THE R.A.P.I.D. SYSTEM

-Rapid Response in Detection of Biological Agents

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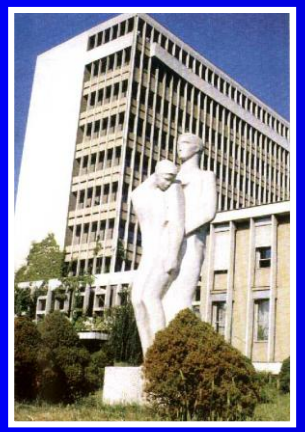


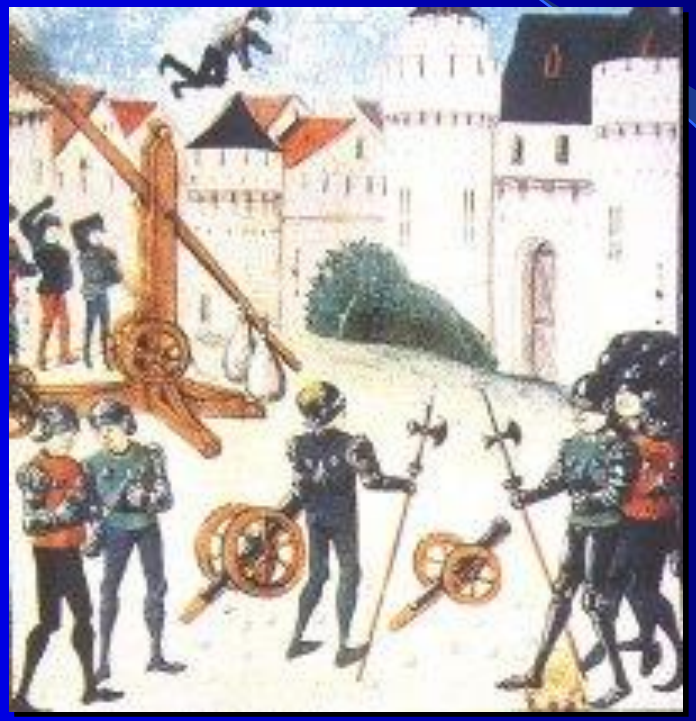
Biological Agents - History

- Oldest of the WMD Agents
- Used for > 2,000 Years

❖ Biological Agents Used By The Romans, Using Dead Animals To Foul The Water Supply Of Their Enemies

❖ Smallpox Blankets Given to Native Americans by Spanish Conquistadors

Middle Ages



- **1346 - Infected Bodies Catapulted Into City During the Siege of Kaffa**

18th Century

- Russian Troops Reportedly Used Plague-Infected Corpses Against the Swedes
- The English in North America Provided Blankets to the Native Population Infected With Smallpox

World War I

- German Agents Use Anthrax to Infect Livestock and Feed for Export to Allied Forces

Between the World Wars

- 1937 – Japan Begins Its Offensive Biological Weapons Program
- 1939 – First Use of Biological Weapons by Japanese

World War II

- The Japanese Army Sprayed Parts of China With Bubonic Plague

World War II

- 1942 – The United States Began Studying Methods to Produce Biological Agents As Weapons
- 1943 – The First Biological Bomb Is Made by the US With the Help of the British

1979

The City of Sverdlovsk

- Anthrax Was Released Killing an Estimated 200 to 1000 People

- 1985-1991 – Iraq Develops an Offensive Biological Weapons Capability

•During October 19-21, 2001, four postal workers at the Brentwood Mail Processing and Distribution Center in Washington, D.C. were hospitalized with inhalational anthrax. This post office was believed to have been contaminated by letters sent to Senators Daschle and Leahy, which were discovered to contain *Bacillus anthracis* spores and to have contaminated the Hart Senate Office building.

Epidemiologic Clues

Biological Agents - Types and Characteristics

- Viruses
- Bacteria
- Toxins

✓ **Anti Human**
✓ **Anti Plants**
✓ **Anti Animal**

- Large Epidemic With High Illness and Death Rate
- HIV(+) Individuals May Have First Susceptibility
- Respiratory Symptoms Predominate
- Infection Non-Endemic for Region
- Multiple, Simultaneous Outbreaks
- Multi-Drug-Resistant Pathogens
- Sick or Dead Animals
- Delivery Vehicle or Intelligence Information

❖BW EXPOSURE:

❖BACTERIAL AGENTS

- –LETHAL: Anthrax, Tularemia, Plague
- –NON-LETHAL: Q Fever, Brucellosis

❖VIRAL AGENTS

- –LETHAL: Smallpox/Monkeypox, Viral Hemorrhagic Fevers
- –NON-LETHAL: Venezuelan Equine Encephalitis

❖TOXINS

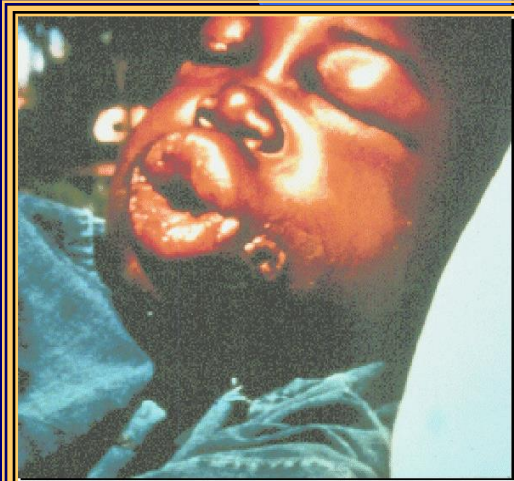
- –LETHAL: Ricin, Botulinum Toxin A
- –NON-LETHAL: Staphyloccal Enterotoxin B

- **Highly pathogenic Avian influenza virus is on every 'top ten' list available for potential agricultural bio-weapon agents, generally following Foot and mouth disease virus and Newcastle disease virus at or near the top of the list.**

