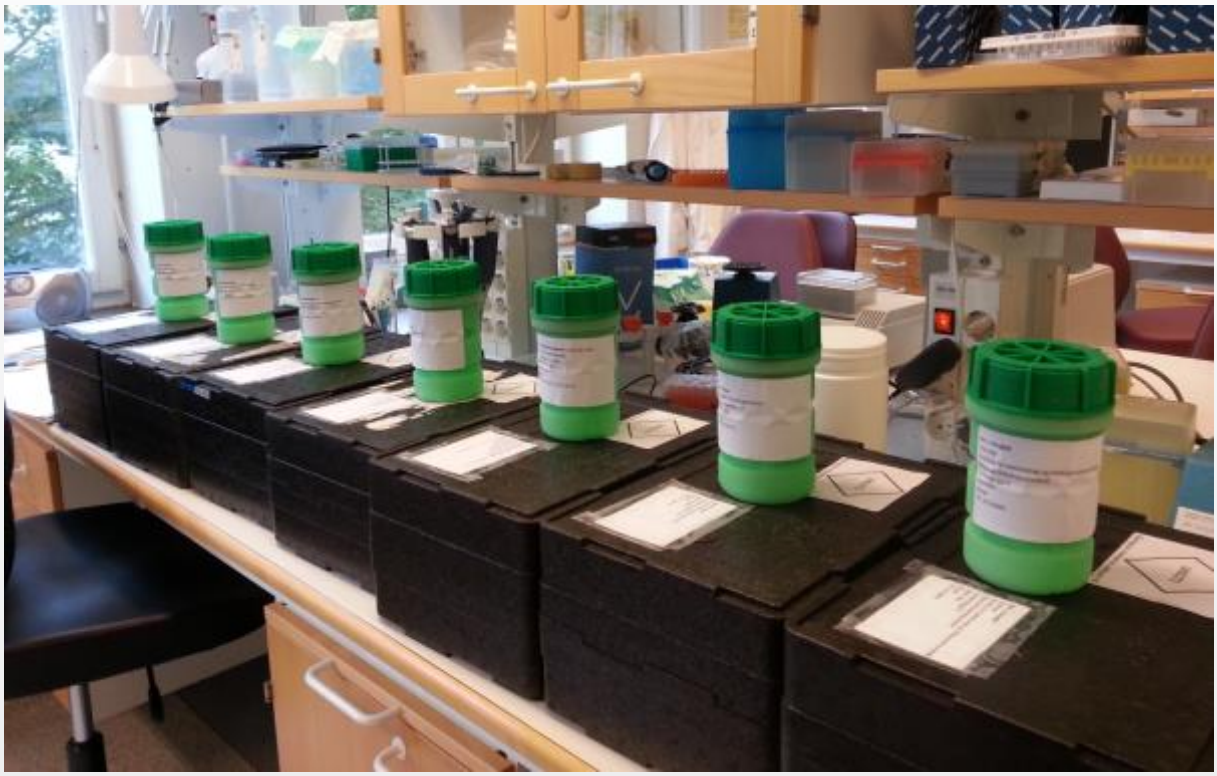
FORUM FÖR
BEREDSKAPSDIAGNOSTIK

PROJECT REPORT

Quality Assurance of Biosafety Level 3 Laboratories



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FBD 2016/17

ABSTRACT

The Forum for Biopreparedness Diagnostics (FBD) is a collaborative effort between four Swedish governmental agencies – the National Food Agency (NFA), the National Veterinary Institute (SVA), the Public Health Agency of Sweden (PHAS), and the Swedish Defence Research Agency (FOI). The aim of this effort is to strengthen the diagnostic capability and capacity for selected agents in Sweden. This is achieved by harmonisation of methods and equipment; optimisation of the national capacity and competence for effective and endurable diagnostics in case of large-scale spread of highly pathogenic microorganisms; and joint exercises to increase the level of bio-preparedness in Sweden.

In this project within the framework of FBD we focused on quality assurance from various perspectives by:

1. Assessing the quality of the existing PCR systems (primers and probes) within FBD by *in silico* validation which rendered new diagnostic tools that increased laboratory capacity.

2. Performing two biopreparedness EQAs (External Quality Assessment) to assess critical steps in the processing of samples; The EQAs evaluated the ability to detect risk group 3 bacteria by culturing methods and molecular techniques. The methods used in the EQA were previously harmonised, validated and implemented by all laboratories belonging to the FBD network. In addition to the Swedish laboratories, three laboratories from Norway also participated in the EQA. Lessons learnt of shortcomings from the first EQA were adapted at the agencies and resulted in improved results in the second EQA.

3. Performing quality audits and a biosafety audit at the Swedish Biosafety Level (BSL3) laboratories based on ISO/IEC 17025:2005 and CWA15793.

The main lesson learnt from the project was that the participating laboratories were of high quality, and still improvements could be done, now indicating a good preparedness for the detection of biological agents in the event of biological threats and extensive natural outbreaks.

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SCOPE OF THE FBD

The overall aim of the Forum for Biopreparedness Diagnostics (FBD) is to strengthen the capability and capacity to identify microbial high consequence agents (i.e. agents that require biosafety level 3 laboratories) in various sample types and enable the authorities to share the sample load during crisis. To achieve this, the FBD strives to harmonise methods, equipment and quality assurance to ensure that results emanating from the participating authorities are comparable. The multisectoral laboratory network enables diagnostic work applied to different sample types e.g. tissue (human and animal), food, feed, drinking water and environmental samples. FBD is a collaborative effort of four Swedish governmental agencies: the National Food Agency (NFA), the National Veterinary Institute (SVA), the Swedish Defense Research Agency (FOI) and the Public Health Agency of Sweden (PHAS).

FBDS ARBETE

Det övergripande målet med Forum för beredskapsdiagnostik (FBD) är att skapa och förbättra förutsättningar för ett mer effektivt utnyttjande av landets samlade kapacitet och kompetens för diagnostik av biologiska riskklass 3 agens (det vill säga patogener som kräver skyddsnivå 3 laboratorier). Genom sådan samordning ska myndighetslaboratorierna kunna utföra jämförbar och kvalitetssäkrad diagnostik med god kapacitet och uthållighet i händelse av storskalig spridning av allvarlig smitta. Forum för beredskapsdiagnostik (FBD) är ett samarbete mellan fyra svenska myndigheter: Livsmedelsverket, Statens Veterinärmedicinska Anstalt (SVA), Totalförsvarets forskningsinstitut (FOI) och Folkhälsomyndigheten (FOHM), som tillsammans täcker kompetensområdena humanmedicin, veterinärmedicin, foder, livsmedel inklusive dricksvatten, miljöprover samt expertis med avseende på miljöprovtagning och bioforensisk analys.

SAMMANFATTNING

Forum för Beredskapsdiagnostik (FBD) är ett samarbete mellan fyra svenska myndigheter, Livsmedelsverket, Statens veterinärmedicinska anstalt, Folkhälsomyndigheten och Totalförsvarets forskningsinstitut. Målet med samarbetet är att stärka förmågan att hitta farliga mikroorganismer i olika provtyper vid ett utbrott eller en antagonistisk handling. En förutsättning för att FBD-myndigheterna laborativt skall kunna samverka vid en större B-händelse är att samtliga myndigheter upprätthåller samma nivå gällande kvalitetssäkring av diagnostiken för de smittämnen som omfattas av samarbetet. I det syftet har FBD sedan nätverkets upprättande till stor del fokuserat på harmonisering av metodik och utrustning samt utveckling av myndighetsgemensamma rutiner och krav för kvalitetssäkring. Kvalitetsarbetet har bland annat mynnat ut i en kvalitetshandbok som dels beskriver övergripande arbetssätt inom samarbetet, men även detaljerade rutiner för bl.a. validering av nya molekylärbiologiska metoder. Inom detta kvalitetsprojekt har vi utfört kvalitetskontroll av de befintliga PCR systemen inom FBD, anordnat två ringtester med både levande och icke levande risk klass 3 bakterier samt genomfört internrevisioner baserade på ISO/IEC 17025:2005[1] respektive biosäkerhetsrevision CWA15793[2].

1. GLOSSARY OF TERMS AND ABBREVIATIONS

BSL3	Biosafety Level 3
CWA 15793:2011	CEN Workshop Agreement, a reference document describing laboratory biorisk management; Ref. No. CWA 15793:2011 D/E/F[2]
EMERGE	Efficient Response to Highly Dangerous and Emerging Pathogens at EU Level
EQA	External Quality Assessment
FBD	Swedish Forum for Biopreparedness Diagnostic
FFI	The Norwegian Defence Research Establishment
FHI	The Norwegian Institute of Public Health
FOI	Swedish Defence Research Agency
IAC	Internal Amplification Control
ISO/IEC 17025:2005	Standard of the International Organization for Standardization describing general requirements for the competence of testing and calibration laboratories[1]
IPC	Internal Positive Control
LIMS	Laboratory Information Management System
MALDI-TOF MS	Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry
NCBI	National Center for Biotechnology Information
NVI	The Norwegian Veterinary Institute
PHAS	Public Health Agency of Sweden
QUANDHIP	Quality Assurance Exercises and Networking on the Detection of Highly Infectious Pathogens
RG3 agent	Risk group 3 agent
R&D	Research and Development
NFA	National Food Agency, Sweden
SmiPrimer	PHAS's in-house software for control of primer and probe
SVA	National Veterinary Institute, Sweden
Tm	Melting temperature

2. BACKGROUND

Background of the project:

The Forum for Biopreparedness Diagnostics (FBD) is a collaborative effort between four Swedish governmental agencies – the National Food Agency (NFA), the National Veterinary Institute (SVA), the Public Health Agency of Sweden (PHAS), and the Swedish Defence Research Agency (FOI). The aim of this effort is to strengthen the diagnostic capability and capacity for selected agents in Sweden. This is achieved by harmonisation of methods and equipment; optimisation of the national capacity and competence for effective and endurable diagnostics in case of large-scale spread of highly pathogenic microorganisms; and joint exercises to increase the level of bio-preparedness in Sweden.

A corner stone for successful laboratory cooperation between FBD agencies during a larger B treat event is that participating agencies are maintaining the same level of quality assurance for diagnostics of infectious agents covered by the cooperation. FBD, since its initiation, has largely focused on harmonisation of methodology and equipment, as well as development of common procedures and requirements for quality assurance at the joint agencies. Quality work has, among other effects, resulted in a quality assurance manual which describes the overall management of the cooperation, and detailed laboratory procedures, including validation of new molecular biological methods as well as how to perform different exercises.

Exercises aimed at testing laboratory capability at each agency is a way to assess the national capacity to deal with major outbreaks of infectious agents that would require the three national BSL3 laboratories. For example, these exercises should include quality assurance of diagnostic methods, as External Quality Assessments (EQAs). Methodology to evaluate laboratory capability, developed within the framework of EU projects such as QUANDHIP and EMERGE, should be transferred and adapted to the national level.

Regular EQAs and laboratory exercises are required to achieve and maintain adequate analytical quality assurance. Risk group 3 (RG3) infectious agents are the primary object of cooperation within FBD. The limited availability of reference material and quality panels for these RG3 agents limits opportunities for external quality assurance exercises; this means that cooperation between BSL3 laboratories is crucial. Quality audits at each high-containment laboratory are also necessary in order to jointly develop and improve procedures and methods, both in terms of quality and biosafety.

The present project is in line with the CBRN Action Plan B12 which stipulates that member states should enhance and support: cooperation among laboratories assigned to deal with unknown pathogens and toxins at national level; establish and support networking among existing laboratories which are competent and have capacity across the EU specialising in high risk biological agents and toxins.

Effective quality management is built on the concept of continual improvement through a cycle of planning, implementing, reviewing and improving the processes and through actions that the organization undertakes to meet goals. This is known as the PDCA (Plan-Do-Check-Act) principle (Figure 1).

Identified deficiencies from the exercises and audits within this project can subsequently be suggested as improvements for the participating agencies.

Figure 1. Figure 1. The Plan-Do-Check-Act principle

3. AIM OF THIS PROJECT

The overall objective of this project was to assure the quality of the analytical process at the individual agencies in accordance with FBD routines and to assure the comparability of the analytical results from the respective laboratories.

Specific aims of this project were:

- To *in silico* validate/control existing FBD molecular techniques and new molecular methods potentially developed within another FBD project ("Improved methods and ability for laboratory biopreparedness"). Tools for the *in silico* validation was the SmiPrimer system from the Public Health Agency of Sweden (PHAS)
- To assess the quality of already established diagnostics as well as newly introduced methodologies at the respective participating agencies by means of External Quality Assessments (EQA).
- To improve the procedures at BSL3 laboratories from a biosafety and biosecurity perspective. This was to be achieved through a review of the analytical process of the risk group 3 bacteria against the FBD quality manual[3]. For example, a sample would be followed from arrival in the laboratory through to response to the results (vertical audit). The audit was based on the laboratory management systems CWA 15793[2] and ISO/IEC 17025:2005[1] concerning relevant parts of the project.

4. QUALITY CONTROL OF PRIMERS AND PROBES

4.1 ACTIVITIES WITHIN THE PROJECT

The *in silico* validation of primer- and probe sequences was initiated by making an inventory of the existing PCR systems at the different agencies. The inclusion criteria was PCR systems used for risk group 3 bacteria in routine diagnostics and within FBD (project report 15)[4]. Specificity and sensitivity for primers and probes were evaluated for the following:

PCR detection at the genus level for: B*acillus, Francisella, Yersinia, Brucella, Burkholderia* and *Coxiella*.

