Gülhane Mikrobiyoloji Günleri

20 - 22 Nisan 2010

Antimikrobik Kemoterapi
Laboratuvar Uygulamaları ve Yenilikler

KLİMUD

GÜLHANE ASKERİ TIP AKADEMİSİ 1898

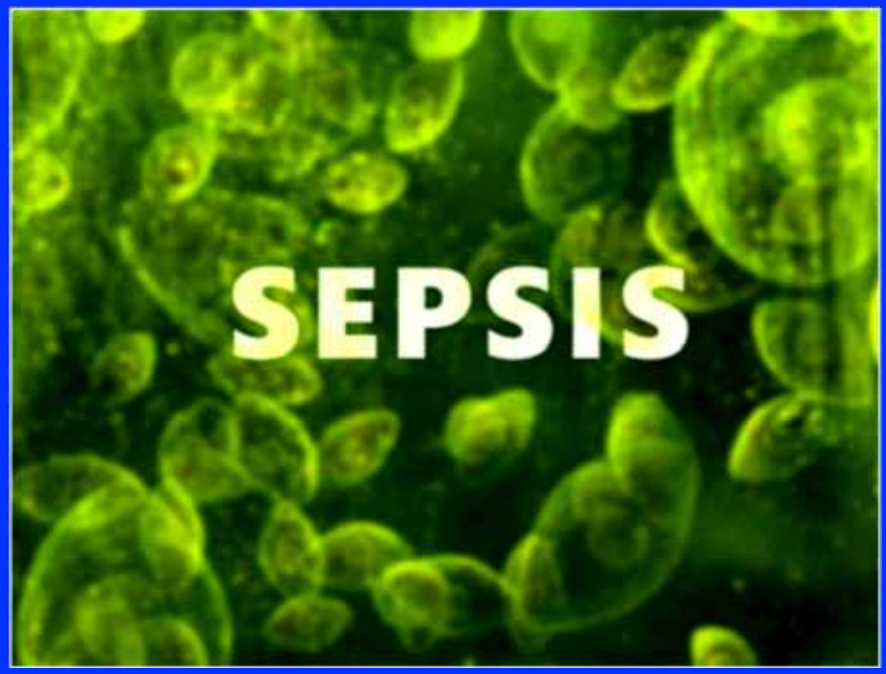
TÜRK MİKROBİYOLOJİ CEMİYETİ

Molecular Niches for Laboratory Diagnosis of Bloodstream Infections: State-of-the-art

Yi-Wei Tang, MD, PhD, F(AAM), FIDSA
Professor of Pathology and Medicine
Director, Molecular Infectious Diseases Laboratory

Outline

- Start with a case presentation
- Background and techniques
- PCR mass spectrometry
- Ehrlichia study
- Take home message



Case Presentation

- A 13-year-old previously healthy boy, presented with severe hypotension, tachycardia, and impending respiratory failure

Case Presentation – cont.

- The patient reported ten days ago a tick bite on his right medial ankle and an ATV accident resulting in mild right leg pain
- Five days prior to admission, hip and knee pain progressively worsened and evolved into full body arthralgia without fever
- 24 hours prior to admission, the patient experienced nausea and vomiting, again without reported fever

Case Presentation – cont.

- On the morning of admission, the patient continued having episodes of nausea and vomiting with chest pain. He looked pale and blue, and collapsed in the parking lot
- On admission, he was hypotensive and tachycardic and increased work of breathing
- Blood cultures were drawn and the patient was given doxycycline and ceftriaxone
- The patient was intubated secondary to severe shock and impending respiratory failure and transported via Life Flight to VCH

Case Presentation – cont.

- The patient was sedated on arrival to the PCCU
- Vancomycin and gentamycin were added per infectious disease consult
- Blood cultures revealed coagulase-positive cocci in clusters at the second hospitalization day
- An ultrasound of the hip and knee were done to assess for joint abscess, but revealed no source of infection
- At the third hospitalization day, blood, trach and pleural fluid cultures were positive for MRSA
- The patient was continued vancomycin, clindamycin and ceftriaxone

Case Presentation – cont.

- Ceftriaxone was discontinued and rifampin was added at hospital day 4
- Sedation was discontinued for complete neurological exam
- The patient developed multiple organ failure, blood cultures remain positive for MRSA
- In the afternoon of hospital day 6, he developed a fixed and dilated pupils
- Brain death was documented and cardiopulmonary support was withdrawn

Case Presentation – cont.

- Autopsy indicated *S. aureus* sepsis, with pre- and post-mortem cultures positive for MRSA
- Severe diffuse necrotizing pneumonia with multifocal fresh infarcts
- Shock-induced myocardial and hepatic injury
- Shock-related changes of spleen, nodal lymphoid hyperplasia, hemorrhage and splenic subcapsular infarctions
- Early infection of right hip, presumed secondary to pulmonary infection
- The isolate was PVL positive and SCC*mec* type IV

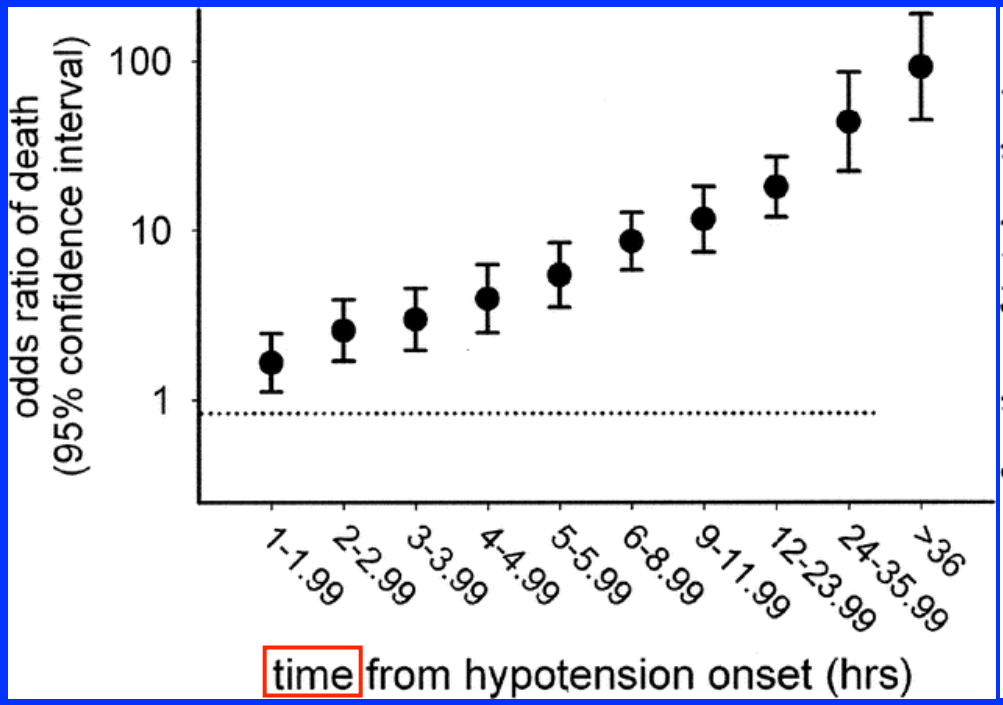
Case Presentation – End

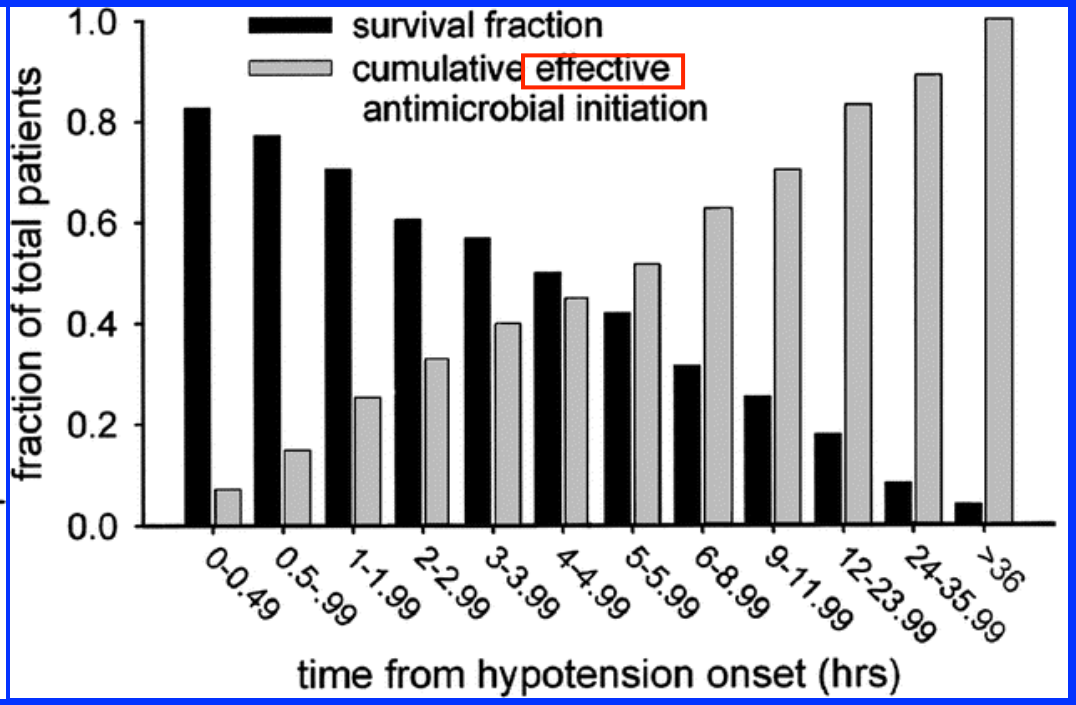
- Can we do better, next time?

