FFIBM/719/144

Godkjent
Kjeller 26 June 2000

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Forskningssjef

Deltagelse i NATO/PFP- interlaboratorieøvelse for deteksjon/identifikasjon av biologiske stridsmidler

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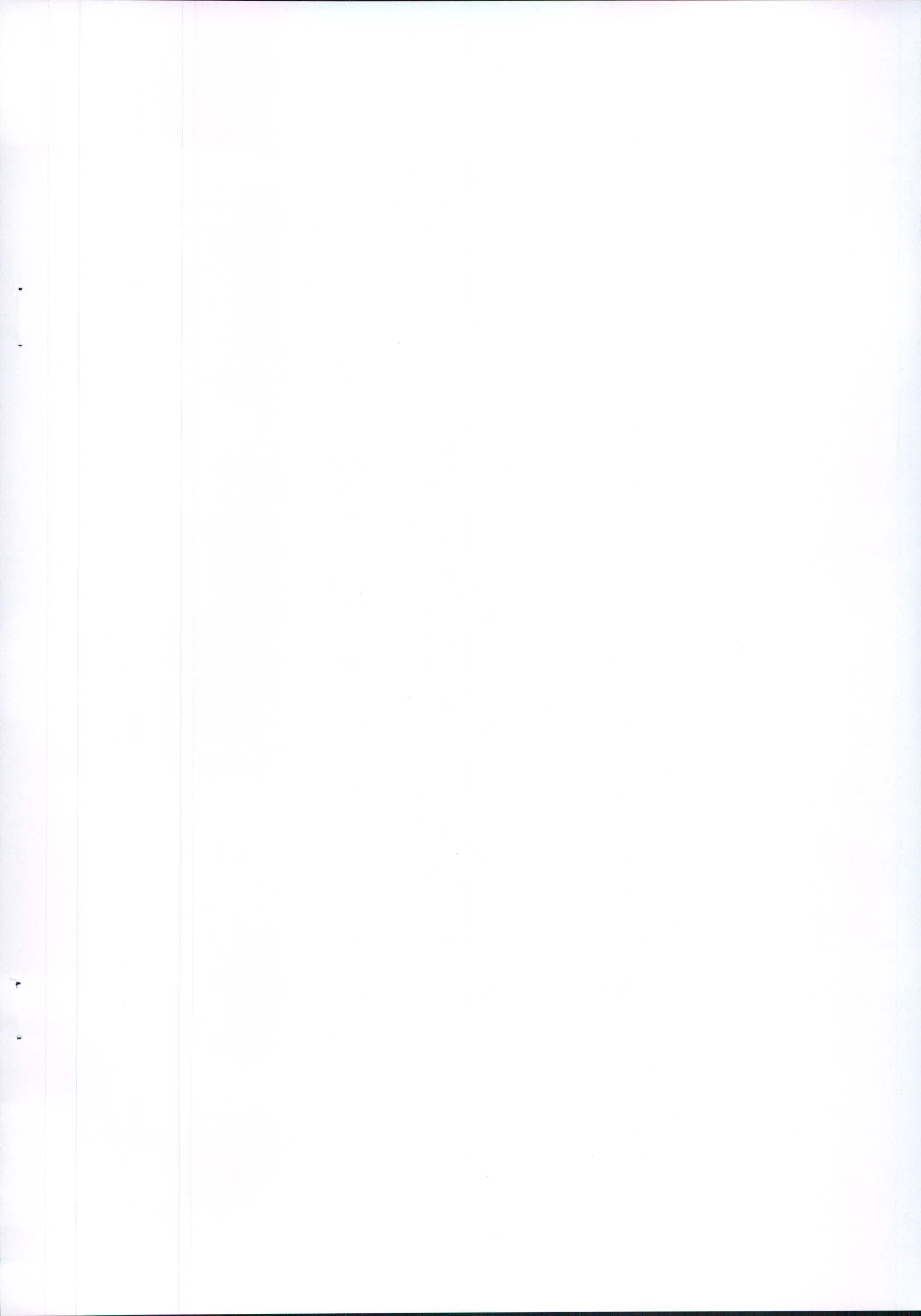
FFI/RAPPORT-2000/03995

FORSVARETS FORSKNINGSINSTITUTT
Norwegian Defence Research Establishment
Postboks 25, 2027 Kjeller, Norge



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Norwegian Defence Research Establishment

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UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE
(when data entered)

REPORT DOCUMENTATION PAGE

1) PUBL/REPORT NUMBER	2) SECURITY CLASSIFICATION	3) NUMBER OF PAGES
FFI/RAPPORT-2000/03995	UNCLASSIFIED	42
1a) PROJECT REFERENCE	2a) DECLASSIFICATION/DOWNGRADING SCHEDULE	
FFIBM/719/144	-	

4) TITLE

Deltagelse i NATO/PFP- interlaboratorieøvelse for deteksjon/identifikasjon av biologiske stridsmidler

Participation in NATO/PFP- interlaboratoryexercise within detection/identification of biological warfare agents

5) NAMES OF AUTHOR(S) IN FULL (surname first)

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6) DISTRIBUTION STATEMENT

Approved for public release. Distribution unlimited. (Offentlig tilgjengelig)

7) INDEXING TERMS

	IN ENGLISH:	IN NORWEGIAN:
a)	PCR	PCR
b)	Biological warfare agents	Biologiske våpen
c)	Detection	Deteksjon
d)	Identification	Identifikasjon
e)	Bacteria, virus, rickettsia	Bakterier, virus, rickettsier

THESAURUS REFERENCE:

8) ABSTRACT

NATO styrker, enten i fredsbevarende operasjoner eller i annen type strid, kan bli utsatt for angrep/sabotasje med biologiske våpen. I en slik situasjon er det viktig at en hurtig evaluering av situasjonen er mulig for at vernetiltak raskt kan iverksettes. For at en slik evaluering skal kunne finne sted må ulike NATO-laboratorier være i stand til å analysere prøver som kan inneholde biologiske våpen. Hensikten med de to SIBCA-øvelsene har derfor vært å evaluere NATO-laboratoriene's evne til å detektere og identifisere potensielle biologiske stridsmidler i ukjente prøver.

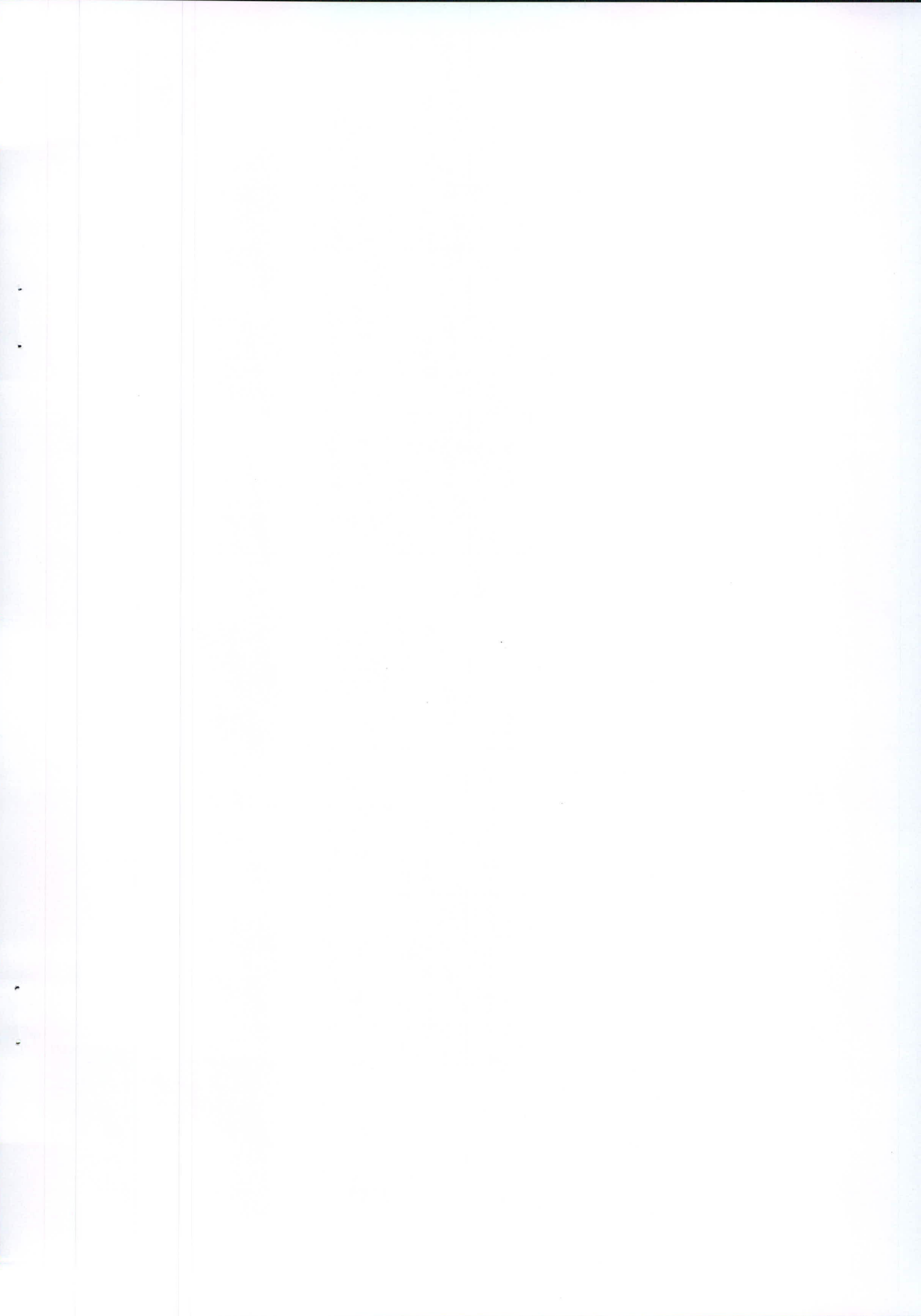
NATO forces in peacekeeping or in other kinds of war operations can be exposed for biological warfare agents during combat or sabotage. In such a situation it is important with a rapid evaluation of the situation to initiate protective measures. To do such an evaluation NATO-laboratories have to be able to analyse samples containing biological warfare agents. The objectives with the two SIBCA-exercises have therefor been to evaluate NATO-laboratories capability to detect and identify potential biological warfare agents in unknown samples.

