
Detection of pathogens in sepsis: what is the role of molecular techniques?
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Content

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- Why could molecular methods be useful?
- FISH for identification of microorganisms in blood cultures
- Pro's and con's of PCR for detection of BSI
- Clinical potential of the bacterial DNA load (BDL) in blood
- Future perspectives

History

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Centralblatt f. Bakteriologie Abt. I. Bd. XXII. 12. August 1897.

Nachdruck verboten.

II. Weitere Mitteilungen über die Streptokokken-Enteritis bei Säuglingen.

Im Anschluß ar
Gelegenheit, 2 weite
nach meiner Ankunft
zu veröffentlichen u
Thatsachen mitzuteil

Detection of bloodstream infections — Review

New developments in the diagnosis of bloodstream infections

Remco P H Peters, Michiel A van Agtmael, Sven A Danner, Paul H M Savelkoul, and Christina M J E Vandenbroucke-Grauls

New techniques have emerged for the detection of bacteria in blood, because the blood culture as gold standard is slow and insufficiently sensitive when the patient has previously received antibiotics or in the presence of fastidious new era in the detection of bacteraemia, which necessitates a thorough reassessment of current concepts and ingrained beliefs on how and when the newer molecular techniques might make a clinical difference.

Blood culture

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Advantages
Confirms diagnosis
Deep-seated infections
Evaluation febris e.c.i.
Epidemiological tool

Disadvantages
Prior use of antibiotics
Turnaround time
Fastidious microorgansims
High a priori chance

Peters RP et al. *Lancet Infect Dis* 2004

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Antibiotics and blood cultures

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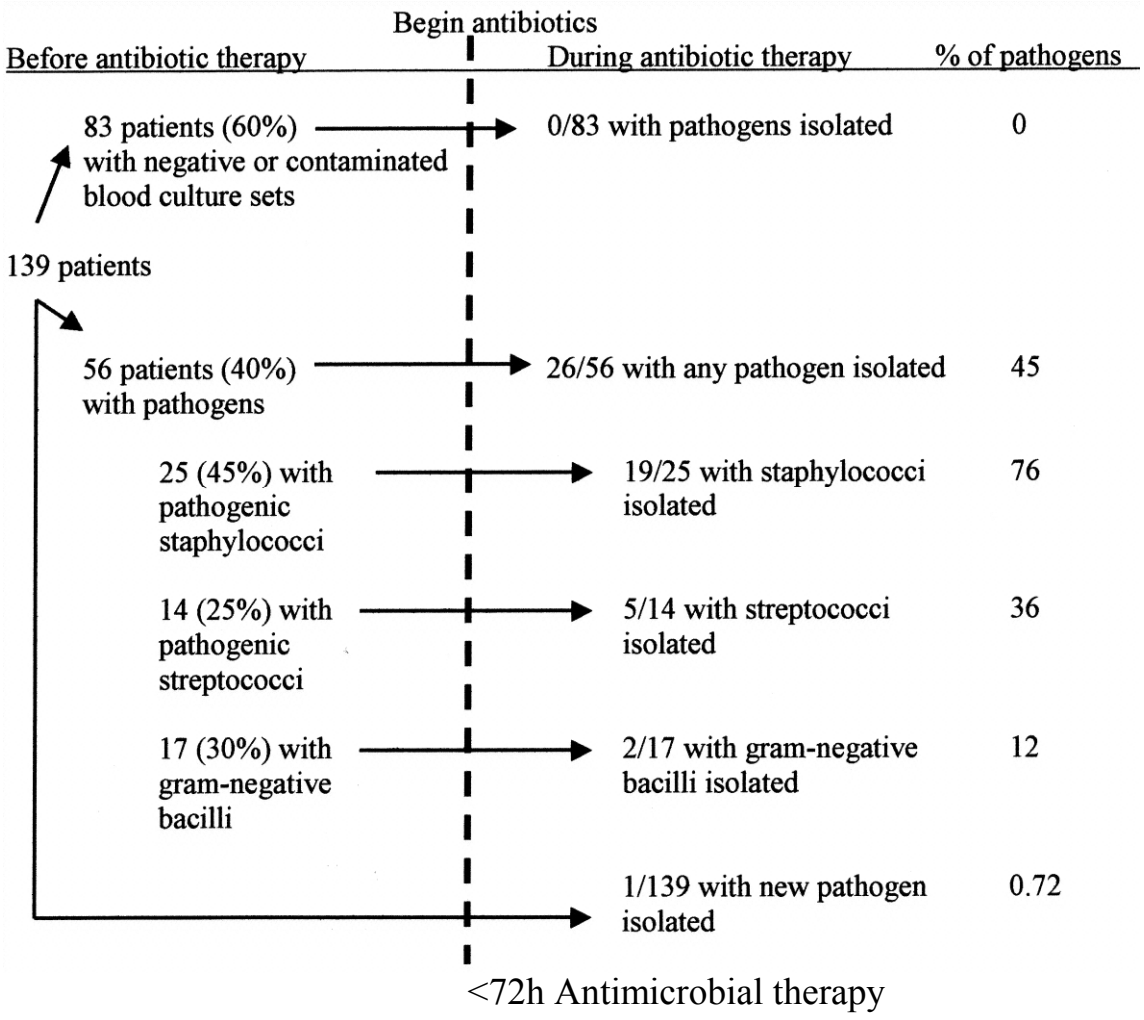

