods
3. Microbiological methods

## Quality assurance

- Parameters: Sterility, purity, cross contamination, recovery, storage stability
- Steps controlled:
  - Preparation and inactivation
  - Inactivated cells after adding different media
  - Portioned DNA and bacterial samples, 3 tubes out 30
  - Repeated analysis after the deadline for results (4 weeks)

# Molecular Genetic and Immunological Analysis

## Final results on characterisation of the reference material:

- In spot tests, DNA concentrations were highly consistent (9-24 ng/μl by Nano Drop for different bacteria)
- copy numbers were approx.  $10^6/\mu\text{l}$
- no cross contaminations observed
- ELISA and IFA applicable

## Performed analyses for quality assurance:

- Number of DNA isolations: **340**
- Number of Real-time PCRs: **80** x 96 well plates
- Number of sequencings: **~200** (only 16S rDNA)
- Number of conventional PCRs: **~100** (plus VNTR, MLVA, MLST)
- Number of immunological tests: **several hundreds**

# Outcomes of 1<sup>st</sup> EQAE

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1. Duration of the transport: average 20.4 h
2. Duration of analyses:  
DNA samples: 13 labs 6h , 5 labs 12h, 5 labs up to 50h
3. Sensitivity (detection limit by end-point titration)  
mean deviation about  $\pm 100$  copies, much higher in particular labs

# Results of 1<sup>st</sup> EQAE: DNA Detection

## Laboratories n=21

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$$\begin{aligned}\text{\% Correct positive} &= [n \text{ correct pos} / n \text{ all target (9x21)}] * 100 \\ &= \mathbf{93 \%} \text{ (accepted F.t., Burkholderia)}\end{aligned}$$

$$\begin{aligned}\text{\% False negative} &= [(n \text{ false neg} + \text{not tested}) / n \text{ all target (8x21)}] * 100 \\ &= \mathbf{7 \%}\end{aligned}$$

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$$\begin{aligned}\text{\% Correct negative} &= [n \text{ correct neg} / n \text{ all non-target (6x21)}] * 100 \\ &= \mathbf{86 \%}\end{aligned}$$

$$\begin{aligned}\text{\% False positive} &= [n \text{ false pos} / n \text{ all samples (15x21)}] * 100 \\ &+ \text{\% incomplete excluded} \\ &= \mathbf{14 \%}\end{aligned}$$

# Results of 1<sup>st</sup> EQAE: Bacteria Detection

Laboratories n=21

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% Correct positive = [n correct pos/ n all target (9x19)]\*100

**= 92 % (accepted F.t.)**

% False negative = [(n false neg + incorrect tested)/ n all target (9x19)]\*100

**= 8 %**

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% Correct negative = [n correct neg/ n all non-target (6x19)]\*100

**= 82 %**

% False positive = [n false pos/ n all samples (15x21)]\*100

**= 18 %**

**Major problem: Bacteria in complex sample matrices!**

# Best Performance

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## 100% correct results:

**DNA**            **10/21**

**Bacteria**      **11/19**

**Both**            **8/19**

## 2<sup>nd</sup> EQAE

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- **Beginning of November 2009**
- **15 samples with viable bacteria including mixtures with environmental contaminants**
- **15 samples with inactivated bacteria in serum, milk, lake water**
- **Arrangements for shipment**

# Interim Summary

- The grade of **laboratory preparedness** for the detection of BT-agents varies at (national and) international levels. Primarily the correct identification of samples including more complex matrices should be improved.
- During the first exercise, no problems in terms of **transportation** occurred, most labs provided time crucial results within hours.
- Recommendations from the first exercise and subsequent **training** are necessary to improve results which will be obtained in the second and third exercises.
- There is a need for comparable **evaluation** of existing in-house and commercial assays and instruments for the detection of selected agents.
- The project is collecting experiences on **biosafety, biosecurity, and transportation** issues throughout Europe.
- The project is **linked** with GHSAG and WHO initiatives, and is announced to ECDC, open for further collaboration.

# Recommendations

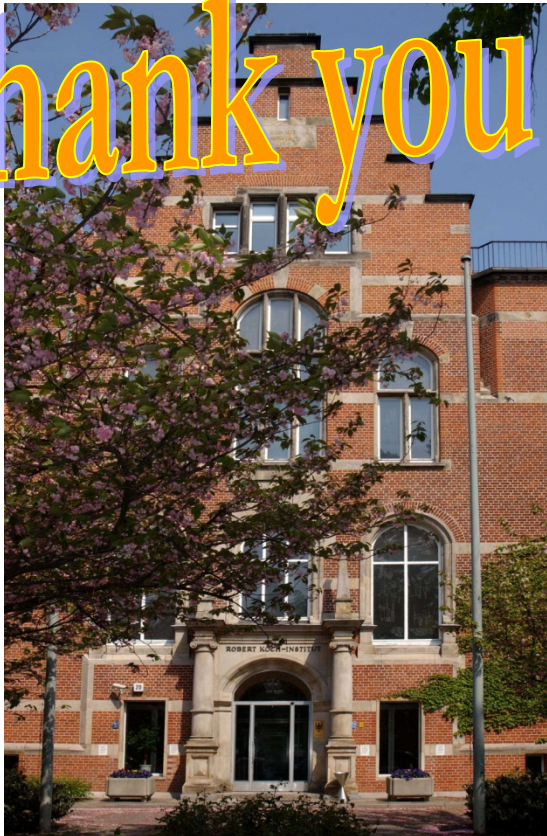
- **The questionnaire on biosafety and biosecurity** is offered to the EU for further development and implementation as a standardised document and recommendation for safe and secure exchange of pathogenic material between European Member States and EFTA as well as other countries.
- **Proficiency tests** for diagnostics of highly pathogenic bacteria are recommended for long term, as most EU Member States do probably not possess it at national levels.
- **A repository for reference material** of highly pathogenic microorganisms is required and should be maintained on long term.
- **A network of laboratories** responsible for the diagnostic of highly pathogenic bacteria is required on long term, as these bacteria also occur naturally with often unknown and underestimated prevalence in the environment.  
The network could also be **linked** with other networks, e.g. on viruses and toxins, but should not be merged due to the complexity of each of the fields.
- **European Reference Laboratories** should be appointed which organise and perform quality assurance exercises and provide appropriate reference materials for validation of diagnostic methods and instruments.

# Acknowledgments

- Thanks to the organisers of the meeting.
- Thanks to all **participants of the project**

and my colleagues:

Thank you for your attention



H. Nattermann  
A. Jenzora  
S. Klee  
K. Lemmer  
A. Roder  
S. Dupke  
S. Volkmar

S. Becker  
T. Franz  
R. Heinrich  
S. Howaldt  
I. Klein  
U. Klein  
R. Krueger  
P. Lochau  
B. Meister  
H. Ranisch

This work was supported by the EU,  
EAHC Agreement - No 2007 204