9) DATE	AUTHORIZED BY This page only	POSITION
26 june 2000	Bjørn Arne Johnsen	Director of Research

ISBN 82-464-0433-4

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE
(when data entered)



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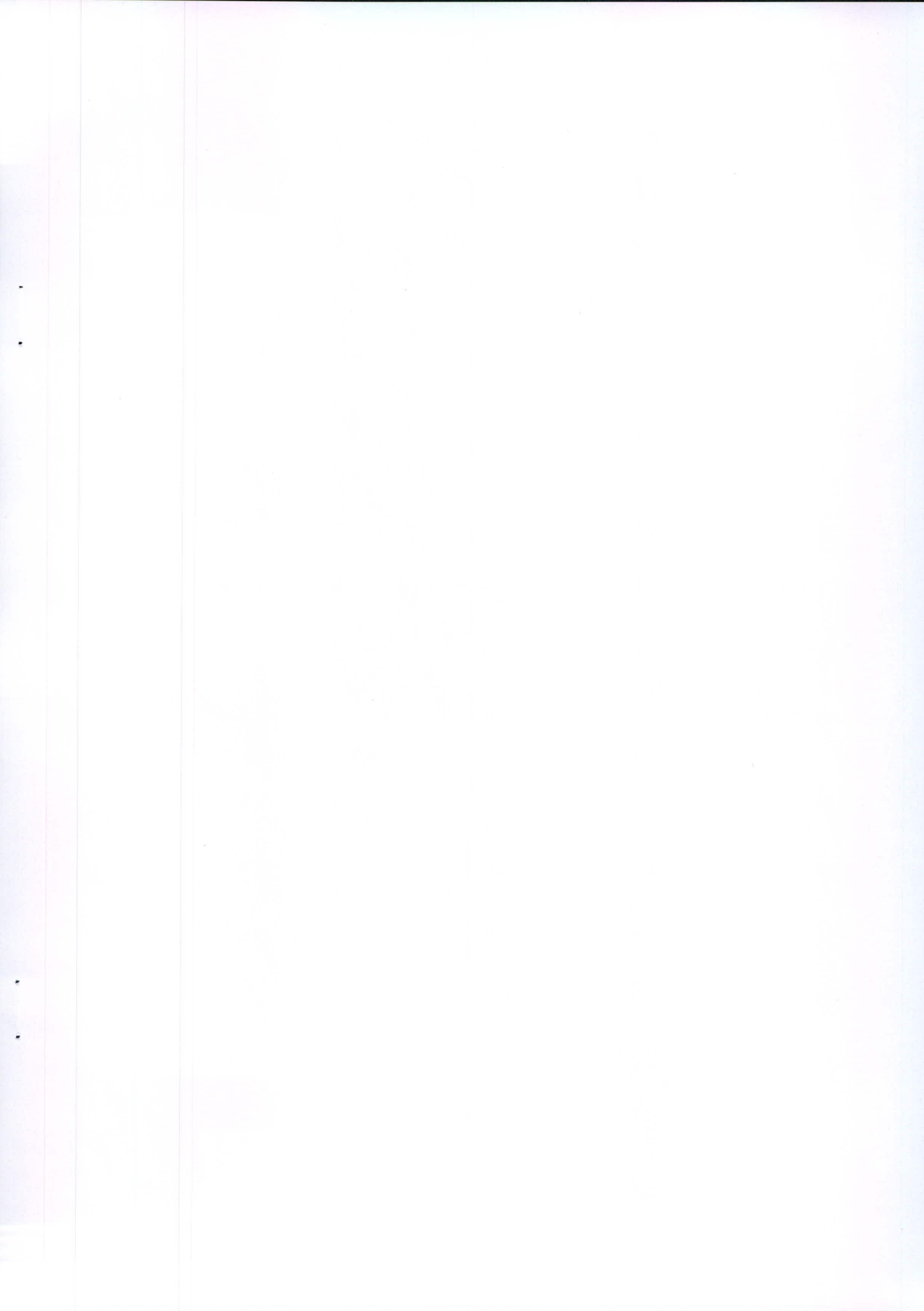

DELTAGELSE I NATO/PFP- INTERLABORATORIEØVELSE FOR DETEKSJON/IDENTIFIKASJON AV BIOLOGISKE STRIDSMIDLER

1 HENSIKT

NATO styrker, enten i fredsbevarende operasjoner eller i annen type strid, kan bli utsatt for angrep/sabotasje med biologiske våpen. I en slik situasjon er det viktig at en rask evaluering av situasjonen er mulig slik at vernetiltak hurtig kan iverksettes. For at en evaluering skal kunne finne sted må ulike NATO-laboratorier være i stand til å analysere prøver som kan inneholde biologiske våpen. Hensikten med SIBCA-øvelsen har derfor vært å evaluere ulike NATO-laboratorier's evne til å detektere og identifisere potensielle biologiske stridsmidler i ukjente prøver.

2 INTRODUKSJON

NAAG/LG-7/SIBCA (Sampling and Identification of Biological and Chemical Agents) er en NATO-gruppe som arbeider med utvikling av prøvetakings- og analysemetoder for biologiske og kjemiske våpen. Gruppen arrangerte i mars 1999 og februar 2000 to laboratorieøvelse der deltagerlandene fikk tilsendt ukjente prøver som kunne innehold en eller flere potensielle biologiske stridsmidler. Prøvene var kobolt-bestrålt og sertifisert døde (Appendix A og B). De kunne inneholde en eller flere organismer hentet fra en liste med 10 ulike mikroorganismer (Tabell 1). Mikroorganismene kunne være bakterier, ricketsier (intracellulære bakterier) eller virus.

Organisme	Sykdom
Bacillus anthracis	miltbrann
Yersinia pestis	pest
Vibrio cholerae	kolera
Coxiella burnetii	Q-feber
Francisella tularensis	harepest
Brucella melintensis	dyresykdom (bruselose)
Burkholderia mallei	hestesykdom (snive)
Yellow Fever Virus	gulfeber
VEE virus	hestesykdom (hjernebetennelse)
Vaccinia virus	nært beslektet med koppevirus

Tabell 1 Tabell over organismer som kunne være i de ukjente prøvene

Deltagerlandene kunne bruke enhver analysemetodene de selv ønsket. Øvelsen måtte utføres og rapporteres innen 10 arbeidsdager. I 1999 deltok det 11 laboratorier mot 12 i 2000. I Norge ble

analysene utført ved Forsvarets forskningsinstitutt, avdeling for beskyttelse og materiell (FFIBM).

3 MATERIALER OG METODER

Ved FFIBM ble det benyttet molekylærbiologiske metoder, PCR (polymerase kjedereaksjon) (1, 2) og sekvensering av PCR-produktene, til analysene. Ved PCR benyttes et enzym (3) og spesifikke primere (små deler av arvematerialet) slik at artsspesifikke deler av organismes arvestoff blir kopiert mange hundre tusen ganger. På den måten kan innholdet av bakterier/virus i prøven bestemmes. Dette gir en meget spesifikk og sensitiv metode. Mange av laboratoriene brukte, i tillegg til PCR, en del andre metoder under testene (Tabell 2). En rekke av disse metodene benytter artsspesifikke antistoffer. Dette er proteiner som gjenkjenner og binder seg til andre spesifikk proteiner i en organisme (4).