Grace CJ et al. *Clin Infect Dis* 2001

Time to diagnosis and mortality

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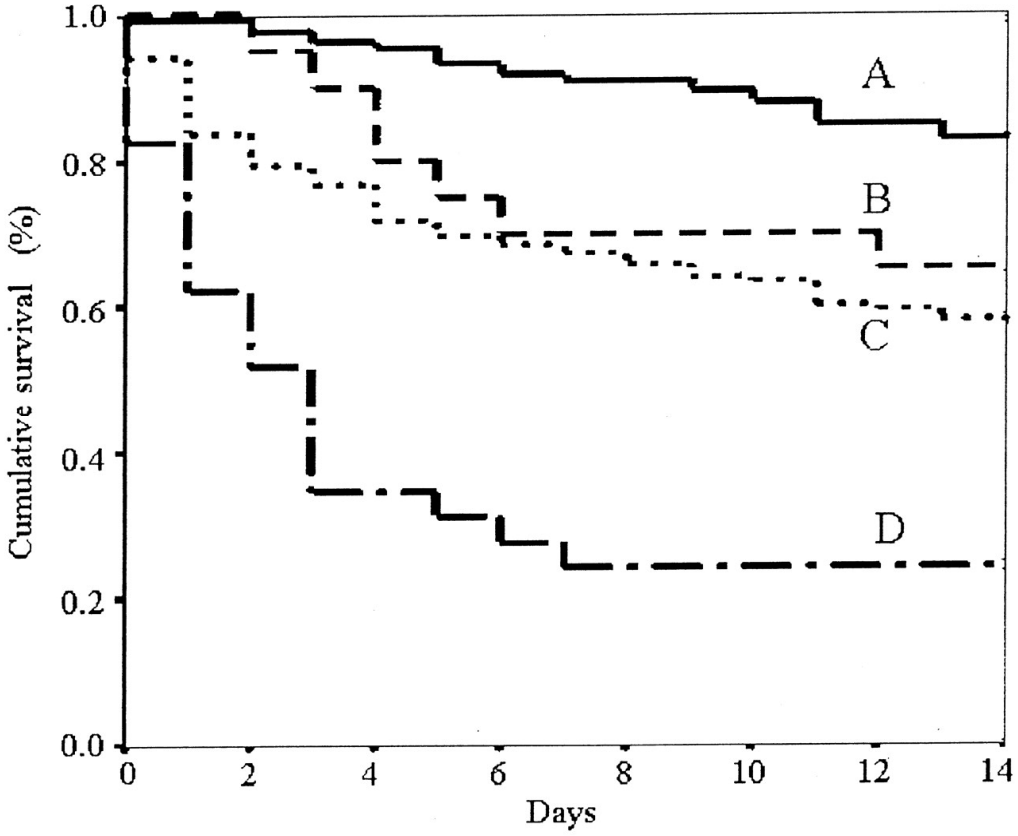
Adequate therapy is associated with better prognosis



Sepsis and adequate treatment

Sepsis and inadequate treatment

Septic shock and inadequate treatment

Septic shock and inadequate treatment

Valles J et al. *Chest* 2003

Time to diagnosis and mortality

VU medisch centrum

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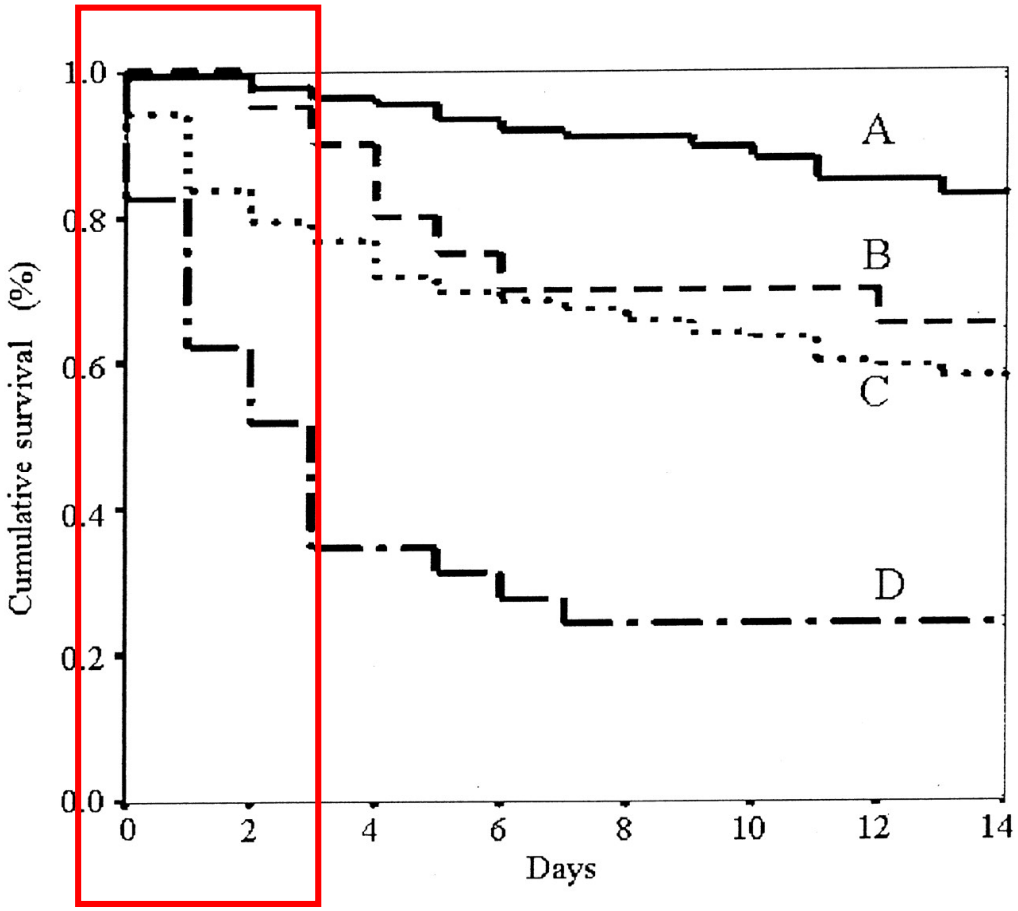

Sepsis and adequate treatment

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Valles J et al. *Chest* 2003