Bloodstream Infections Caused by CA-MRSA and Others Can be Lethal

"Relax – MRSA will get you before the Asian Flu"

Antimicrobial Initiation Associated Survival Following Onset of Septic Shock





Kumar et al. *Crit. Care Med.* **34**:1589-96, 2006

Bloodstream Infection Detection: Automated Blood Culture Instruments with Continuous Monitoring

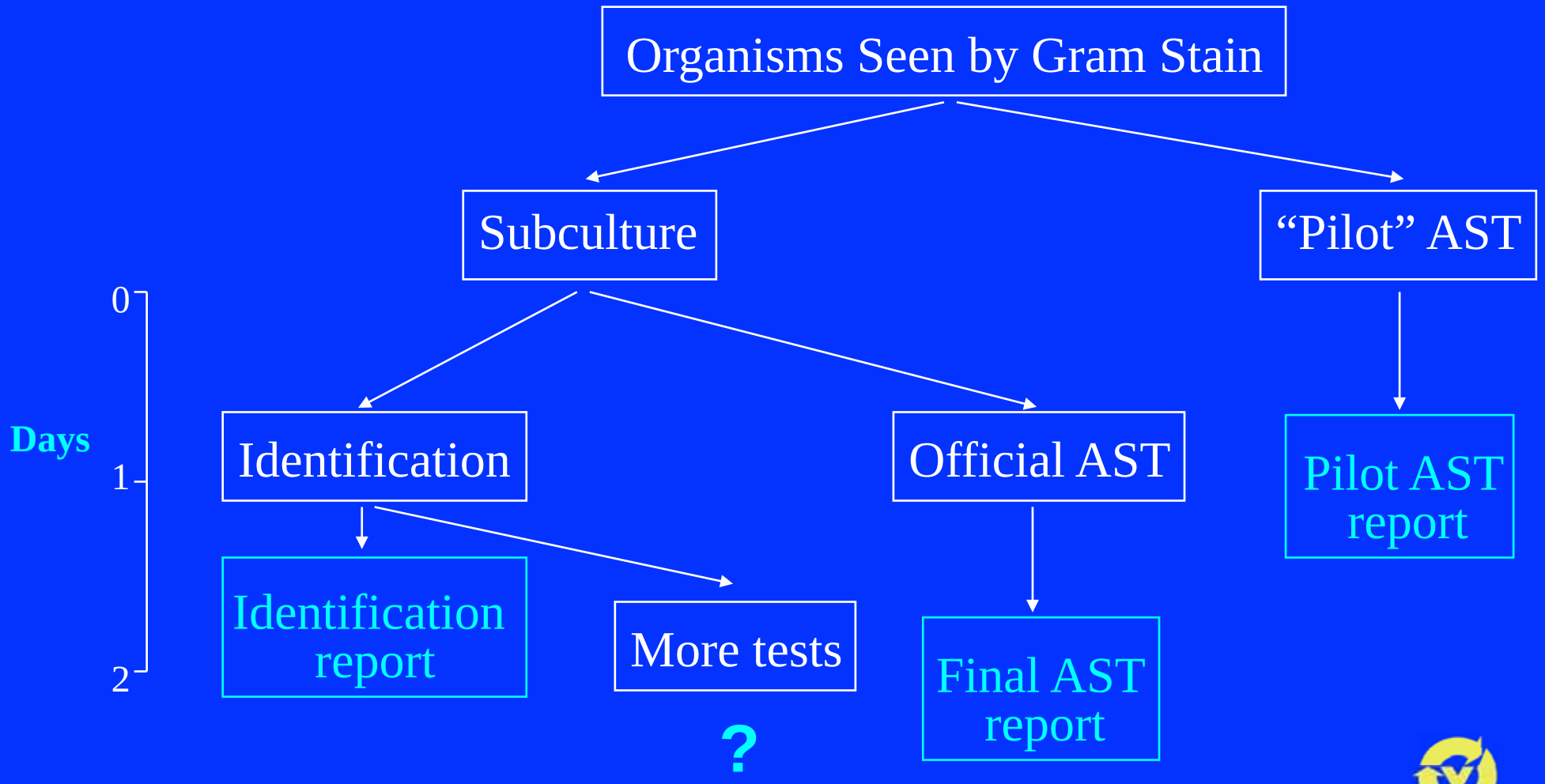
It works well, but it still takes an overnight growth, and …

BD BACTEC

BacT/Alert 3D

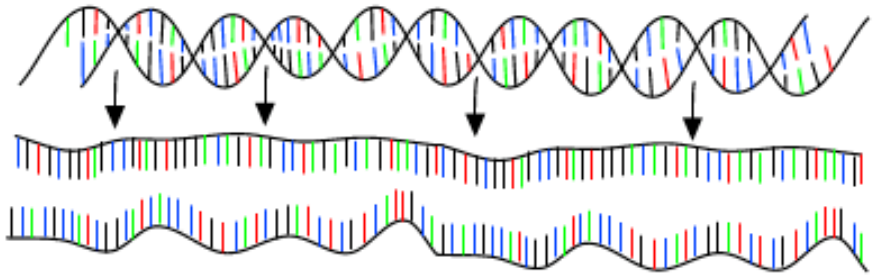
Trek ESP

Identification and Antimicrobial Resistance Determination: Current Procedures



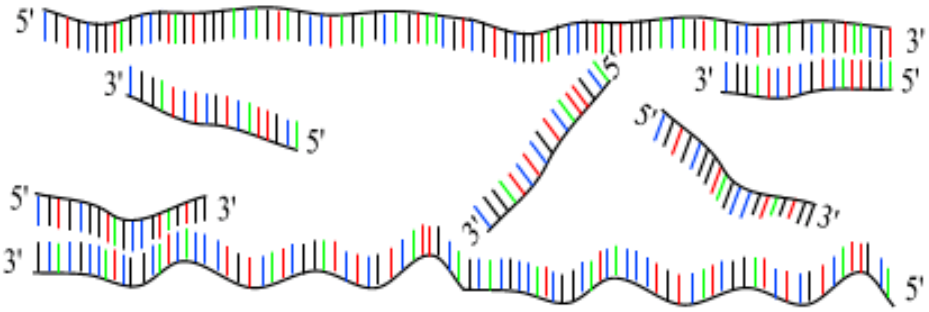
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

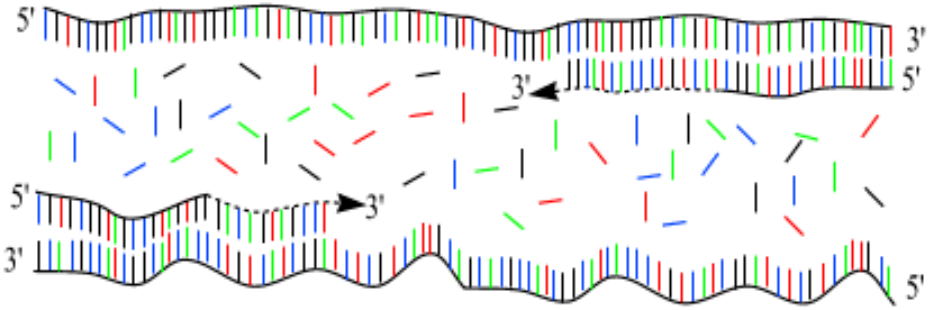
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!

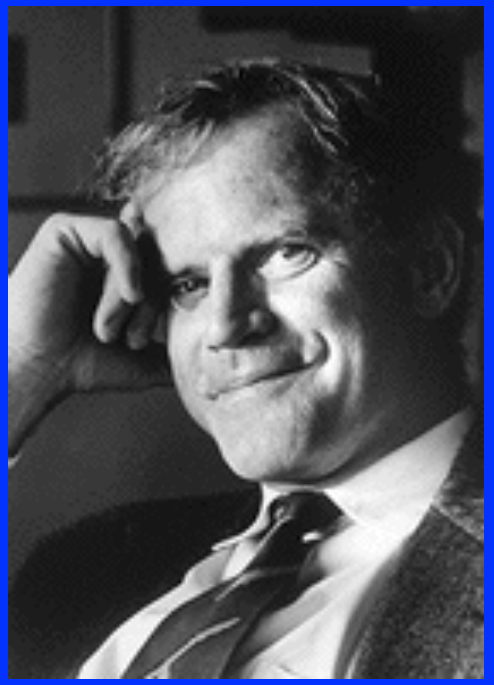


Step 3 : extension

2 minutes 72 °C

only dNTP's

(Andy Vierstraete 1999)

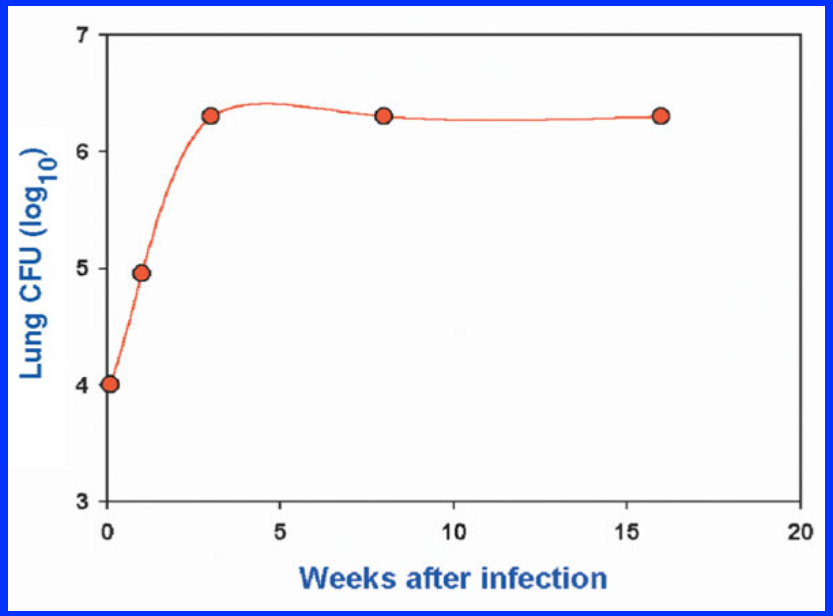


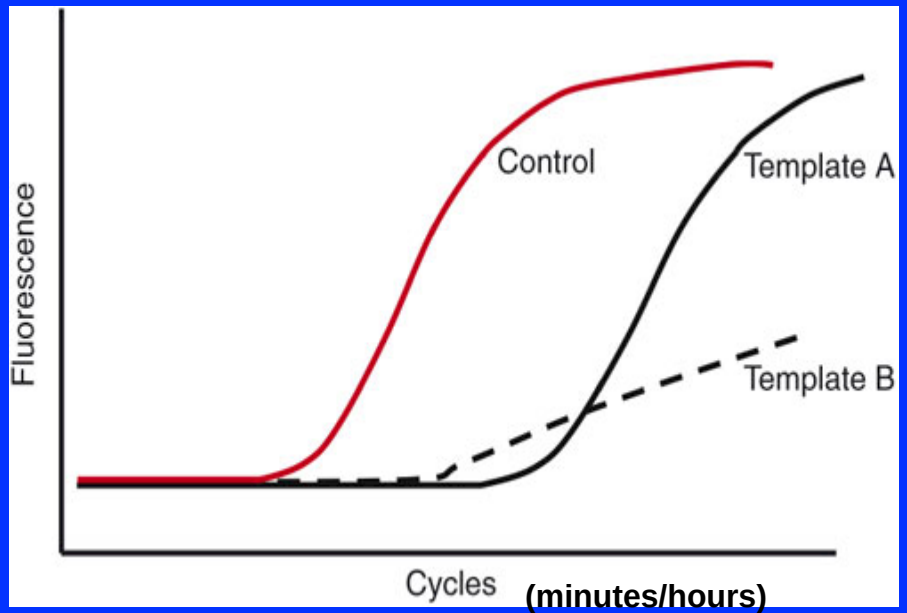
Kary B. Mullis

(1993 Nobel Laureate in Chemistry)