PCR detection at the species level for: *Bacillius anthracis, Francisella tularensis, Yersinia pestis, Brucella abortus, Burkholderia mallei* and *Burkholderia pseudomallei.*

PCR detection at the subspecies level for: *F. tularensis* ssp. tularensis (type A) and *F. tularensis* ssp. *holarctica* (type B).

A list of the PCR systems was forwarded to a bioinformatic expert at PHAS who evaluated these with the in-house SmiPrimer. The National Center for Biotechnology Information (NCBI) was used as the source of reference sequences unless stated otherwise.

4.2 RESULTS

The majority of the PCR systems showed good specificity and sensitivity for most bacterial targets. In the report, primers and probes were numbered along the amplicon direction (5') so that the reverse primer was numbered from the 3' end. Confidence values were included in the report. The validation revealed the following:

- The genus specific PCR for *Yersinia* would not detect *Yersinia enterocolitica*

- A specific PCR for *Brucella melitensis* was completely missing.

- The subspecies specific PCR for *Francisella tularensis* ssp. *tularensis* were not fully specific and would incorrectly also detect other *Francisella tularensis* subspecies.

Several PCR methods were predicted to have a very low melting temperature, around 50 °C, which can result in inefficient or no binding of primers and probes if a too high annealing temperature is used.

4.3 DISCUSSION

The *in silico* validation and quality control of primers and probes within the FBD predicted a good specificity and sensitivity for most of the PCR systems.

Some predictions from the SmiPrimer report did not correspond with the experience from the laboratory processing. For example, the genus PCR of *Bacillus* sp. should theoretically not detect *Bacillus cereus* according to the report, but in practice it did. In the same manner, the genus PCR for *Francisella* sp. should theoretically not detect *Francisella novicida*, but in practice it did. Few published references genomes available at the time of the *in silico* analysis may have contributed to this deviation. A further example of the lack of concordance between the experiences from the laboratory and the *in silico* analysis was that the latter predicted low melting temperatures that theoretically could have a negative effect on the PCR-systems. But, interestingly, no reduced sensitivity was noted by the laboratories using these PCR-systems, this in spite usage of higher annealing temperatures.

As a result of the above, development of two new PCR systems were initiated within the FBD ("Improved methods and capacity for laboratory biopreparedness"). One genus specific PCR for Yersinia genus including *Yersinia enterocolitica* and one specific PCR for *Brucella melitensis*.

5. EXTERNAL QUALITY ASSESSMENT 2014

5.1 ACTIVITIES WITHIN THE PROJECT

Planning

Planning was started in April 2014 and the external quality assessment (EQA) was distributed to all participants on the 11th of September. Upon arrival, each participant had three weeks to pre-form and report their results. Assembling and analysis of data from the EQA was done in October to November.

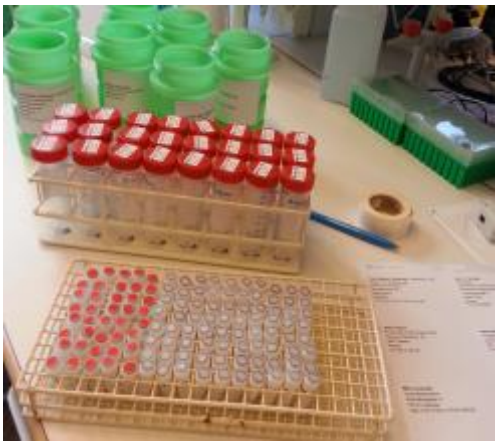
The EQA comprised four assessments:

- Correct and quick transport of the samples
- Correct analysis of 15 inactivated samples
- Turnaround time of analysis for five (highlighted in red) of the 15 samples (i.e. time dependent)
- Reporting of results and methodology, used within the specified time and predefined reporting format.

Besides the FBD agencies, the Protective Centre from Umeå and three laboratories in Norway – the Norwegian Defence Research Establishment (FFI), the Norwegian Institute of Public Health (FHI) and the Norwegian Veterinary Institute (NVI) – participated in the EQA, i.e., a total of 8 laboratories.

Preparation

The EQA consisted of 15 inactivated bacterial samples of risk groups 2 and 3 in three different matrices, i.e. milk, serum and water. The preparation of the samples was done at the PHAS (Figure 2). At the BSL3 laboratory at PHAS, all bacteria were cultured on agar plates. Species and subspecies were confirmed by MALDI-TOF and real-time PCR. After the isolates were inactivated by heating, sterility culture control was performed for each inactivated material before this was relocated from the BSL3 to the BSL2 laboratory. Subsequently different matrices were spiked with inactivated RG3 and RG2 bacteria according to a strict protocol. Before shipment, the concentration of the DNA was quantified by real-time PCR to correspond to a cycle (Cq) value between 20 and 25.



Figures 2. Preparation of the samples, sample tubes and the two transportation vials

Performance

Participating laboratories were informed approximately three months in advance about the anticipated number of samples and date for the EQA. Instructions for the EQA (Appendix 1) with individual laboratory codes, together with a form for reporting the result, were sent by e-mail to the contact person at each laboratory one week before the samples. The EQA instructions and the form for reporting the results were also sent with the samples. The recipients were instructed to use molecular methods for the identification of risk group 3 bacteria at species and subspecies levels. Each laboratory analysed the samples according to the local routines and therefore used different target sequences to some extent. Analysis of five (highlighted in red, Table 1) of the 15 samples was time-dependent, i.e., to be performed as fast as possible. For the remaining 10 samples, the participating laboratory had an additional three weeks for analysis. The correct answer of the EQA and the evaluation questionnaire were sent to the laboratories after the deadline for the analysis and all the participating laboratories had submitted their results.

For each sample the participants were to properly identity or to exclude the following bacteria:

- *Bacillus anthracis*
- *Francisella tularensis* ssp. *holarctica*
- *Burkholderia pseudomallei*
- *Brucella abortus*
- *Coxiella burnetii*
- *Yersinia pestis*
- *Francisella tularensis* ssp. *tularensis*
- *Burkholderia mallei*
- *Brucella melitensis*

Transportation

The samples were transported by courier (Special Delivery Service). They were picked up on 11 September 2014 and were to be delivered before the end of the next working day to a contact person at each laboratory. Samples were transported as category B infectious substances in a box containing cooling packs and according to the shipment agreement to be kept at 4-8 °C. After delivery, some samples were found to be of insufficient quality. Therefore, the delivery of the samples was stopped and the laboratories that already had received the samples were informed to discard the samples. Information of the cancellation was sent to all participating laboratories. Three days later, a second distribution was performed.

5.2 RESULTS

In total, eight laboratories participated in the EQA, five from Sweden and three from Norway.

Transport

The transport of the samples was acceptable but not perfect. The samples were delivered within 24 hours, as contracted, to five of the eight participating laboratories. Three laboratories received the samples after 48 hours. The packages arrived without damage at all participating laboratories.

Laboratory results

Seven of the eight participating laboratories reported their results. One laboratory was not able to report the final results within the given time frame and was therefore excluded from this report. One laboratory reported correct test results for all 15 samples (FBD-01-FBD-15) included in the EQA. The other six laboratories reported correct test results from 53 to 83 percent for all 15 samples. Samples FBD-01 - FBD-05 were in addition analyzed on time (Table 1).

Four different methods for the DNA extraction were used by the seven participating laboratories. Inhibition control was used by six of seven laboratories, while extraction control was only used by four.

The sample (FBD-09) rendering most incorrect answers (four out of seven) was containing *F. tularensis* ssp. *novicida* and it was predominantly incorrectly identified as *F. tularensis* ssp. *tularensis*. For sample FBD-12, *Brucella* spp., not *B. abortus*, was considered a correct answer because of the general lack of molecular methods to detect *B. melitensis*. In spite this, two laboratories did correctly identify *B. melitensis*. Three laboratories could not detect *Burkholderia pseudomallei* correctly and one laboratory had a contamination in the same sample (FBD-14).

Table 1. Results of the FBD external quality assessment 2014

			Laboratory code							
Sample no.	Agent	Sample type	RS	AC	KL	MS	RQ	AB	UF	Comments
FBD-01	*Bacillus anthracis* (pXO1 och pXO2 positive)	Tap water								
FBD-02	*Yersinia. pseudotubeculosis*	Milk[1]								Not risk group 3 bacterium. Incorrect answer with a comment is given to laboratories that reported the wrong bacteria.
FBD-03	*E. coli*	Distilled water								Not risk group 3 bacterium.
FBD-04	*Francisella tularensis* ssp *holarctica*	Distilled water								
FBD-05	*Brucella abortus*	Milk[1] (lactose free)								
FBD-06	*F. tularensis* ssp *tularensis*									
FBD-07	*Yersinai pestis*	Tap water								
FBD-08	*B. anthracis* (pXO2 negative)	Tap water								
FBD- 09	*F. tularensis* ssp *novicida*	serum								The laboratories with incorrect answers (red boxes) reported *F. tularensis* ssp. t*ularensis*. The laboratory with yellow box had answered *F. tularensis ssp. tularensis/ holartica.*
FBD-10	*Burkholderia mallei*	Milk[1] (lactose free)								
FBD-11	*Coxiella burnetii*	Milk (lactose free)								
FBD-12	*Brucella melitensis*									The laboratories that reported *Brucella* sp. and could not specify the species *B. melitensis* were given a green box (correct answer).
FBD-13	*Bacillus cereus*	Milk[1]								Not risk group 3 bacterium. The laboratories that did not specify *Bacillus* genes were given an incorrect answer with a comment (yellow box).
FBD-14	*Burkholderia pseudomallei*	Milk[1]								
FBD-15	H_2O	Distilled water								
		Correct (%)	13 (87)	15 (100)	11 (80)	8 (53)	13 (87)	13 (87)	12 (80)	
		Errors (%)	2 (13)	0	4 (20)	7 (47)	2 (13)	2 (13)	3 (20)	

A green box indicates a correct answer, a red box indicates an incorrect one and yellow boxes indicate incorrect answers with comments

[1] The sample had a natural contamination of C. burnetii from the milk and therefore did not result in an incorrect answer.

The time it took for each laboratory to analyse the five time-dependent samples ranged from 2 hours and 20 minutes to 9 hours and 25 minutes (Table 2).

Table 2. Time for analysis of the five time-dependent samples (FBD-01 – FBD-05)

Lab code	Time for analysis of sample FBD-01 – FBD-05
AC	2 h 20 min
AB	3 h 8 min
KL	3 h 30 min[1]
RQ	4 h 20 min
UF	6 h 24 min
RS	8 h
MS	9 h 25 min
NB	Did not complete the EQA

[1] Calculated time. The laboratory could not perform the analysis on time because of other, more urgent tasks.

5.3 DISCUSSION

The ability to analyse risk group 3 bacteria by molecular methods varied between the participating laboratories in the EQA 2014. Only one laboratory reported 100 percent correct results. The two most challenging analyses were to distinguish *F. tularensis* ssp. *tularensis* from *F. tularensis* ssp. novicida and to detect *Burkholderia pseudomallei* (Table 1). Additionally, several participating laboratories lacked a molecular method to detect *Brucella melitensis* at the time of the EQA 2014. As a consequence of the difficulties in detecting *B. melitensis*, FBD developed and implemented a species specific molecular method in 2016.

The time it took for each laboratory to analyse the time-dependent samples (FBD-01 – FBD-05) varied greatly. The two laboratories that reported the shortest turnaround time also reported 100 percent correct results for the five samples. In general, short turnaround time correlated well with high accuracy of the results i.e. the four laboratories that reported 100% correct results for the five time-dependent samples were all placed in the top 5 in shortest turnaround time. However, it is difficult to compare times between different laboratories because some laboratories performed their analyses in BSL 3 laboratories and others in a BSL 2.

The transportation of the samples during the EQA 2014 was acceptable but not perfect. The samples were delayed to two laboratories in Norway and there were some troubles with the communication between the shipper and the operator. The evaluation questionnaire for EQA 2014 revealed that some of the participating laboratories thought that the instructions for the EQA were somewhat unclear on the performance part, for example if it was a task to identify risk group 2 bacteria for species and subspecies or not.

In 2015 an EQA was distributed by the Norwegian BLS3 laboratories and all the FBD agencies participated. The Norwegian colleagues were supported with our experiences acquired from the EQA 2014, e.g. how to perform spiking with the DNA/inactivated bacterial suspension in different materials and how to assure the quality of the samples at different temperatures.

The results of the EQA 2014 were presented at the Medical Biodefence conference in Munich, Germany (Boskani et al, 2016) (Appendix 2)[5].

6. EXTERNAL QUALITY ASSESSMENT 2016

6.1 ACTIVITIES WITHIN THE PROJECT

Planning

Planning of the second external quality assessment (EQA) started in November 2015, the exercise was performed in September 2016, and the results were compiled in October. In the EQA there were seven participating laboratories – three in Norway (FFI, FHI and NVI) and all four Swedish FBD agencies.

The EQA comprised four assessments:

- Correct and quick transport of the samples
- Correct analysis of five samples containing viable bacteria
- Time-dependent reporting of preliminary results
- Reporting of results and methodology, used within the specified time and predefined reporting format.

Preparation

The exercise consisted of five samples containing viable risk group 3 and risk group 2 bacterial pathogens in solid agar tube. All samples were prepared at the PHAS, who also checked stability, i.e. that they were able to grow after 10 days' storage at 4-8 °C.