Molecular techniques

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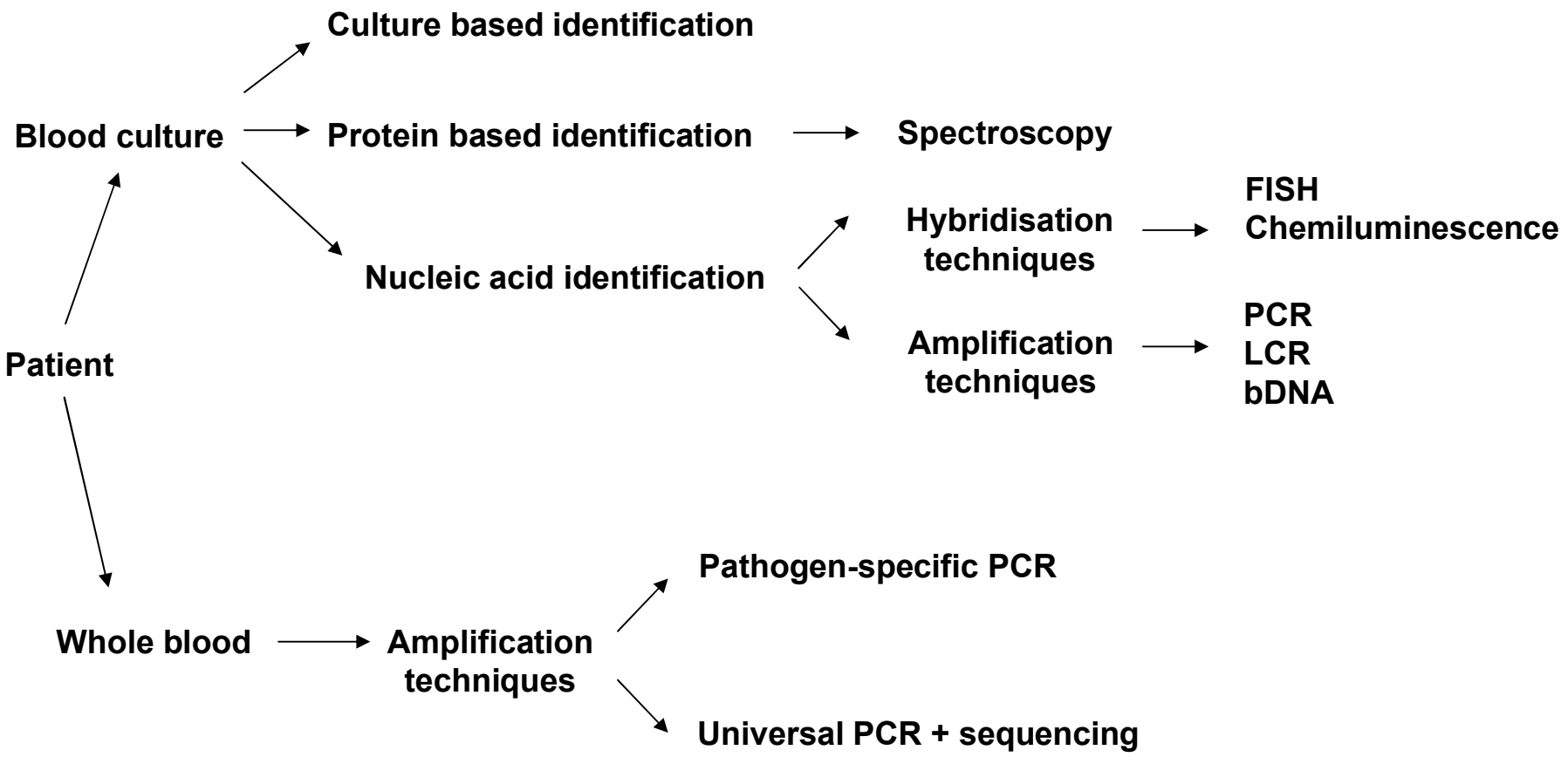

Peters RP et al. *Lancet Infect Dis* 2004

Molecular techniques

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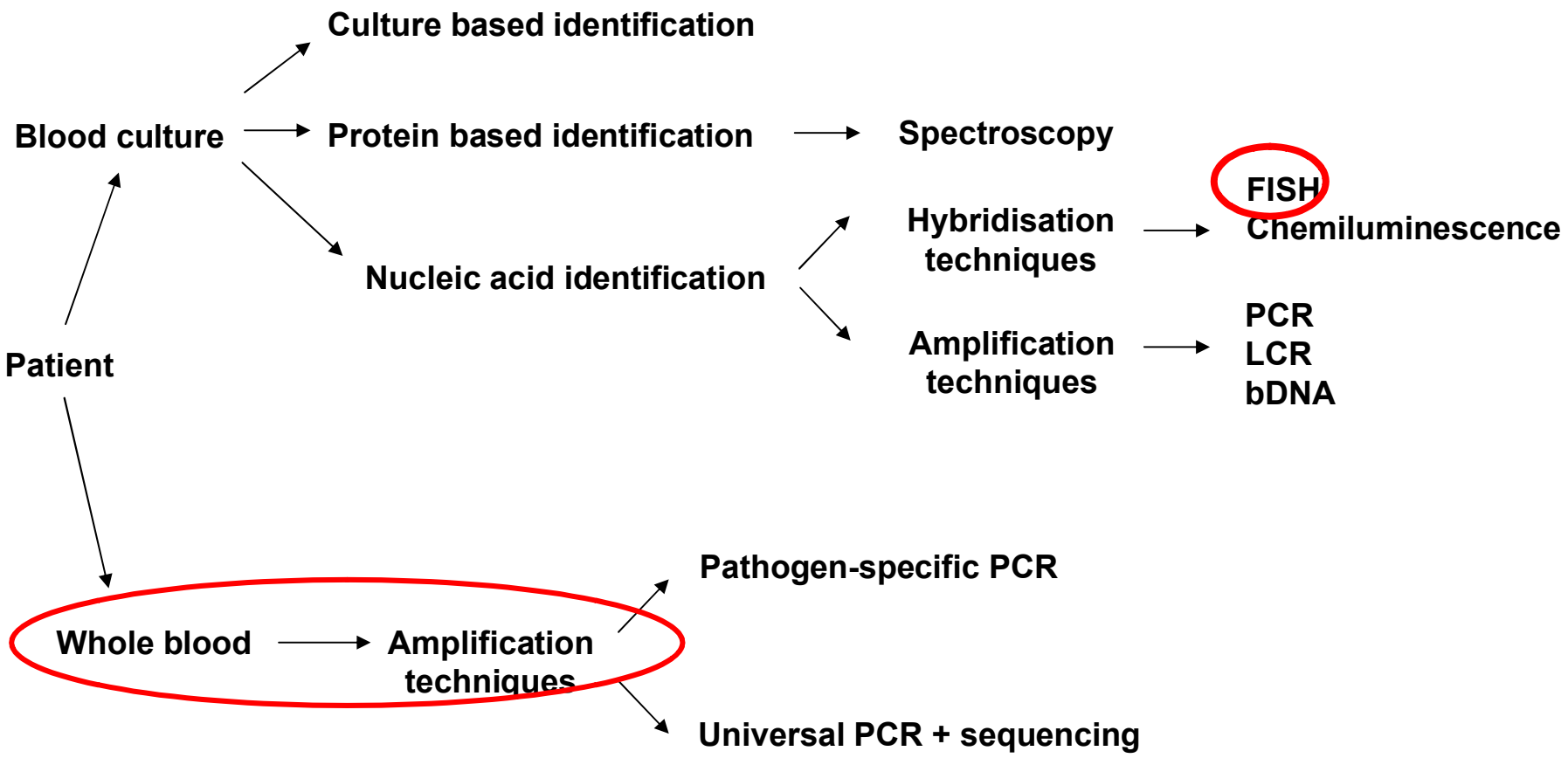