Anthrax - Pathogenesis

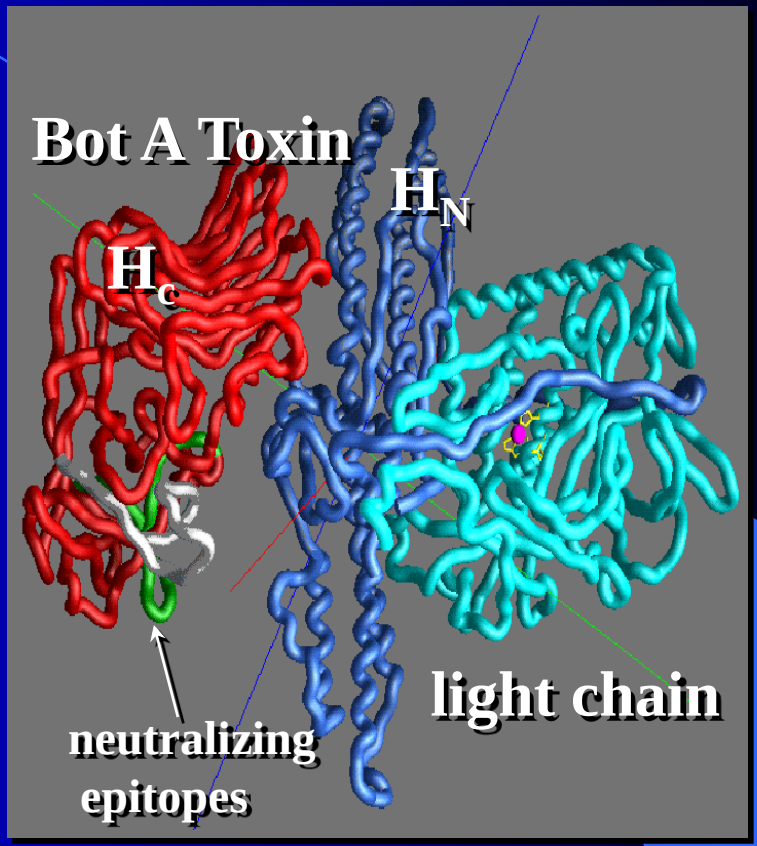
- **Inoculation, Ingestion, or Inhalation of Spores Which May Travel to the Regional Lymph Nodes**
- **Vegetative Bacteria Produce Edema Factor and Lethal Factor (Toxins)**
- **Inhalation Route Has Highest Mortality and Is Most Likely Route to Be Used Intentionally**
- **Inhaled Anthrax Causes a Mediastinitis Rather Than a Pneumonia**

ID 8000 do 50 000 Spores
Mort. 100% T / NT 99%
P>P NO
Inc 1-6 days



Botulinum Toxin - Characteristics

- Neurotoxin Produced by *Clostridium Botulinum* - Botulism
- Most Lethal Compound Per Weight (15,000 Times More Toxic Than the Nerve Agent VX)
- Different Toxicity If Inhaled or Ingested



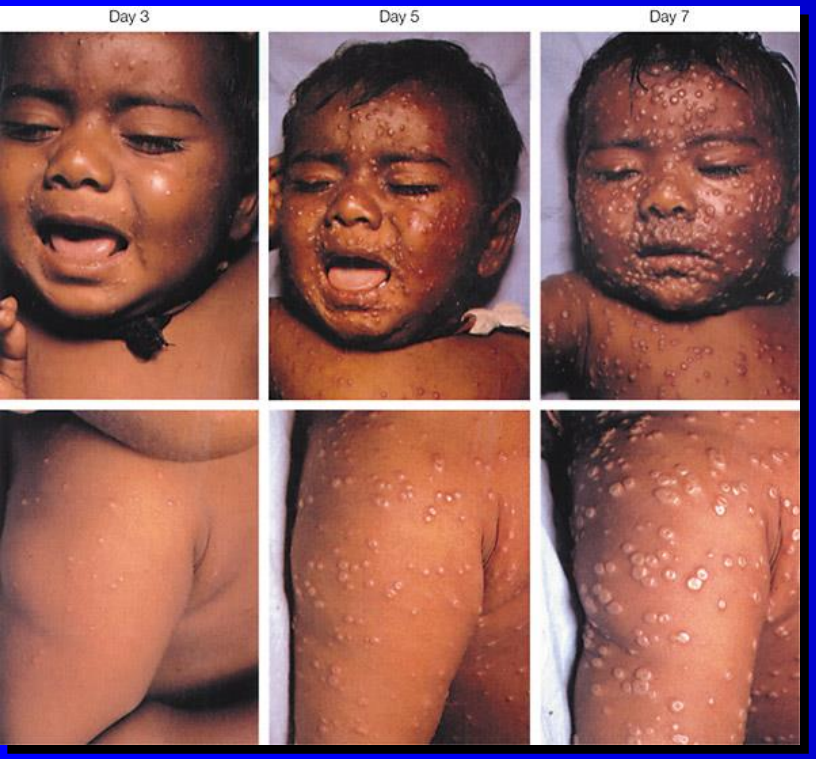
ID 0,001 µg/kg

Mort. 5-10%

P>P NO

Inc 6h to 10 days

Smallpox



- *Variola* (Var-ï-óla) Virus, an Orthopox Virus, Both Minor and Major Forms of Smallpox Exist
- Structure Is a Large DNA Virus
- Declared Eradicated in 1980

ID 10-100 mo

Mort. 20-50%

P>P YES

Inc 7-17 days

Plague

Plague - Acral Gangrene

- *Yersinia Pestis* - Gram(-), Non-Motile, Non-Spore Forming Bacillus
- Fleas Living on Infected Rodents Spread Infection to Humans
- Recovery Offers Temporary Immunity

ID <100 m.o.