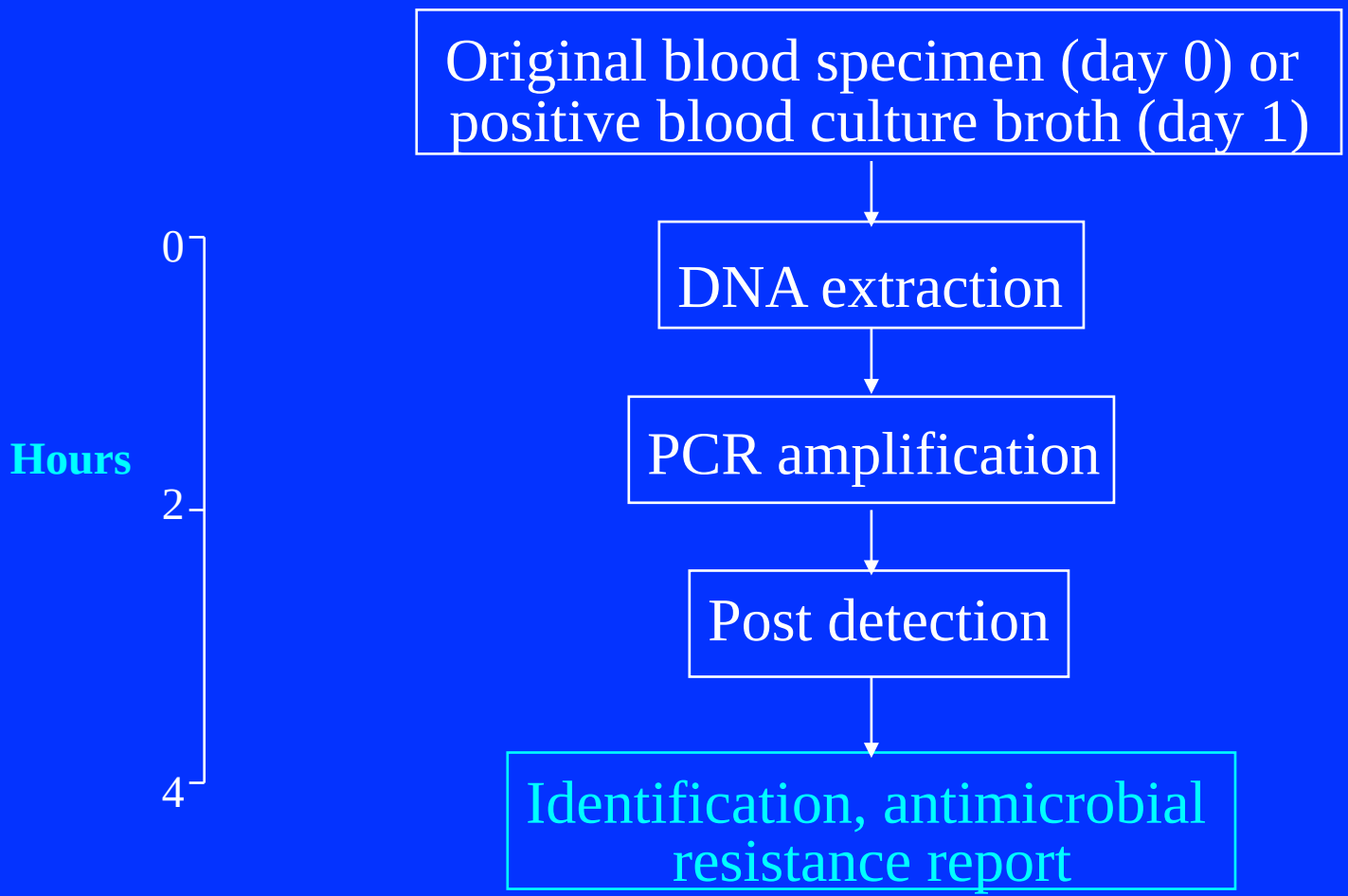
"One spring night …
I came across PCR …
It was the first day of the rest of my life"

Switch from Biological to Enzyme-Mediated Amplification Shortens Test Turnaround Time

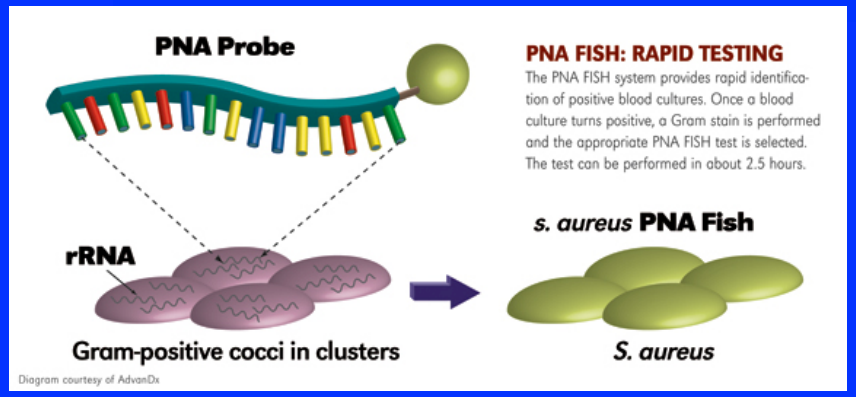


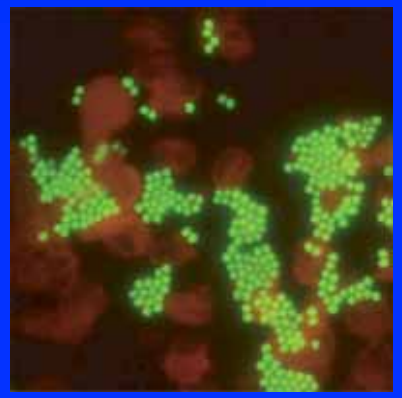


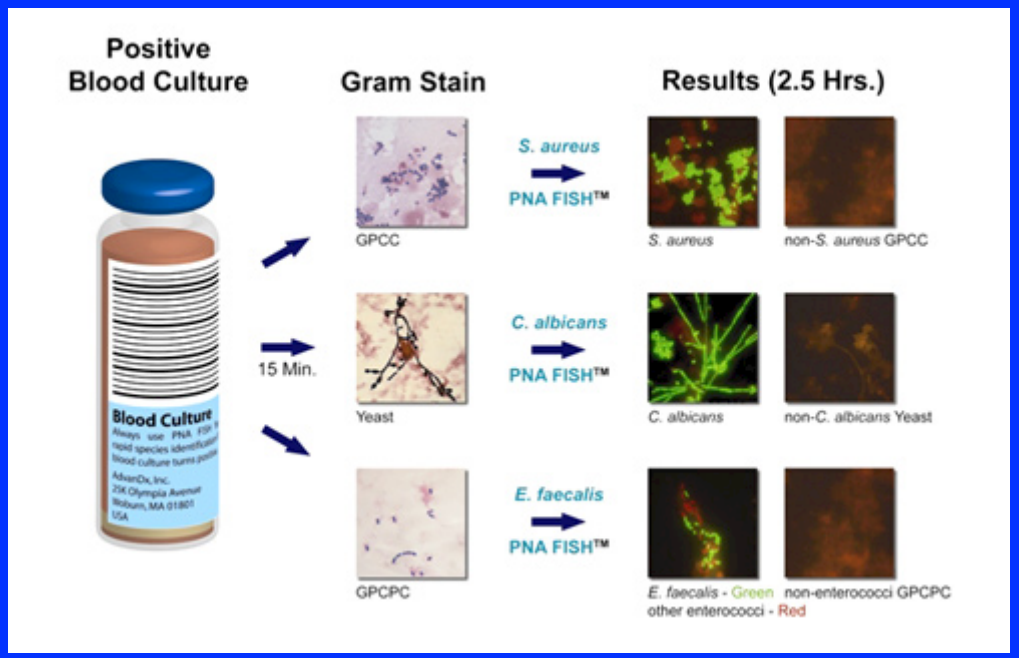
Molecular Niches for Rapid Detection and Identification of Pathogens Causing Sepsis