Peters RP et al. *Lancet Infect Dis* 2004

FISH technology

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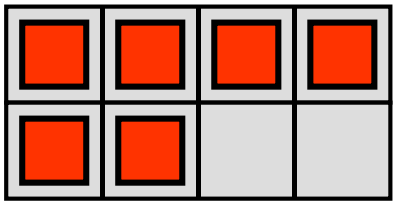
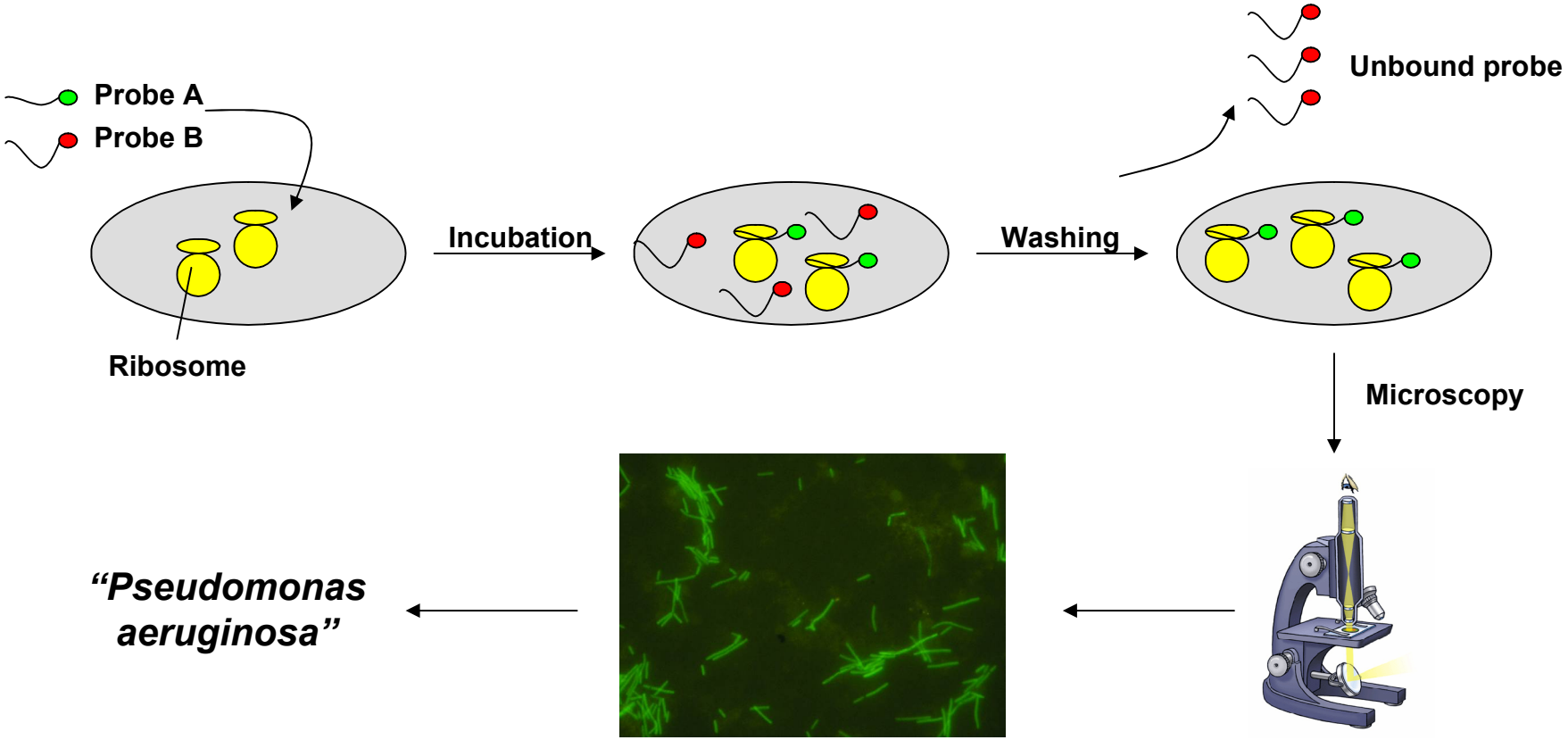


Application of blood culture fluid to slide
Fixation and permeabilisation of microorganisms



FISH probes

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Oligonucleotide probes

Eubacterial/panfungal
Enterobacteriaceae
Staphylococcus genus
Streptococcus genus
Enterococcus genus
Staphylococcus aureus
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus agalactiae
Enterococcus faecalis

Enterococcus faecium
Enterococcus galinarum
Escherichia coli/Shigella spp.
Klebsiella pneumoniae
Haemophilus influenzae
Bacteroides/Prevotella spp.
Neisseria meningitidis
Pseudomonas aeruginosa
Proteus/Morganella spp.
Helicobacter pylori

Candida albicans
Candida tropicalis
Candida glabrata
Candida krusei
Candida dubliensis
Candida parapsilosis
Clostridium difficile
Brucella spp.
Fusobacterium nucleatum
Fusobacterium necrophorum

PNA probes

Staphylococcus aureus
Escherichia coli
Pseudomonas aeruginosa
Klebsiella pneumoniae
Klebsiella pneumoniae

Campylobacter ssp.
Candida albicans
Candida krusei
Candida tropicalis
Candida parapsilosis

Candida glabrata
Mycobacterium tuberculosis
Mycobacterium avium
Mycobacterium kansasii
Helicobacter pylori

Performance of FISH

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- Probe sensitivity and specificity >95% for target microorganism
- Identification dependent on the probes included in the assay

Study	No. of blood cultures	Identification of family/genus	Identification of species	Probes not included
Kempf VA *et al.* (JCM 2000)	115	97%	66%	3%
Jansen GJ *et al.* (JCM 2000)	182	89%	65%	9%
Peters RP *et al.* (JCM 2006)	200	91%	79%	8%
Sogaard M *et al.* (JCM 2005)*	1231	-	46%	53%

*Only probes specific for *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* were included in this study

Time to diagnosis by FISH

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- The use of oligonucleotide FISH results in faster identification of microorganisms than with culture techniques in routine practice

Microorganism	FISH TTI (h)	Provisional identification		Final identification	
		TTI (h)	Time gain (h)[b]	TTI (h)	Time gain (h)
Staphylococci	4.1	5.5	1.4 ± 1.2	22.9	18.8 ± 1.6
Streptococci	4.5	5.8	1.3 ± 1.1	23.1	18.6 ± 1.3
Enterococci	4.7	7.1	2.4 ± 1.3	23.1	18.4 ± 1.7
Enterobacteriaceae	3.8			23.1	19.3 ± 1.5
Pseudomonas aeruginosa	3.6	5.7	2.1 ± 0.9	23.7	20.1 ± 1.1
Yeasts	4.9			47.0	42.1 ± 1.4

[a] TTI, mean time to identification.
[b] Difference in times to identification in hours (means ± standard deviations) between FISH and provisional culture.