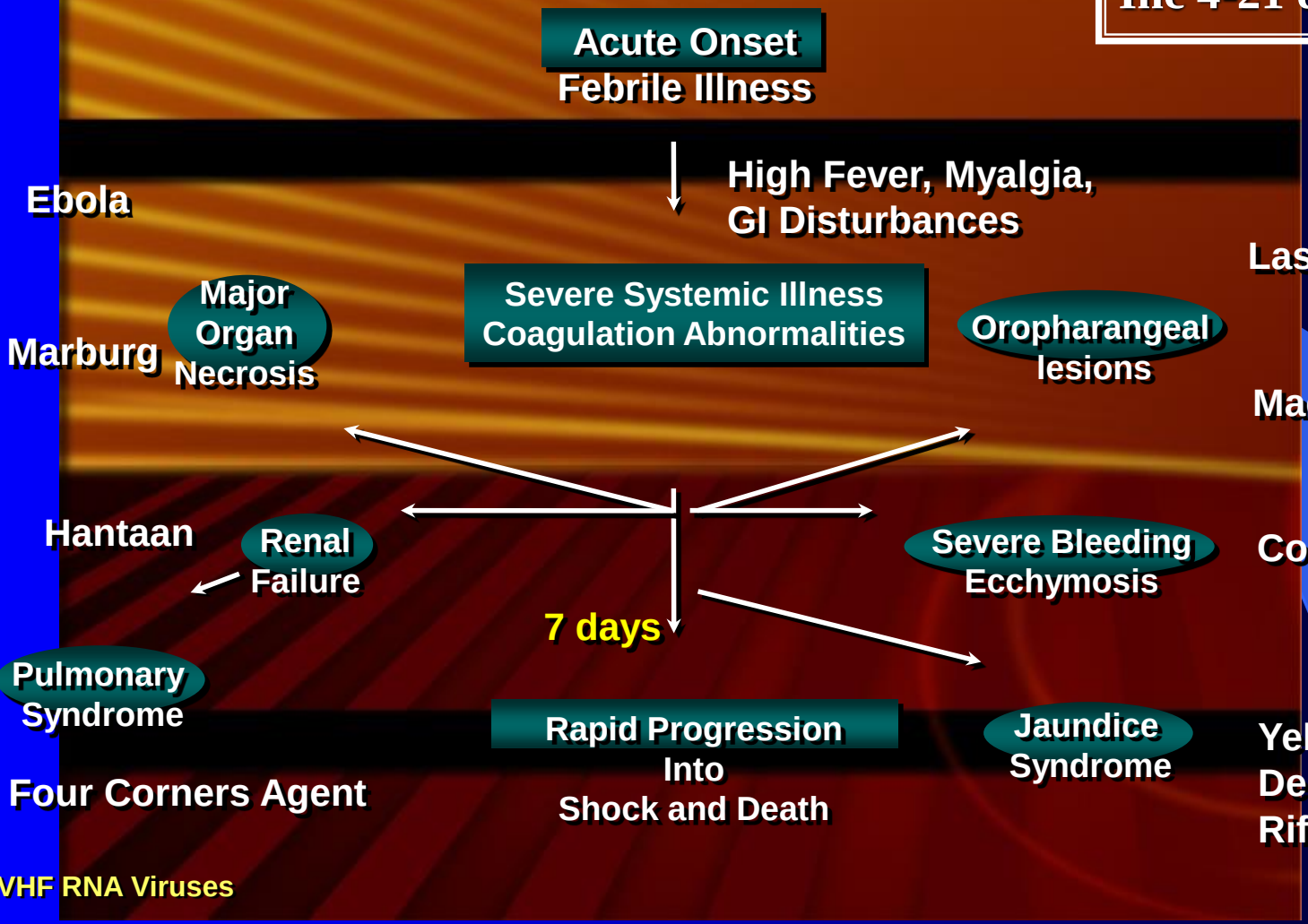
Mort. T 5% NT 40-70%

P>P YES

Inc 2-3 days

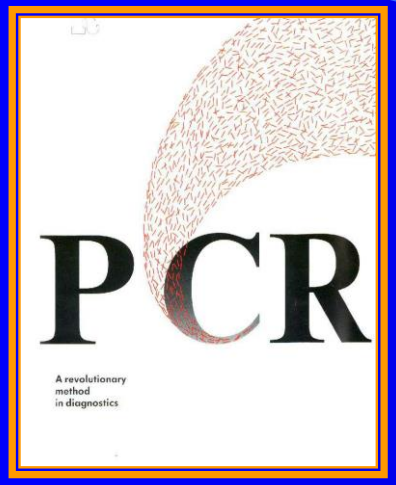
The VHF RNA Viruses

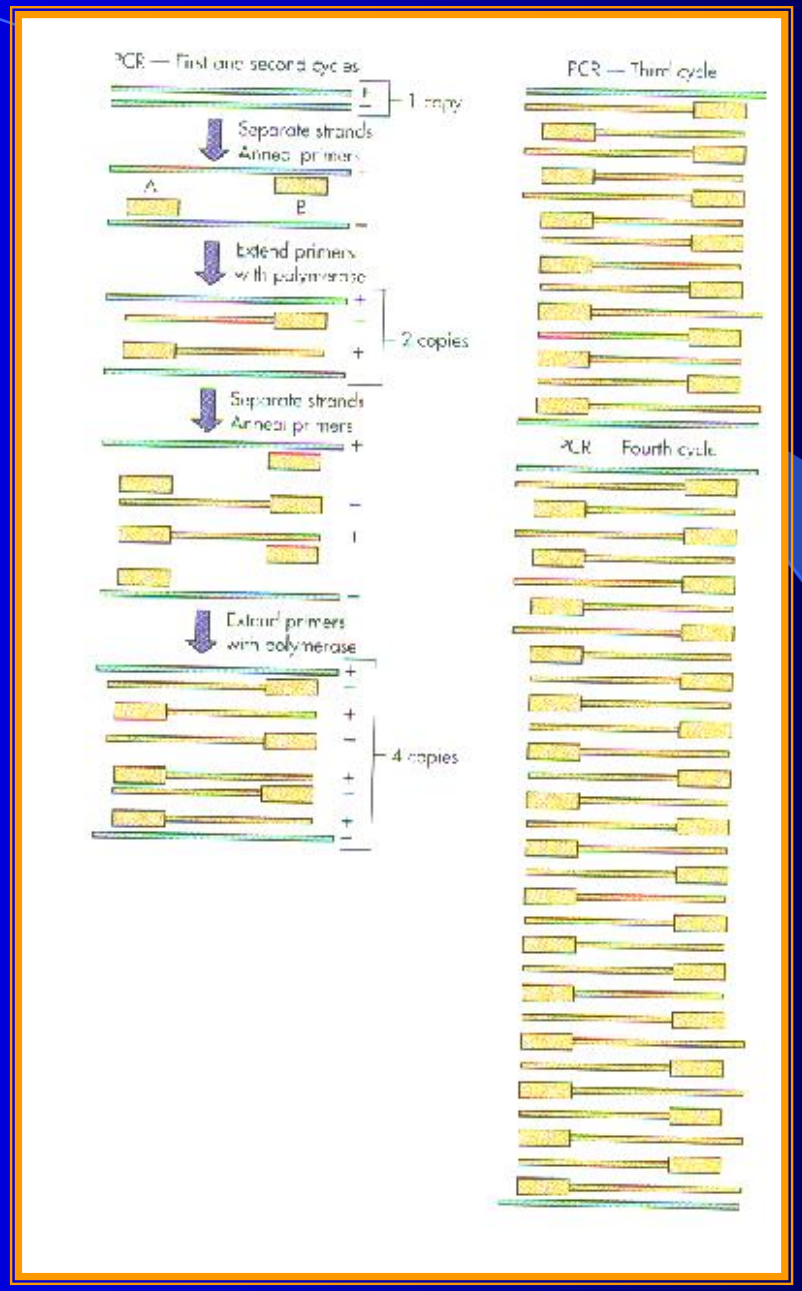
ID 1-10 m.o.
Mort. 53-88%
P>P YES
Inc 4-21 days