AdvanDx PNA FISH for Rapid Identification from Positive Blood Cultures

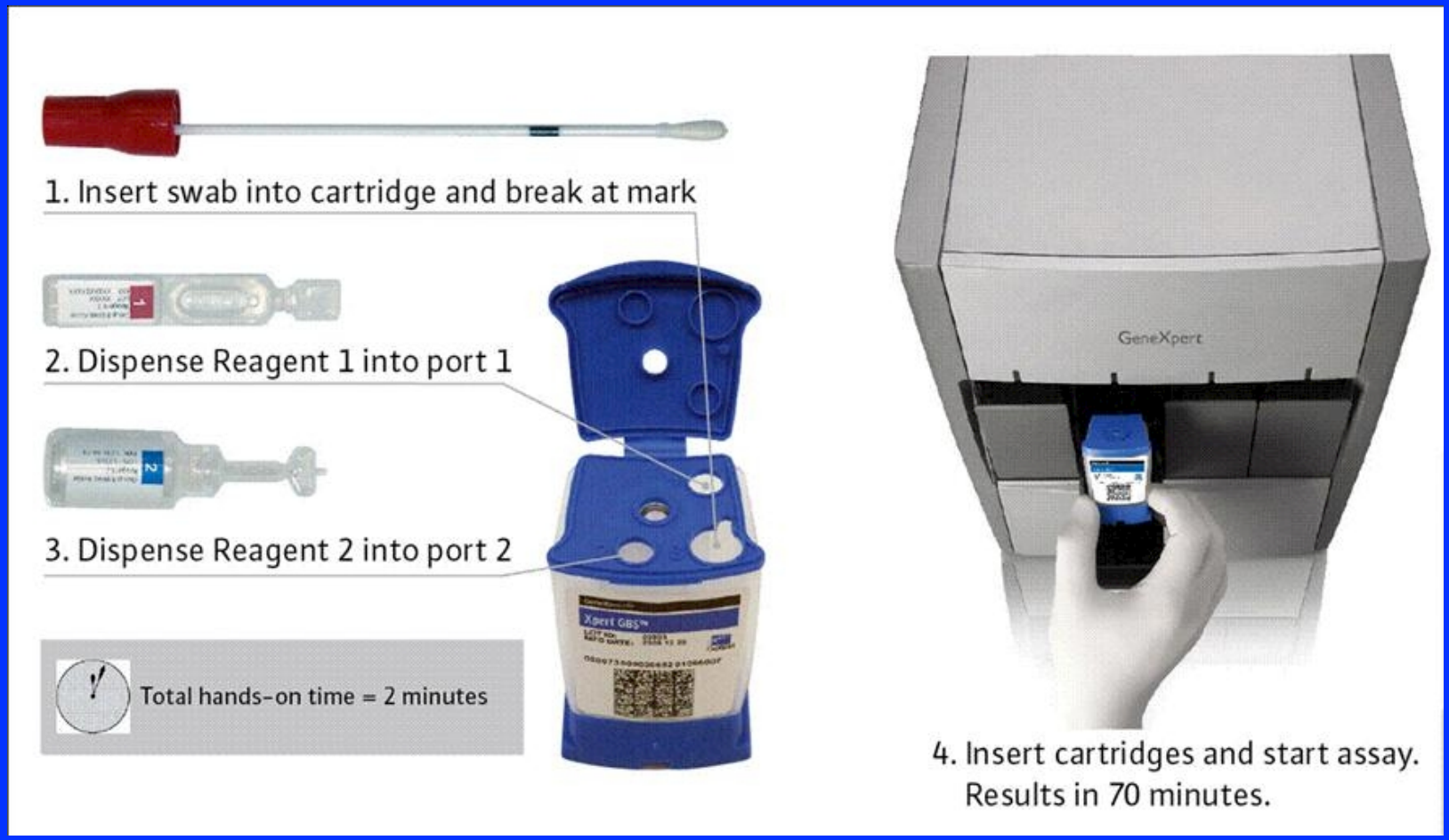






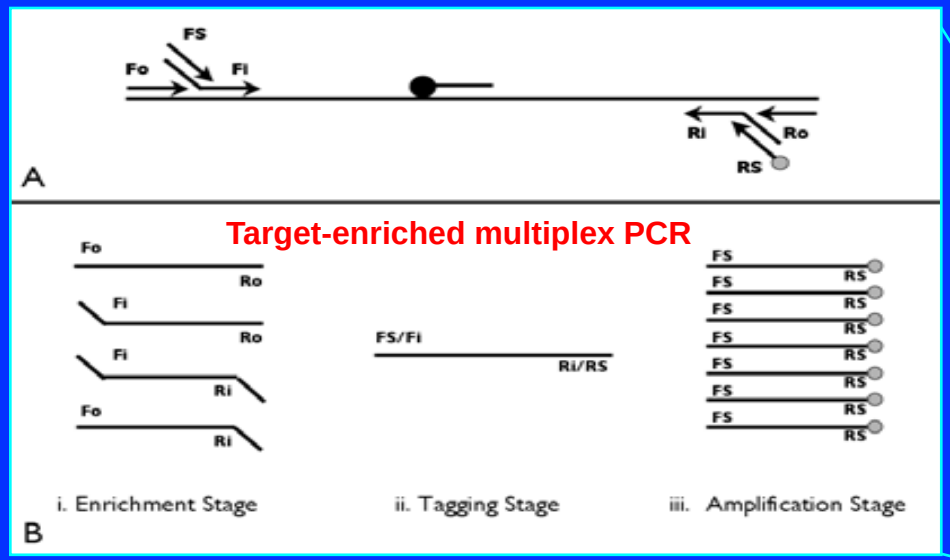
Oliveira et al. *J. Clin. Microbiol.* **40**:247-51, 2002

Cepheid GeneXpert MRSA for Rapid Identification from Positive Blood Cultures



Parta et al. *J. Clin. Microbiol.* **47**:1609-10, 2009

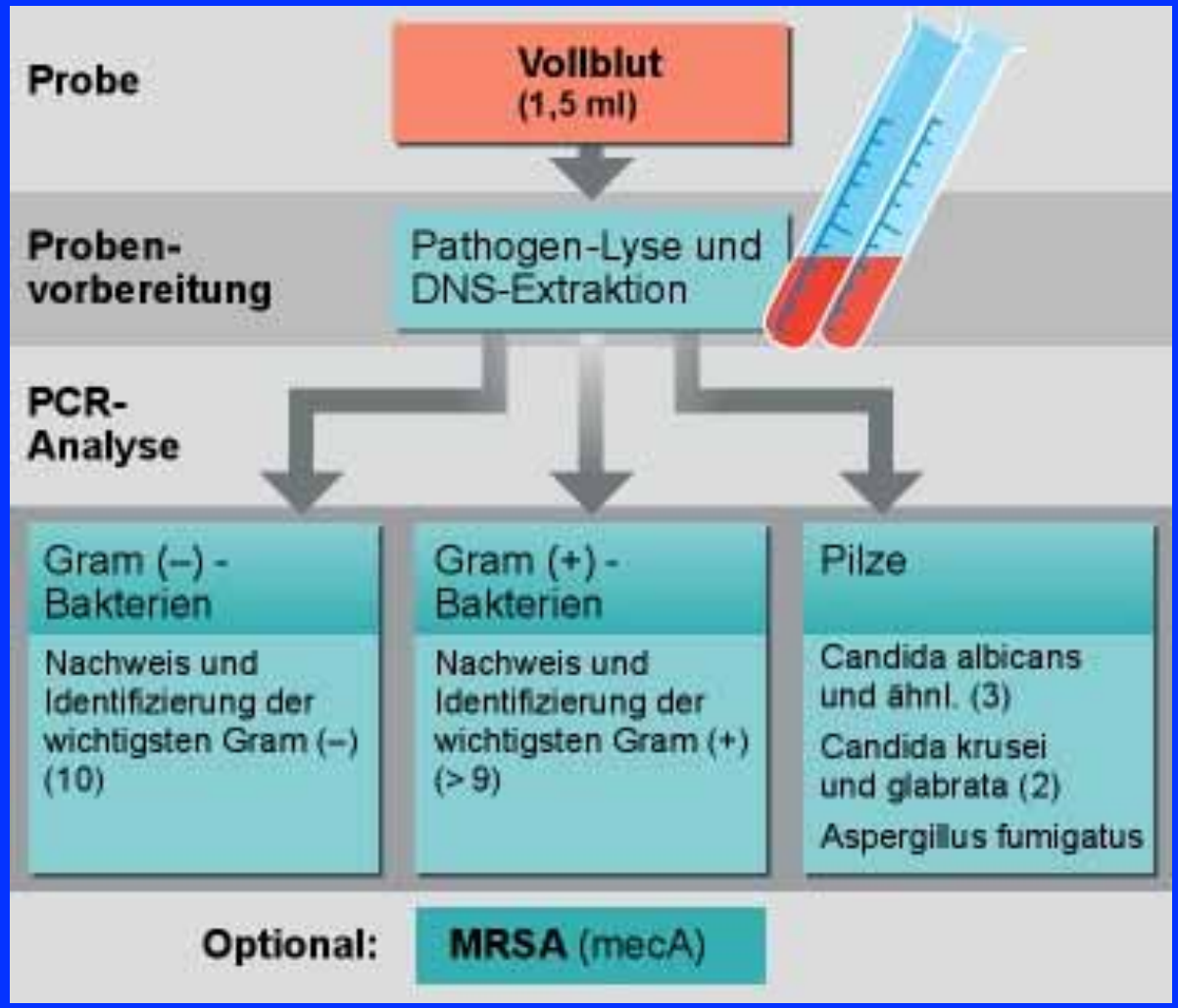
Qiagen StaphPlex for Rapid Identification from Positive Blood Cultures



No.	Samples	epi	cons	haem	lug	homi	sim	nuc	mecA	ccrBI	ccrBII	ccrBIII	ccRBIV	pvl	aacA	ermA	ermC	tetM	tetK	IDS
01	*S. epidermidis*	1089	1870	20	33	26	28	10	2134	13	1833	3163	29	19	3093	2084	1894	4	9	16
02	*S. haemolyticus*	26	2959	2859	39	30	39	24	21	24	18	42	28	21	8	22	17	40	29	25
03	*S. lugdunensis*	45	3762	54	651	38	39	38	39	51	41	57	40	29	39	40	35	48	42	41
04	*S. hominis*	18	1772	27	27	756	14	20	2204	26	25	3812	31	24	2281	21	2268	3	35	25
05	*S. simulans*	13	1565	25	21	17	714	16	11	25	20	86	32	15	15	21	18	37	547	20
06	*S. capitus*	53	2519	52	54	41	34	55	78	32	36	54	57	50	40	56	33	47	25	48
07	*S. warneri*	17	3896	31	20	20	14	24	41	22	44	55	36	28	23	31	37	30	17	32
08	*S. saprophyticus*	31	2369	34	54	34	44	31	24	33	32	30	37	33	33	33	33	31	16	38
09	*S. aureus* (MSSA)	17	40	27	11	16	25	2229	53	23	41	40	29	24	34	26	17	35	19	20
10	HA-MRSA	20	44	41	40	25	20	2464	3557	17	3132	32	29	43	2900	3270	29	4461	38	26
11	HA-MRSA	25	51	40	24	7	34	990	3480	2845	25	27	28	27	17	18	14	4664	3499	25
12	HA-MRSA	1	43	22	20	12	19	924	3265	21	15	5357	35	20	658	2401	4120	4373	1132	20
13	CA-MRSA	14	30	15	15	28	9	1351	3563	36	39	22	4174	1874	22	14	29	30	17	44
14	Blank	10	21	8	2	9	11	4	13	14	7	17	22	11	3	8	12	18	8	10
15	CutOff	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250