- With a modified FISH procedure, the time to identification is reduced to less than 1 hour

Peters RP et al. *J Clin Microbiol* 2006 (1)
Peters RP et al. *J Clin Microbiol* 2006 (11)

Cost-effectiveness of FISH

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- PNA FISH for identification of *Candida albicans* in blood cultures reduces use of caspofungin

Drug (administration method)	n=31				n=41			
	C. albicans				Non-*C. albicans*			
	DDD/patient		Cost ($)/patient		DDD/patient		Cost ($)/patient	
	2003	2004	2003	2004	2003	2004	2003	2004
Fluconazole (oral)	11.4	14.9	23	29	8.2	8.7	16	17
Fluconazole (i.v.)	7.4	8.8	696	827	5.7	2.7	536	254
Caspofungin	8.7	3.2[a]	2,871	1,056	11.9	8.7	3,927	2,871
ABLC	9.8	10	1,540	1,570	10	9.7	1,570	1,522
Total[b]			169,290	107,942			260,107	191,224

[a] Statistically significant ($P < 0.05$).
[b] The last row shows the total overall cost per year.

- Total saving of PNA FISH identification is $1700 per patient
- Similar data in other study by Alexander and colleagues and for discrimination between Staphylococcus aureus and CNS

Forrest AN et al. *J Clin Microbiol* 2006
Forrest AN et al. *J Antimicrob Chemother* 2006
Alexander BD et al. *Diagn Microbiol Infect Dis* 2006

Discussion of FISH

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- Fast identification of majority of microorganisms in blood cultures
- Panel of probes based on local epidemiology
- Time-gain compared to culture identification
- FISH is cost-effective in situations with clear consequences for clinical management

Discussion of FISH

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--> Does the time-gain to identification have sufficient impact on clinical management to warrant implementation of FISH in routine practice?

Therapeutic decisions

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- The majority of therapeutic decisions related to bloodstream infections are taken shortly after presentation

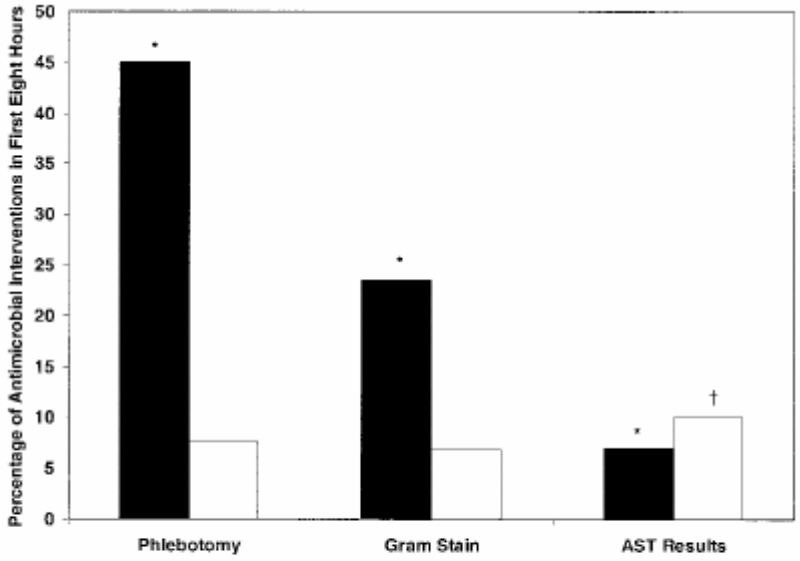

FIG. 1. Percentage of all antimicrobial interventions occurring within the first 8 h (initiations, solid bars; discontinuations, open bars) after each event of interest. *, $P < 0.001$ for differences noted in therapy initiations. †, $P < 0.05$ for differences noted in therapy discontinuations.

Munson EL et al. *J Clin Microbiol* 2003

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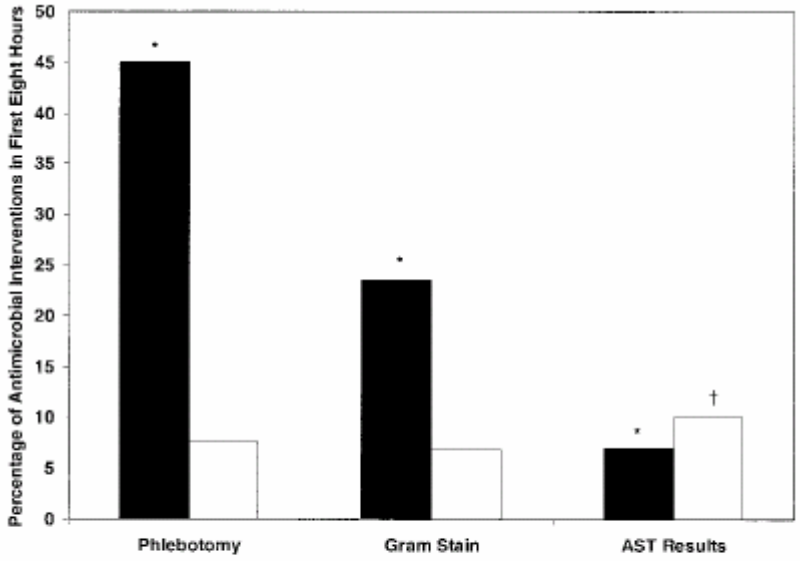

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Does PCR directly on blood samples provide a fast and reliable alternative to the blood cultures?