A list of bacteria was selected in order to test target bacteria and background flora. Each bacterial species was specifically cultured on appropriate agar plates, and incubated in appropriate environment (aerobic or CO2) and at appropriate time. Separate bacterial suspensions were made for each agents and diluted to turbidity 0.5 McFarland, from these different volumes were inoculated in tubes containing 1 ml soft agar according to a preset protocol. Agar tubes were incubated overnight to ensure the growth of bacteria and then stored at 4-8 °C. To test the stability, culturing, PCR and MALDI-TOF were performed on the first bacterial suspension after one day of incubation at 37 °C, and then after 2, 7 and 10 days' storage at 4-8 °C. The samples were cultured on blood agar, chocolate agar, and special agar for *F. tularensis* (Figure 3).

Figure 3. Preparation of the samples, different agar plates were used for different bacteria.

Performance

Approximately four months before the EQA, the participating laboratories were informed when the samples would be sent. One month later, a Material Transfer Agreement MTA (Appendix 3) was sent by e-mail for each laboratory to sign for the transfer of live material between PHAS and the participants. Instructions of the EQA (Appendix 4) with more detailed information were sent out via mail two weeks prior to the test; the e-mail also included a result form and an evaluation questionnaire.

Each set of samples was marked with individual laboratory codes. Every set of the five samples possibly containing viable risk group 3 bacteria in 1 ml solid agar tube was placed in 50 ml tubes with absorbent material. These tubes were then packed in a leak proof secondary packaging, according to the general requirements for packaging of category A infectious substances (Figure 4). The leak proof secondary packaging was then transferred from BSL3 laboratory to BSL2 and subsequently placed in rigid outer packaging. All packages were handed over to the operator (World Courier) for transport to the participating laboratories. A copy of the EQA instruction were also sent in the shipment of the samples. The samples were expected to be analysed using both molecular methods and cultivation to identify risk group 3 bacteria at species and subspecies levels. For each sample the participants were to properly identity or to exclude the following bacteria:

- *Bacillus anthracis*
- *Francisella tularensis* ssp. *holarctica*
- *Burkholderia pseudomallei*
- *Brucella abortus*
- *Yersinia pestis*
- *Francisella tularensis* ssp. *tularensis*
- *Burkholderia mallei*
- *Brucella melitensis*

(N.B. *Coxiella burnetii* was not included in this EQA)

Two of the samples contained background flora, i.e., a risk group 2 bacteria per sample. Risk group 2 bacteria was not compulsory to be characterized at species level, but background flora was expected to be reported from the processing of the samples. Each laboratory was free to analyse the samples according to their local routines. A preliminary result was to be sent in as quickly as possible and a final result before the set deadline one month after the shipment of the EQA.

Figures 4. Preparation for transport of the samples

6.2 RESULTS

Among the seven laboratories that participated in the EQA, six responded with a final result within the given time frame. One laboratory did not reported the final result due to problems in receiving reagents.

Transport

The samples were picked up at PHAS by World Courier on September 12 for delivery at 4-8 °C during office hour the following day, to a contact person at each laboratory. The transport took longer time (two days) than agreed to three of the laboratories. These three packages were delayed in the customs due to missing details in the "Shipper's declaration", caused by World Courier. At one of the Norwegian laboratories the package was delivered to the goods reception instead of the specific contact person, the package was picked up later by the contact person. All the packages and samples, including the delayed samples, were in good condition and within the accepted temperature interval when they arrived.

Results

The time it took for the laboratories to report a preliminary result varied from 2 hours and 40 minutes to 28 hours (Table 3). All the participating laboratories reported correct preliminary results as well as final results for the five samples that were included in the EQA (Table 4).

Table 3. Time for analysis of the all samples (FBD-01 – FBD-05)

Lab code	Time for analysis of sample FBD-01 – FBD-05
G	2 h 40 min
B	3 h
D	6 h 15 min
A	6 h 20 min
C	6 h 45 min
F	28 h
E	Did not complete the EQA

Table 4. Results of the FBD external quality assessment 2016

		Laboratory code							
Sample no.	Agent	A	B	C	D	E[1]	F	G	Comments
FBD-01	*Bacillus anthracis*								pXO1 pos, pXO2 neg
	Burkholderia pseudomallei								
FBD-02	*Francisella tularensis* ssp *holarctica*								
	Streptococcus pneumoniae								
FBD-03	*Brucella melitensis*								*Brucella* spp. gave correct answer
FBD-04	*Yersinia pestis*								
	Haemophilus influenzae								
FBD-05	*Water*								

[1] Did not complete the EQA
Light yellow box indicates that mixed cultures were not reported by the responder

Analyses

The participating laboratories had slightly different approaches in analysing the samples in the EQA. All performed direct PCR on the material for the preliminary result. The cultivation was performed on different agar plates but all came to the same results in the end. Some laboratories only used MALDI-TOF to identify cultivated bacteria, while others used both MALDI-TOF and PCR.

6.3 DISCUSSION

The 2016 EQA was the first ever prepared by FBD containing live, risk group 3 bacteria and the participating laboratories performed very well. All laboratories could detect and isolate correctly all the risk group 3 bacteria. The laboratory that did not complete the EQA reported that this was due to problems in receiving reagents. Two of the samples included background flora. Identification of background flora at species level was not included as a task in this EQA; however, it was expected that participating laboratories should mention the presence of mixed cultures. Some of the laboratories had difficulties in determining the presence of mixed cultures (Table 4).

The laboratories considered that the EQA was just enough difficult, probably because it was the first time that isolation of strains was a part of the EQA. The laboratories also considered that it was good to include background flora in the samples. The transport to certain laboratories was not optimal and resulted in delays of delivery to all laboratories in Norway. Moreover, one package was not delivered to the contact person. The operator will be contacted regarding these complaints to avoid similar events in the future.

In the evaluation of EQA 2014 some participating laboratories complained about unclear instructions. During the preparation of the EQA 2016, the working group therefore tried to be as clear as possible in the instructions. There were no comments about unclear instructions in the evaluation after EQA 2016.

During the spring of 2016, the European network EMERGE performed their first EQA, including five coded inactivated samples, in which both PHAS and FOI participated. An aliquot of the five inactivated samples was forwarded by PHAS to SVA, NFA and Protective Centre in Umeå for analysis. These results were presented at the FBD closing meeting in November 2016.

7. QUALITY AUDITS

7.1 ACTIVITIES WITHIN THE PROJECT

Preparation

Annual quality audits are usually not available for non-accredited methods used in BSL3 laboratories. For this reason, it was important to perform an audit program, including all agencies authorities within FBD.

Training of auditors
A course on how to perform quality audits according to ISO/IEC 17025:2005[1] was held in May 2015 at the PHAS. Participants were all the members of the project group, as well as one or two additional persons from each agency. The course was led by Gunn-Mari Löfdahl from SP Technical Research Institute of Sweden. The course comprised the steps in the audit process from planning and implementation to follow-up of the results.

Audit documentation
Two checklists were produced by the project group – one for biosafety audits (FBD 010, Appendix 5), and one for quality audits (FBD 011, Appendix 6).

Performance

During the autumn of 2015, the project group conducted quality audits at the three agencies with BSL3 laboratories – PHAS, FOI and SVA. The NFA also participated in the quality audit at the SVA since they perform their analyses in this laboratory. In addition, a biosafety audit based on relevant parts of the laboratory management system CWA 15793[2] was performed at FOI during the spring of 2015.

The quality audits were based on relevant parts (laboratory operations) of the FBD quality manual (FBD 001)[3] and the standard ISO/IEC 17025:2005[1] with requirements for an accredited laboratory. The two checklists produced by the project group for quality and biosafety audits were given to the participating laboratories in advance, together with information on what the auditors would focus on (for example, which bacterial agent). The laboratories were requested to send relevant information to the auditors beforehand. Each audit was performed by two auditors from two different agencies. Each laboratory was given three months to respond to the report. The suggested improvements from all audits was not compulsory to implement, instead they were agreed to be seen as recommendations for improvements.

7.2 RESULTS

All revised laboratories had implemented quality system, but the level varied in-between the participants. The laboratories had high competence for the analyses performed and had well-established procedures for working in BSL3 laboratories. Positive examples and suggestions for improvement from each audit were communicated to the respective agency. All laboratories responded within the three-month limit. A compilation of the findings from all four audits can be found below.

Positive findings:

- The overall impression was very positive. All laboratories show good order.
- Use of quality systems for document management.
- The quality system contained templates for methods, standard operation procedures, forms, etc. The quality system tracked how many copies were printed and these were printed in exclusively reserved colours.
- Training in the BSL3 laboratory environment was performed with a well-established mentor system.
- Detailed competence levels were available for work in BSL3 (A, AB, AC, D). Competence certificates are saved in the quality system and are electronically available.
- Availability of manual DNA-extraction methods as a redundant alternative to automated DNA-extraction. This is especially valuable if the automated extraction system (EZ1 Advanced) is malfunctional.
- Laboratory Information Management System (LIMS) may reduce the risks of errors and keeps all information regarding the samples collected in one and the same place.

Weaknesses to be addressed:

- The following observations or deviations were found in one or more of the participating laboratories:
- The quality system for document management was not traceable (versions) and had no archive function.
- Full descriptions of methods were missing, e.g., what to do if a sample is inhibited. Instructions for interpretation of the test results was missing. Reports of validation were missing.
- Traceability of reagents and substrates was missing.
- The Excel template used as protocol for real-time PCR was not included in the quality system.
- No personal alarm was in place when working in a BSL3 environment.
- Documents should be clarified, e.g., regarding storage of positive samples. Old information was present in some documents. The names of former staff members at the agency should be removed when updating documents. If needed, they can be mentioned as external reference persons.
- Instructions regarding competence, training and vaccination procedures were lacking.
- Competence certificates were available only in paper format. Electronic certificates provide better accessibility and overview of which persons can perform a particular method.
- Complete titles and report numbers of FBDs reports should be used when referring to FBD reports in SOP.
- Abbreviations regarding controls should be reviewed to avoid misunderstanding and explained in the document in which they are used e.g., IPC, IAC etc. Primers and probes in an assay should have the same target name.
- Safety equipment was in good order and everything was well planned, but the Fan-assisted breathing protection with helmet were not calibrated or checked routinely for proper function at one laboratory.
- Information regarding which bacterial agent, respectively activity, is currently being carried out in the laboratory was missing.
- There were uncertainties in the general safety instruction of BSL3 laboratory work regarding which disinfectant (ethanol, virkon, chlorine, etc.) should be used for the respective agents.

7.3 DISCUSSION

To achieve and maintain reliable and comparable results, it is essential that the quality of the diagnostic analysis chain is assured. This can be achieved, for example, by quality audits. Both quality and biosafety audits were performed in this project and the general impression from the audits was positive. The representation from the agencies was broad, the commitment of the participants was high, the laboratory order was good, and so was the knowledge of the analytical operations. Each agency had to reply to their respective audit report within a given time limit, which everyone did. However, the responsibility for the follow-up was handed over to the respective agency since FBD has no mandate to require any measures. Sharing the results for both quality and biosafety audits between laboratories is a good way to learn from each other and therefore recommended on a regular basis.

The advantage of an external over an internal audit is the opportunity it provides to identify positive aspects and suggestions for improvement where deficiencies are observed. These measures help revise and thereby improve laboratory activities.

8. CONCLUSIONS

Within a network of laboratories, it is important with quality assurance especially for highly pathogenic bacteria since there is seldom commercial proficiency tests available. Also, many high-consequence pathogens are rarely, or have never been, encountered in many laboratories. In this project, Quality Assurance Exercises (EQA) were used, Inter-agency audits were performed at the BLS-3-laboratories at the agencies and primers and probes for PCR systems were validated *in silico*.

Regular EQAs are a good way to quality-assure diagnostics at the laboratories. Two different EQAs, one in 2014 (inactivated bacteria) and one in 2016 (live bacteria) were prepared and distributed to the participating agencies. The laboratories participating in the second EQA reported more correct results than in the first EQA, indicating that the ability to detect risk group 3 bacteria had been improved. It should be noted that the two EQAs were not fully comparable as they were different in their designs. For instance, different bacterial species were included respectively and cultivation methods were included which led to supplementary analyses and confirmation of the molecular results.

The EQA evaluation questionnaires revealed that it is an advantage to be at least two laboratory personnel who perform the EQA, so that e.g. methods and results can be discussed. An important experience was that the planning of an EQA, preparation of samples, compiling and reporting of results were very time-consuming. Both EQAs showed weaknesses regarding sample transport, underlining the challenges in choosing an appropriate carrier. Most difficulties occurred with the transport from Sweden to Norway, but as all obstacles were identified, the exercises have given the experiences to circumvent these in future shipments.

The *in silico* validation and quality control of primers and probes predicted a good specificity and sensitivity for most of the used PCR systems. The major need for improvements that were identified was redesign of the genus specific Yersinia PCR and a new specific PCR for *Brucella melitensis*. As a consequence, these two PCR systems were designed cooperatively within the network during 2015 and 2016. At the time of EQA 2016, in spite some but not all of the FBD laboratories had implemented this PCR, this work likely contributed to increase the correct answers from 86% to 100% in samples containing *Brucella melitensis*.

Inter-agency audits by BSL3 staff members were performed on quality assurance of diagnostic methods and biosafety at the BSL-3 laboratories, using ISO 17025[1] and relevant parts of the CWA 15793[2]. The general impression from the audits was positive. These audits led to improved practical solutions and facilitated the harmonisation of good practice between the laboratories. The audits were not only appreciated by the visited laboratory, but also gave the possibility for the auditors to bring back good examples to improve routines in their own laboratory.

In conclusion, quality assurance of the analytical process and to assure the comparability of the analytical results from the respective BSL3 laboratories is of great importance for detecting weaknesses in the analysis process and an important way to improve the national capacity.