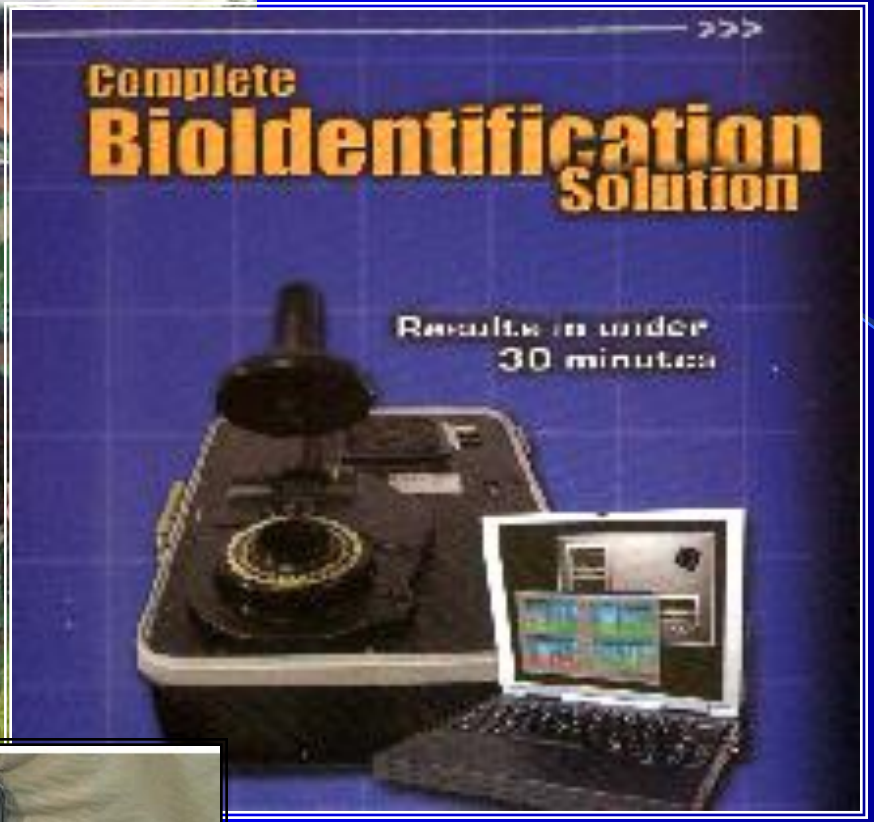


Bioterrorism – Are we Prepared?

- Rapid detection techniques for bio-weapon agents are a critical need for the first-responder community, on a par with vaccine and antiviral development in preventing spread of disease.
- The most promising existing approach is real-time fluorescent PCR analysis in a portable format using exquisitely sensitive and specific primers and probes.
- Laboratories experience in handling or identifying BW ???
- Recognising a bioterrorism incident


PCR
A revolutionary
method
in diagnostics


PCR — First and second cycles
1 copy
Separate strands
Anneal primers
A
B
Extend primers
with polymerase
2 copies
Separate strands
Anneal primers
Extend primers
with polymerase
4 copies
PCR — Third cycle
PCR Fourth cycle



The R.A.P.I.D. SYSTEM (IT)

- ✓1996 LightCycler
- ✓1999 R.A.P.I.D. (IT+US Air Forces)
- ✓2001 (US Patent)
- ✓2004 RAZOR System

Conventional PCR

I. 1 cycle	$94C^0$	5’ (10’)
II. 30 cycles:	$94C^0$	1’
	$60C^0$	1’
	$72C^0$	1’ (1-3’)
III. 1 cycle	$72C^0$	3’ (10’)

2-5 h

R.A.P.I.D-LightCycler- PCR

I. 1 cycle	$94C^0$	2’
II. 40 cycles:	$60C^0$	20”
	$94C^0$	0’

6-30min

R.A.P.I.D - PCR-Setup MASTER MIX

dH2O	x 6µl	
10 X PCR Buffer	x 2µl	500mM Tris-Hcl, 50 mM MgCl
10 X dNTP	x 2µl	2mM of each dNTP
Forward primer	x 2µl	5µM
Reverse primer	x 2µl	5µM
*Taq*Man Probe	x 2µl	0.3µM
Platinum *Taq* DNA Polimerase	x 2µl	0.4 units
Template DNA (1ng/µl)	x 2µl	2 ng
TOTAL VOLUME	20 µl	

500 bp/10 sec

Conventional PCR-Setup MASTER MIX

dH2O	x 15µl	
10 X PCR Buffer	x 5µl	500mM Tris-Hcl, 50 mM MgCl
10 X dNTP	x 4µl	2mM of each dNTP
Forward primer	x 10µl	5µM
Reverse primer	x 10µl	5µM
Gold *Tag* DNA Polimerase	x 1µl	0.4 units
Template DNA (1ng/µl)	x 5µl	2 ng
TOTAL VOLUME	50 µl	