Munson EL et al. *J Clin Microbiol* 2003

PCR detection of BSI

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Target	Population	Sensitivity	Specificity
Eubacterial 16S	Neonates Neonates with sepsis	96% 96%	66% 99%
Panfungal	Haemato-oncology patients	75%	70%
Streptococcus pneumoniae	Patients with CAP Meningitis patients Retrospective case-control study	75% 92% 69%	96% 98% -
Staphylococcus aureus	ICU admissions	75%	93%
Enterococcus faecalis	ICU admissions	73%	96%
Neisseria meningitidis	Meningitis patients Children with meningitis	88% 100%	98% 17%
Haemophilus influenzae	Meningitis patients	100% (n=9)	99%
Salmonella typhi	Patients suspected of typhoid fever	64%	79%
Aspergillus species	Patients suspected of invasive aspergillosis	92% 67%	95% 100%

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Sensitivity issues PCR

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- Sensitivity of PCR assays on whole blood for BSI: 64-75%
- Higher sensitivity: blood samples of neonates or children (96-100%) or from patients suspected of meningitis (88-100%)
- To improve sensitivity:

  -Increase volume of blood in DNA isolation

  -Purification steps prior to DNA isolation

  -Test multiple blood samples

  -Adequate processing controls

Specificity issues PCR

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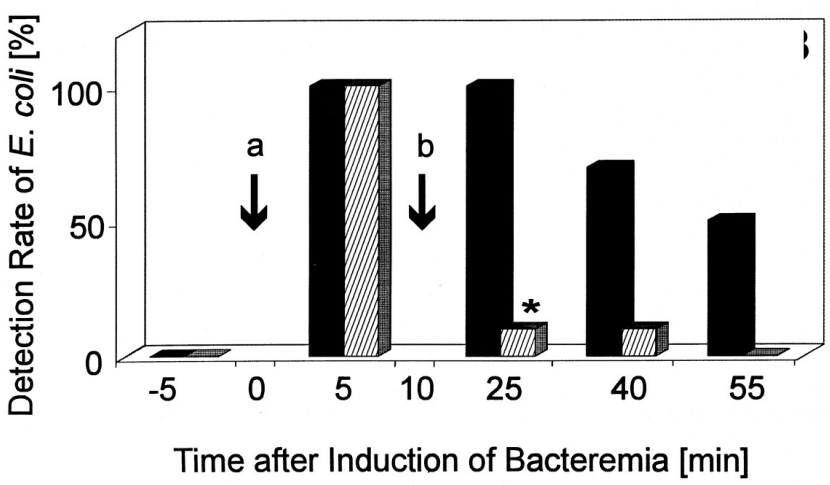
- Additional cases identified by PCR, blood culture negative

  -PCR false-positive: contamination, aspecific PCR reactions

  -Blood culture false-negative: use of antibiotics, sampling error



a. Induction of E. coli bacteraemia
b. Treatment with cefotaxim

Heininger A et al. *J Clin Microbiol* 1999

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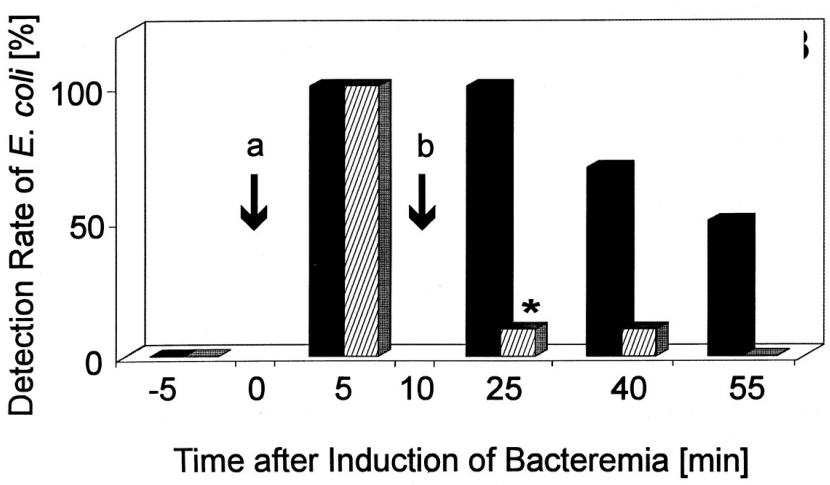

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- **Difficulty with 'Gold standard': Bacteraemia vs. DNAemia**

Heininger A et al. *J Clin Microbiol* 1999

Specificity issues PCR

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- Positive *S. aureus* or *E. faecalis* DNAemia but negative blood cultures:

  -6/12 (50%): likely related to infection (clinical & microbiological data)

  -5/12 (42%): clinical data consistent with *S. aureus*/*E. faecalis* infection

  -1 case unlikely related to infection: other focus found

- Clinical interpretation of a positive DNAemia: a potential role for the bacterial DNA load (BDL)?

	Invasive SP pneumonia	**Non-invasive SP pneumonia**	**Other pneumonia**	**P-value**
BDL (cfu equiv./mL)	350 (<125-2350)	210 (<125-900)	<125	<0.001

Peters RP et al. *J Clin Microbiol 2007*
Peters RP et al. *Submitted*

BDL as marker of severity

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BDL and severity of infection

- Higher *S. aureus* / *S. pneumoniae* BDL for invasive vs. localised infection
- Higher BDL in patients with *S. pneumoniae* meningitis vs. pneumonia
- Higher BDL on admission for nonsurviving vs. surviving children with pneumococcal or meningococcal meningitis
- Correlation of *S. pneumoniae* BDL with C-reactive protein, IL-6, IL-10 and TNFα levels

Carrol ED et al. *Pediatr Infect Dis J* 2007
Hackett SJ et al. *Arch Dis Childh* 2002
Peters RP et al. *Submitted*

Discussion use of PCR

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- PCR: fast results, no culture step required
- Improvement of sensitivity is essential for routine application
- Validation of other PCR assays for panels or algorithms on blood
- Sensitivity of the assays is related to patient category and condition
- A positive DNAemia is related to infection with the microorganism in the majority of cases
- The possible association of BDL with clinical and microbiological severity of infection warrants further study

Future perspectives (1)