Instument/metode	**Type identifikasjon**
Light addressable potentiometric Sensor (LAPS)	immunologisk
Immunochromatographic Assays (IAC)	immunologisk
Polymerase Chain Reaction (PCR)	genetisk
Imunofluorescens	immunologisk
Lysmikroskopiering	visuell
Gram - farging	fargereaksjon
Transmisjon elektronmikroskopiering	visuell
Enzymelinked Immunosorbent Assay (ELISA)	immunologisk

Tabell 2 Oversikt over benyttede metoder under SIBCA-øvelsene

Strategien som FFI benyttet for å påvise de ulike organismene var først å anvende generelle primere som gjenkjenner deler av 16S-rRNA-genet. Dette er et gen som finnes i alle bakterier, men ikke i virus, og kan derfor brukes som en generell markør for påvisning av bakterier. Etter denne "screeningen" ble det brukt artsspesifikke primere. For å kontrollere at det ikke var flere enn en organisme i hver prøve ble alle prøvene undersøkt med samtlige artspesifikke primere. PCR-produktene fra *C. Burnetii* og *B. anthracis* ble kontrollert med hybridiseringsprober. Dette er korte enkelt trådete DNA-biter som vil binde seg (hybridisere) til PCR-produktet hvis sekvensen til produktet er det riktige. I tillegg til PCR-analysene ble utvalgte PCR-produkter sekvensert. Sekvensering er en metode som kan benyttes for å kontrollere at PCR-produktene som er dannet er korrekte. Ved sekvensering bestemmes rekkefølgen av basene i DNA'et (adenin, guanin, cytosin og thymin) (6). Etter sekvenseringen ble produktene kontrollert i Genbanken (National Center for Biotechnology Information (NCBI)). For ytterligere informasjon vedrørende materialer og metoder, se materialer og metoder i (2) og Appendiks C.

4 RESULTATER OG DISKUSJON

Laboratoriet ved FFI identifiserte samtlige ukjente prøver under øvelsen i 1999 og 2000 (2, Tabell 3). Denne rapporten vil hovedsakelig diskutere resultatene fra testen i 2000. Resultater fra testen i 1999 er beskrevet i (2).

Vi fikk tilsendt seks prøver. Alle prøvene ble først testet med de generelle primerne (16S-rRNA) for å undersøke om de inneholdt bakterier. Fem prøver inneholdt bakterier. I den siste prøven ble Vaccinia-virus identifisert med bruk av Vaccinia-spesifikke primere. Det ble også brukt artsspesifikke primere til å identifisere hvilke bakterier som var tilstede de fem 16S-rRNA-positive prøvene (Appendix C). Ingen av prøvene ble funnet å inneholde mer enn en organisme. De falske positive resultatene omtalt i tabell 3 skyldes at enkelte land påviste flere organismer i hver enkelt prøve. Det ble sagt av arrangøren at hver prøve kunne inneholde flere enn en organisme, men det var ingen av deltagerlandene som fikk prøver som inneholdt mer enn en organisme.

Under øvelsen i 1999 brukte FFI ti dager på å analysere de fem ukjente prøvene som var tilsendt. Under årets test brukte vi kun fire timer på å bestemme alle seks organismene. Dette skyldes blant annet innkjøp av en ny hurtig PCR (LightCycler™ fra Roch Diagnostics) hvor resultatet kan leses av direkte under eller etter analysen (real-time, on-line). Metoder og rutiner har i tillegg blitt bedre innarbeidet ved laboratoriet. Før øvelsen i 1999 var det kun utført enkle innledende forsøk med PCR på bakterien *Escherichia coli*. I tiden fremover vil det bli viktig å få etablert metoder for analyser av miljøprøver. Dette er prøver som ikke kan analyseres direkte slik det er blitt gjort i de to første SIBCA-testene. Det skyldes at jord og vannprøver inneholder forurensninger, blant annet humusstoffer, som effektivt vil inhibere PCR-reaksjonen. I slike prøver må bakterier eller DNA/RNA fra prøvene isoleres. Dette er ofte tidkrevende operasjoner hvor tap av bakterier kan være høyt. FFI har innledet arbeidet med å utvikle metoder for prøveopparbeidelse og målet er at metodene blir tilstrekkelig etablert innen neste SIBCA-øvelse.

Organisme påvist	Riktige	Falske positive	Falske negative[a]
B. melitensis	12	1 (Ft[b])	1
B. anthracis	12	1 (Bm[b])	0
C. burnetii	10	1 (Ba[b])	1
V. cholerae	12	3 (Cb, Bm, Ba[b])	0
Vaccinia	11	0	0
F. tularensis	9	0	3

[a] Kun markert negativ hvis laboratoriet hadde en metode men kunne ikke detektere
[b] Ft- *F. Tularensis*, Bm- *B. mallei*, Ba- *B. anthracis*, Cb- *C. burnetii*

Tabell 3 Resultater fra SIBCA II (2000). Tallene angir antall laboratorier.

Alle PCR-produktene som ble sekvensert ble kontrollert i Genbanken. Dette er en database som inneholder de aller fleste rapporterte DNA-sekvenser fra ulike mikroorganismer. Tabell 4 viser sekvensen av PCR-produktene samt referansenummer (Accession nr.) i Genbanken. Sekvensering er sannsynligvis en av de beste metodene for å oppnå en mest mulig sikker identifisering. Sekvenseringen vil gi informasjon om art, men kan også gi nyttig informasjon om hvilken stamme man har av en art. Metoden er fremdeles tidkrevende, men utviklingen av nye og hurtigere systemer kommer hele tiden ut på markedet.

Organisme	Primer	Sekvenser	Accession nr.[a]
Bacillus anthracis	BAlef-1/2*	1 ttactgtaga aaatactgaa aaggcactga acgtttatta tgaaataggt aagatattat 61 caagggatat tttaagtaaa attaatcaac catatcagaa atttttagat gtattaaata 121 ccattaaaaa tgcatctgat tcagatggac aagatc	AF065404
Coxiella burnetii	CB-1*/2	1 tgcctgctag ctgtaacgaa ttgagctaat tggaagttat cacgcagttg tttttggata 61 gcaggattat ccatgtcttt tttgagctga gcaacattta atcctacttt ttctgcggtt 121 tgaagggtga tttgttctga taattggccg tcgacactc	M88613
Vibrio cholerae	VCctx-1/2	1 agtttgagaa gtgcccactt agtgggtcaa actatattgt ctggtcattc tacttattat 61 atatatgtct gatagccact gcacccaaca tgtttaacgt taatgatgta ttaggggcat 121 acagtcctca tccagatgaa caagaagttt ctgctttagg tgggattcca tactcccaaa 181 tatatggatg gtatcgagtc a	X58785
Francisella tularensis	TUL-1/2	<u>TUL-1:</u> 1 gatgatgcaa aagcttcagc taaagatact gctgctgctc agacagctac tactgagcaa 61 gctgctgctg tatctaagcc aactgcaaaa gtaattxaaa taaacttggt caggataaaa 121 taaaagcaac tgtatataca acatacaata ataac	M32059
		<u>TUL-2:</u> 1 gcctgccatt gtaatcttac acttccttgt gggtxattat tgtatgtctg tatatacagt 61 tgccttttac txtctgatcc tgaccaagtx atttaaact	M32059
Vaccinia virus	VACha-1/2	1 ataaggxgcc caattccatt attcttttac gtcgtaaaag cgacgtcttg tattttgata 61 attataccaa ggataaaata tcttacgact ctccatacga tgatctagtt acactatcac 121 ataatcatga ctgctagaga tgcgtactag ag	AF095689
Brucella melitensis	BAB4/ BAB5	1 acgcgcaacg atatggatcg tttccgggta aagcgtcgcc agaaggcgca aatcttccac 61 cttgcccttg ccatcataaa ggtcccggtg ctc	M20404

[a] Referanse til sekvenstreff i GenBanken

Tabell 4 Resultater fra sekvenseringsanalysene

Konsentrasjonene av de ulike bakterier og virus som var tilsendt ble etter testene oppgitt av arrangøren (Appendix D og E). Verdiene ble benyttet til å bestemme det minste antall organismer som kunne påvises ved PCR (deteksjonsgrense) (Tabell 5). De relativt store variasjonene mellom ulike organismer skyldes forskjellig effektivitet av primere som ble benyttet. Dette kan skyldes primerenes GC-innhold, GC-innhold i 3' ende, primerlengde, antall mismatc, smeltepunkt og komplimenteritet til seg selv (looping).

Analysetidene som er oppført i tabell 5 er omtrentlige tider. Disse kan fremdeles reduseres noe med vi har ikke prioritert dette arbeidet. En måte å få ned deteksjonstiden ytterligere er å

redusere lengden på PCR-produktet, ettersom DNA- polymerasen trenger tid til å syntetisere nytt DNA under PCR-reaksjonen. Lengdene av våre produkter ligger mellom 150-600 baser. Det optimale er mellom 120 og 180 baser. Analysetidene vil da kunne komme ned i 10-20 minutter.