*Tag*Man Probe

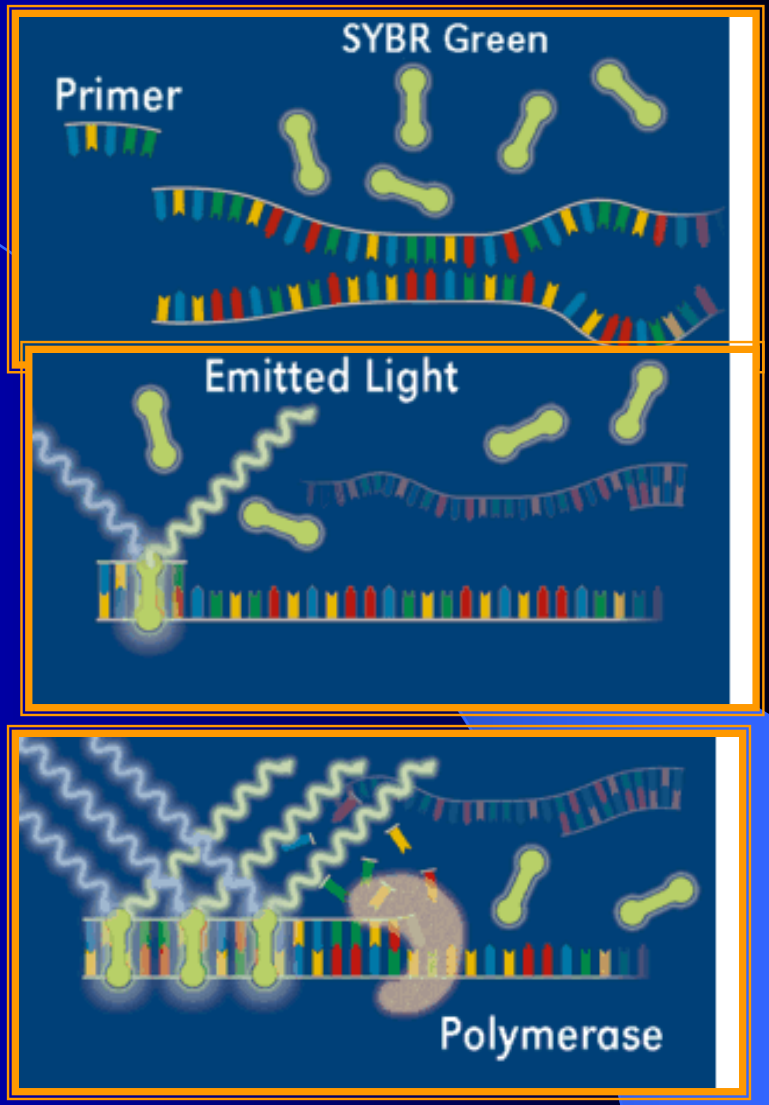
6FAM-CGG TTG CAC AGG CCC CGA CA-TAMRA

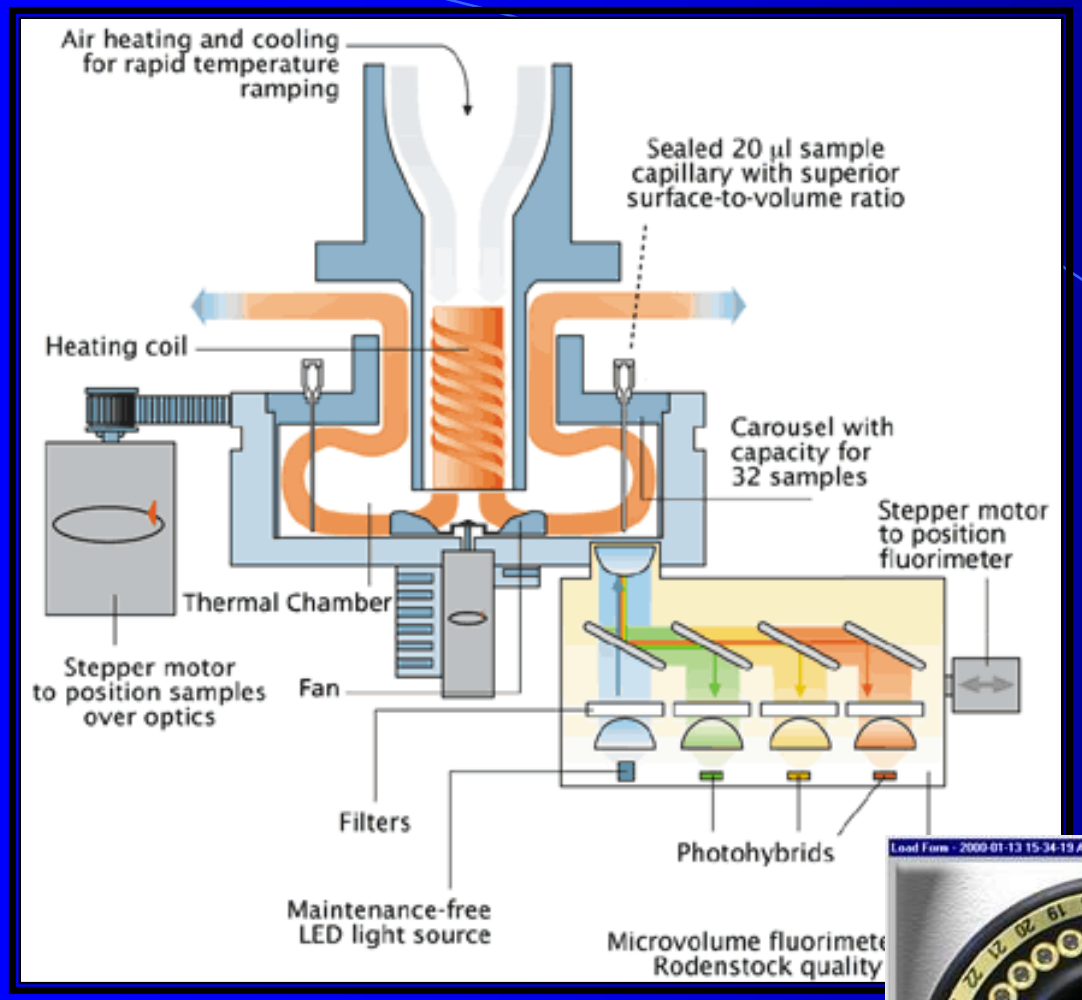
ABI PRISM

FLUORESCENT DYE INFORMATION CARD

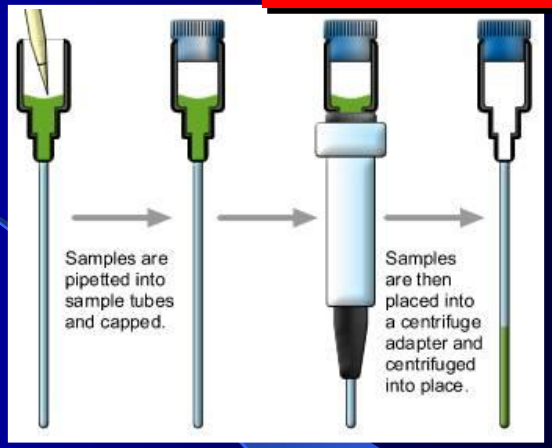
DYE	Absorption MAX(nm)	Emission MAX(nm)
5-FAM	494	530
6-FAM	494	522
TET	521	538
JOE	528	554
HEX	535	553
VIC	538	554
NED	546	575
TAMRA	560	582
ROX	587	607

