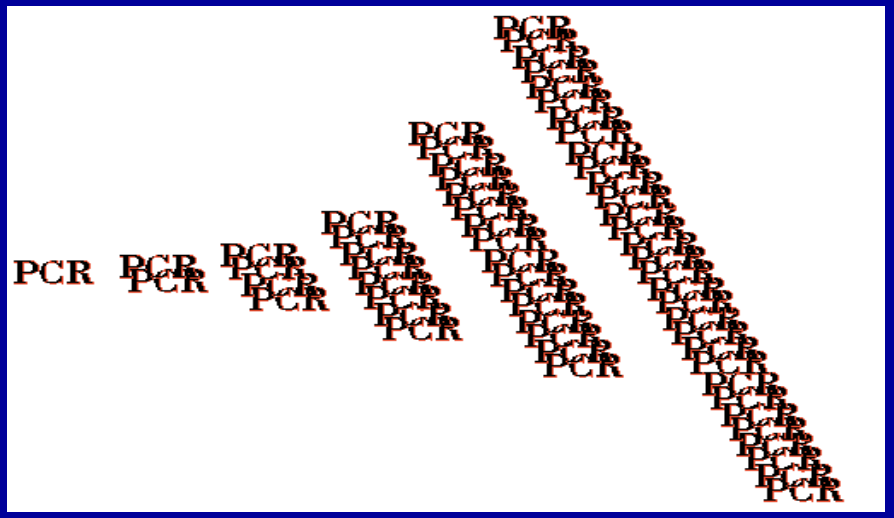
16S PCR til diagnostik af infektioner problemer og muligheder



Marianne Voldstedlund

Lisbeth Nørum Pedersen og Kurt Fuursted

KMA, Skejby Sygehus

Oversigt

- Indledning
- 16S PCR til klinisk brug. Generelle betragtninger.
- Erfaringer med prøver modtaget på KMA, Skejby:
  - Endocardit projekt
  - Spondylit projekt
  - Pacemaker pilot studium
  - Diverse ortopædkirurgiske prøver
  - Andre typer prøver
  - ID af isolater fra Dyrkningslaboratoriet
- Sammenfatning.

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1990, p. 1942–1946 Vol. 28, No. 9
0095-1137/90/091942-05$02.00/0

Amplification of Bacterial 16S Ribosomal DNA with Polymerase Chain Reaction

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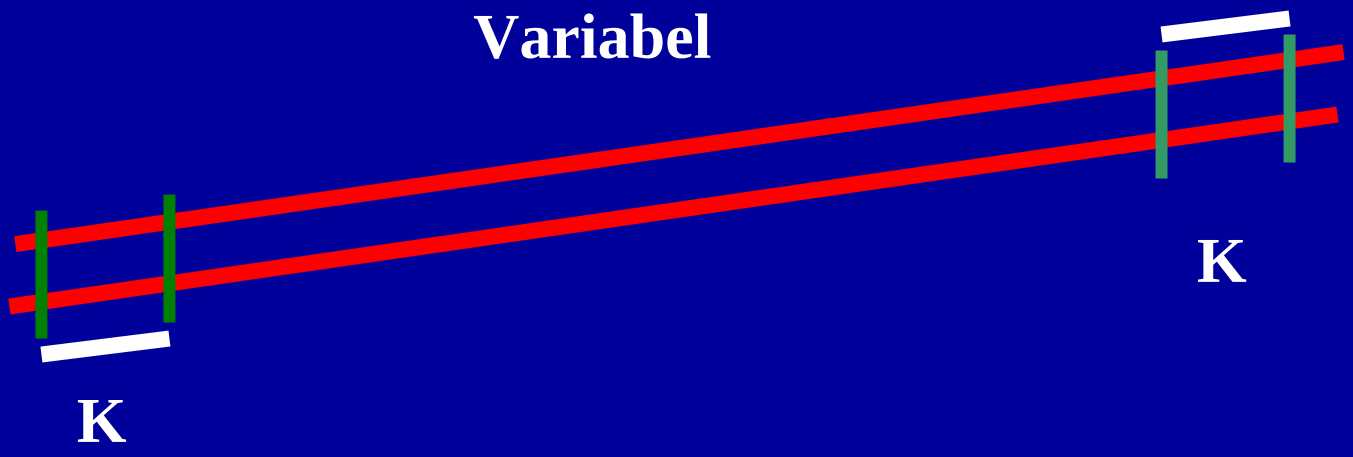
Received 15 December 1989/Accepted 21 May 1990

The sequence of small-subunit rRNA varies in an orderly manner across phylogenetic lines and contains segments that are conserved at the species, genus, or kingdom level. By directing oligonucleotide primers at sequences conserved throughout the eubacterial kingdom, we amplified bacterial 16S ribosomal DNA sequences with the polymerase chain reaction. Priming sites were located at the extreme 5′ end, the extreme 3′ end, and the center of 16S ribosomal DNA. The isolates tested with these primers included members of the genera *Staphylococcus*, *Coxiella*, *Rickettsia*, *Clostridium*, *Neisseria*, *Mycobacterium*, *Bilophila*, *Eubacterium*, *Fusobacterium*, and *Lactobacillus* and the family *Enterobacteriaceae*. Initially, the yields from the reactions were erratic because the primers were self-complementary at the 3′ ends. Revised primers that were not self-complementary gave more reproducible results. With the latter primers, 0.4 pg of *Escherichia coli* DNA consistently gave a visible band after amplification. This method should be useful for increasing the amounts of bacterial 16S ribosomal DNA sequences for the purposes of sequencing and probing. It should have a broad range of applications, including the detection and identification of known pathogens that are difficult to culture. This approach may make it possible to identify new, nonculturable bacterial pathogens.