Organisme	Deteksjonsgrense	Analysetid
B. cereus	< 20 cfu*	~ 25 min
B. anthracis	< 10 cfu*	~ 30 min
C. burnetii	< 10 cfu*	~ 20-30 min
Y. pestis	700-1500 cfu*	~ 30-40 min
B. melintensis	~300 cfu*	~ 30 min
V. cholerae	~10 cfu*	~ 30-40 min
F. tularensis	< 10 cfu*	~ 30-40 min
Vaccinia virus	< 10 cfu*	~ 30 min

*Cfu: colony forming units

Tabell 5 Oversikt over deteksjonsgrenser og analysetider

5 KONKLUSJON

Deltagerlandene, under SIBCA-møtet i Munster 8-10 mai 2000 i Tyskland, var enige om at testene i 1999 og 2000 hadde vært en gode øvelser for å evaluere laboratorienes kapasitet for deteksjon av biologiske stridsmidler. Øvelsene har vært et steg i riktig retning av å kartlegge kompetansen som finnes innen B-deteksjon. Etter testene blir det lettere for hvert enkelt laboratorium å vurdere hvor begrensningene ligger for deretter å kunne rette innsatsen inn på disse problemområdene.

Resultatene i 2000 var bedre enn for testen i 1999. Generelle problemer i 1999 var at en rekke laboratorier manglet gode spesifikke antistoffer for de aktuelle mikroorganismene. Det var også problemer med å skaffe til veie tilstrekkelig gode primere for de genetiske metodene. Disse problemene var imidlertid, i stor grad, løst til øvelsen i 2000. Ettersom resultatene har bedret seg fra 1999 ønsket deltagerene og øke vanskelighetsgraden på den kommende øvelsen i 2001. I denne testen vil prøvene bestå av jord og luft (luften føres gjennom en bufferløsning). De resterende prøvene skal være i en bufferløsning slik de har vært i de to første øvelsene, men med lavere konsentrasjoner av mikroorganismer. Hensikten er å gjøre prøvene mest mulig autentiske med miljøprøver, slik de vil være når de blir samlet inn fra felten i en reell situasjon.

APPENDIKS

A SERTIFIKAT PÅ INAKTIVERTE MIKROORGANISMER UNDER SIBCA I

STEDP-WD-L February 25, 1999

MEMORANDUM FOR RECORD

SUBJECT: Certificate of Non-Viability

1. This package contains one tube that is a buffer blank (PBS) and four tubes that contain one of the following materials. Due to the nature of this test, the tubes are not identified. However, the materials were inactivated (cobalt irradiation) according to the following radiation doses and exposure time:

a. Spores *(Bacillus anthracis)* 4.0×10^6 rads total dose, over a period of 376 mins.
b. Vegetative bacteria *(Yersinia pestis, Vibrio cholerae, Francisella tularensis, Brucella melintensis, Burkholderia mallei)* 3.0×10^6 rads total dose, over a period of 226 mins.
c. Virus (Venezuelan equine encephalomyeltis, Yellow fever, Vaccinia) 8.0 x 106 rads total dose, over a period of 711 mins.
d. Rickettsia *(Coxiella bumettii)* 3.0×10^6 rads total dose, over a period of 114 mins.

The above values are actual doses for a given sample. The samples were irradiated at the U.S. Army Medical Research Institute of Infectious Diseases, Radiation Safety Office.

2. The samples were certified non-viable (killed or inactivated) with the following protocols:

a. Spores and vegetative bacteria: The following procedure describes the method used at the Life Sciences Division of the West Desert Test Center of U.S. Army Dugway Proving Ground, Utah, to determine sterility of bacterial suspensions which have been inactivated:

1. Ensure that the suspension is thoroughly mixed to homogeneity. This may be done by vortex mixing or using a sterile stir bar or some other method (depending on sample size).

2. Aseptically transfer 0.1 ml of concentrated suspension into each of three tubes of sterile broth that is suitable for the bacterium of question.

3. Mix and incubate at the temperature appropriate for the bacterium for 72 hours.

4. Mix the contents of each tube and aseptically transfer I ml of each tube's fluid to each of three agar plates (previously shown to be clear of contamination) and incubate for 48 hours.

5. If no growth occurs on the agar plates, then the suspension is considered free of viable bacteria of the type in question. (Positive and negative controls are included in the assay for validation of the protocol).

The materials commonly assayed at the Life Sciences Division are concentrated to the order of 10^{10} cfu/ml. Consequently, 0.1 ml would contain 10^{9} cfu and a result of no growth in the sterility test would indicate a minimum of a 9logarithmic reduction in viability.

b. Viruses. Vaccinia and VEE are certified dead with a plaque assay. The virus is diluted 1:10 in MEM without serum. A 0.4 ml sample is added to each of two wells in a 6 well micro-titer plate (confluent cell sheet BSC-1 cells for vaccinia assay, and Vero cells for VEE virus assay). The virus is adsorbed for I hr (with rocking) and overlayed with soft agar. After 72 hrs, the overlay is removed, and the cell sheet stained with crystal violet. Positive and negative controls are included in the assay for validation of the protocol. No plaques are observed in the cobalt-irradiated samples or negative controls.

The virus samples were greater than 10^{10} pfu/mI. A 0.4ml sample of a 1:10 dilution would indicate greater than 8 log reduction in virus viability.

Coxiella. *Coxiella bumetii* is certified deadthrough passage of the cobalt treated material in mice (twice), followed by an infectious dose assay in ntice. A 0.1 ml sample of cobalt irradiated material is injected (i.p.) into each of 5 mice. After 28 days, the spleens are harvested, minced, and the material injected again into mice (i.p., 0.5 ml each). After 28 days, the spleens are harvested again, and injected into a 3rd set of mice. The serum is collected after 28 days, and tested for coxiella antibodies using ELISA. An increase of 2 standard deviations above negative controls is considered a positive antibody response (meaning the injected material has viable coxiella organisms). Positive and negative controls are included in the assay for validation of the protocol.

This coxiella sample tested negative for infectious organisms. The concentration of coxiella in the material is greater than 10^{11} organisms/ml (based on OD of purified coxiella). This test shows greater than 10 log reduction in viable organisms.

3. Based on our knowledge of the sterilization procedure used in paragraph 1, and based on the results of the confirmation procedure used in paragraph 2, we certify, to the best of our knowledge, that the above listed organisms (of which some of them are included in this package) are non-viable.

BRUCE G. HARPER
Project Officer

STEPHEN L. PARKER
Biosafety Officer
Life Sciences Division

ALAN JEFF MOHR
Chief, Life Sciences Divisioin

B SERTIFIKAT PÅ INAKTIVERTE MIKROORGANISMER UNDER SIBCA II

CSTE-DTC-DP-WD-L January 27, 2000

MEMORANDUM FOR RECORD

SUBJECT: Certificate of Non-Viability

1. This package contains six tubes (10 mls each) that contain one or more of the following materials. Due to the nature of this test, the tubes are not identified. However, the materials were inactivated (cobalt irradiation) according to the following radiati6n doses and exposure time:

a. Spores *(Bacillus anthracis)*. 4.0 x 10^6 rads total dose, over a period of 376 mins.
b. Vegetative bacteria *(Yersinia pestis, Vibrio cholerae,. Francisella tularensis, Brucella melintensis, Burkholderia mallei)*. 3.0 x 10^6 rads total dose, over a period of 226 mins.
c. Virus (Venezuelan equine encephalomyeltis, Yellow fever, Vaccinia). 8.0 x 10^6 rads total dose, over a period of 7 11 mins.
d. Rickettsia *(Coxiella bumettii)*. 3.0 x 10^6 rads total dose, over a period of 114 mins.
e. Environmental sample. No irradiation, this material is non-infectious.

The above values are actual doses for a given sample. The samples were irradiated at the U.S. Army Medical Research Institute of Infectious Diseases, Radiation Safety Office.

2. The samples were certified non-viable (killed or inactivated) with the following protocols:

a. Spores and vegetative bacteria: The following procedure describes the method used at the Life Sciences Division of the West Desert Test Center of U.S. Army Dugway Proving Ground, Utah, to determine sterility of bacteria] suspensions which have been inactivated:

1. Ensure that the suspension is thoroughly mixed to homogeneity. This may be done by vortex mixing or using a sterile stir bar or some other method (depending on sample size).
2. Aseptically transfer 0. 1 ml of concentrated suspension into each of three tubes of sterile broth that is suitable for the bacterium of question.
3. Mix and incubate at the temperature appropriate for the bacterium for 72 hours.
4. Mix the contents of each tube and aseptically transfer I ml of each tube's fluid to each of three agar plates (previously shown to be clear of contamination) and incubate for *48* hours.
5. If no growth occurs on the agar plates, then the suspension is considered free of viable bacteria of the type in question. (Positive and negative controls are included in the assay for validation of the protocol).