PE Biosystems

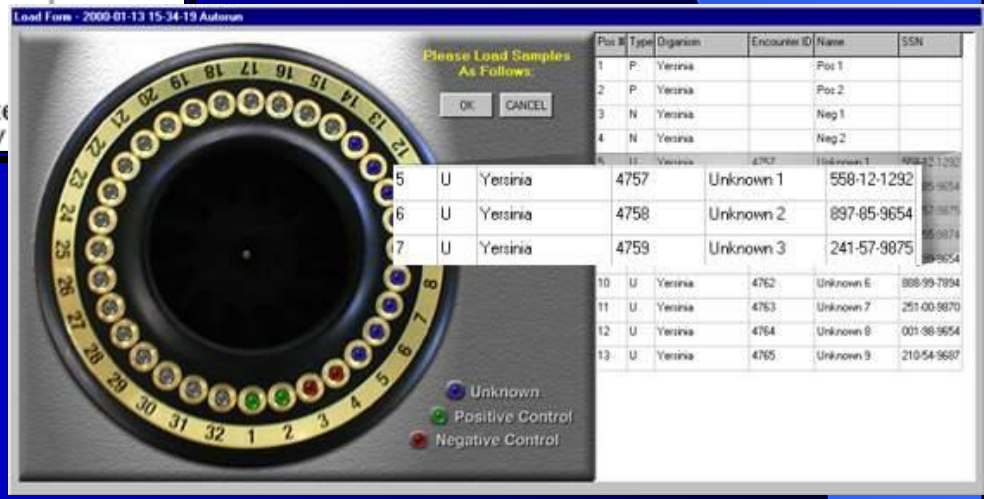


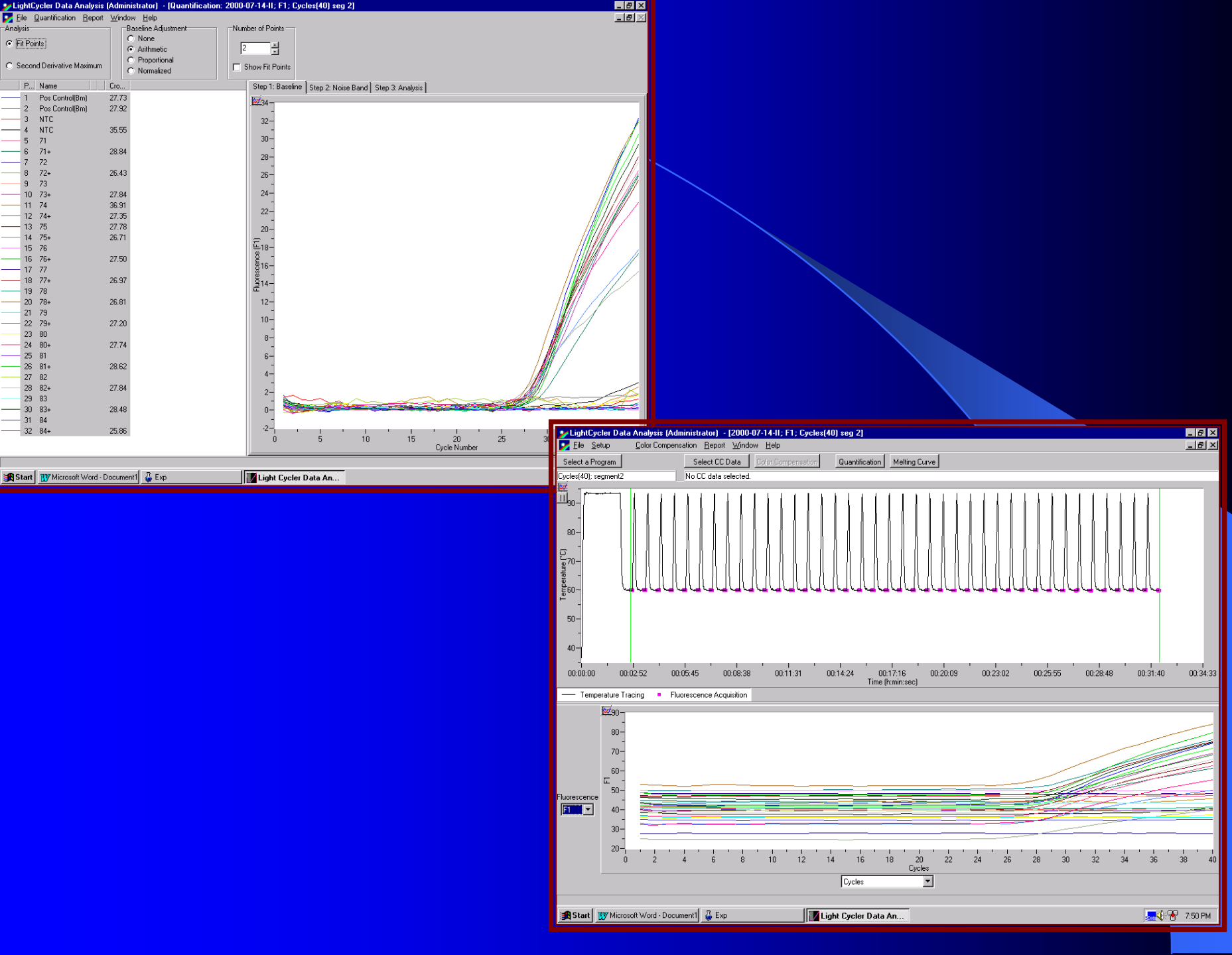


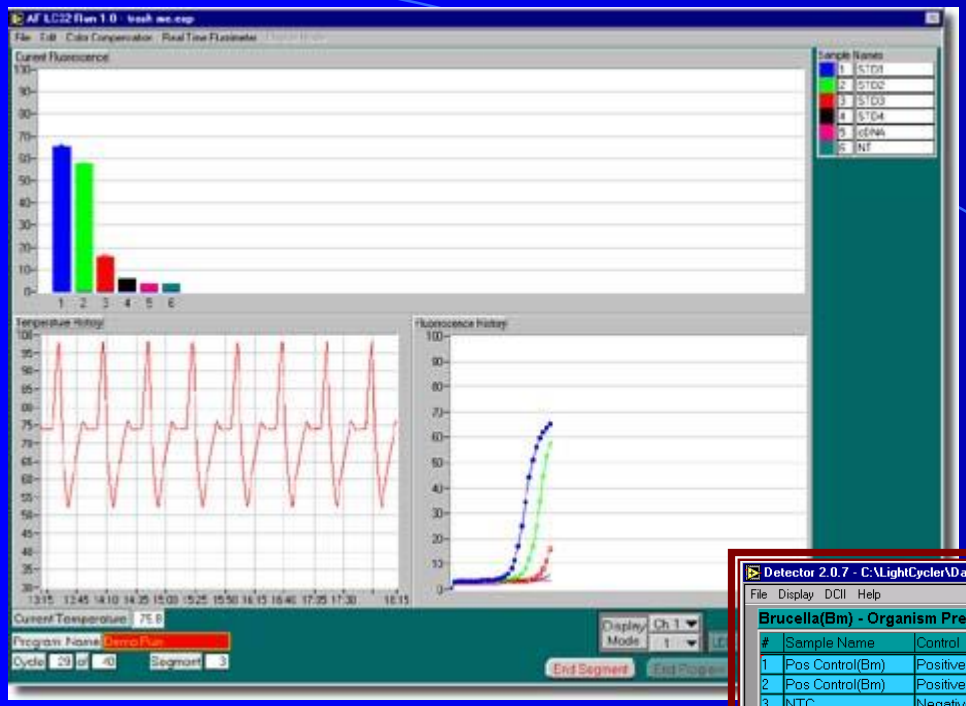
Surface/Volume



5-20 µl




LightCycler Data Analysis (Administrator) - [Quantification: 2000-07-14-II; F1; Cycles(40) seg 2]
File Quantification Report Window Help
Analysis
Fit Points
Second Derivative Maximum
Baseline Adjustment
None
Arithmetic
Proportional
Normalized
Number of Points
2
Show Fit Points
P... Name Cro...
1 Pos Control(Bm) 27.73
2 Pos Control(Bm) 27.92
3 NTC
4 NTC 35.55
5 71
6 71+ 28.84
7 72
8 72+ 26.43
9 73
10 73+ 27.84
11 74 36.91
12 74+ 27.35
13 75 27.78
14 75+ 26.71
15 76
16 76+ 27.50
17 77
18 77+ 26.97
19 78
20 78+ 26.81
21 79
22 79+ 27.20
23 80
24 80+ 27.74
25 81
26 81+ 28.62
27 82
28 82+ 27.84
29 83
30 83+ 28.48
31 84
32 84+ 25.86
Step 1: Baseline
Step 2: Noise Band
Step 3: Analysis
Fluorescence (F1)
Cycle Number
Start
Microsoft Word - Document1
Exp
Light Cycler Data An...
LightCycler Data Analysis (Administrator) - [2000-07-14-II; F1; Cycles(40) seg 2]
File Setup Color Compensation Report Window Help
Select a Program
Select CC Data
Color Compensation
Quantification
Melting Curve
Cycles(40); segment2
No CC data selected.
Temperature (°C)
Time (h:min:sec)
Temperature Tracing
Fluorescence Acquisition
Fluorescence
F1
Cycles
Start
Microsoft Word - Document1
Exp
Light Cycler Data An...
7:50 PM



Detector 2.0.7 - C:\LightCycler\Data\2000-07-14-III.ABT

File Display DCII Help

Brucella(Bm) - Organism Present

#	Sample Name	Control	Score	Result	SSN	Enc. ID
1	Pos Control(Bm)	Positive	1130	Present		
2	Pos Control(Bm)	Positive	333	Present		
3	NTC	Negative	0	Not Detected		
5	58	Unknown	-2	Not Detected		
6	58+	Unknown	165	Present		
7	85	Unknown	-1	Not Detected		