Tang, Kilic et al. *J. Clin. Microbiol.* **45**:1867-73 , 2007

Roche SeptiFast for Detection and Identification Pathogens Directly from Blood



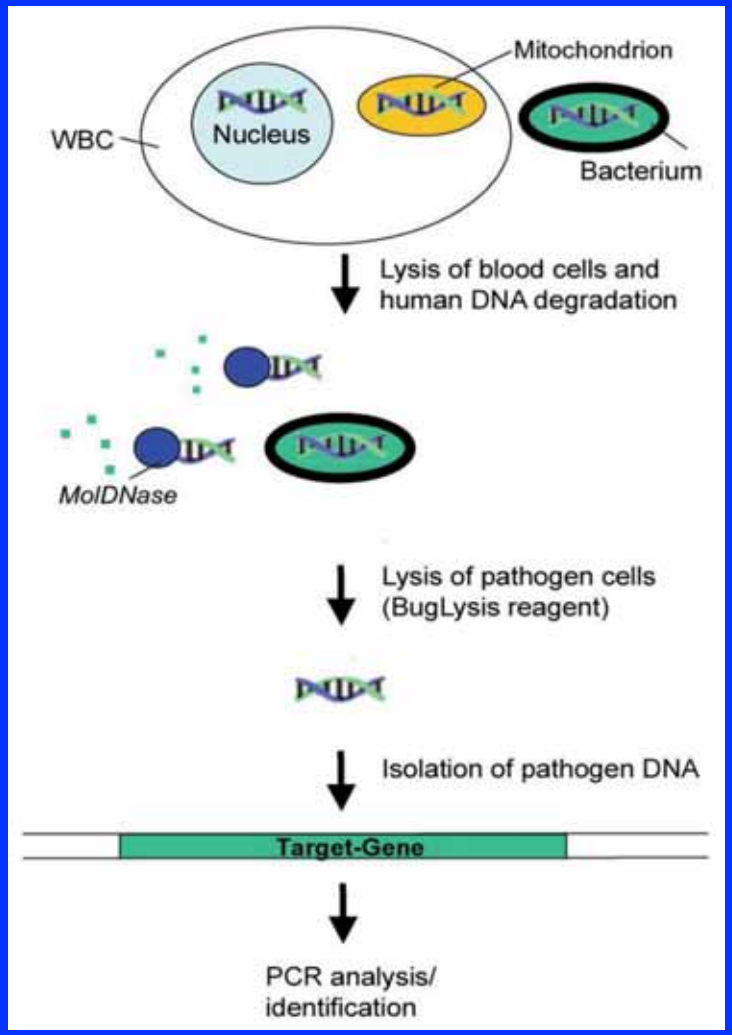
Gram negative
Escherichia coli
Klebsiella (pneumoniae/oxytoca)
Serratia marcescens
Enterobacter (cloacae/aerogenes)
Proteus mirabilis
Pseudomonas aeruginosa
Acinetobacter baumannii
Stenotrophomonas maltophilia

Gram positive
Staphylococcus aureus
ConS
Streptococcus pneumoniae
Streptococcus spp.
Enterococcus faecium
Enterococcus faecalis

Fungi
Candida albicans
Candida tropicalis
Candida parapsilosis
Candida krusei
Candida glabrata
Aspergillus fumigatus

Lehmann et al. *Med. Microbiol. Immunol.* **197**: 313-24, 2008

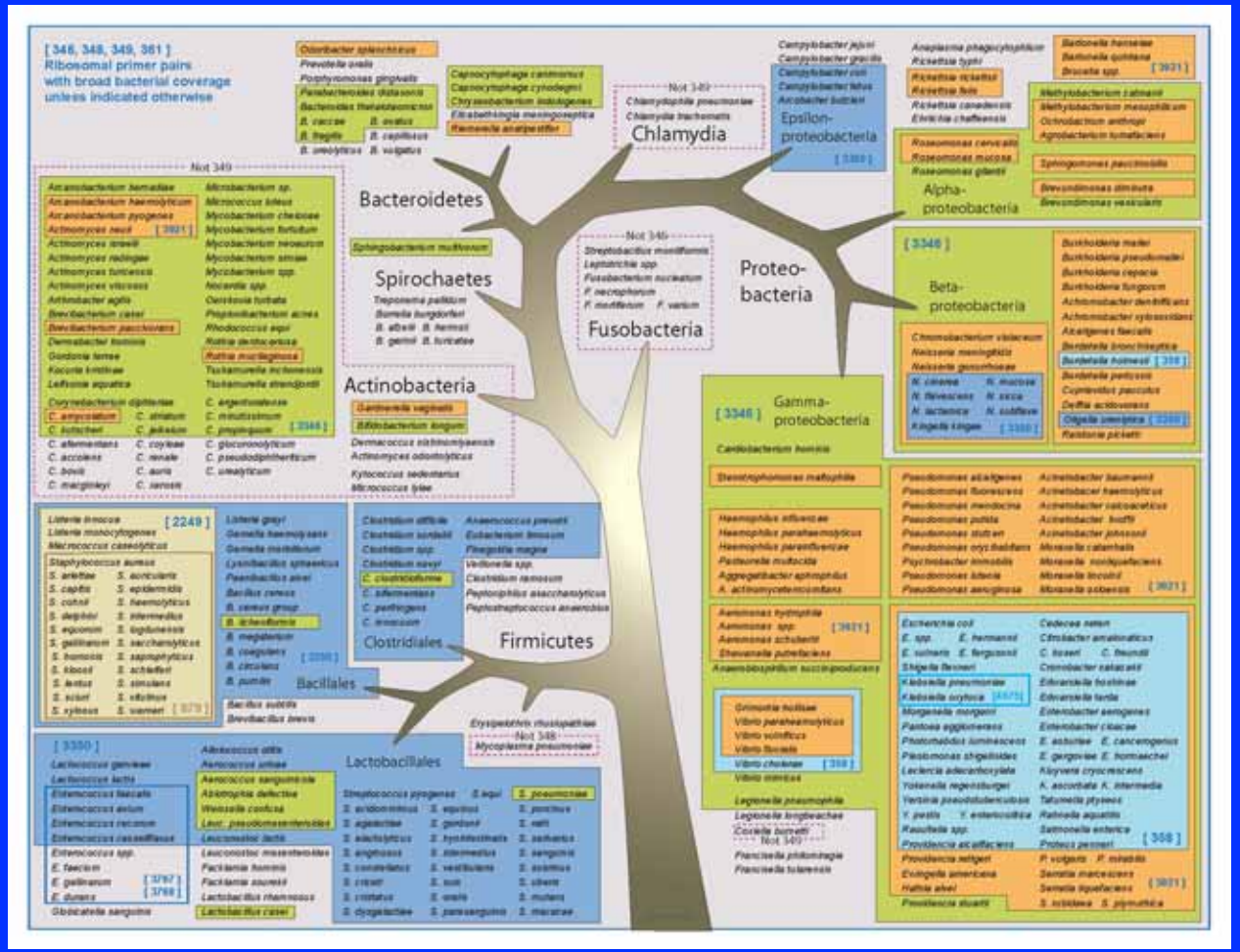
Molzym SepsiTest for Detection and Identification Pathogens Directly from Blood



Bacteria						Yeasts
Gram-negative	Species	**Gram-negative**	Species	**Gram-positive**	Species	
Achromobacter	1	*Methylobacterium*	1	*Actinomyces*	1	*Candida albicans*
Acinetobacter	5	*Moraxella*	2	*Aerococcus*	1	*Candida glabrata*
Actinobacillus	2	*Morganella*	1	*Arthrobacter*	1	*Candida krusei*
Aeromonas	7	*Neisseria*	2	*Bacillus*	20	*Candida parapsilosis*
Aggregatibacter	3	*Ochrobactrum*	2	*Clostridium*	2	*Candida tropicalis*
Alcaligenes	2	*Pantoea*	2	*Corynebacterium*	2	*Cryptococcus neoformans*
Alteromonas	1	*Pasteurella*	1	*Enterococcus*	7	
Anaplasma	1	*Photobacterium*	1	*Erysipelothrix*	1	
Bacteroides	2	*Plesiomonas*	1	*Exiguobacterium*	1	
Bifidobacterium	9	*Proteus*	2	*Geobacillus*	2	
Brevibacterium	1	*Pseudomonas*	18	*Frankia*	1	
Brevundimonas	2	*Rahnella*	1	*Kocuria*	1	
Burkholderia	6	*Ralstonia*	3	*Lactobacillus*	28	
Butyrivibrio	1	*Riemerella*	1	*Lactococcus*	3	
Campylobacter	2	*Salmonella*	2	*Leifsonia*	1	
Chlamydophila	2	*Serratia*	4	*Leuconostoc*	2	
Citrobacter	1	*Shewanella*	3	*Microbacterium*	2	
Comamonas	1	*Sphingomonas*	1	*Micrococcus*	1	
Delftia	1	*Stenotrophomonas*	1	*Mycobacterium*	10	
Ehrlichia	2	*Vibrio*	7	*Nocardia*	3	
Enterobacter	6	*Xanthomonas*	1	*Paenibacillus*	3	
Escherichia	1	*Yersinia*	2	*Pediococcus*	2	
Flavobacterium	3			*Propionibacterium*	1	
Haemophilus	4			*Rhodococcus*	3	
Hafnia	1			*Staphylococcus*	19	
Helicobacter	1			*Streptococcus*	6	
Klebsiella	2			*Streptomyces*	2	
Leptospira	5			*Weissella*	2	
Megasphaera	1	**Sum:**	**114**	**Sum:**	**128**	