The materials cornmonly assayed at the Life Sciences Division are concentrated to the order of 10^{10} cfu/ml. Consequently, 0.1 ml would contain 10^9 cfu and a result of no growth in the sterility test would indicate a minimum of a 9 logarithmic reduction in viability.

b. Viruses. Vaccinia and VEE are certified dead with a plaque assay. The virus is diluted 1:10 in MEM without serum. A 0.4 ml sample is added to each of two wells in a 6 well micro-titer plate (confluent cell sheet BSC-1 cells for vaccinia assay, and Vero cells for VEE virus assay). The virus is adsorbed for I hr (with rocking) and overlayed with soft agar. After 72 hrs, the overlay is removed, and the cell sheet stained with crystal violet. Positive and negative controls are included in the assay for validation of the protocol. No plaques are observed in the cobalt-irradiated samples or negative controls.

The virus samples were greater than 10^8 pfu/ml. A 0.4ml sample of a 1: 10 dilution would indicate greater than 6 log reduction in virus viability.

Coxiella. *Coxiella bumetii* is certified dead through passage of the cobalt treated material in mice (twice), followed by an infectious dose assay in mice. A 0.1 ml sample of cobalt irradiated material is injected (i.p.) into each of 5 mice. After 28 days, the spleens are harvested, minced, and the material injected again into mice (i.p., 0.5ml each). After 28 days, the spleens are harvested again, and injected a 3rd set of mice. The serum is collected after 28 days, and tested for coxiella antibodies using ELISA. An increase of 2 standard deviations above negative controls is considered a positive antibody response (meaning the injected material has viable coxiella organisms). Positive and negative controls are included in the assay for validation of the protocol.

This coxiella sample tested negative for infectious organisms. The concentration of coxiella in the material is greater than 10^{11} organisms/ml (based on OD of purified coxiella). This test shows greater than 10 log reduction in viable organisms.

3. Based on our knowledge of the sterilization procedure used in paragraph 1, and based on the results of the confirmation procedure used in paragraph 2, we certify, to the best of our knowledge, that the above listed organisms (of which some of them are included in this package) are non-viable.

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Project Officer

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Biosafety Officer
Life Sciences Division

ALAN JEFF MOHR
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C MATERIALER FRA SIBCA II

Sekvenser og annealingstemperaturer til primerne anvendt under SIBCA II

Primer	Organisme	Gen	Annealing temp.[a]	Sekvens
16S rRNA-1	Bacteria	16S rRNA, universal region	68°C	5'- gaa cgc tgg cgg cag gcc taa -3'
16S rRNA-2	Bacteria	16S rRNA, universal region	68°C	5'- ggt aag gtt ctt cgc gtt gca t -3'
UP16S-1	Bacteria	16S rRNA, universal region	68°C	5'- gag ttt gat cct ggc tca g-3'
UP16S-2	Bacteria	16S rRNA, universal region	68°C	5'- aga aag gag gtg atc cag cc -3'
VCet-1	*Vibrio cholerae*	Enterotoxin	58°C	5'- ggc aga ttc tag acc tcc t -3'
VCet-2	*Vibrio cholerae*	Enterotoxin	58°C	5'- tcg atg atc ttg gag cat tc -3'
VCctx-1	*Vibrio cholerae*	Vibrio cholerae toxin	55°C	5'- ctc aga cgg gat ttg tta ggc acg -3'
VCctx-2	*Vibrio cholerae*	Vibrio cholerae toxin	55°C	5'- tct atc tct gta gcc ggt att acg -3'
FT16s-1	*Francisella tularensis*	16S rRNA	62°C	5'- cct ttt tga gtt tcg ctc c -3'
FT16s-2	*Francisella tularensis*	16S rRNA	62°C	5'- tac cag ttg gaa acg act gt -3'
FT-1	*Francisella tularensis*	T-cell epitopes of membr. prot.	55°C	5'- tag gat ccc att agc tgt cca ctt acc -3'
FT-2	*Francisella tularensis*	T-cell epitopes of membr. prot.	55°C	5'- gga att cgt tag gtg gct ctg atg at -3'
TUL-1	*Francisella tularensis*	17kDa lipoprotein	59°C	5'- tat caa tcg cag gtt tag c-3'
TUL-2	*Francisella tularensis*	17kDa lipoprotein	59°C	5'- tcg ttc ttc tca gca tac tta g-3'
FNA8L	*Francisella tularensis*	Major outer membrane protein	55°C	5'- cga gga gtc tca atg tac taa ggt ttg ccc-3'
FNB2L	*Francisella tularensis*	Major outer membrane protein	55°C	5'- cac cat tat cct gga tat tac cag tgt cat -3'
FNA7L	*Francisella tularensis*	Major outer membrane protein	55°C	5'- ctt gag tct tat gtt tcg gca tgt gaa tag -3'
FNB1L	*Francisella tularensis*	Major outer membrane protein	55°C	5'- cca act aat tgg ttg tac tgt aca gcg aag-3'
BA813-1	*Bacillus anthracis*	BA813	58°C	5'- tta att cac ttg caa ctg atg gg -3'
BA813-2	*Bacillus anthracis*	BA813	58°C	5'- aac gat agc tcc tac att tgg ag -3'
BAlef-1	*Bacillus anthracis*	Lethal factor	60°C	5'- gga tat gaa ccc gta ctt gta a -3'
BAlef-2*	*Bacillus anthracis*	Lethal factor	60°C	5'- aag tct gtg gga tgt tcc tt -3'
BM23s-1	*Burkholderia mallei*	23S-rDNA	47°C	5'- aaa ccg aca cag gtg g -3'
BM23s-2	*Burkholderia mallei*	23S-rDNA	47°C	5'- cac cga aac tag ca -3'
BM16s-1	*Burkholderia mallei*	16S-rDNA	68°C	5'-gcc cgt cac acc atg gga g-3'
BM16s-2	*Burkholderia mallei*	16S-rDNA	68°C	5'- tcg cct (gc)tg (ag) (ag)g cca agg c-3'
YPpla-1	*Yersinia pestis*	Plasminogen activator	60°C	5'- tgc ttt atg acg cag aaa cag g -3'
YPpla-2	*Yersinia pestis*	Plasminogen activator	60°C	5'- ctg tag ctg tcc aac tga aac g -3'
YPcaf-1	*Yersinia pestis*	Antigen fraction I	58°C	5'- cag ttc cgt tat cgc cat tgc-3'
YPcaf-2	*Yersinia pestis*	Antigen fraction I	58°C	5'- tat tgg tta gat acg gtt acg gt-3'
Bspp16s-1	*Brucella melitensis*	16S rRNA	54°C	5'- tcg agc gcc cgc aag ggg -3'
Bspp16s-2	*Brucella melitensis*	16S rRNA	54°C	5'- aac cat agt gtc tcc act aa -3'
BMomp-1	*Brucella melitensis*	Outer membrane protein	60°C	5'- gcg ctc agg ctg ccg acg caa -3'
BMomp-2	*Brucella melitensis*	Outer membrane protein	60°C	5'- acc agc cat tgc ggt cgg ta -3'
BaB4	*Brucella melitensis*	31 kDa antigen	60°C	5'- tgg ctc ggt tgc caa tat caa-3'
BaB5	*Brucella melitensis*	31 kDa antigen	60°C	5'- cgc gct tgc ctt tca ggt ctg-3'
CB-1	*Coxiella burnetii*	27-kDa OMP	60°C	5'- agt aga agc atc cca agc att-3'
CB-1*	*Coxiella burnetii*	27-kDa OMP	60°C	5'- agt gtc gac ggc caa tta tc -3'
CB-2	*Coxiella burnetii*	27-kDa OMP	60°C	5'- tgc ctg cta gct gta acg att -3'
YFns3-1	Yellow fever virus	Non-structural protein	50°C	5'- ggc act tca gga tct cc -3'
YFns3-2	Yellow fever virus	Non-structural protein	50°C	5'- atg gat gaa gcc cat tt -3'
VACha-1	Vaccinia virus	Hemaglutinin	55°C	5'- atg caa ctc tat cat gta a -3'
VACha-2	Vaccinia virus	Hemaglutinin	55°C	5'- cat aat cta ctt tat cag tg -3'
VACati-1	Vaccinia virus	Acidophilic inclusion protein	40°C	5'- aat aca agg agg atc t -3'
VACati-2	Vaccinia virus	Acidophilic inclusion protein	40°C	5'- ctt aac ttt ttc ttt ctc -3'
VEETC83-1	VEE virus	none structural gene (nsP1)	58°C	5'- ata atg acc atg cta atg cc-3'
VEETC83-2	VEE virus	none structural gene (nsP1)	58°C	5'- ttc agc tta gtt gca tac tt -3'