8	85+	Unknown	326	Present		
9	86	Unknown	0	Not Detected		
10	86+	Unknown	359	Present		
11	87	Unknown	-17	Not Detected		
12	87+	Unknown	743	Present		
13	88	Unknown	-26	Not Detected		
14	88+	Unknown	372	Present		
15	89	Unknown	-4	Not Detected		
16	89+	Unknown	74	Present		
17	90	Unknown	-6	Not Detected		
18	90+	Unknown	476	Present		
19	91	Unknown	41	Present		
20	91+	Unknown	255	Present		
21	92	Unknown	-0	Not Detected		
22	92+	Unknown	259	Present		
23	93	Unknown	5	Not Detected		
24	93+	Unknown	179	Present		
25	94	Unknown	-5	Not Detected		
26	94+	Unknown	732	Present		
27	95	Unknown	12	Present		
28	95+	Unknown	368	Present		
29	96	Unknown	-0	Not Detected		
30	96+	Unknown	441	Present		
31	97	Unknown	14	Present		
32	97+	Unknown	539	Present		

Brucella - Please Repeat

#	Sample Name	Control	Score	Result	SSN	Enc. ID
4	NTC	Negative	5	Not Detected		

SHOW GRAPH EXIT

Start | Microsoft Word - Document1 | Exp | Detector | 7:30 PM

FEATURAS of R.A.P.I.D.

(Ruggedized Advanced Pathogen Identification Device)

FAST: Fastest and most sensitive BioIdentification System
(Up to 32 samples may be amplified and analyzed in less than 30 min.)

Easy-to-use:

Commercially Available Freeze-Dried-Reagents (6 months Shelf life)
Optimized and Validated

Simple software:

Minimal Operator Training Required
Automated Data Analysis and Reporting
Secure Online Acces Worldwide (Real time monitoring)
Lab-proven and Field tested in Real World Situations (Anth. 5 mil tests)

Portable and Rugged:

> Air, Land and Sea Transportable
> Total Weight 22.7 kg
> Withstand 1 Meter Drop test

Batch mode tests up to thirty samples for a single pathogen.

Screen mode tests for multiple organisms in a single run. Up to 10 different organisms may be tested at one time.