8.1 SYNERGY EFFECTS WITH OTHER PROJECTS/AGENCIES

Cooperation with the FBD project 2016/16 ("Nordic Biopreparedness Forum") in order to establish contact with the Norwegian laboratories.

Cooperation with the FBD project 2016/18 ("Improved methods and ability for laboratory biopreparedness") in order to develop new PCR which was missing or unspecific during the quality control by SmiPrimer.

9. SUGGESTIONS FOR FURTHER STUDIES

Future studies should consider EQAs in various matrices with both clinical materials and environmental samples similar to mimic real samples. More advanced studies should be considered for assessments, for example, when there is a low concentration of target agents in the samples.

Furthermore, regular quality audits in BSL3 laboratories and control of PCR systems performed on a regular basis would be of great value.

The FBD quality manual[3] should be updated periodically.

10. APPENDICES

Appendices are not published in this report but are available (in Swedish) for download at https://www.msb.se/

Appendix 1: EQA instructions, FBD September 2014

Appendix 2: Poster at the Medical Biodefence Conference, Munich 2016

Appendix 3: Material Transfer Agreement (MTA)

Appendix 4: EQA instructions, FBD September 2016

Appendix 5: Checklist biosafety audit

Appendix 6: Checklist quality audit

11. REFERENCES

1. ISO/IEC 17025:2005; Standard of the International Organization for Standardization describing general requirements for the competence of testing and calibration laboratories
2. CWA15793:2011, Laboratory biorisk management; CEN Workshop Agreement, a reference document describing laboratory biorisk management; Ref. No. CWA 15793:2011 D/E/F
3. FBD 001 Quality manual (Kvalitetsmanualen)
4. FBD-project 2014/15 (Quality assurance of the real-time PCR and laboratory safety for the analysis of risk group 3 bacteria in FBD)
5. T Boskani, M Granberg, S Frosth, S Bereczky1 and C Flink. Quality Assurance of BSL3 laboratory capacity in Sweden. Poster no. PG08 presented at: 15th Medical Biodefense Conference, April 2016, Munich, Germany

Appendix 1:

EQA instructions, FBD September 2014

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

SmiPrimer:
FBD bakt. qPCR

Folkhälsomyndigheten 2014

2014-03-03

Detta dokument innehåller en validering av primer- och probsekvenser utförd in-silico. Där inget annat anges har NCBI använts som källa för referenssekvenser. Notera att numreringen i primrarna och proberna sker längs amplikonets riktning, så att reverse-primern blir numrerad från 3'-änden. Vid utlåtandet för varje primer/probe finns en färgmarkering där grön betyder låg risk för problem med systemet, gul måttlig risk och röd hög risk. Det finns även en färgmarkering för konfidens som beskriver hur tillförlitliga resultaten är. Vid frågor, kontakta erik.alm@folkhalsomyndigheten.se.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Sammanfattning av egenskaper (beräknade in-silico)

System	Kemiska egenskaper	Specificitet/Känslighet
Brucella genus	50 grader	Ok
Brucella abortus	60 grader	Ok
Brucella abortus, BruAb2_0168	50 grader	Ok
Francisella genus	50 grader	Tar troligen inte dessa arter: Noatunensis, Novicidia, Strain tx077308. Mediasiatica kan vara ett gränsfall.
F. tularensis	50 grader	Matchar även Novicidia.
F. tularensis typB	50 grader. Mkt låg Tm på proben (52 grader).	Oklart vad systemet ska detektera, det matchar F. tul. hol.
F. tul. tul.	50 grader, men proberna är 70-graders MGB. Problematiskt system där man nog enklast borde designa om primrarna till 60 grader och ha båda proberna med MGB.	Ok. Diskriminerar bara med en bas i proben så denna assay ska köras som en allelic discrimination assay.
F. tul. hol.	60 grader, proberna ska vara MGB.	Diskriminerar med en bas i båda proberna, ska köras som allelic discrimination assay, men osäkert om det fungerar eftersom proberna inte ligger på samma plats.
F. tul. Derived/Ancester	50 grader på primrarna, 70 grader på proberna. Samma problem som F. tul. tul.	Oklart vad systemet ska göra så inget utlåtande kan lämnas.
Coxiella	50 grader	En shotgunsekvens har 3 mismatches i reverse-primern. Annars inga problem.
Yersinia genus	60 grader	Ok
Y. pestis, YPO1091	50 grader	Ok

System	Kemiska egenskaper	Specificitet/Känslighet
Y. pestis, pla	50 grader	Ok
Y.pestis, caf1	50 grader	En avvikelse precis vid 3’ i reverse-primern för en strain, i övrigt ok.
Burkholderia genus	50 grader	Detekterar inte följande arter: *B. cepacia, cenocepacia, glumae* och strain rpe64
B. mallei	60 grader	Ok
B. pseudomallei	60 grader	Ok
Bacillus genus	50 grader	Systemet tar inte *B. megaterium* och *cereus*. Annars ok.
B. anthracis, chrom	50 grader	Ok
B. anthracis, pagA	50 grader	Ok
B. anthracis, capD	50 grader	Fyra avvikelser i reverse-primern i två olika shotgunsekvenser. Borde inte vara något större problem.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Brucella genus, IS711	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-02-27 15:44
Primer file: Brucella.fasta
Database file: talar/Brucella/234.fasta, 18 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C

Fwd primer●	Inga avvikelser. Tm=49.9. OBS Tm!
Rev primer ●	Inga avvikelser. Tm=46.8. OBS Tm!
Probe ●	Inga avvikelser. Tm=59.8 utan modifierare i 3'. OBS Tm!
Konfidens ●	18 sekvenser, ganska låg tillförlitlighet, men det finns ingen antydan till att det skulle finnas några mutationer i detta område. OBS! Denna assay ska köras på 50 grader.

Primersystem	Brucella abortus, BruAb	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-02-27 16:02
Primer file: Babortus_bruab.fasta
Database file: talar/Babortus/235.fasta, 4 sequences.

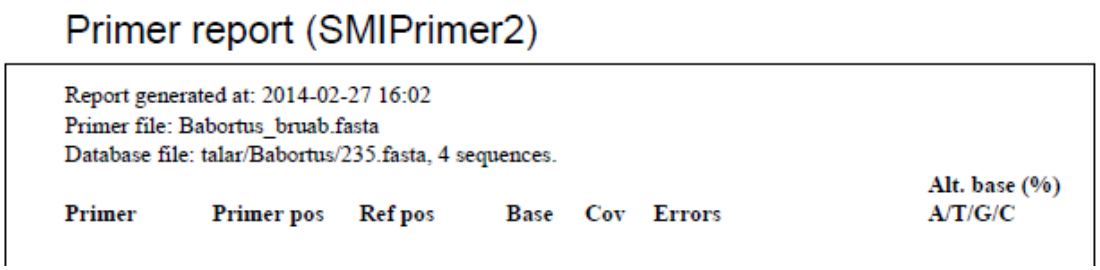
Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C

Fwd primer●	Inga avvikelser. Tm=59.6
Rev primer ●	Inga avvikelser. Tm=59.7
Probe ●	Inga avvikelser. Tm=69.8 utan modifierare i 3'.

Konfidens ●	4 sekvenser, låg tillförlitlighet, men det finns ingen antydan till att det skulle finnas några mutationer i detta område.

Primersystem	Brucella abortus, BruAb2_0168	Källa	FBD

Översikt



Primer report (SMIPrimer2)

Report generated at: 2014-02-27 16:02
Primer file: Babortus_bruab.fasta
Database file: talar/Babortus/235.fasta, 4 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C

Fwd primer●	Inga avvikelser. Tm=47.2. OBS Tm!
Rev primer ●	Inga avvikelser. Tm=46.3. OBS Tm!
Probe ●	Inga avvikelser. Tm=57.6 utan modifierare i 3'. OBS Tm!
Konfidens ●	4 sekvenser, låg tillförlitlighet, men det finns ingen antydan till att det skulle finnas några mutationer i detta område. OBS Tm! 50 graders PCR.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Francisella genus	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-02-28 14:27
Primer file: Francisella.fasta
Database file: talar/Francisella/F.fasta, 38 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C
F	1	1661796	C	38	7.89%	0/7.9/0/0
F	2	1661797	C	38	13.16%	10.5/2.6/0/0
F	6	1661801	A	38	10.53%	0/10.5/0/0
F	11	1661806	C	38	13.16%	0/0/13.2/0
F	16	1661811	A	38	2.63%	0/2.6/0/0
F	17	1661812	G	38	10.53%	10.5/0/0/0
F	19	1661814	C	38	5.26%	0/5.3/0/0
R	1	1722973	C	35	5.71%	0/0/5.7/0
R	2	1722974	T	35	11.43%	0/0/0/11.4
R	4	1722976	G	35	5.71%	0/5.7/0/0
R	5	1722977	G	35	11.43%	11.4/0/0/0
R	6	1722978	T	35	5.71%	0/0/5.7/0
R	15	1722987	T	35	5.71%	5.7/0/0/0
R	17	1722989	A	35	5.71%	0/0/0/5.7
R	20	1722992	A	35	5.71%	5.7/0/0/0
R	22	1722994	A	35	5.71%	0/0/0/5.7
R	24	1722996	C	35	5.71%	5.7/0/0/0
P	1	1661820	T	33	6.06%	0/0/0/6.1
P	4	1661823	T	33	6.06%	0/0/0/6.1
P	14	1661833	G	33	6.06%	6.1/0/0/0
P	22	1661841	A	33	6.06%	0/0/6.1/0

Fwd primer ●	Noatunensis har 4 avvikelser (pos 2, 6, 11, 17). Novicidia har 4 avvikelser (pos 1, 2, 11, 16). tx077308 har 2 avvikelser (pos 1, 19). Tm=45.6. OBS Tm!
Rev primer ●	Noatunensis har 5 avvikelser. Mediasiatica har 4 avvikelser (pos 1, 2, 4, 6). Novicidia har 4 avvikelser (pos 2, 20, 22, 24). Philomiragia har 1 mismatch (pos 5). Tm=48.6. OBS Tm!
Probe ●	Noatunensis och Novicidia har 6 avvikelser. tx077308 har 4 avvikelser (pos 1, 4, 14, 22). Systemet kommer inte detektera de ovan nämnda

	arterna och troligen inte Mediasiatica. Tm=58.9 utan modifierare i 3'. OBS Tm!
Konfidens ●	Baserat på 38 genom som ger en samstämmig bild, ganska god tillförlitlighet. OBS Tm! 50 graders PCR.

Primersystem	Francisella tularensis	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-02-28 14:05
Primer file: Ftularensis.fasta
Database file: talar2/Ftul/F.fasta, 38 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C

Fwd primer ●	Inga avvikelser. Tm=48.3. OBS Tm!
Rev primer ●	Inga avvikelser. Tm=51.7. OBS Tm!
Probe ●	Inga avvikelser. Systemet har också perfekt match på *F. novicidia*, så den kan ge falskt positivt utslag. Tm=59.3 utan modifierare i 3'. OBS Tm!
Konfidens ●	OBS Tm! 50 graders PCR.

Primersystem	Francisella tularensis typB	Källa	FBD

Översikt

Fwd primer	Oklart vad systemet ska targeta, proben har perfekt match på F. tul. hol. Tm=45.5. OBS Tm!
Rev primer	Inga avvikelser. Tm=49.3. OBS Tm!
Probe ●	Inga avvikelser. Tm=52.1 utan modifierare i 3'. OBS Tm! Låg Tm. 73 grader med MGB.
Konfidens ●	OBS Tm! 50 graders PCR.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Francisella tularensis subsp. tularensis	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-02-28 11:06
Primer file: Ftularensis_tularensis.fasta
Database file: talar3/Ftultul/Ftultul.fasta, 9 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C
P_VIC	13	1199457	C	9	100.00%	100.0/0/0/0

Fwd primer●	Inga avvikelser. Tm=42.5. OBS Tm!
Rev primer ●	Inga avvikelser. Tm=44.8. OBS Tm!
Probe 1 (FAM-BHQ) ●	Tm=65.0 med MGB. OBS! Probe-Tm matchar inte primrarna. Om detta är allelic discrimination bör båda proberna ha samma 3'-modifierare. Proben har inga avvikelser och diskriminerar med en SNP på pos 13. Om denna inte körs som allelic discrimination assay är den troligen inte specifik för F. tul. tul.
Probe 2 (VIC-MGB) ●	Tm=66.0 med MGB. OBS! Probe-Tm matchar inte primrarna. Om detta är allelic discrimination bör båda proberna ha samma 3'-modifierare.
Konfidens ●	13 sekvenser som alla ger en entydig bild.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Francisella tularensis, supsp. holarctica	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-02-28 12:44
Primer file: Ftularensis_hol.fasta
Database file: talar4/Ftul_hol/Ftulhol.fasta, 11 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C
P_VIC	15	1047989	T	11	100.00%	0/0/0/100.0