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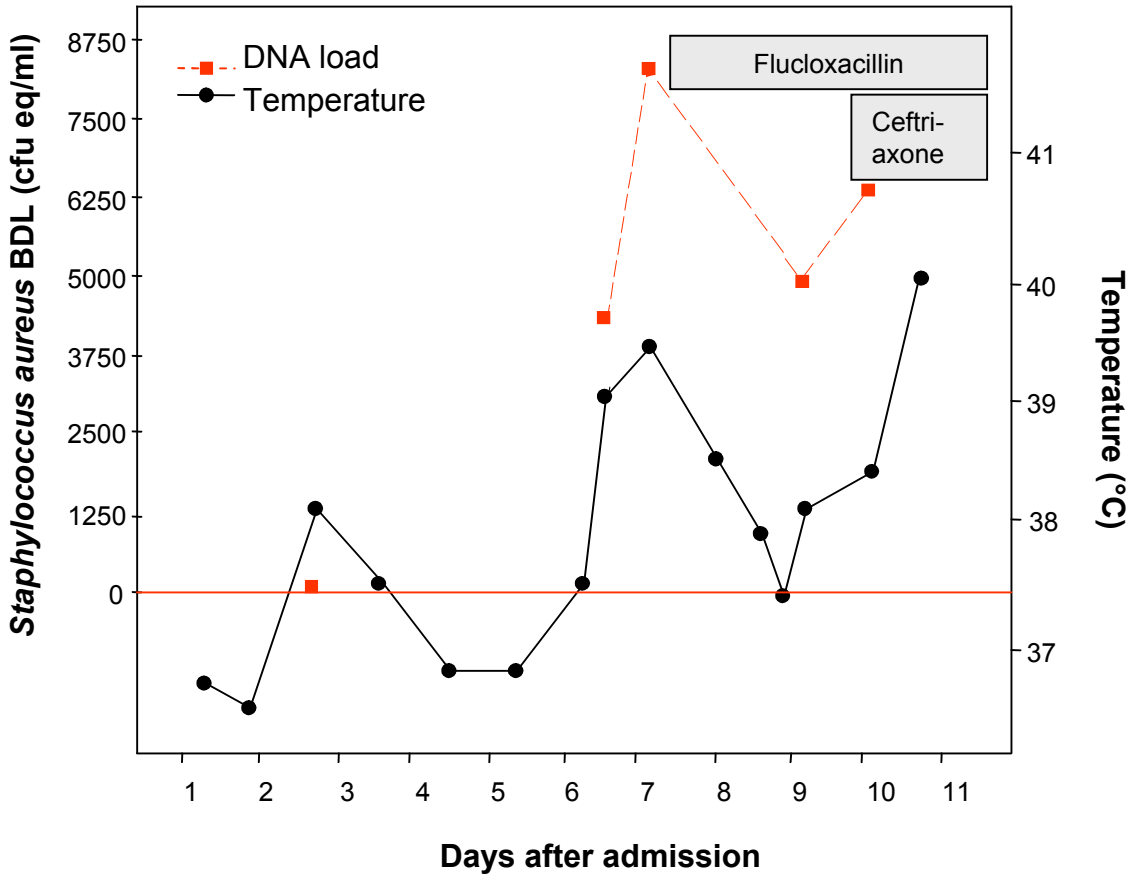
- BDL for monitoring of infection? Kinetics of DNA in blood?



Peters RP et al, *J Clin Microbiol* 2007

Future perspectives (2)

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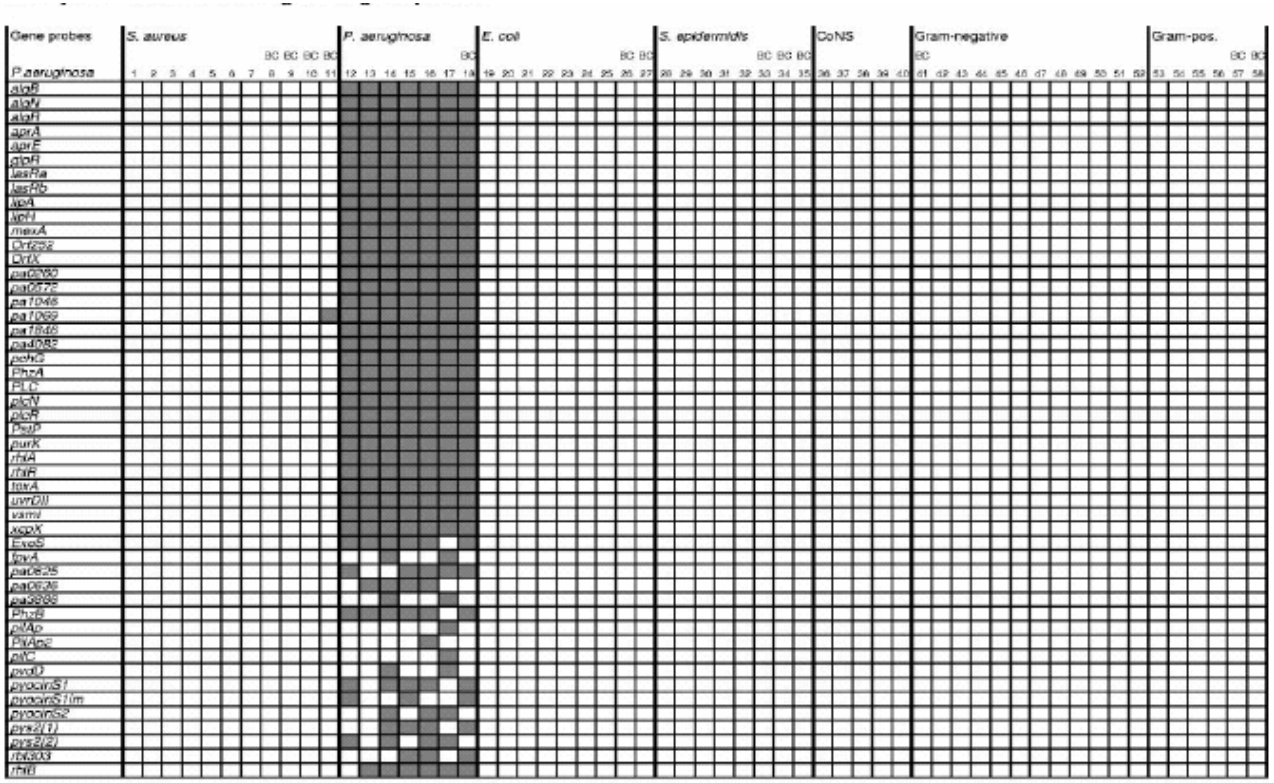
- Simultaneous molecular detection, identification, quantification and susceptibility determination by micro-array analysis