Sekvensene til hybridiseringsprobene anvendt under SIBCA II

Organisme	Primer	Sekvens
Bacillus anthracis	Balef-FL	cct att tca taa taa acg ttc agt gcc
Bacillus anthracis	Balef-705	ttt cag tat ttt cta cat aat ctt ccg aag
Bacillus anthracis	Ba813-FL	ata gaa cct ggc att aaa aga ctc att ga
Bacillus anthracis	Ba813-640	aac tcg tta atg ctt caa att ctg tgt tt
Coxiella burnetii	CB-FL	cca tgt ctt ttt tga gct tga gca aca t
Coxiella burnetii	CB-640	aat cct act ttt tct gcg gtt tga agg
Coxiella burnetii	bchem-FL	cta ata aac tcg gtt gat tgt ctg caa
Coxiella burnetii	bchem-640	agc ttt att ggc aag ttg tac agc tc

Lengden til PCR-produktene

Organisme	Primer	Lengde (bp)
Bacteria	16S rRNA-1/2	950
Bacteria	UP16S-1/2	1550
Vibrio cholerae	VCet-1/2	563
Vibrio cholerae	VCctx-1/2	302
Francisella tularensis	FT16s-1/2	1142
Francisella tularensis	FT-1/2	347
Francisella tularensis	TUL-1/2	300
Francisella tularensis	FNA8L/ FNB2L	900
Francisella tularensis	FNA7L/ FNB1L	409
Bacillus anthracis	BA813-1/2	152
Bacillus anthracis	BAlef-1/2*	223
Burkholderia mallei	BM23s-1/2	526
Burkholderia mallei	BM16s-1/2	603
Yersinia pestis	YPpla-1/2	344
Brucella melitensis	Bspp16s-1/2	905
Brucella melitensis	BMomp-1/2	193
Brucella melitensis	BAB4/BAB5	223
Coxiella burnetii	CB-1/2	501
Coxiella burnetii	CB-1*/2	157
Yellow fever virus	YFns3-1/2	470
Vaccinia virus	VACha-1/2	273
Vaccinia virus	VACati-1/2	1603
VEE-virus	VEETC83-1/2	200

Kjemikalier benyttet til PCR, RT-PCR og DNA sekvensering under SIBCA II

Kjemikalium	Leverandør
10 X PCR-buffer	Stratagene
25 mM $MgCl_2$	Roche Molecular Biochemicals
100 mM dNTP	Stratagene
5 U/µl Taq2000™ DNA polymerase	Stratagene
Primers	Eurogentec
GeneAmp RNA PCR kit	Perkin Elmer
Lamda DNA-BstE II Digest (500 µg/ml)	New England Biolabs
Ethidium bromide	KEBOlab
Xylenecyanol	Merck
Bromophnol blue	KEBOlab
EDTA	Sigma
Agarose	KEBOlab
Sucrose	KEBOlab
Tris base	Sigma
Boric acid	Merck

Acrylamide Bis-Acrylamide (19:1) Solution	Bio-Rad
Thermo Sequenase Cycle Sequencing Kit	USB
Thermo Sequenase Radiolabelled Terminator Cycle Sequencing Kit	USB
Redivue dideoxyribonucleotide ^{33}P triphosphates	USB
Redivue [α ^{35}S] dideoxyribonucleotide triphosphates	USB
Concert Rapid PCR Purification System	Life Technologies
Light Cycler™ – DNA Master SYBR Green I	Roche Molecular Biochemicals
Light Cycler™ – DNA Master Hybridization probes	Roche Molecular Biochemicals
Light Cycler™ – RNA Amplification Kit SYBR Green I	Roche Molecular Biochemicals
Taurine	Bio-Rad
Urea	Life Technologies
APS (ammonium persulfat)	Bio-Rad
TEMED (N, N, N, N'-tetrametylendiamine)	Sigma
Non-toxic Gel Plate Coating	FMC
100% Acetic acid	Merck
Methanol	Merck

Standard reaksjonsblanding til "real-time" og konvensjonell PCR

	Konsentrasjon
"Real-time" PCR:	
Light Cycler™ – DNA Master SYBR Green I buffer[a]	1 X
$MgCl_2$	3,5 mM
Primers	1 µM (each)
Conventional PCR:	
PCR-buffer[b]	1 X
$MgCl_2$	2 mM
dNTP[c]	200 µM (each)
Taq DNA polymerase	1,5 U
Primers	0,2 µM (each)

[a] 10 X Light Cycler™ – DNA Master SYBR Green I buffer contain Taq DNA polymerase, reaction buffer, dNTP (with dUTP), SYBR Green I dye and 10 mM $MgCl_2$
[b] 10 X PCR-buffer contain 100 mM Tris-HCl (pH 8.8), 500 mM KCl, 15 mM $MgCl_2$, 0,01% (w/v) gelatine
[c] dNTP=dATP, dCTP, dGTP dTTP

Standard temperaturprogram benyttet til "real-time" og konvensjonell PCR

	Temperatur	Tid	Sykluser
"Real-time" PCR:			
(for products shorter than 350 bp)			
Initial denaturation	94°C	3 min.	1
Denaturation	94°C	0 sek.	30-40
Annealing	58°C	5 sek.	30-40
Extension	72°C	15 sek.	30-40
"Real-time" PCR:			
(for products between 350 and 1000 bp)			
Initial denaturation	94°C	3 min.	1
Denaturation	94°C	0 sek.	30-40
Annealing	58°C	10 sek.	30-40
Extension	72°C	30 sek.	30-40
Conventional PCR:			
Initial denaturation	94°C	3 min.	1
Denaturation	94°C	1 min.	35
Annealing	58°C	1 min.	35
Extension	72°C	1 min, 30 sec.	35
Final extension	72°C	7 min.	1

Reaksjonsblandinger for hybridiseringsprobene

Organisme	Probe FL (µM)	Probe LC-640/705 (µM)	Primer (µM)	$MgCl_2$ (mM)	LC Hybprobe mix	Annealing temp. (°C)
Bacillus anthracis (BA813)	0,2	0,2	1	3,5	1 X	58
Bacillus anthracis (BAlef)	0,2	0,2	1	3,5	1 X	58
Coxiella burnetii (CB)	0,2	0,2	1	3,5	1 X	58

Optimale PCR-betingelser benyttet for ulike primerpar

Primer	PCR-buffer	$MgCl_2$ (mM)	SYBR Green I	Taq DNA polymerase (U)	Primer (µM)	dNTP[b] (µM)	Annealing temp.(°C)
VCet-1/2	-	3,5	1 X	-	1	-	55
FNA8L/FNB2L	-	3,5	1X	-	1	-	55
FNA8L/FNB2L	-	3,5	1X	-	1	-	55
UP16S-1/2	1X	4,5	-	1,5	1	0,4	68
BM16S-1/2	-	3,5	1X	-	1	-	68
BM23S-1/2	-	3,5	1X	-	1	-	47
FT16S-1/2	1X	3,5	-	1,5	0,2	0,4	62
VACati-1 and 2	1X	2,5	-	1,5	1	0,4	40/50

Reaksjonsblanding til transkripsjonen av RNA. Totalvolumet var 20 µl

Reagens	Konsentrasjon
LightCycler™ – RT-PCR enzyme mix[a]	50 X dilution of stock
LightCycler™ – RT-PCR reaction mix. SYBR Green I	1 X
$MgCl_2$	3 mM
Primers	1 µM (each)

[a]Concentration not specified by the manufacturer of the kit

Temperature program used for RT-PCR

	Temperatur	Tid	Sykluser	Steg
Reverse transcription	55°C	10 min.	1	RT
Denaturation	95°C	3 min.	1	RT
Denaturation	95°C	0 sek.	50	PCR
Annealing	55°C	10 sek.	50	PCR
Extension	72°C	20 sek.	50	PCR

D ARRANGØR-RAPPORT FRA SIBCA I

SIBCA Round Robin Exercise

U.S. Army Dugway Proving Ground

Bruce G. Harper, Ph.D.

West Desert Test Center

Objective

- 1. To evaluate the capability of NATO laboratories to detect and identify BW materials (killed).
- 2. To compile a data report of instrumentation and techniques used in the exercise, both those that worked, and those that did not.