Wellinghausen et al. *J. Clin. Microbiol.* **47**:2759-65, 2009

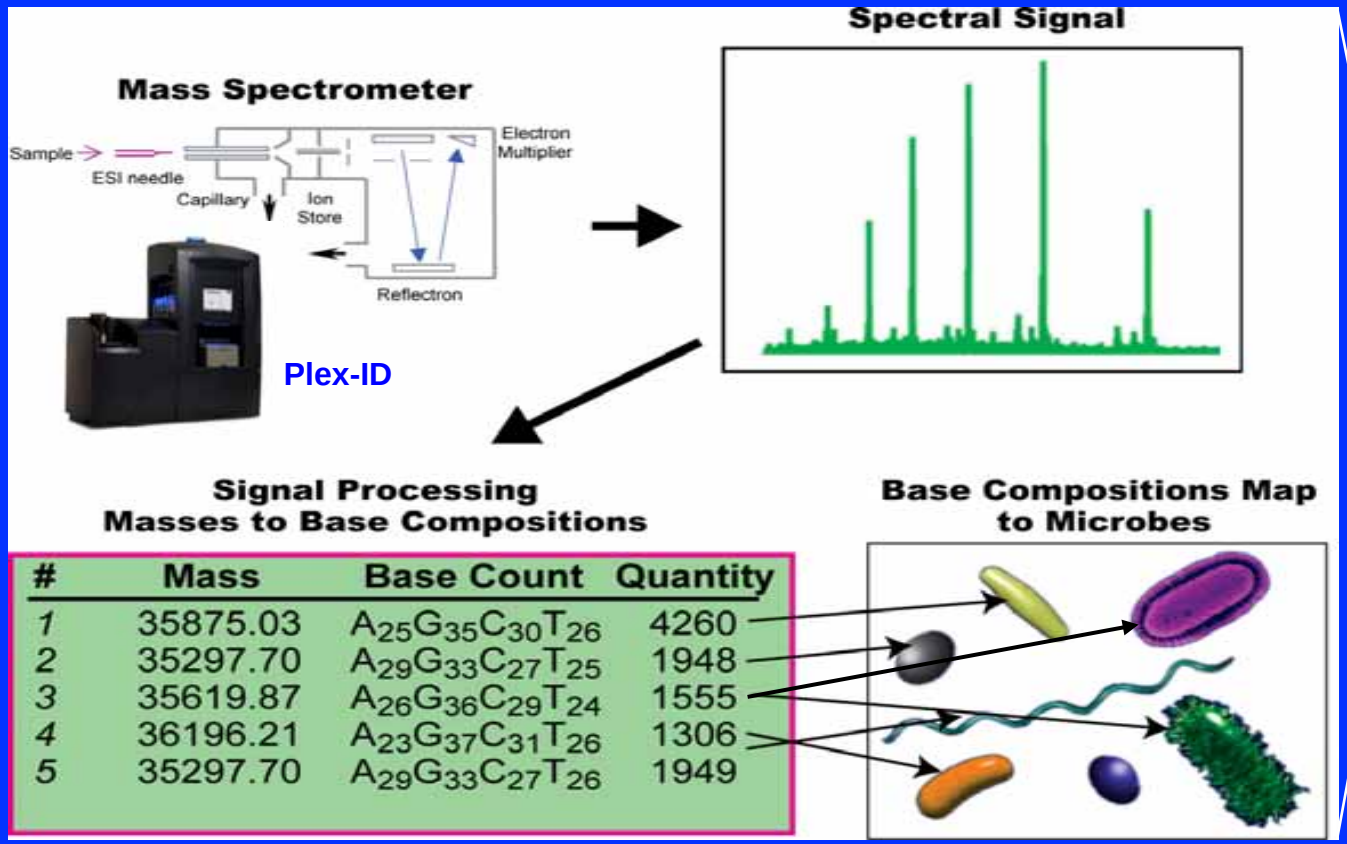
A Universal, Rapid and Accurate System for Detection and Identification of Bacterial Pathogens



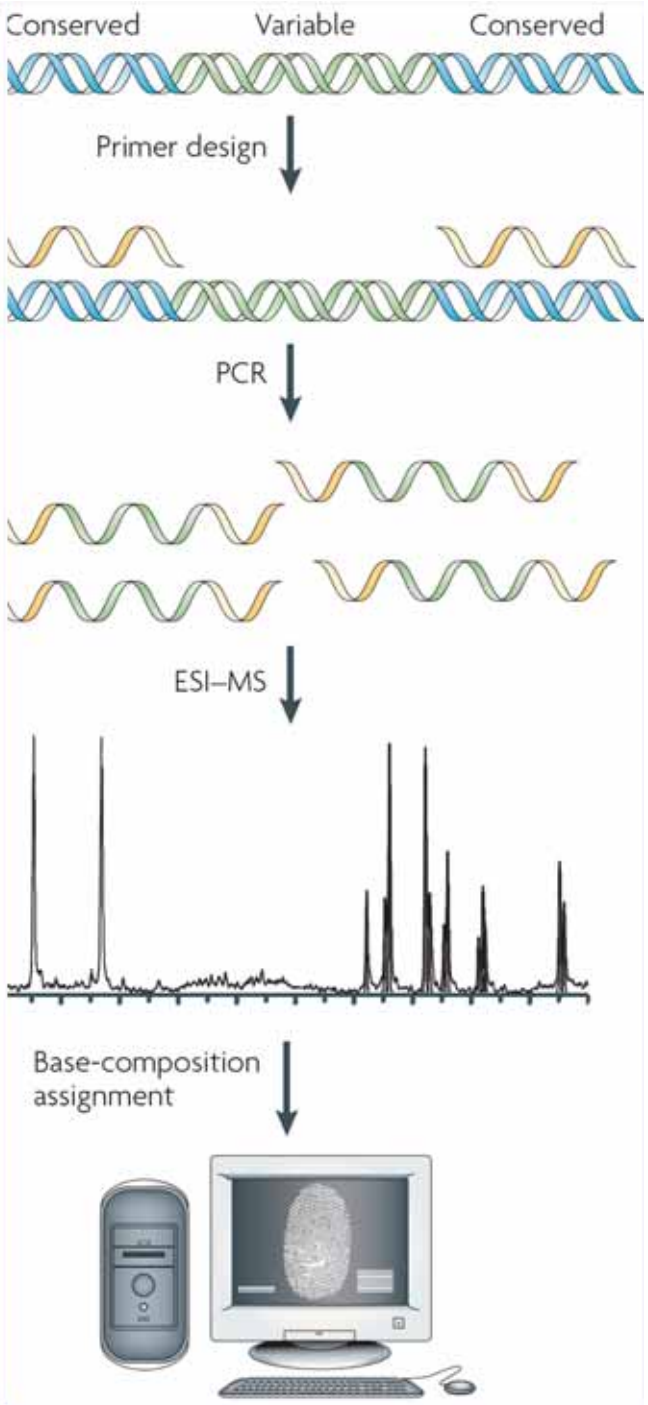
ibis Biosciences™

Abbott Molecular

PCR-MS Combines Sensitivity (PCR) and Specificity (MS)



#	Mass	Base Count	Quantity
1	35875.03	$A_{25}G_{35}C_{30}T_{26}$	4260
2	35297.70	$A_{29}G_{33}C_{27}T_{25}$	1948
3	35619.87	$A_{26}G_{36}C_{29}T_{24}$	1555
4	36196.21	$A_{23}G_{37}C_{31}T_{26}$	1306
5	35297.70	$A_{29}G_{33}C_{27}T_{26}$	1949



Ecker et al. *Nat. Rev. Microbiol.* **6**:553-8, 2008

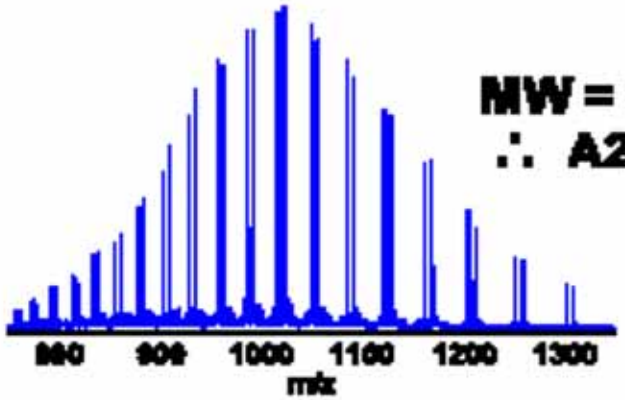
Masses to Base Composition

Penny = 2.500 g
Nickel = 3.950 g
Dime = 2.268 g
Quarter = 6.670 g

Scale

Weight = 377.33 g
∴ 28 Pennies
29 Nickels
25 Dimes
24 Quarters

A = 313.0576 amu
G = 329.0526 amu
C = 289.0464 amu
T = 304.0461 amu



Mass spectrum

MW = 32,596.90 amu
∴ A28 G29 C25 T24

You can distinguish any change, even a single nucleotide

Ecker et al. *Nat. Rev. Microbiol.* **6**:553-8, 2008

Detection and Identification of Ehrlichia species in Blood Using PCR/ESI Mass Spectrometry

- Directly from whole blood specimens
- Rapid procedure done within six hours
- Multiple organisms covered in one single reaction
- Simultaneous detection and identification

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0095-1137/10/$12.00 doi:10.1128/JCM.01669-09

Detection and Identification of *Ehrlichia* Species in Blood by Use of PCR and Electrospray Ionization Mass Spectrometry▽†

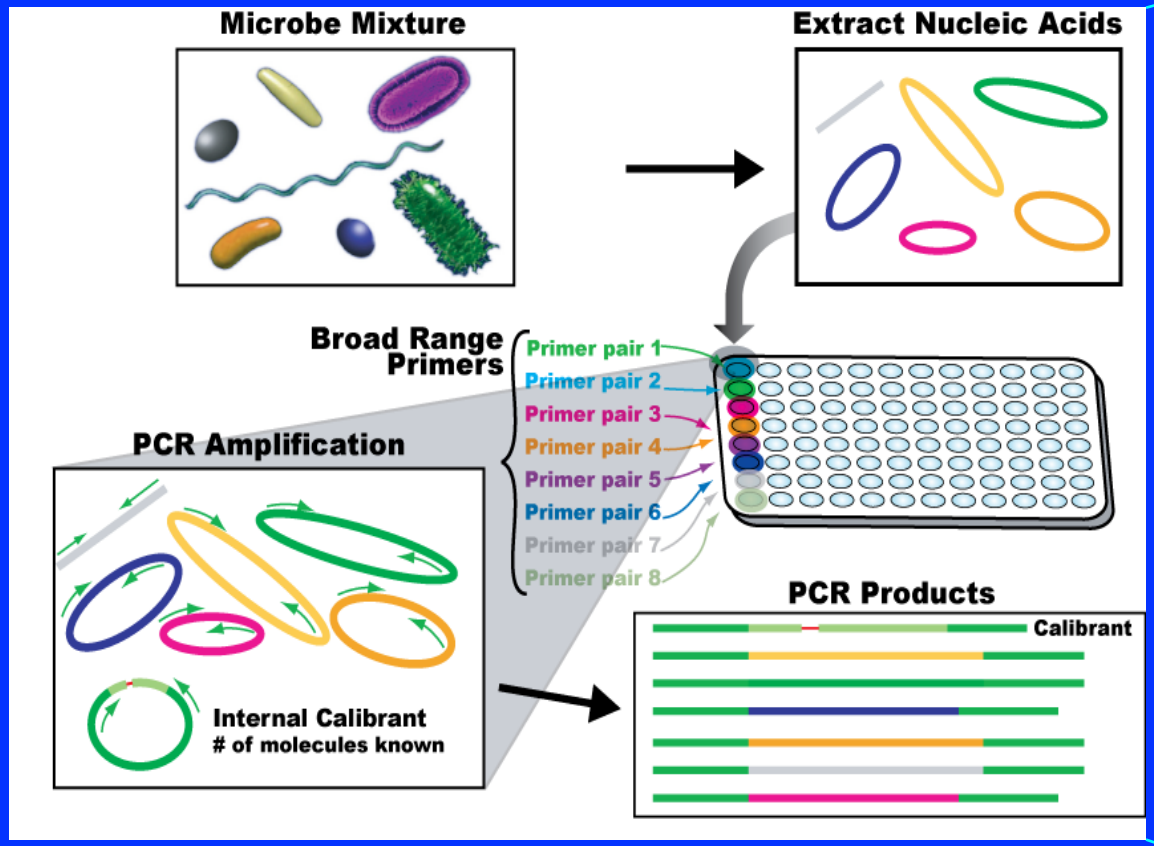
Mark W. Eshoo,[1] Chris D. Crowder,[1] Haijing Li,[2] Heather E. Matthews,[1] Shufang Meng,[2] Susan E. Sefers,[2] Rangarajan Sampath,[1] Charles W. Stratton,[2,3] Lawrence B. Blyn,[1] David J. Ecker,[1] and Yi-Wei Tang[2,3]*