Fwd primer●	Inga avvikelser. Tm=58.2
Rev primer ●	Inga avvikelser. Tm=55.9
Probe 1 (FAM) ●	Inga avvikelser. Tm=67.0 med MGB. OBS! Denna prob ska vara MGB eller BHQplus, inte BHQ1.
Probe 2 (VIC) ●	Inga avvikelser. Tm=64.0 med MGB. OBS! Denna prob ska vara MGB eller BHQplus. Denna probe diskriminerar med 1 bas i pos 15. Proberna ligger inte på samma site och har inte jämförbar bindningsstyrka, så detta multiplex måste man kalibrera in väldigt noggrant för att man ska kunna dra rätt slutsats av resultaten.
Konfidens ●	11 sekvenser som alla ger en entydig bild.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Francisella tularensis, multiplex ancester/derived	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-02-28 17:43
Primer file: Ftul2.fasta
Database file: talar5/Ftul_multiplex/F.fasta, 38 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C
F	2	1928059	A	38	13.16%	0/0/13.2/0
R	15	1388176	T	38	5.26%	5.3/0/0/0
R	16	1388177	C	38	5.26%	0/0/5.3/0
R	17	1388178	T	38	5.26%	5.3/0/0/0
R	18	1388179	T	38	5.26%	5.3/0/0/0
P_FAM	5	1928106	T	38	18.42%	13.2/5.3/0/0
P_FAM	8	1928109	A	38	13.16%	0/0/13.2/0
P_VIC	5	1928105	A	38	47.37%	0/47.4/0/0
P_VIC	8	1928108	G	38	47.37%	47.4/0/0/0
P_VIC	11	1928111	G	38	5.26%	5.3/0/0/0
P_VIC	16	1928116	G	38	39.47%	0/39.5/0/0
P_VIC	17	1928117	A	38	34.21%	0/0/0/34.2

Fwd primer●	En avvikelse långt ifrån 3', borde inte ge problem. Tm=50.0. OBS! Tm
Rev primer ●	Francisella tx077308 har 4 avvikelser, men systemet ska troligen inte ta denna. Tm=49.5. OBS! Tm
Probe 1 (FAM) ●	Det är lite oklart vad proberna ska åstadkomma, denna prob verkar ta det mesta förutom *F. noatunensis orientalis* och tx077308. Tm=69.0 med MGB. OBS! Denna prob ska vara MGB eller BHQplus, inte BHQ1. OBS! Probe Tm matchar inte primrarnas Tm. Vid allelic discrimination måste man ha samma 3'modifierare på båda proberna.
Probe 2 (VIC) ●	Igen lite oklart vad systemet ska göra, denna prob tar de varianter som fam-proben inte tar, *F. noatunensis orientalis* och tx077308. Tm=71.0 med MGB. OBS! Denna prob ska vara MGB eller BHQplus. OBS! Probe Tm matchar inte primrarnas Tm. Vid allelic discrimination måste man ha samma 3'modifierare på båda proberna.
Konfidens ●	38 sekvenser Francisella. OBS! 50 graders PCR. Fel Tm på proberna.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Coxiella	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-02-28 13:05
Primer file: Coxiella.fasta
Database file: talar6/coxiella/cox.fasta, 12 sequences.

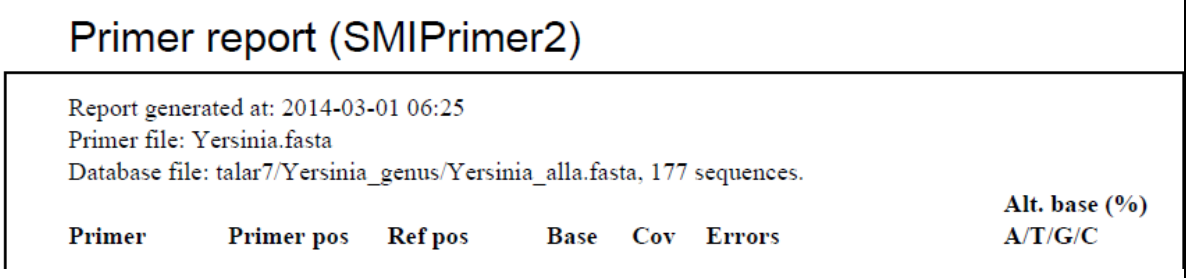
Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C
R	2	466724	A	12	8.33%	0/0/8.3/0
R	10	466732	C	12	8.33%	0/8.3/0/0
R	13	466735	T	12	8.33%	0/0/0/8.3

Fwd primer●	Inga avvikelser. Tm=45.7. OBS Tm!
Rev primer ●	En sekvens av 12 har tre avvikelser. Detta är en shotgunsekvens (gi|533736734, coxiella burnetii z3055) och är därmed lite mindre tillförlitlig än övriga. Om denna variant dyker upp kan den dock påverka systemet negativt då den har mismatch nära 3'. Tm=47.0. OBS Tm!
Probe ●	Inga avvikelser. Tm=58.2 utan modifierare i 3'. OBS Tm!
Konfidens ●	OBS Tm! 50 graders PCR.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Yersinia genus	Källa	FBD

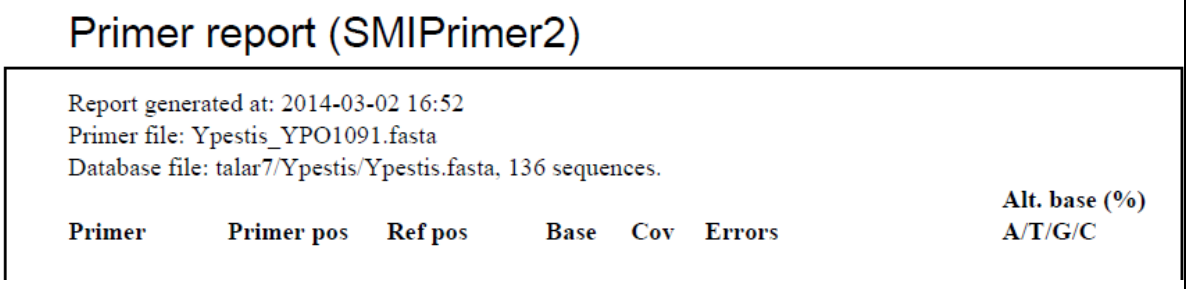
Översikt



Fwd primer●	Detta system tar alla *pestis, pseudoturberculosis* och *microti* helt utan avvikelser men tar inte *enterocolitica*. Tm=58.9
Rev primer ●	Se fwd. Tm=58.3
Probe ●	Se fwd. Tm=69.1 utan modifierare i 3'.
Konfidens ●	Datan är baserad på 34 genomsekvenser. Anledningen till att det står 177 i rapporten är på grund av att några shotgungenom inkluderades.

Primersystem	Yersinia pestis YPO1091	Källa	FBD

Översikt



Fwd primer●	Inga avvikelser. Tm=46.6. OBS Tm!
Rev primer ●	Inga avvikelser. Tm=47.6. OBS Tm!
Probe ●	Inga avvikelser. Tm=57.7 utan modifierare i 3'. OBS Tm!
Konfidens ●	26 sekvenser, ser ut som 177 på grund av shotgungenom. OBS! 50 graders PCR.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Yersinia pestis, pla	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-03-03 09:50
Primer file: Ypestis_pla.fasta
Database file: talar10/Ypestis_pla/Ypestis_pla_sekv.fasta, 27 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C

Fwd primer ●	Inga avvikelser. Tm=46.7. OBS Tm!
Rev primer ●	Inga avvikelser. Tm=47.0. OBS Tm!
Probe ●	Inga avvikelser. Tm=61.7 utan modifierare i 3'. OBS Tm!
Konfidens ●	27 sekvenser. OBS! 50 graders PCR.

Primersystem	Yersinia pestis, caf1	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-03-03 09:44
Primer file: Ypestis_caf1.fasta
Database file: talar10/Ypestis_caf1/Ypestis_caf1_sekv.fasta, 13 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C
R	1	4800	G	11	9.09%	0/9.1/0/0
R	19	4818	G	11	9.09%	9.1/0/0/0

Fwd primer ●	Inga avvikelser. Tm=42.8. OBS Tm! Lågt Tm
Rev primer ●	En avvikelse i sista basen vid 3' i strain 1392g vilket eventuellt kan ge problem. Tm=46.9. OBS Tm!
Probe ●	Inga avvikelser. Tm=57.4 utan modifierare i 3'. OBS Tm!
Konfidens ●	13 sekvenser av plasmiden, ok konfidens. OBS! 50 graders PCR.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Burkholderia genus, fliC	Källa	FBD

Översikt

All isolates (SMIPrimer2)

F hits:

gi|528314160|dbj|ab775209.1| burkholderia glumae flic gene for flagellin, complete cds, strain: pg-10, 2 mismatches (pos 8, 9)
gi|546197632|dbj|ab810225.1| burkholderia sp. rpe64 flic gene for flagellin, complete cds, 4 mismatches (pos 3, 4, 8, 10)
gi|482613000|gb|kc763156.1| burkholderia cenocepacia strain k56-2 flagellin (flic) gene, complete cds, 2 mismatches (pos 8, 9)
gi|1628639|gb|u73848.1|bpu73848 burkholderia pseudomallei ribosomal protein s21 (rpsu) and flagellin (flic) genes, complete cds, 0 mismatches
gi|1773064|gb|u82287.1|bpu82287 burkholderia pseudomallei flagellin (flic) gene, complete cds, 0 mismatches
gi|1773062|gb|u82286.1|bpu82286 burkholderia pseudomallei flagellin (flic) gene, complete cds, 0 mismatches
gi|5070225|gb|af084815.1| burkholderia mallei strain atcc23344 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, complete cds, 0 mismatches
gi|5070222|gb|af084814.1| burkholderia mallei strain atcc15310 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, complete cds, 0 mismatches
gi|3834672|gb|af098793.1| burkholderia mallei flagellin (flic) gene, complete cds, 0 mismatches
gi|3550356|gb|af084813.1| burkholderia pseudomallei strain atcc23343 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, comple..., 0 mismatches
gi|3550352|gb|af084812.1| burkholderia pseudomallei strain atcc15682 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, comple..., 0 mismatches
gi|3420942|gb|af081500.1| burkholderia thailandensis flagellin (flic) gene, complete cds, 0 mismatches
gi|2935156|gb|af011371.1| burkholderia cepacia e242 type i flagellin (flic) gene, complete cds, 0 mismatches
gi|2935154|gb|af011370.1| burkholderia cepacia e243 type ii flagellin (flic) gene, complete cds, 4 mismatches (pos 1, 7, 8, 9)

F misses:

R hits:

gi|528314160|dbj|ab775209.1| burkholderia glumae flic gene for flagellin, complete cds, strain: pg-10, 2 mismatches (pos 8, 10)
gi|546197632|dbj|ab810225.1| burkholderia sp. rpe64 flic gene for flagellin, complete cds, 4 mismatches (pos 3, 4, 8, 10)
gi|482613000|gb|kc763156.1| burkholderia cenocepacia strain k56-2 flagellin (flic) gene, complete cds, 2 mismatches (pos 3, 10)
gi|1628639|gb|u73848.1|bpu73848 burkholderia pseudomallei ribosomal protein s21 (rpsu) and flagellin (flic) genes, complete cds, 0 mismatches
gi|1773064|gb|u82287.1|bpu82287 burkholderia pseudomallei flagellin (flic) gene, complete cds, 0 mismatches
gi|1773062|gb|u82286.1|bpu82286 burkholderia pseudomallei flagellin (flic) gene, complete cds, 0 mismatches
gi|5070225|gb|af084815.1| burkholderia mallei strain atcc23344 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, complete cds, 0 mismatches
gi|5070222|gb|af084814.1| burkholderia mallei strain atcc15310 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, complete cds, 0 mismatches
gi|3834672|gb|af098793.1| burkholderia mallei flagellin (flic) gene, complete cds, 0 mismatches
gi|3550356|gb|af084813.1| burkholderia pseudomallei strain atcc23343 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, comple..., 0 mismatches
gi|3550352|gb|af084812.1| burkholderia pseudomallei strain atcc15682 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, comple..., 0 mismatches
gi|3420942|gb|af081500.1| burkholderia thailandensis flagellin (flic) gene, complete cds, 0 mismatches
gi|2935156|gb|af011371.1| burkholderia cepacia e242 type i flagellin (flic) gene, complete cds, 2 mismatches (pos 8, 10)
gi|2935154|gb|af011370.1| burkholderia cepacia e243 type ii flagellin (flic) gene, complete cds, 1 mismatches (pos 3)

R misses:

P hits:

gi|528314160|dbj|ab775209.1| burkholderia glumae flic gene for flagellin, complete cds, strain: pg-10, 4 mismatches (pos 1, 4, 13, 21)
gi|482613000|gb|kc763156.1| burkholderia cenocepacia strain k56-2 flagellin (flic) gene, complete cds, 4 mismatches (pos 7, 13, 18, 19)
gi|1628639|gb|u73848.1|bpu73848 burkholderia pseudomallei ribosomal protein s21 (rpsu) and flagellin (flic) genes, complete cds, 0 mismatches
gi|1773064|gb|u82287.1|bpu82287 burkholderia pseudomallei flagellin (flic) gene, complete cds, 0 mismatches
gi|1773062|gb|u82286.1|bpu82286 burkholderia pseudomallei flagellin (flic) gene, complete cds, 0 mismatches
gi|5070225|gb|af084815.1| burkholderia mallei strain atcc23344 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, complete cds, 0 mismatches
gi|5070222|gb|af084814.1| burkholderia mallei strain atcc15310 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, complete cds, 0 mismatches
gi|3834672|gb|af098793.1| burkholderia mallei flagellin (flic) gene, complete cds, 0 mismatches
gi|3550356|gb|af084813.1| burkholderia pseudomallei strain atcc23343 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, comple..., 0 mismatches
gi|3550352|gb|af084812.1| burkholderia pseudomallei strain atcc15682 ribosomal protein s21 (rpsu) gene, partial cds; and flagellin (flic) gene, comple..., 0 mismatches
gi|3420942|gb|af081500.1| burkholderia thailandensis flagellin (flic) gene, complete cds, 0 mismatches
gi|2935156|gb|af011371.1| burkholderia cepacia e242 type i flagellin (flic) gene, complete cds, 0 mismatches

Fwd primer ●	Mismatches mot *B. cepacia, cenocepacia, glumae* och *rpe64*. Dessa arter detekteras inte. Tm=45.2. OBS Tm!
Rev primer ●	Se fwd. Tm=48.7. OBS Tm!
Probe ●	Se fwd. Tm=58.3 utan modifierare i 3'. OBS Tm!
Konfidens ●	OBS! 50 graders PCR.