Approach

- Prepare samples of killed (gamma irradiation) BW
  - 5 unknowns (one is a buffer blank)
  - 10 ml sample
    - Bacteria: 10^6-10^7 cfu/ml
    - Viruses/Rickettsia: 10^7-10^8pfu/ml

List of Possible Test Organisms

- *Bacillus anthracis*
- *Coxiella burnetii*
- *Yersinia pestis*
- *Francisella tularensis*
- *Vibrio cholerae*
- *Brucella melintensis*
- VEE virus
- *Burkholderia mallei*
- Vaccinia virus
- Yellow Fever Virus

Time Schedule

- Samples were Fed Exp 2 March (and 22 March for Canada)
- Samples were received 5, 8, 10, 24 Mar)
- First Results reported 8 March (TNO)

Organisms Used

- *Coxiella burnetii,* 9 Mile, phase I
  - 2.1×10^7 ID_{50}/ml
  - 2.3 *u*g/ml (optical density)
- *Yersinia pestis,* Lapaz strain
  - 3.7×10^7 cfu/ml

Organisms Used

- VEE virus, vaccine strain TC83
  - 7.5×10^{8} $TCID_{50}$/ml
  - 0.5 *u*g/ml (protein)
- *Bacillus anthracis*, vollum strain
  - 8.7×10^{6} cfu/ml
  - 6.1 ug/ml (protein)

Test Participants

- Canada (DRES)
- France (CEB)
- Germany (WIS ABC)
- Germany II (IMSB)
- Hungary
- Italy (CTM)
- Netherlands (TNO)
- Norway (NDRE)
- Poland (MIHE)
- United Kingdom (CBD)
- United States (NMRC)

Test Results (10 Labs)

	Correct	False Pos	False Neg
PBS	9	1 (vaccinia)	0
B.anthracis	10	0	0
C.burnetii	9	1 (vaccinia)	1*
VEE	2	2 (vaccinia)	6*
Y.pestis	9	1 (vaccinia)	1

* No tests for VEE and Coxiella

Technologies Used

Canada

- LAPS
  - Not available for Vibrio, VEE, YFV, Coxiella
  - Pos ID for B.*anthracis* and Y.*pestis*
- HHTK (New Horizons)
  - Not available for VEE, YFV, Coxiella
  - No positive ID's
- HHTK (ARISTA)
  - Not available for VEE, YFV, Coxiella
  - Pos ID for *B.anthracis* and *Y.pestis*

Data Report

- ✦ Methods and Protocols
- ✦ Results (both Positive and Negative)
- ✦ Lessons Learned

Issues

- – shipping and permits
- – cobalt irradiation
- – temperature
- – death certificates
- – concentration

Technologies Used
United States (NMRC)

- Antigen capture ELISA
  - not available for YFV
  - Pos ID for *B.anthracis*, *Y.pestis*, Coxiella
- Hand Held Assay
  - not available for YFV and Burkholderia
  - Pos ID for *B.anthracis*, *Y.pestis*, Coxiella

Technologies Used
Poland (WIHiE)

- Not Available at this time
  - *Pos ID for B.anthracis, Coxiella, and* Y.pestis
  - VEE sample identified as vaccinia

Technologies Used

Italy (CTM)

- PCR (16s rRNA, RT-PCR, and specific)
  - Pos ID for *B.anthracis* and Coxiella

Technologies Used

Hungary

- Gram stain
- Direct IF Microscopy
  - Still working on the samples

Technologies Used
Netherlands (TNO)

- Gram Stain
- Immunoblot (available for YFV, VEE, Vaccinia, Burkholderia, Vibrio, and Brucella)
  - Pos ID for vaccinia
- ELISA (available for VEE, vaccinia, *Y.pestis*, *F.tularensis*, Coxiella, and *B.anthracis*)
  - Pos ID for *Y.pestis* and *B.anthracis*

Technologies Used
Netherlands (TNO)

- Immunofluorescence (available for Brucella, Burkholderia, Coxiella, *Y.pestis*, and Vibrio)
  - Pos ID for *Y.pestis*
- PCR (protocol for all test organisms)
  - Pos ID for *B.anthracis*, Coxiella, *Y.pestis*, and vaccinia

Technologies Used

France (CEB)

- Immunofluorescence
  - Pos ID for *B.anthracis*, Coxiella, *Y.pestis*
- PCR (Bacteria only)
  - 16s rRNA: Pos ID for *B.anthracis*, Coxiella
  - specific DNA sequences: Pos ID for Coxiella, *Y.pestis, B.anthracis,*
- RT-PCR (Alphavirus and Flavivirus)
  - neg for all samples
- Fluorescence Microscopy (Hoechst 33258)
  - Pos ID for vaccinia

Technologies Used

Norway (NDRE)

- PCR (16s rRNA)
  - Pos ID for *Y.pestis*, Coxiella, *B.anthracis*
- PCR and RT-PCR (specific primers)
  - Pos ID for VEE, Coxiella, *Y.pestis, B.anthracis*

Technologies Used
Germany II (IMSB)

- ELISA
  - Pos ID for Coxiella, *B.anthracis, Y.pestis*
- PCR
  - Pos ID for Coxiella, *B.anthracis, Y.pestis*, and VEE (seminested RT-PCR)
- HHTK (Hand Held Ticket)
  - Pos ID for *B.anthracis, Y.pestis*

Technologies Used
United Kingdom (CBD)

- PCR
  - Pos ID for vaccinia (unvalidated), Coxiella, *B.anthracis, and Y.pestis*
- ELISA (only tested VEE, Vaccinia, and Coxiella)
  - Pos ID for Coxiella (very weak vaccinia signal)

E ARRANGØR-RAPPORT FRA SIBCA II

SIBCA Round Robin Exercise

Bruce G. Harper, Ph.D.
West Desert Test Center

Dugway Proving Ground

Objective

- **1. To evaluate the capability of NATO laboratories to detect and identify BW materials (killed).**
- **2. To compile a data report of instrumentation and techniques used in the exercise, both those that worked, and those that did not.**

Dugway Proving Ground

Approach

- Prepare samples of killed (gamma irradiation) BW
  - 6 unknowns
  - 2 samples with Environmental Interferants
    - Burning Vegetation
    - Burning Diesel Fuel
  - 10 ml sample
    - Bacteria: 10^6-10^7 cfu/ml
    - Viruses/Rickettsia: 10^7-10^8pfu/ml

Dugway Proving Ground

List of Possible Test Organisms (same as last year)

- *Bacillus anthracis*
- *Coxiella burnetii*
- *Yersinia pestis*
- *Francisella tularensis*
- *Vibrio cholerae*
- *Brucella melintensis*
- VEE virus
- *Burkholderia mallei*
- Vaccinia virus
- Yellow Fever Virus

Dugway Proving Ground

Time Schedule

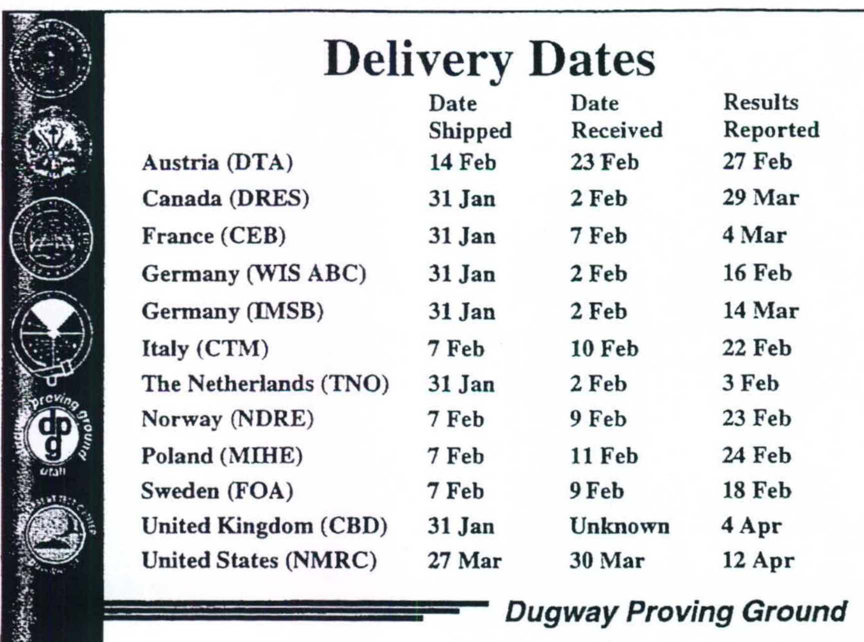
- Samples were sent Fed Exp (with dry ice) on Jan 31 (and 14 Feb for Austria, 27 Mar for USA-NMRC)
- Samples were sent Fed Exp (with ice packs) on Feb 7th (Italy, Norway, Sweden, and Poland)
- First Results reported 3 February (TNO)

Dugway Proving Ground

Test Participants

- Austria (DTA)
- Canada (DRES)
- France (CEB)
- Germany (WIS ABC)
- Germany II (IMSB)
- Italy (CTM)
- The Netherlands (TNO)
- Norway (NDRE)
- Poland (MIHE)
- Sweden (FOA)
- United Kingdom (CBD)
- United States (NMRC)

Dugway Proving Ground



Delivery Dates

	Date Shipped	Date Received	Results Reported
Austria (DTA)	14 Feb	23 Feb	27 Feb
Canada (DRES)	31 Jan	2 Feb	29 Mar
France (CEB)	31 Jan	7 Feb	4 Mar
Germany (WIS ABC)	31 Jan	2 Feb	16 Feb
Germany (IMSB)	31 Jan	2 Feb	14 Mar
Italy (CTM)	7 Feb	10 Feb	22 Feb
The Netherlands (TNO)	31 Jan	2 Feb	3 Feb
Norway (NDRE)	7 Feb	9 Feb	23 Feb
Poland (MIHE)	7 Feb	11 Feb	24 Feb
Sweden (FOA)	7 Feb	9 Feb	18 Feb
United Kingdom (CBD)	31 Jan	Unknown	4 Apr
United States (NMRC)	27 Mar	30 Mar	12 Apr