Ibis Biosciences, Carlsbad, California 92008,[1] and Departments of Pathology[2] and Medicine,[3] Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Received 27 August 2009/Returned for modification 29 October 2009/Accepted 20 November 2009

- When: May 1 to August 1, 2009
- Where: Vanderbilt University Medical Center
- What: 213 whole blood specimens suspected of *Ehrlichia* infections
  - PCR-EIA assay detects *E. chaffeensis*, *E. ewingii* and *Anaplasma phagocytophilum*
  - PCR/ESI MS detects and differentiate tick-borne bacterial species

Detection and Identification of Bacterial Pathogens in Blood: Boom/PCR/ESI-MS



PCR/ESI/MS Primers and Bacterial Gene Targets

Primer pair	Primer ID[a]	Primer sequence	Target	Target clade/genus
BCT3517	BCT8241F BCT8242R	TGCTGAAGAGCTTGGAATGCA TACAGCAATTGCTTCATCTTGATTTGC	Flagellin	All *Borrelia* spp.
BCT3515	BCT8237F BCT8238R	TCCACAAGGTGGTGGTGAAGG TCGGCTGTCCCCAAGGAG	*rplB*	All *Spirochaetes*
BCT1083	BCT2764F BCT2763R	TAAGAGCGCACCGGTAAGTTGG TCAAGCGATCTACCCGCATTACAA	*RNaseP*	All *Rickettsia* spp.
BCT1084	BCT2765F BCT2763R	TCCACCAAGAGCAAGATCAAATAGGC TCAAGCGATCTACCCGCATTACAA	*RNaseP*	All *Rickettsia* spp.
BCT3569	BCT8334F BCT8335R	TGCATGCAGATCATGAACAAAATGC TCCATGTGCTGGTCCCCA	*gltA*	*Bartonella*, *Anaplasma*, and *Ehrlichia*
BCT3575	BCT8346F BCT8347R	TGCATCACTTGGTTGATGATAAGATACATGC TCACCAAAACGCTGACCACCAAA	*rpoB*	*Bartonella*, *Anaplasma*, and *Ehrlichia*
BCT3570	BCT8336F BCT8337R	TGCATGCAGATCATGAACAGAATGC TCCACCATGAGCTGGTCCCCA	GLTA	*Bartonella*, *Anaplasma*, and *Ehrlichia*
BCT3571	BCT8338F BCT8339R	TAAGGTTGGTGGATCTAGTGAAGTTGA TACACCTTCCTCAACAGCAGC	*groEL*	*Anaplasma* and *Ehrlichia*
BCT3573	BCT8342F BCT8343R	TGTGGAAGGTGAAGCTTTGGCAAC TAACATGGCTTTACGGCGATCACC	*groEL*	*Bartonella*
BCT3574	BCT8344F BCT8345R	TTCTGACTATGACCGTGAGAAATTGCAAG TCACCAACACGGATAACAGCAACACC	*groEL*	*Bartonella* and *Anaplasma*
BCT2328	BCT5602F BCT5603R	TGAGGGTTTTATGCTTAAAGTTGGTTTTATTGGTT TGATTCGATCATACGAGACATTAAAACTGAG	*asd*	*Francisella tularensis*
BCT1079	BCT2717F BCT2718R	TCGCCGTGGAAAAATCCTACGCT TAGCCTTTTCTCCGGCGTAGATCT	*icd*	*Coxiella burnetii*
BCT346	BCT1366F BCT1367R	TAGAACACCGATGGCGAAGGC TCGTGGACTACCAGGGTATCTA	16S rRNA gene	All bacteria
BCT348	BCT1393F BCT1370R	TTTCGATGCAACGCGAAGAACCT TACGAGCTGACGACAGCCATG	16S rRNA gene	All bacteria
BCT360	BCT1386F BCT1402R	TCTGTTCTTAGTACGAGAGGACC TTTCGTGCTTAGATGCTTTCAG	23S rRNA gene	All bacteria
BCT361	BCT1396F BCT1403R	TTTAAGTCCCGCAACGAGCGCAA TTGACGTCATCCCCACCTTCCTC	16S rRNA gene	All bacteria

Eshoo et al. *J. Clin. Microbiol.* **48**:472-8, 2010

Base Composition Signatures for Detection and Identification of Bacterial Pathogens

Primer pair	No. of each base (A, G, C, T) in the PCR amplicon						
	E. chaffeensis	*E. ewingii*	*R. rickettsii*	*Bacteroides* sp.	*N. meningitidis*	*P. aeruginosa*	*S. aureus*
BCT1083	DNP[a]	DNP	40, 34, 30, 31	DNP	DNP	DNP	DNP
BCT1084	DNP	DNP	25, 22, 21, 23	DNP	DNP	DNP	DNP
BCT3569	29, 32, 22, 39	34, 29, 24, 35	DNP	DNP	DNP	DNP	DNP
BCT3575	28, 30, 17, 37	29, 30, 17, 36	DNP	DNP	DNP	DNP	DNP
BCT3570	27, 35, 22, 41	32, 32, 24, 37	28, 30, 32, 35	DNP	DNP	DNP	DNP
BCT3571	30, 30, 11, 26	DNP	DNP	DNP	DNP	DNP	DNP
BCT346	28, 32, 21, 18	28, 32, 21, 18	28, 31, 24, 16	30, 28, 22, 19	29, 28, 26, 16	30, 31, 23, 15	27, 30, 21, 21
BCT348	26, 31, 30, 32	27, 30, 29, 33	25, 32, 32, 31	29, 31, 27, 28	26, 34, 30, 26	26, 32, 29, 29	30, 29, 30, 29
BCT360	35, 32, 23, 32	35, 32, 23, 32	33, 38, 27, 24	27, 37, 26, 32	34, 37, 25, 26	31, 36, 27, 28	31, 38, 24, 29
BCT361	33, 29, 26, 22	33, 29, 26, 22	31, 32, 25, 22	29, 31, 24, 26	27, 31, 26, 24	27, 33, 29, 20	29, 30, 25, 24

[a] DNP, does not prime; no PCR amplicon was generated using the specified primer pair.

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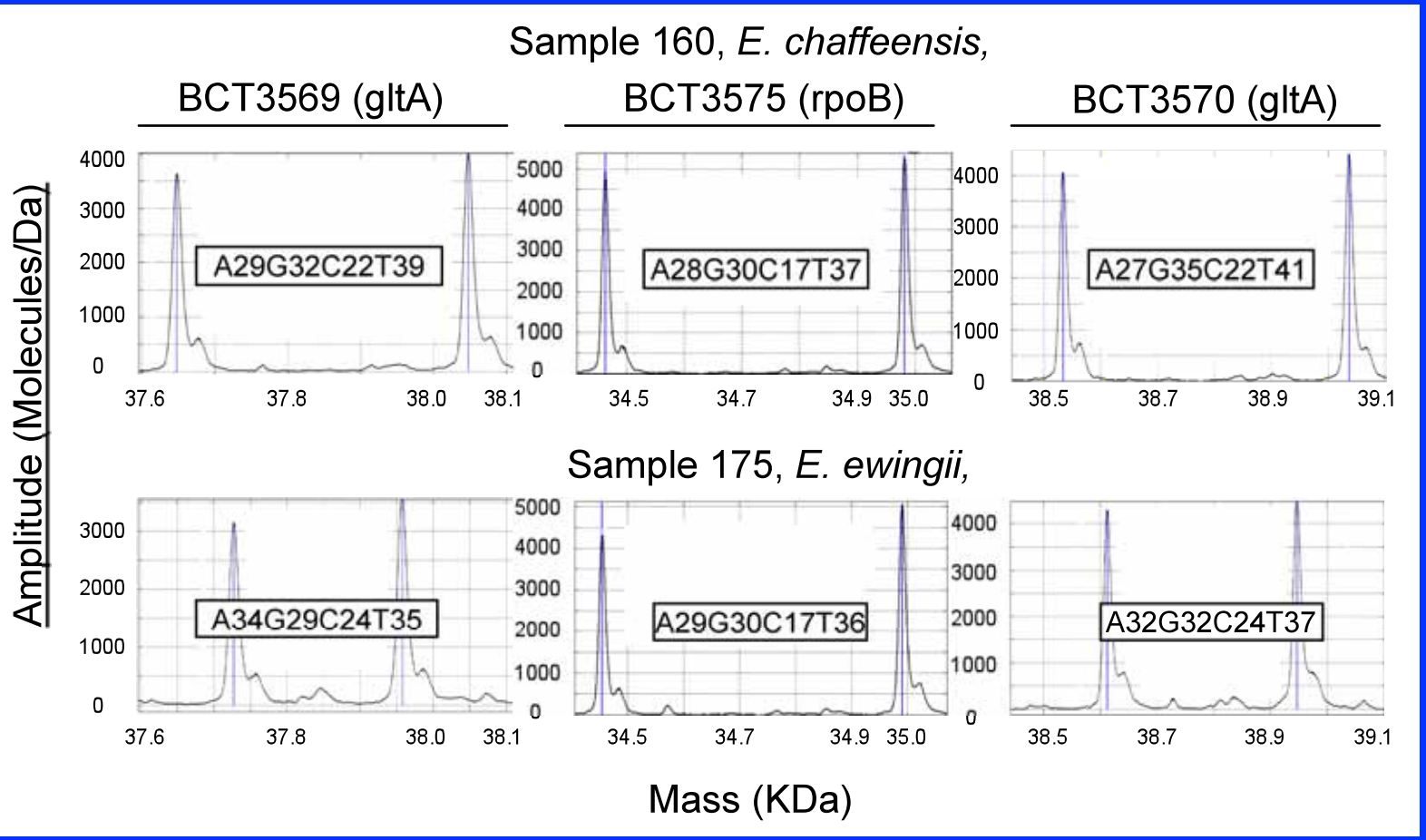
Mass Spectra from PCR/ESI-MS Analysis of Patient Specimens