Primersystem	Burkholderia mallei	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-02-27 15:29
Primer file: Bmallei.fasta
Database file: talar/Bmallei/13373.fasta, 10 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C

Fwd primer●	Inga avvikelser. Tm=58.9
Rev primer ●	Inga avvikelser. Tm=58.0
Probe ●	Inga avvikelser. Tm=67.0 utan modifierare i 3'
Konfidens ●	10 sekvenser, ganska låg tillförlitlighet, men det finns ingen antydan till att det skulle finnas några mutationer i detta område.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	B. pseudomallei, mrpA	Källa	FBD
Översikt			
Fwd primer ●	Inga avvikelser. Tm=56.5		
Rev primer ●	Inga avvikelser. Tm=56.2		
Probe ●	Inga avvikelser. Tm=67.1 utan modifierare i 3'.		
Konfidens ●	13 genom. Intressant att binding site ligger på chromosom 1 för strain MSHR305 men på chromosome 2 för alla andra strains.		

Primersystem	Bacillus genus, rpoB	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-03-01 03:24
Primer file: Bacillus_rpob.fasta
Database file: talar9/bacillus/bacillus_genom.fasta, 51 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C
F	1	5151151	A	51	5.88%	0/0/5.9/0
F	3	5151153	C	51	5.88%	5.9/0/0/0
F	8	5151158	C	51	5.88%	5.9/0/0/0
F	15	5151165	G	51	3.92%	3.9/0/0/0
F	16	5151166	T	51	3.92%	0/0/3.9/0
F	17	5151167	G	51	3.92%	0/3.9/0/0
R	4	5151216	T	51	9.80%	0/5.9/0/3.9
R	14	5151226	A	51	5.88%	5.9/0/0/0
P	1	5151172	C	51	5.88%	0/5.9/0/0
P	3	5151174	T	51	5.88%	5.9/0/0/0
P	9	5151180	G	51	5.88%	5.9/0/0/0
P	15	5151186	G	51	5.88%	5.9/0/0/0

Fwd primer ●	Systemet tar inte *B. megaterium* och *B. cereus*, i övrigt inga avvikelser. Tm=42.6. OBS Tm! Lågt Tm
Rev primer ●	Se fwd. Tm=46.4. OBS Tm!
Probe ●	Se fwd. Tm=62.8 utan modifierare i 3'. OBS Tm!
Konfidens ●	51 genom. OBS! 50 graders PCR.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Bacillus anthracis, chrom (5345)	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-03-01 08:09
Primer file: Banthracis_chrom.fasta
Database file: talar9/Banthracis/Banthracis_genome.fasta, 24 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C

Fwd primer ●	Inga avvikelser. Tm=48.2. OBS Tm!
Rev primer ●	Inga avvikelser. Tm=45.1. OBS Tm! Lågt Tm.
Probe ●	Inga avvikelser. Tm=56.6 utan modifierare i 3'. OBS Tm!
Konfidens ●	5 genom, det ser ut som fler pga. shotgungenom. OBS! 50 graders PCR.

Primersystem	Bacillus anthracis, pXO1 pagA	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-03-03 10:13
Primer file: Banthracis_paga.fasta
Database file: talar12/Banthracis_pxo1/Banthracis_pxo1.fasta, 89 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C

Fwd primer ●	Inga avvikelser. Tm=46.1. OBS Tm!
Rev primer ●	Inga avvikelser. Tm=49.1. OBS Tm!
Probe ●	Inga avvikelser. Tm=61.9 utan modifierare i 3'. OBS Tm!
Konfidens ●	42 sekvenser, ser fler ut pga. shotgungenom. OBS! 50 graders PCR.

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Primersystem	Bacillus anthracis, pXO2 capD	Källa	FBD

Översikt

Primer report (SMIPrimer2)

Report generated at: 2014-03-03 10:14
Primer file: Banthracis_capd.fasta
Database file: talar12/Banthracis_pxo2/Banthracis_pxo2.fasta, 64 sequences.

Primer	Primer pos	Ref pos	Base	Cov	Errors	Alt. base (%) A/T/G/C
R	5	85854	A	41	4.88%	4.9/0/0/0
R	8	85857	A	41	4.88%	0/0/0/4.9
R	10	85859	T	41	4.88%	0/4.9/0/0
R	17	85866	C	41	4.88%	0/0/4.9/0

Fwd primer ●	Inga avvikelser. Tm=43.4. OBS Tm! Lågt Tm.
Rev primer ●	4 avvikelser i reverse-primern i två shotgunsekvenser. Inte så hög konfidens på dessa men de har samma 4 avvikelser vilket stärker risken att de är verkliga. Tm=46.8. OBS Tm!
Probe ●	Inga avvikelser. Tm=59.7 utan modifierare i 3'. OBS Tm!
Konfidens ●	OBS! 50 graders PCR.

Appendix 2:

Poster at the Medical Biodefence Conference, Munich 2016

Quality assurance of BSL3 laboratory capacity in Sweden

Talar Boskani[1], Malin Granberg[2], Sara Frosth[3], Sándor Bereczky[1], Catarina Flink[4]
[1]Public Health Agency of Sweden, Tomtebodavägen 12B, SE-17182 Solna, Sweden. [2]Swedish Defence Research Agency, Cementvägen 20, SE-16490 Umeå, Sweden.
[3]National Veterinary Institute, Ulls väg 2B, SE-75189 Uppsala, Sweden. [4]National Food Agency, Hamnesplanaden 5, SE-75319 Uppsala, Sweden.

Introduction

External Quality Assurance Exercises (EQAE) are used for quality assurance of laboratory methods. Within the Swedish national BSL3 laboratory network we have arranged ring trials for quality assurance, because it is difficult to find external quality panels for BSL3 bacteria.
In the most recent ring trial participated also the following collaborating partners: Norwegian Defence Research Establishment, Norwegian Institute of Public Health and Norwegian Veterinary Institute.

Objective

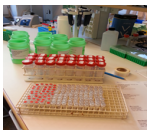
To test and improve the network´s capability for preparedness diagnostic by arranging ring trial. Specific objectives were:

- To test rapid and correct molecular diagnostic methods.
- To ensure the best diagnostic strategies to support and strengthen the Swedish response strategy in case of biological threats or natural outbreaks of highly pathogenic infectious agents (bacteria).

Methodology

For the ring trial we used different spiked matrices to test the detection range of BSL3 bacteria, each sample potentially containing inactivated bacteria from one or several of the following agents:

- *Bacillus anthracis*
- *Yersinia pestis*
- *Francisella tularensis tularensis*
- *Francisella tularensis holarctica*
- *Burkholderia mallei*
- *Burkholderia psudomallei*
- *Brucella melitensis*
- *Brucella abortus*
- *Coxiella burnetii*



The analysis of the first 5 (of total 12) unknown samples were time critical.
Results of the ring trial were evaluated with regards to:

- Speed of analyzes.
- Specificity of applied methods.

Results

- The detection of target bacteria was good, besides one laboratory the ring trial showing a high proportion of correct answers, 80 to 100 % (see table 1).
- Samples containing milk as matrix generated a weak background signal of *Coxiella*.
- Some laboratories had difficulties identifying subspecies of *Francisella tularensis.*
- The delivery of samples to two of the laboratories exceeded the time included in the agreement with the courier (see table 2).

FBD-nr	Type of samples	RS	AC	KL	MS	RQ	AB	UF
FBD-01	tap water							
FBD-02	milk							
FBD-03	d. water							
FBD-04	d. water							
FBD-05	milk							
FBD-06	tap water							
FBD-07	tap water							
FBD-08	tap water							
FBD-09	serum							
FBD-10	milk							
FBD-11	milk							
FBD-12	d. water							
FBD-13	milk							
FBD-14	milk							
FBD-15	d. water							
	Correct res.	13(87 %)	15(100 %)	12(80 %)	7(47 %)	13(87 %)	12(80 %)	12(80 %)
	Fault res.	2(13 %)	0	3(20 %)	8(53 %)	2(13 %)	3(20 %)	3(20 %)

Table 1: Compilation of the results Green mark correct answers, red marks wrong answers and yellow marks wrong answers for non target bacteria.

Lab.code	Transport (h)	First result (h)
1	13,5	8
2	9,5	2,5
3	11,5	3,5
4	13	9,5
5	16	4,5
6	15,5	3,5
7	11	6,5

Table 2: Duration of transport (h) and time to obtain the first results for time-critical samples(h)

Discussion

- Detection could likely be improved if participating laboratories received live bacteria instead of only inactivated.
- The ability to detect risk class 3 bacteria differed between the participating laboratories, mainly explained by the absence of proper detection methods at certain partners.
- The EQAE has obtained experience regarding transport of inactivated biological material between the Swedish laboratories, as well as between Sweden and Norway.

Conclusions and perspectives

A network of laboratories is important for the quality assurance of methods for highly pathogenic bacteria. In this quality assurance work EQAE is an important component for detecting weaknesses in analysis capability.

Acknowledgment

This work was supported by "2:4 krisberedskapsmedel" from the Swedish Civil Contingencies Agency. We would like to thank all partners for good cooperation.
This EQAE was part of the ongoing project "Quality assurance of laboratory capacity in Sweden" performed within the Forum for Biopreparedness Diagnostics (FBD), involving four governmental institutes: the Public Health Agency of Sweden (FOHM), the Swedish Defence Research Agency (FOI), the National Veterinary Institute (SVA) and the National Food Agency (NFA).

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

SVA NATIONAL VETERINARY INSTITUTE

FOI

Livsmedelsverket
NATIONAL FOOD AGENCY, SWEDEN

Appendix 3:

Material Transfer Agreement (MTA)

Folkhälsomyndigheten

Registration number

Date

[Viktigt att varje gång "aktivt" läsa igenom MTAn för att värdera om ev. strykningar, ändringar och tillägg behöver göras för att anpassa det till den aktuella situationen. Framförallt gulmarkeringar behöver ändras/strykas.]

MATERIAL TRANSFER AGREEMENT

Between

Folkhälsomyndigheten, The Public Health Agency of Sweden, organisation number 202100-6545 (=The Sender), SE-171 82 Solna, Sweden

and

[Namn] [organisationsnummer] (=The Recipient), [adress].

1. The Sender hereby agrees to transfer [specificera vad som omfattas av avtalet och antal, t ex kvalitetspanelprover], containing [XX/dna/etc from Genus species], the Material listed below, to the Recipient. The Material may only be used for [specificera syftet, t ex kvalitetssäkring/specificerade studier av relevans för svenskt smittskydd].

   Specification of the Material:
   [Bacillus anthracis, FOHM-2011-02 (eller den beteckning isolaten har eller ges)]

2. Last date of transfer of the Material is [datum].

3. The costs for the preparation of the Material before transport in the form of [specificera vad, t ex uppodling av isolat, frystorkning, preparation eller inköp av transportmedium, emballering etc] shall be paid by the [Sender/Recipient].

4. [Om ersättning är överenskommen, specificera för vad och hur mycket. Radera annars hela denna punkt].The Sender is entitled to a compensation for [xx,yy och zz] amounting to [xx] sek. An invoice will be sent after delivery.

   The Recipient's invoice address and reference:
   [Mottagarens fulla namn]
   [Mottagarens fakturareferens]
   [Mottagarans fakturaadress]

5. The Material may only be used for the above mentioned purpose and only at the Recipient´s laboratory located at [fullständig adress till mottagaren].

6. The Sender shall have no liability for the use of the Material, or the consequences by its use, by the Recipient or by any other person.

7. The Recipient shall use the Material in accordance with good laboratory practice and the highest standards of skill and care and shall ensure compliance with any applicable laws, regulations and administrative guidelines governing the transportation, keeping or use of the Material. This includes that the Material is handled under suitable biosafety and containment conditions.

8. The Recipient shall not permit the Material or any portion thereof to be conveyed or transported outside of its control or to a third party without the prior written consent of the Sender.

9. The Recipient agrees to provide Folkhälsomyndigheten with results generated using the Material in the event that the Recipient makes or observes any new discovery, improvement or invention relating to the examination of the Material.

10. The Recipient agrees that it will not use the Material or any derivatives or progeny thereof for commercial purposes.

11. The Recipient shall not acquire any proprietary rights to the Material and no intellectual property license is granted or implied by this Agreement.

12. In the event that the Recipient wishes to publish results that can be derived from the Material the Sender shall first take part of the writing to be able to comment on how the Material is described. The Recipient shall provide the Sender with a copy of the final publication.

13. The Recipient shall acknowledge the Sender as the source of the Material in any publication containing results that can be derived from the Material by the following statement: *The [isolates/strains/material, specificera Materialet] has/have been provided by The Public Health Agency of Sweden to improve diagnostics relevant for infectious disease control and/or for studies of relevance for infectious disease control.*

14. A disclosure of confidential information concerning the Material is permitted only when such disclosure is an obligation according to the principle of public access to official records and Swedish secrecy legislation or by a court order.

15. Upon request the Recipient shall promptly return the Material to the Sender or destroy it in accordance with instructions given by the Sender.