Dugway Proving Ground

Organisms Used

- *Coxiella burnetii,* 9 Mile, phase I
  - $3.6x10^6$ ID_{50}/ml
  - 0.4 *ug*/ml (optical density)
  - Mixed with environmental sample (burning vegetation)
  - (last year $2.1x10^7$, 2.3 *ug*/ml)
- *Vibrio cholerae,* Inaba strain
  - $1.1x10^7$ cfu/ml
  - 6.7 *ug*/ml
- Vaccinia virus, Lister strain
  - $6.7x10^6$ pfu/ml

Dugway Proving Ground

Organisms Used

- *Bacillus anthracis*, Strain Vollum 1B
  - 8.7×10^6 cfu/ml (same as last year)
  - 6.1 ug/ml
  - Mixed with environmental sample (burning vegetation)
- *Francisella tularensis*, Strain Schu S4
  - 5.2×10^6 cfu/ml
  - 6.7 ug/ml
- *Brucella melitensis melitensis*, biovar 1
  - 2.9×10^7cfu/ml
  - 6.7 ug/ml

Dugway Proving Ground

Test Results 2000

(12 Labs Participating)

	Presumptive/ Confirm	False Pos	False Neg*
B.melitensis	12/3	$1^{(Ft)}$	1
B.anthracis	12/7	$1^{(Bm)}$	0
C.burnetii	10/3	$1^{(Ba)}$	1
V. cholerae	12/6	$3^{(Cb,Bm,Ba)}$	0
Vaccinia	11/6	0	0
F. tularensis	9/6	0	3

* Only marked neg if the country had an assay, and could not detect

Dugway Proving Ground

Technologies Used

- Austria
  - Molecular
    - 16S rRNA
    - Agent-specific PCR
- Canada
  - Imunological
    - ELISA
    - Light Addressable Potentiometric System (LAPS)
  - Molecular
    - 16sRNA
    - Agent-specific PCR

Dugway Proving Ground

Technologies Used

- France
  - Imunological
    - Immunofluresecence
  - Molecular (Bacteria only)
    - 16sRNA
    - Agent-specific PCR

Dugway Proving Ground

Technologies Used

- Germany (WIS-ABC)
  - Light Microscopy (different stains)
  - Transmission Electron Microscopy
  - Immunological
    - ELISA, Immunoflurescence, Immunoblot, Hand-Held-Test-Kit
  - Molecular
    - Agent-specific PCR

Dugway Proving Ground

Technologies Used

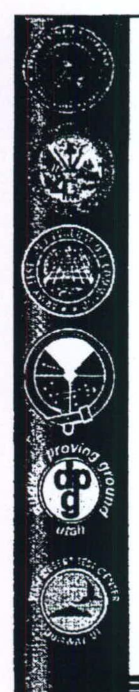
- Germany II (IMSB)
  - Imunological
    - ELISA
    - Immunochromatographic (Hand Held Test Kits, HHTK)
    - Fluorescent Activated Cell Sorter (FACS)
  - Molecular
    - Agent-specific PCR Immunological
- Italy
  - Molecular
    - 16sRNA
    - Agent-specific PCR

Dugway Proving Ground

Technologies Used

- Norway
  - Molecular
    - 16S rRNA
    - Agent-specific PCR
- Poland
  - Imunological
    - ELISA
    - Immunochromatographic (SMART ticket)
  - Molecular
    - Agent-specific PCR

Dugway Proving Ground



Technologies Used

- Sweden
  - Immunological
    - ELISA
    - Immunoblotting
    - Fluorescent Activated Cell Sorter (FACS)
  - Molecular
    - 16S rRNA
    - Agent-specific PCR
- United Kingdom
  - Imunological
    - Yes...awaiting report
  - Molecular
    - Agent-specific PCR

Dugway Proving Ground

Technologies Used

- United States (NMRC)
  - Imunological
    - ELISA
    - Immunochromatographic (SMART ticket)
  - Molecular
    - Agent-specific PCR

Dugway Proving Ground

Data Report

- Methods and Protocols
  - Still waiting for formal reports from a few countries
- Results (both Positive and Negative)
- Lessons Learned

Dugway Proving Ground

Issues

- shipping and permits
  - Canada permits were OK this year
  - Austria, need to improve
- cobalt irradiation
  - We have started Beta propriolactone inactivation, especially for viruses
- temperature
  - 3 countries received warm samples
    - France, Austria,
  - Dry Ice is a problem into some countries
  - DHL service
    - company has a -20 C box, lasts 80 hrs

Dugway Proving Ground

Issues

- Next Year
  - Live vrs Dead
    - Live will introduce a whole different set of obstacles for shipping...
  - Field sampling exercise?
    - Battle field scenario
    - Use of killed pathogens outdoors
      - special environmental permits required

Dugway Proving Ground

Litteratur

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(4) Campbell, N. A. (1990): The Immune System In: *Biology*, The Benjamin/Cummings Publishing Company, Inc., California, 849-873.

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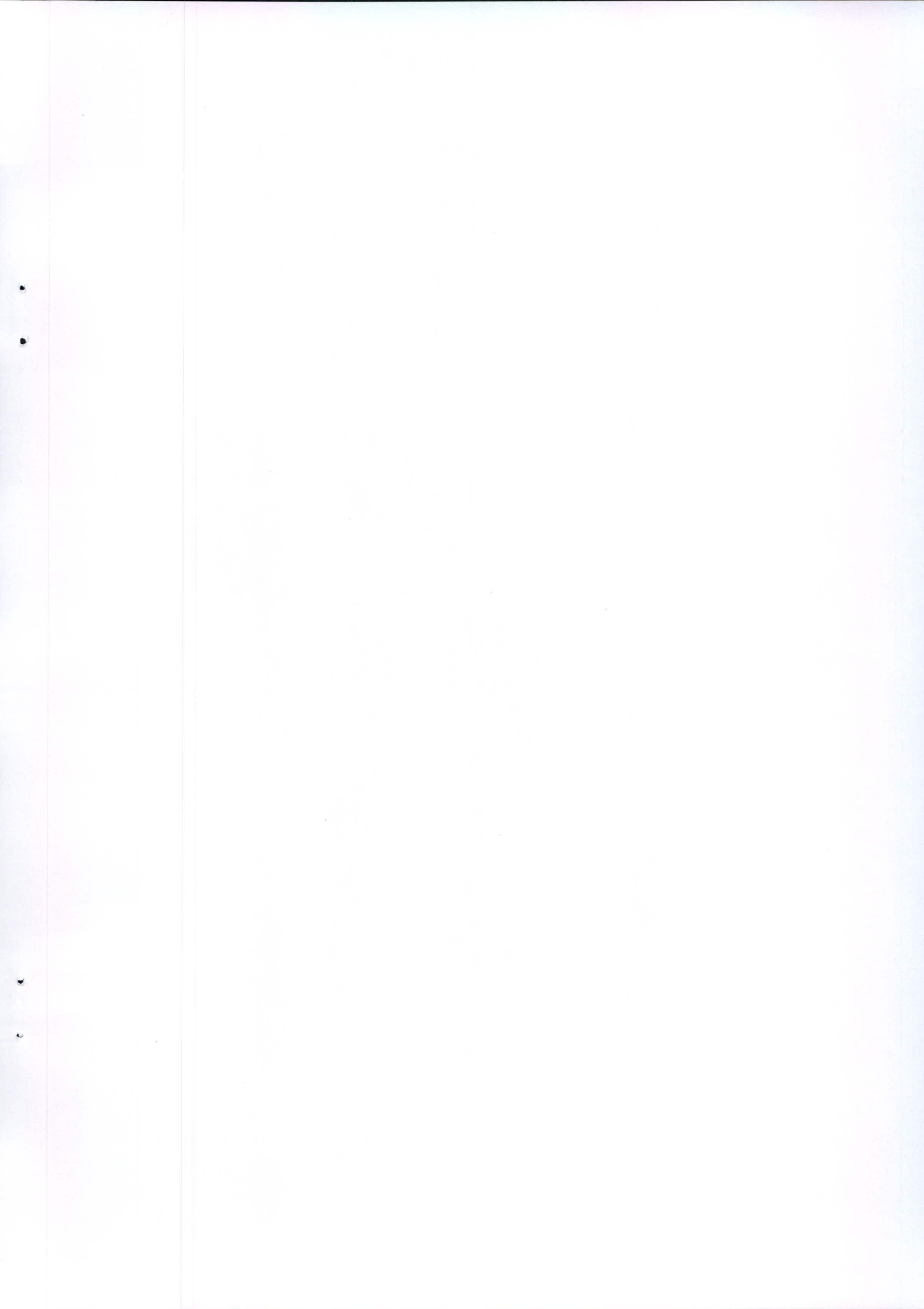

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RAPPORTENS BESKYTTELSESGRAD		ANTALL EKS UTSTEDT	ANTALL SIDER
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RAPPORTENS TITTEL		FORFATTER(E)	
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