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Diagnostic Performance of PCR/ESI-MS Assay for Detection of Ehrlichia Species in Blood

Pathogen	No. of samples[a]:				Diagnostic sensitivity (%)	Diagnostic specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	$S^+ T^+$ (true positive)	$S^+ T^-$ (false negative)	$S^- T^+$ (false positive)	$S^- T^-$ (true negative)				
E. chaffeensis	35	2	2	174	94.6	98.9	94.6	98.9
E. ewingii	3	0	0	210	100.0	100.0	100.0	100.0
Ehrlichia species	38	2	2	171	95.0	98.8	95.0	98.8

[a] S, PCR-EIA; T, PCR/ESI-MS; +, positive; −, negative.

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Detection and Identification of Additional Bacterial Pathogens by PCR/ESI-MS

Specimen(s)	Gender	Age (yr)	T5000 result	No. of genomes/ml of blood	Clinical diagnosis	Laboratory confirmation
61	Female	11	*R. rickettsii*	8.3×10^3	Likely tick-borne illness with dehydration and myalgia	Serum collected at acute phase positive for rickettsial IgM and negative for IgG. *R. rickettsii*-specific PCR-EIA was positive.
73	Male	49	*R. rickettsii*	$\geq 2.8 \times 10^5$	Clinical findings consistent with RMSF	Serum rickettsial IgG titer of 1:512. *R. rickettsii*-specific PCR-EIA was positive.
83, 81	Female	<1 (9 mo)	*R. rickettsii*	9.9×10^4, 3.0×10^4	Sepsis with multiorgan failure secondary to RMSF	Acute-phase serum was negative, and convalescent-phase serum was positive, for rickettsial IgG and IgM. *R. rickettsii*-specific PCR-EIA was positive.
58	Male	71	*P. aeruginosa*	$\geq 6.9 \times 10^4$	Bacteremia, aspergillosis	*Pseudomonas aeruginosa* recovered from blood culture
9	Female	19	*N. meningitidis*	$\geq 6.9 \times 10^4$	Bacterial meningitis, sepsis	*Neisseria meningitidis* recovered from blood culture
2	Male	47	*Bacteroides* spp.	$> 3.9 \times 10^4$	Diverticulitis, retroperitoneal abscess	Blood culture was negative
138	Male	2	*S. aureus*	1.7×10^4	Sepsis, septic arthritis, osteomyelitis	MRSA[a] recovered from blood culture

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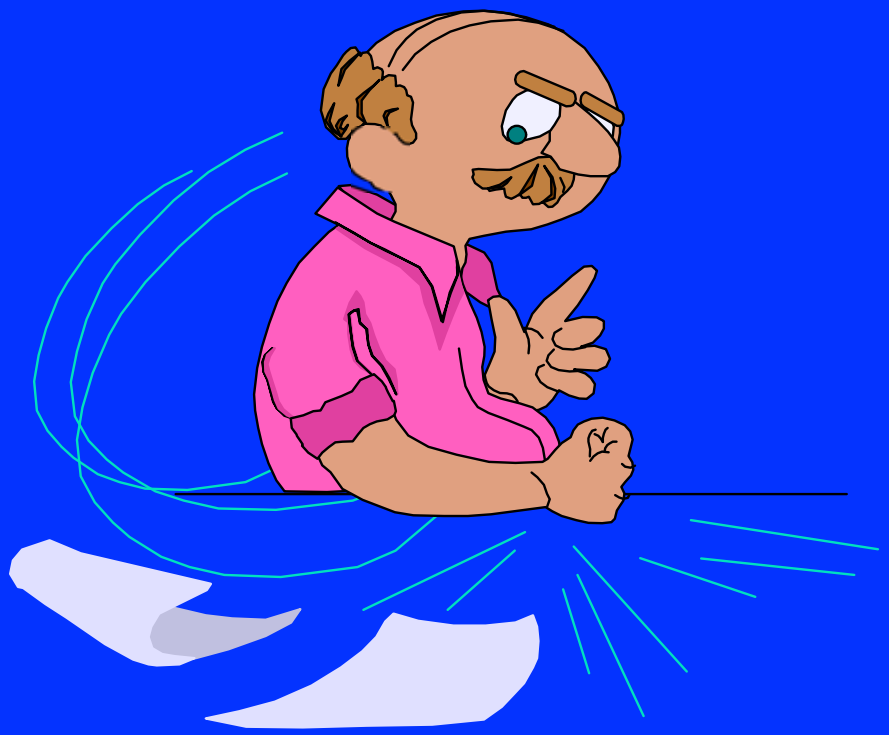
Summary

- The PCR/ESI-MS assay possessed sensitivity, specificity and positive and negative predictive values of 95.0%, 98.8%, 95.0%, and 98.8%, respectively
- The PCR/ESI-MS assay had a perfect speciation for 38 *Ehrlichia*-positive specimens.
- *Rickettsia rickettsii* was detected by PCR/ESI-MS from four specimens which were confirmed retrospectively by serology and PCR-EIA
- The PCR/ESI-MS assay identified *Pseudomonas aeruginosa*, *Neisseria meningitidis*, *and Staphylococcus aureus* from three specimens; these were confirmed by culture and/or clinical diagnosis
- From specimen processing to result reporting, the PCR/ESI-MS assay can be completed within six hours



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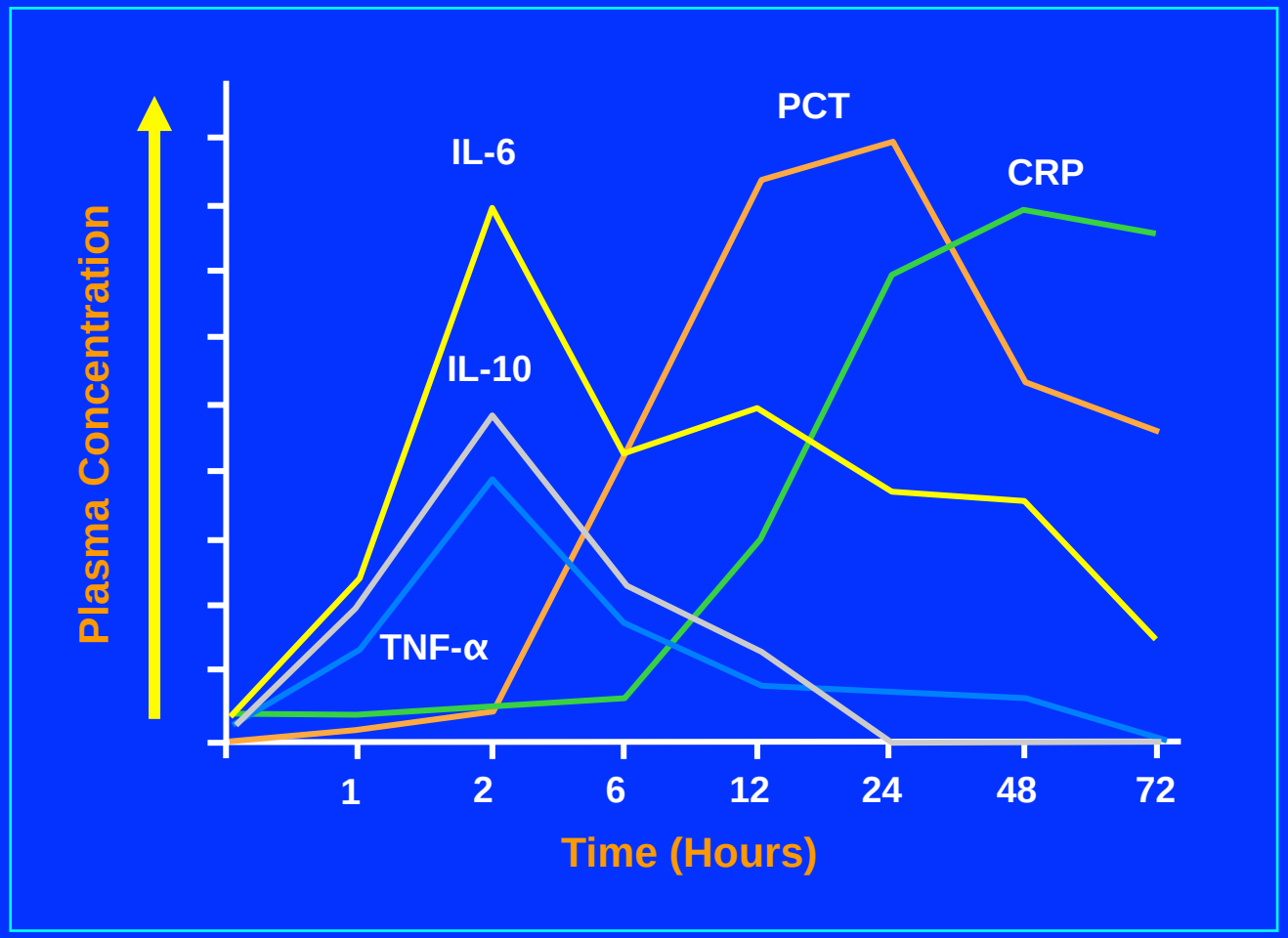
Beyond Bugs: Look for Host Responses



VANDERBILT UNIVERSITY

University Ranking: 19 (2009)
School Ranking: 15 (2009)
Hospital Ranking: 16 (2009)

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Gülhane Mikrobiyoloji Günleri

20 - 22 Nisan 2010

Antimikrobik Kemoterapi
Laboratuvar Uygulamaları ve Yenilikler

KLİMUD

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