Cleven BE et al. *J Clin Microbiol* 2006

Conclusion

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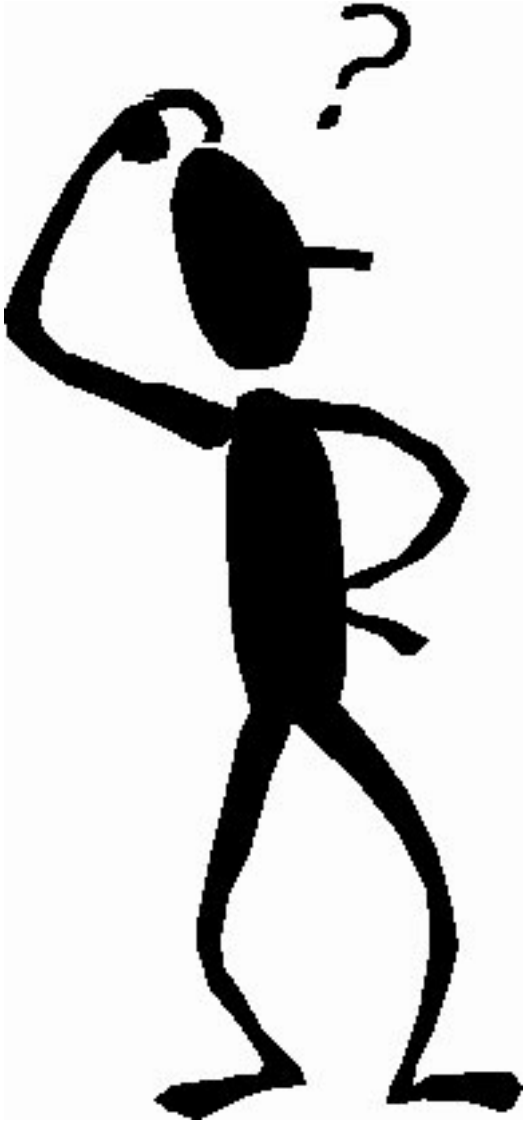
- Molecular techniques emerge for faster and more sensitive detection of bloodstream infections
- FISH identification of microorganisms in positive blood cultures is reliable and cost-effective
- PCR on whole blood is a promising tool for rapid detection of BSI
- Potential value of BDL in blood to identify high-risk patients and to monitor infection



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Case report (1)

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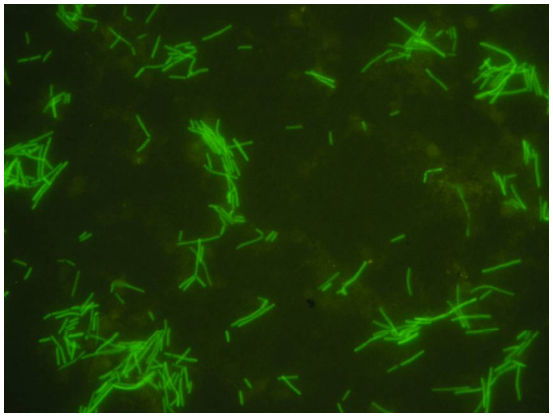
Patient M.: an 86-years old man

- History of relapsing urinary tract infections
- Presentation with fever, pollakisuria and mental status alterations
- Urine cultures negative, blood culture: Gram-negative rods
- Ceftriaxone and gentamicin were started empirically for urosepsis
- FISH/VITEK: *Pseudomonas aeruginosa* --> Therapy change



Peters RP et al. *J Clin MIcrobiol* 2006 (11)

Cost-effectiveness of FISH (2)

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- PNA FISH for discrimination between *Staphylococcus aureus* and CNS in blood cultures: reduction in use of vancomycin and hospital costs

	n=84	n=119	
	Control ($)	PNA FISH ($)	Savings ($)
Average bed cost/patient	9002	6298	2704
Average pharmacy costs/patient	3371	2386	985
Average laboratory costs/patient[a]	1248	932	316
Total costs/patient	13 621	9616	4005

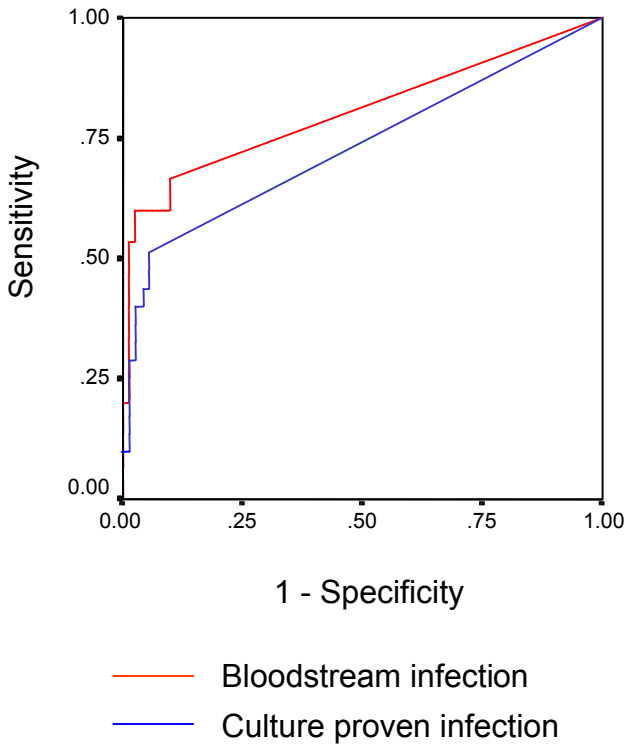
[a]Laboratory costs include radiology, chemistry and hematology costs.

- Retrospective study --> prospective confirmation warranted
- Oligonucleotide probes are cheaper than PNA probes

Forrest AN et al. *J Antimicrob Chemother* 2006

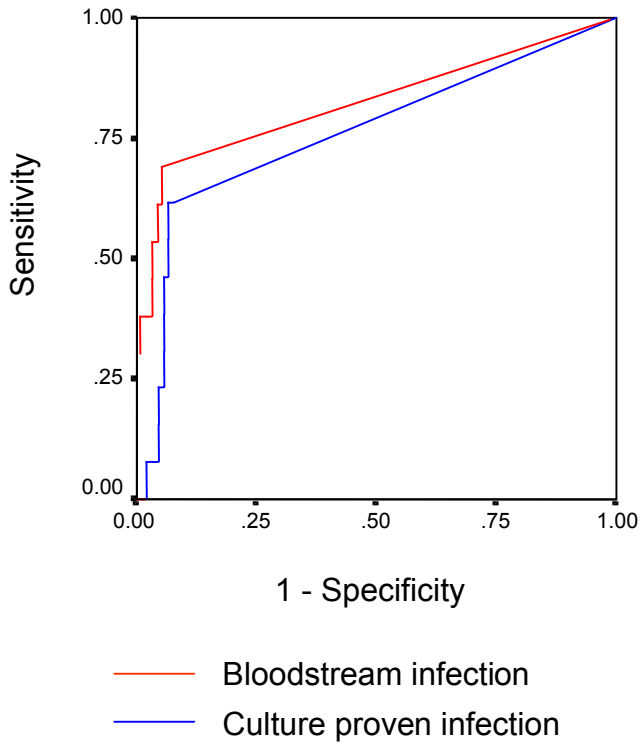
Figure 1. Receiver operating characteristic curves of bacterial DNA load for bloodstream infection and culture proven infection.

Staphylococcus aureus



	AUC	P-value
Bloodstream infection	0.80	<0.001
Local microbial infection	0.73	<0.001

Enterococcus faecalis



	AUC	P-value
Bloodstream infection	0.82	<0.001
Local microbial infection	0.76	<0.001