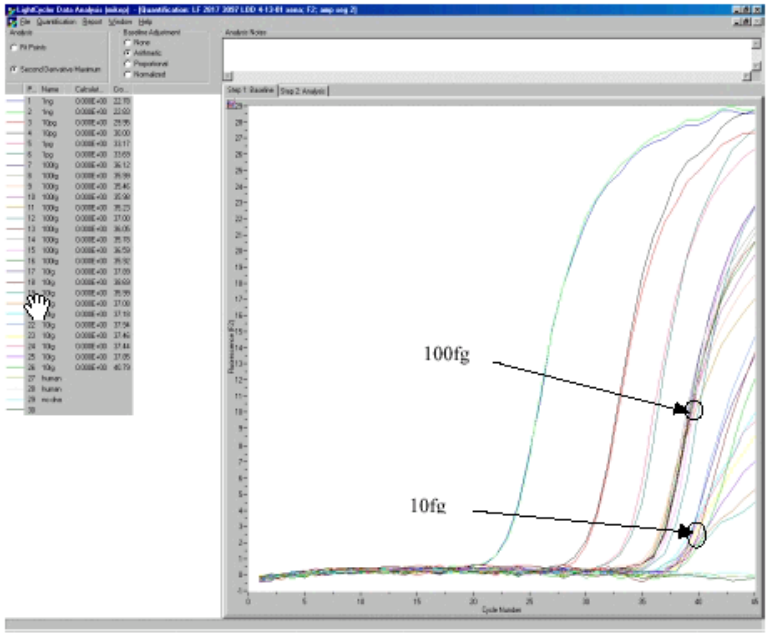
Advanced mode, where automatic pathogen detection protocols are created and refined.

Sensitivity

- The R.A.P.I.D. - **B. anthracis Lethal Factor** Detection Kit is able to reliably detect up to 100 fg of amplifiable DNA material in a single reaction, this is approximately 20 copies of bacterial DNA.

- If at least **100 spores** are present on the swab amount of DNA recovered from these spores is sufficient for detection.

Testing probes and LOD determination



DNA Concentration	Cycle Threshold	Percent Detected
1ng	22.8	100
1pg	33.4	100
100fg	36	100
10fg	37.8	100

Brucella spp. As a Biological Agent

- ❑ BSL-3
- ❑ Aerosol infection
- ❑ No human vaccine

Hypotetical bio-warfare attack:

(50 kg of agent by aircraft along a 2 km line

- ➢ **Upwind of a population center of 500.000**

Agent reach downwind 10 km,

- ➢ **500 dead, 125.000 incapacitated**

(Biological Weapons FAQ v. 0.43)

Brucella spp.	Human virulence	No of organisms
B. melitensis	HIGH	1-10
B. suis	High-Moderate	1.000-10.000
B. abortus	Moderate	100.000
B. canis	Low/immunosuppresed	>1.000.000

Costs (billions $):

- livestock industry – production, abortions, lowered milk production, eradication costs, unrealized export, animal vaccination,
- absenteeism and treatment of patients.

Primers :

- ***IS711*** - Insertion sequence (845 bp 198 A 237 C 222 G 188 T) (*amplicon 134 bp*)

B.melitensis **(Bm F-167)**
5'-AGC GTG ACG AAG CAC TGT CT-3', 20 nucleotides,
B.melitensis **(Bm R-301)**
5'-TAT CGT CGT ATT GCG CTG C-3', 19 nucleotides,

Taqman probe (230):
6FAM-CGG TTG CAC AGG CCC CGA CA-TAMRA, 20 nucleotides.

- ***BCSP 31*** (1037 bp 233 A 273 C 263 G 268 T) (*amplicon 60 bp*)

BCSP 31 **F-622** **5'-GCG TTG GGA GCG AGC TTT-3', 18 nucleotides,**
BCSP 31 **R-681** **5'-GCC AGT GCC GAT ACG GAA-3', 18 nucleotides,**

Taqman probe (640) :
6FAM-CGG TTG CAC AGG CCC CGA CA-TAMRA, 20 nucleotides.

Sample Purification Kits

Whole Blood:
DNA, RNA
Air in PBS-DNA, RNA
Nasal Swabs-DNA,RNA
Surface Swabs-DNA,RNA
Culture-DNA,RNA
Powder-DNA,RNA
Sputum-DNA,RNA

Milk-DNA
Salad-DNA
Meat-DNA
Tuna salad-DNA
Stool-DNA
Soil-DNA
Pus Swabs-DNA
Gastric Washing-DNA
Lymph Node Aspirates-DNA

VALIDATED REAGENT KITS

Anthrax (3 Targets)
Brucella
Tularemia (2 Targets)
Plague (2 Targets)
Botulism
Cryptosporidium (2 Targets)
Variola
Listeria
E.coli 0157
Campylobacter
Salmonella
Vibrio cholerae
VEE
West Nile
Yellow fever
Staphylococcal Enterotoxin

Protocols for:

- **Clinical specimens**
- **Air samples**
- **Food samples**
- **Water samples**

CONCLUSION

- **The RAPID system , as a fully automated, molecular diagnostic method, based on nucleic acid amplification and detection, is a promising tool in rapid identification of biological agents, which is essential for rapid response to BW attack.**

The RAZOR instrument

- Designed to operate in the most extreme environ[es]
- **Hand-portable**, pathogen detection closer to the
- Simple sample preparation makes it the ideal instrument for pathogen detection for military and homeland defense personnel.
- **Analyze up to twelve 100 µl Samples in less than 30 min.**
- Push Button User Interface
- Battery Operated - Rechargeable
- **Weight: 4.1 kg**
- **Size: 17 x 11 x 23 cm** (h x d x w)
- Capable of performing thermocycling, analysis and identification without an external power source or attached to a computer

Freeze-dried Sample Pouch

- Revolutionary new RAZOR™ pouch technology.
- Each pouch is target specific and contains all of the probes, primers, buffers, etc. necessary for real-time fluorescent detection.
- Reaction set-up does not need centrifugation and requires no pipetting.

RAZOR Instrument Software

- Performs system self-test diagnostics upon start-up
- Stores the run-time protocols needed for the available reagent pouches.
- Prompts the user on the LCD display and guides them through reaction setup and testing.
- After a test is finished the instrument displays positive and negative results in an easy-to-read format.
- The results and real-time data are saved in the instrument's memory and can be downloaded to the RAZOR desktop software for further analysis.