16. Agreed by the parties through their authorized signatories:

For the Sender	For the Recipient
Karin Tegmark-Wisell Head of Department for Microbiology The Public Health Agency of Sweden	NN XX YY
Date: [Datum]	Date: [Datum]

Appendix 4:

EQA instructions, FBD September 2016

FORUM FÖR
BEREDSKAPSDIAGNOSTIK

FBD

Ringtest inom FBD - 2016

VÄNLIGEN LÄS IGENOM HELA INSTRUKTIONEN INNAN NI PÅBÖRJAR ANALYSERNA.

Proverna skickas från:
Folkhälsomyndigheten
Avdelning för Mikrobiologi
Enheten för Beredskap och Smittskyddsdiagnostik
Nobels väg 18
171 82 Solna

Samordnare/Ansvarig
Talar Boskani (talar.boskani@folkhalsomyndigheten.se, tfn: +46 – 102052428)
Sandor Bereczky (sandor.bereczky@folkhalsomyndigheten.se, tfn: +46 – 102052527)

Uppgift
Ringtestet omfattar flera delar:

- Korrekt och snabb leverans av provförsändelse.
- Analys av fem prover innehållande levande material.
- Rapportering av preliminärsvar och slutsvar.

Laboratoriet får brådskande prover (5 st) med en begäran om att påvisa eller utesluta en uppsättning av misstänkta högpatogena bakterier (species och subspecies) enligt nedan:

- *Bacillus anthracis (B.a)*
- *Yersinia pestis (Y.p)*
- *Francisella tularensis ssp. holarctica (F.th)*
- *Francisella tularensis ssp. tularensis (F.tt)*
- *Burkholderia pseudomallei (B.psm)*
- *Burkholderia mallei (B.m)*
- *Brucella spp.*

Observera att det kan förekomma andra bakterier i proverna men dessa behöver inte identifieras.

Uppdragsgivaren behöver ett preliminärsvar på proverna så snart som möjligt. Den tid som krävs från analysstart till dess att det preliminära svaret svaras ut är en del av ringtestet. Alla laboratorier ombeds att tillämpa sina vanliga diagnostiska metoder.

Säkerhetsaspekter

Proverna ska hanteras under BSL-3 förhållanden. Sekundärförpackningen får endast öppnas i BSL-3 laboratorium.

Material

Deltagarna kommer att få 5 stycken prover med levande riskklass 2 och 3 bakterier i agarrör. Proverna kan innehålla en eller flera bakteriearter. Materialet får endast användas i samband med detta ringtest.

Proverna är avkodade med lab. kod och provnummer t.ex.
AB-01
AB-02

Transport

Proverna kommer att skickas med transportören World Courier (WC) och förpackas för att säkerställa en temperatur av 4-8°C. Paketet ska tas emot av en förutbestämd person vid respektive laboratorium.

Tillvägagångssätt:

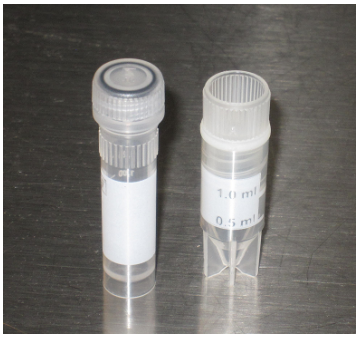
1. Notera datum och tid för ankomst och kontrollera att ytterförpackningen (bild 1) är intakt, det vill säga att den är obruten och att den inte är skadad. Vid eventuella skador, kontakta samordnarna omedelbart.
2. Öppna ytterförpackningen och ta ut det medföljande dokumentet och läs noggrant. Kontrollera och notera temperaturen inuti ytterförpackningen tillsammans med transportören.
3. Fyll i uppgifter angående transport, punkterna 1-4 under "Transport" i resultatblanketten, punkt 5-8 kan besvaras senare. Skicka denna information till samordnarna samtidigt som ni skickar in ert slutsvar.
4. Sekundärförpackningen (bild 2) tas in till BSL-3 labbet enligt lokala rutiner. OBS! Sekundärförpackningen får endast öppnas i säkerhetsbänk inne på BSL-3.
5. Analyserna måste påbörjas senast den 20 september 2016 och ska utföras på BSL-3 lab. Proverna ska förvaras vid 4-8°C till dess att analyserna påbörjas.
6. Kontrollera primärkärlen (bild 3) för att identifiera eventuella skador/läckage och att rätt antal prov har erhållits. Vid eventuella avvikelser, kontakta samordnarna omedelbart.
7. Börja analysera proven för snabb identifiering som i en verklig situation (notera starttid)
8. När ni har ett preliminärsvar, fyll i **preliminärsvarsblanketten** och skicka via e-post till samordnarna.

9. När ni har ett slutsvar fyll i **resultatblanketten** och skicka via e-post till samordnarna senast den 12 oktober.
10. Provmaterialet och erhållna isolat ska förstöras enligt överenskommelse (MTA).

Bild 1. Ytterförpackning

Bild 2. Sekundärförpackning

Bild 3. Primärkärl



FORUM FÖR
BEREDSKAPSDIAGNOSTIK

Ringtest inom FBD - 2016

Preliminärt resultat

Laboratorium:

Rapporterat av:

Ankomst av prover (datum + tid):

Analysstart (datum + tid):

Preliminärt resultat (datum + tid):

Datum och signatur:

Skicka preliminärt resultat via e-post till:

Talar Boskani (talar.boskani@folkhalsomyndigheten.se)

Sandor Bereczky (sandor.bereczky@folkhalsomyndigheten.se)

Målbakterie	Prov 1	Prov 2	Prov 3	Prov 4	Prov 5
Bacillus anthracis (B.a)	Ja / Nej				
Yersinia pestis (Y.p)					
Francisella tularensis ssp. holarctica (F.th)					
Francisella tularensis ssp. tularensis (F.tt)					
Burkholderia pseudomallei (B.psm)					
Burkholderia mallei (B.m)					
Brucella spp.					

Kommentarer:

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Ringtest inom FBD – 2016

Resultatblankett

Laboratorium:

Rapporterat av:

Datum och signatur:

Transport:

1. Ankomst (datum + tid):
2. Ytterförpackningens skick:
3. Om skadat, beskrivning av skadan:
4. Avläst temperatur: _____ °C
5. Tätning (primärkärl som innehåller provmaterialet) intakt:
6. Start analys (datum + tid):
7. Stopp analys (datum + tid):

Kommentarer:

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Prov ID	**Metoder:** (t ex direkt PCR, odling, PCR på kolonimaterial, sekvensering, NGS mm)	**Identifierad(e) målbakterie(r)**	**Ren- eller blandkultur**	**Uteslutna målbakterier**	**Kommentarer:**
Prov 1					
Prov 2					
Prov 3					
Prov 4					
Prov 5					

Slutsvar

Övriga kommentarer:

Appendix 5:

Checklist biosafety audit

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BEREDSKAPSDIAGNOSTIK

Ringtest inom FBD – 2016

Utvärdering – FBD ringtest

Tack för ni tar er tid för att fylla i denna utvärdering!
Resultatet av enkäten är enbart till för utvärderingen och kommer inte att redovisas enskilt.

1. Har instruktionerna för ringtestet varit tillräckligt tydliga?
   Ja ☐ Nej ☐

   Om inte specificera gärna vad som upplevdes som otydligt eller saknades

2. Ringtestets svårighetsgrad?

   Lätt ☐ Lagom ☐ Svårt ☐

3. Antal prov?

   För få ☐ Lagom ☐ För många ☐

4. Vad har gått bra respektive mindre bra? Lärdomar från ringtestet?
5. Tips och råd inför kommande ringtest?
6. Övriga kommentarer

Tack för din medverkan!

Svaret skickas till talar.boskani@folhalsomyndigheten.se

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REVISION – CHECKLISTA - BIOSÄKERHET

FBD 010-1

2015-04-24

Checklista för kartläggning av efterlevnad av rutinerna för bioriskhantering vid mikrobiologisk laboratorieverksamhet samt verksamhet i anslutning till denna. Biosäkerhetsronden är en revision där checklistan ger förslag på frågor som kan ställas. Resultatet av revisionen redovisas på detta formulär. En skriftlig sammanfattning av revisionen görs i anslutning till ronden.

Datum:	
Avdelning och enhet:	
Avdelningschef:	
Enhetschef:	
Skyddsombud:	
Lokala funktionsföreträdare (namn och funktion):	
Övriga deltagare:	
Datum för senast utförda kartläggning:	

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REVISION – CHECKLISTA - BIOSÄKERHET

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2015-04-24

		Ja	Nej	Kommentar	Avvikelse no.
1.	**Förebyggande åtgärder**				
A	Är biosäkerhet och bioskydd integrerat i den dagliga verksamheten? Finns styrdokument för hantering av biorisker?				
B	Är ansvarsfördelning (inklusive mandat/befogenheter) för hantering av biorisker vid laboratorierna dokumenterad och känd inom verksamheten?				
C	Finns SOP **Skyddsinstruktioner för hantering av biologiska ämnen** (liknande)? Är denna SOP förankrad och tillämpad?				
D	Har all personal läst och signerat SOP? Var förvaras dessa listor?				

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		Ja	Nej	Kommentar	Avvikelse no.
E	Finns lokala skyddsinstruktioner med avseende på biosäkerhet och bioskydd? Om *Ja*, vilka?				
F	Om *Ja*, har all personal läst och signerat? Var förvaras dessa listor?				
G	Är personalen vaccinerad enligt myndighetens krav för tillträde till laboratorieverksamhet? Vilka ev. särskilda vaccinationer krävs?				
H	Fungerar rutiner för uppföljning och boosterimmunisering?				
I	Fungerar återkopplingen från företagshälsovården?				

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		Ja	Nej	Kommentar	Avvikelse no.
J	Utförs särskilda hälsokontroller? IGRA?				
K	Finns särskilda tillträdeskrav för verksamheten med avseende på vaccinationsskydd respektive fysiska förutsättningar (syn/hörsel etc)?				
L	Informeras gravida/ammande om särskilda risker i arbetet?				
M	Informeras övriga ev. riskgrupper om särskilda risker i arbetet? (ex. städpersonal och teknisk personal, immunsupprimerade etc.)				
N	Har laborerande personal gått laboratoriesäkerhetskurs (biosäkerhetskurs)? Har alla gått kursen under de senaste 5 åren? Var förvaras listan?				

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		Ja	Nej	Kommentar	Avvikelse no.
O	Hur kommuniceras ny information/nya rön/best practice som rör riskhantering inom verksamheten?				

2. Riskbedömning - biologiska ämnen och GMM					
A	Deltar alla berörda (dvs. laboratoriepersonal, projektledare etc.) vid identifiering och bedömning av risker och val av skyddsåtgärder? När och vid vilka tillfällen görs dokumenterade riskbedömningar?				
B	Var förvaras giltiga riskbedömningar och tillstånd för verksamhet? Har all berörd personal tillgång till riskbedömningarna?				

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		Ja	Nej	Kommentar	Avvikelse no.
C	Beaktas aspekter kring bioskydd i de dokumenterade riskbedömningarna?				
D	Förekommer GMM i verksamheten? Om ja, finns anmälan/tillstånd enl. AFS 2011:02?				
E	Förekommer djurförsök? Om ja, finns tillstånd enl. SJVFS 2012:26?				
F	Utförs särskild riskbedömning med avseende på djurhantering?				
G	Har riskbedömningarna för hantering av smittämnen i riskklass 3 samt vid djurhantering behandlats i Biosäkerhetskommittén?				

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		Ja	Nej	Kommentar	Avvikelse no.
3. Skyddsnivå för laboratorielokaler/djurrum					
A	Vilket/vilka biologiska agens hanteras?				
B	Vilka andra biologiska agens kan materialet tänkas innehålla?				
C	Vilka typer av provmaterial hanteras?				

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		Ja	Nej	Kommentar	Avvikelse no.
D	Är de ventilerade arbetsplatserna överbelamrade med utrustning/material?				
E	Används säkerhetskoppar vid centrifugering?				
F	Hur och hur ofta rengörs centrifuger och annan utrustning såsom ventilerade arbetsplatser?				
G	Är apparatur (såsom ex. ventilerade arbetsplatser) funktionstestad och/eller validerad? Sker detta på regelbundet basis? Hur ofta?				

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		Ja	Nej	Kommentar	Avvikelse no.
4. Tillträde					
A	Finns ett väl fungerande fysiskt skydd (skalskydd, skydd av biologiskt material, avfall, mm)?				
B	Säkerhetskontrolleras personalen? Finns rutiner för rekrytering och kontroll av kompetenser?				
C	Finns rutiner för kontroll av uppförande (tillförlitlighet) och avstängning av personal?				
D	Finns ett väl fungerande informationsskydd (identifiering av känslig information; riktlinjer för att förvara, sända och förstöra)?				

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		Ja	Nej	Kommentar	Avvikelse no.
E	Möts besökare i receptionen och följs tillbaka dit?				
F	Finns särskilda tillträdesrestriktioner för verksamheten?				
G	Finns särskilda rutiner för lokalvård och service av apparatur samt kontroll av leveranser/skyddsservice etc. med avseende på smittrisk? Om ja, var finns dessa dokumenterade?				
H	Finns särskilda restriktioner för hantering av smittämnen, exempelvis tillgång till stamförråd?				

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		Ja	Nej	Kommentar	Avvikelse no.
I	Genomförs årlig inventering av stamförråd för smittämnen i riskklass 3 resp. 4? Om ja, var förvaras dessa? (**Obs! Denna information noteras ej**)				
J	Hur tas besökare in på kvällar/helger? Finns det regler för ensamarbete?				
K	Informeras besökare om ev. smittrisker?				
L	Vistas barn i laboratorierna eller andra lokaler?				
M	Efterlevs förbudet mot mat, dryck, rökning, snus och tuggummi i laboratorielokalerna?				

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		Ja	Nej	Kommentar	Avvikelse no.
N	Informeras och utbildas personal om personligt skydd (vid exempelvis hot)?				

5. Personlig skyddsutrustning					
A	Används laboratorierock (rätt storlek och tillknäppt) vid allt laboratoriearbete? Vilka typer?				
B	Använder all personal rumsbundna skor i laboratorierna där detta är ett krav?				
C	Används annan personlig skyddsklädsel?				

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		Ja	Nej	Kommentar	Avvikelse no.
D	Hur hanteras skyddsklädseln efter användning? Vid återanvändning av skyddsklädsel, hur och var rengörs denna?				
E	Finns kunskap om rengöring/ dekontaminering och lämplig förvaring av återanvändbar personlig skyddsutrustning (PPE)? Hur säkerställs detta?				
F	Har personal tränats i korrekt användning av PPE?				

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		Ja	Nej	Kommentar	Avvikelse no.
G	Används engångsutrustning? Vilken/vilka typer?				
H	Används handskar vid allt arbete med blod, serum och annat humant/animalt material? Vilken typ?				
I	Används handskar vid arbete med kemikalier? Vilken typ?				
J	Används skyddsglasögon, visir eller plexiglasskärm vid risk för stänk eller arbete med UV-ljus? Om ja, vid vilka arbetsmoment?				

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		Ja	Nej	Kommentar	Avvikelse no.
K	Används munskydd? Om ja, när?				
L	Behöver andningsskydd användas? Vilken typ? Är dessa tillpassningstestade för varje medarbetare? Om ja, hur och när? Används PAPR? Om ja, vem ansvarar för filterbyten och kontrollerar luftflöde och batterier?				

6. Rutiner för (person) dekontamination och handdesinficering					
A	Tas armband och ringar av vid laboratoriearbete?				

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		Ja	Nej	Kommentar	Avvikelse no.
B	Efterlevs rutiner kring hår och sår? Hur?				
C	Efterlevs avdelningens telefonrutiner med tanke på hygien? Vilka är rutinerna?				
D	Förvaras privata tillhörigheter på särskild förvaringsplats utanför laboratoriet?				
E	Finns handtvättanordning och desinfektionsmedel i omedelbar anslutning till arbetsområdet?				
F	Hur desinficeras händerna efter avslutat laboratoriearbete?				

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		Ja	Nej	Kommentar	Avvikelse no.
7. Arbetsplatsen					
A	Är det god ordning på laboratoriet?				
B	När utfördes storstädning senast?				
C	Finns det tillräckligt med utrymme?				
D	Finns det krokar för rockar e. dyl.?				
E	Separeras skyddskläder och privata kläder?				

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		Ja	Nej	Kommentar	Avvikelse no.
F	Hanteras remisser och protokoll på laboratoriebänkar? Används datorer på laboratoriebänkar? Används telefoner?				
G	Hur dekontamineras arbetsytor, inkl. tangentbord och mus? Hur ofta?				
H	Vilka desinfektionsmedel används till respektive agens? Finns skyddsinstruktion för förvaring och hållbarhet?				
I	Används kanyler, skalpeller el dyl. och finns behållare/kastburk för skärande/stickande avfall (riskavfall)?				
J	Hur hanteras fyllda kastburk för stickande/skärande avfall?				

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		Ja	Nej	Kommentar	Avvikelse no.
K	Kan arbete med stickande/skärande undvikas?				

8. Olyckshändelse/tillbud och spill					
A	Hur snart sker anmälan om tillbud/olyckor/arbetsskador och på vilket sätt?				
B	Är rutinerna samt roller vid tillbud kända?				
C	Är rutinerna i händelse av exponering för laboratorieassocierad smitta känd?				

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		Ja	Nej	Kommentar	Avvikelse no.
D	Hur sker uppföljningen av incidenter (tillbud, olyckor etc.)?				
E	Vet alla var akutvagnen finns?				
F	Är innehållet på akutvagnen komplett?				
G	Vem ansvarar för påfyllning av akutvagnen?				
H	Genomförs praktiska (tillbuds-) övningar?				
I	Presenteras/diskuteras tidigare olyckshändelser/tillbud? Sker översyn av rutiner?				

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		Ja	Nej	Kommentar	Avvikelse no.
9. Avfallshantering					
A	Finns SOP om avfallshantering? Har all laborerande personal läst denna?				
B	Vilken typ av avfall hanteras (smittförande, stickande/skärande, kadaver etc.)?				
C	Hur separeras fast och flytande avfall?				
D	Hälls avfall ut i vasken?				
E	På vilket sätt sker en säker förvaring innan destruktion/transport? Hur/vem ombesörjer transport av stickande/skärande till destruktion?				

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		Ja	Nej	Kommentar	Avvikelse no.
F	Finns validerade metoder (på plats) för destruktion av olika typer av avfall?				
G	Var autoklaveras avfallet?				
H	Hanteras blod, serum och annat humant/animalt material som potentiellt smittfarligt?				
I	Hur märks resp. avfall upp? På vilket sätt säkerställs att myndighetens märkta avfall kan spåras? Hur säkerställs korrekt märkning före/efter inaktivering (autoklavering)?				

		Ja	Nej	Kommentar	Avvikelse no.
J	Fungerar hantering och hämtning av avfall enligt befintlig SOP?				
K	Vilken dokumentation erhåller myndigheten vid destruktion av riskavfall vid annan anläggning?				

10. Disk och städ					
A	Fungerar hantering av diskgods?				
B	Fungerar städningen utförd av städpersonalen?				

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		Ja	Nej	Kommentar	Avvikelse no.
11. Transporter					
A	Är biologiska ämnen förpackade så att läckage förhindras vid all transport? In/utgående prov				
B	Hur transporteras prover mellan byggnader?				
C	Finns SOP om transport-internt? Är innehållet i denna SOP känt?				
D	Fungerar mottagande av provmaterial bra (med avseende på biorisker)?				
E	Finns dokumentation och ett spårningssystem vid transport?				

REVISION – CHECKLISTA - BIOSÄKERHET

FBD 010-1

2015-04-24

		Ja	Nej	Kommentar	Avvikelse no.
12. Förvaring					
A	Sker säker förvaring av smittämnen i riskklass 2, 3 samt GMM?				
B	På vilket sätt förvaras smittämnen i riskklass 3? (**Obs! *Noteras ej här***)				

13. Övningar					
A	Förekommer regelbundna, dokumenterade tillbudsövningar? Förekommer krisövningar/ simuleringar?				
B	Inkluderar dessa övningar även bioskyddsaspekter?				

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FBD 010-1

2015-04-24

		Ja	Nej	Kommentar	Avvikelse no.
14. Uppföljning och utvärdering					
A	Hur följs resultatet från biosäkerhetsronden upp på enheten/avdelningen?				
B	Hur följs ärenden i biosäkerhetskommittén upp på enheten/avdelningen?				
C	Vilka uppföljningar/kontroller av korrigerande åtgärder sker efter incident eller annan händelse? Hur ofta och i vilka sammanhang utförs sådan uppföljning/ kontroll? Vilka är delaktiga? Hur förmedlas resultat till berörda medarbetare?				

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REVISION – CHECKLISTA - BIOSÄKERHET

FBD 010-1

2015-04-24

		Ja	Nej	Kommentar	Avvikelse no.
15. Övrigt					

FORUM FÖR
BEREDSKAPSDIAGNOSTIK

Avvikelser och förbättringsmöjligheter

Datum för revision:	
FBD revisorer:	
Deltagarna:	
Plats:	

Skriv kort det generella intrycket på besöket. Lyft fram positiva sakar på besöket och revisionen t.ex. bred representation från myndigheten, engagemang från deltagarna, god ordning på laboratoriet etc.

Efter revision (enligt FBD_XXX) vill vi framföra följande förbättringsmöjligheter:

1. Beskriv detaljer förbättringsmöjligheter för avvikelse nummer 1 i checklistan.
2. Avvikelse nr.2
3. Avvikelse nr.3
4. Avvikelse nr.4
5. Avvikelse nr.5
6. Avvikelse nr.6

Rapport utfärd av: Datum:

Appendix 6:

Checklist quality audit

FORUM FÖR
BEREDSKAPSDIAGNOSTIK

KVALITETSREVISION - CHECKLISTA

FBD 011-1
2015-09-02

Kvalitetsrevisionen baseras på FBD´s kvalitetsmanual.

Datum	
Myndighet/område	
Revisorer	
Deltagare	

Tekniska krav	OK	F	P	Kommentarer/noteringar	Förbättrings-förslag
Dokumenthantering Giltiga versioner av metodbeskrivningar, revisionsstatus, formulär, skydda och back-up på elektroniska dokument etc. Inga lösa lappar eller handändringar, riskbedömning (agens/kemikalier), spill och saneringsinstruktion					
Personal Krav på kompetens, kompetensbevis, upplärning ny personal eller efter lång frånvaro, underhåll av upplärd personal (egenkontroll), dokumentation under upplärning/egenkontroll Vaccination rutiner					
Utrustning Spårbar kalibrering, underhåll, instruktioner, instruktioner för skötsel, underhåll och kalibrering, förvaring av dokumentation kring utrustning, loggbok, ansvar för underhåll och kalibrering (funktionskontroll av säkerhetsbänk och autoklav)					
Säkerhetsutrustning Skyddsutrustning, rutiner/instruktioner, underhåll och funktionskontroll, ansvarig Desinfektionsmedel? Agens?					
Lokaler och miljö P3 Lämpliga lokaler, avgränsningar, förändringar, kontamineringsrisker vid molekylära metoder, tillträde till P3, loggning av viktiga parametrar för P3, skriftliga rutiner för städning (labblokaler samt arbetsbänk) och skötsel, rökning, hepafilter					

FORUM FÖR
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KVALITETSREVISION - CHECKLISTA

FBD 011-1
2015-09-02

Metoder och validering Analysprotokoll, tydlighet, finns allt beskrivet? mätosäkerhet, valideringar, verifieringar, avdödning					
Provhantering Registrering, uppackning mm, kontaminationsrisk, provuttag, remittering, märkning, avidentifiering av prov vid skick mellan myndigheter Förvaring av provmaterial, destruktion,					
Spårbarhet Spårbarhet på reagenser och substrat, analysprotokoll					
Reagenser, kontrollmaterial, referensmaterial Förvaring, hållbarhet, utgångsdatum, märkning, rutiner för hantering av kontrollmaterial, certifierade referensmaterial, definierade krav på vilka reagens som ska användas					
Kvalitetssäkring Kontroller (PPC, NPC, PEK, NEK, PTC, NTC), regler för omkörningar och analysavvikelser inkl. inhibering,					
Provsvars rutiner Behörighet, arkivering av provsvar och analysprotokoll, anmälningsplikt,					
Provjämförelse/ringtest* Regelbundet deltagande, rapportering, uppföljning					
Användning av FBDs* **FBD-002,** Kvalitetsdokument, Provremiss					
FBD-003, Protokoll för handhavande och validering av sälherpesvirioner					
FBD-004, Dokumentationsriktlinjer för framtagande av primers och probe					
FBD-005-2, Valideringsmanual för kvalitativ realtids PCR-analys för detektion av bakterier					
FBD-006, Valideringsprotokoll för referens- och kontrollmaterial i matris					
FBD-007, Valideringsprotokoll för referens- och kontrollmaterial (DNA)					
FBD-008, Kvalitetskrav vid datatolkning av realtids-PCR					
FBD-009, Provsvarsmall					
Övning (olycksfallsövning, spill övning)					
Översynrutiner i myndigheten					

KVALITETSREVISION - CHECKLISTA

Övrigt					
Vertikal revision (följa ett hanterat prov)*	**OK**	**F**	**P**	**Kommentarer/noteringar**	**Förbättrings-förslag**
Provregistrering					
Provberedning/Provbearbetning					
Provförvaring					
Spårbara rådata					
Standarder/referensmaterial					
Deltagande i provningsjämförelse					
Kontrollkort					
Utrustning					
Behörighet					
Provsvar					

OK = granskat och bedömt utan anmärkning
F = förbättringsförslag
P = positivt exempel

Signatur bedömare	Signatur myndighetens representant

FORUM FÖR
BEREDSKAPSDIAGNOSTIK

KVALITETSREVISION - CHECKLISTA

FBD 011-1
2015-09-02

Iakttagelseblankett – Kvalitetsrevision

Datum för revision	
Revisionsbedömare	
Deltagare	
Revisionsobjekt	
Plats	

☐ Förbättringsförslag ☐ Positivt exempel

Beskrivning:

Förbättringsförslag:

1. Beskriv detaljer förbättringsmöjligheter för förbättringsförslag nummer 1 i checklistan.
2. Förbättringsförslag nr.2
3. Förbättringsförslag nr.3
4. Förbättringsförslag nr.4
5. Förbättringsförslag nr.5
6. Förbättringsförslag nr.6

Positiv exempel:

1. Beskriv detaljer positiva exemplar P1 i checklistan.
2. P2
3. P3

Återkopplingsdatum: Senast xxxx-xx-xx

STATENS
VETERINÄRMEDICINSKA
ANSTALT

Folkhälsomyndigheten
PUBLIC HEALTH AGENCY OF SWEDEN

Livsmedelsverket