16S rDNA PCR



NCBI

nucleotide–nucleotide BLAST

Nucleotide | Protein | Translations | Retrieve results for an RID

New BLAST design to be released on April 16

New BLAST interface beta release Try it NOW !

Search

Set subsequence From: To:

Choose database
- Human genomic plus transcript
- Mouse genomic plus transcript
- Others (nr etc.):

Human genomic plus transcript

NEW Two new **Human** and **Mouse** databases combine genomic plus transcript alignments in a single report. You can also choose from **Others** to use nr or an existing database.

Now: BLAST! or Reset query Reset all

Options for advanced blasting

Ribosomal Database Project II

Seqmatch - Start

[help]

Did you know you can select sequences from *myRDP* and Hierarchy Browser to do seqmatch?
Percent identity scores will be reported for aligned sequences (limited to 2000).

Please enter your sequences:

Choose a file to upload: Gennemse...

Cut and paste sequence(s) (in Fasta, GenBank, or EMBL format):

16S PCR kan identificere alle bakterier

- De levende, også de nemme
- De farlige
- De kræsne
- De langsomt voksende
- De døde
- - men kun én ad gangen

Analytisk sensitivitet ?
i vand

- I litteraturen :
  1 – 10^5 CFU / PCR rør
  afhænger af bakterie art

- På Skejby:
  Stafylococcus aureus: 40 kopier / µl prøve
  dvs 200 kopier i snit / PCR rør.
  (Højere sensitivitet for de andre bakterier)

JOURNAL OF CLINICAL MICROBIOLOGY, May 2002, p. 1575–1580 Vol. 40, No. 5
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.5.1575–1580.2002

MINIREVIEW

Risk Assessment Models and Contamination Management: Implications for Broad-Range Ribosomal DNA PCR as a Diagnostic Tool in Medical Bacteriology

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Molecular methods have now become established as accepted methods for the detection of causal agents of infection (viral, bacterial, fungal, and protozoal). In particular, the use of a combination of rRNA genes from bacteria, fungi, and protozoa, i.e., universal or broad-range targets, has become popular for detection. However, the laboratory workup of clinical specimens for universal PCR differs significantly from that for specific PCR in that numerous problems, mainly related to

pathogens in bacterial angiomatosis (12) and in Whipple's disease (3, 4).

In bacteria, there are three genes which make up the rRNA functionality: the 5S, 16S, and 23S rRNA genes. The 16S rRNA gene has historically been most commonly employed; however, more recently, employment of the 16-23S rRNA intergenic spacer region, along with the 23S rRNA gene, has become popular

16S PCR prøver på KMA, Skejby.
I perioden 2002-2007

632 prøver analyseret

193 positive (ca 30 %)

Prøvekategorier / antal

1.	Endocarditis	184
2.	Pacemaker	33
3.	Spondylitis	132
4.	Div ortopædkir	178
5.	Div lokalisationer	51
6.	ID af isolater	52

PCR af hjerteklap-prøver 1

- **I alt**: 184 prøver heraf 74 positive (40 %)
  inkludere kontrol patienter og patienter hvor endokardit diagnosen senere blev forkastet.

- **Analyseret**: 87 prøver fra 74 patienter
  16 prøver fra 16 kontrol patienter

PCR af hjerteklap-prøver 2

- Guld standart:

  Resultat af bloddyrkning

  + Duke kriterier (inkl PCR)

- 74 pt ialt:

  56 pt med definitiv endocarditis

PCR af hjerteklap-prøver 3

	16S PCR	Dyrkning
sensitivitet	64 % (36/56)	27 % (15/56)
specificitet	100 % (16/16)	62 % (6/10)
forurening	0 (0/90)	35 % (32/90)

Ingen hæmning af PCR reaktion

PCR af hjerteklap-prøver overvejelser

- Altid plads til forbedringer.

- Makroskopisk vurdering af klapvæv.
  Flere PCR prøver fra samme klap?.

PCR af hjerteklap-prøver 4

- 6 dyrkningsnegative: 3 PCR positive
- 2 Tropheryma whipplei
- 17 cases hvor PCR gav en mere præcis diagnose end bloddyrkning
- Ikke uvæsentligt med en konfirmation af diagnosen fra bloddyrkning

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 6123–6125 Vol. 43, No. 12
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.12.6123–6125.2005

The Role of 16S rRNA Gene Sequencing in Identification of Microorganisms Misidentified by Conventional Methods

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Received 27 July 2005/Returned for modification 20 September 2005/Accepted 5 October 2005

Traditional methods for microbial identification require the recognition of differences in morphology, growth, enzymatic activity, and metabolism to define genera and species. Full and partial 16S rRNA gene sequencing methods have emerged as useful tools for identifying phenotypically aberrant microorganisms. We report on three bacterial blood isolates from three different College of American Pathologists-certified laboratories that were referred to ARUP Laboratories for definitive identification. Because phenotypic identification suggested unusual organisms not typically associated with the submitted clinical diagnosis, consultation with the Medical Director was sought and further testing was performed including partial 16S rRNA gene sequencing. All three patients had endocarditis, and conventional methods identified isolates from patients A, B, and C as a *Facklamia* sp., *Eubacterium tenue*, and a *Bifidobacterium* sp. 16S rRNA gene sequencing identified the isolates as *Enterococcus faecalis*, *Cardiobacterium valvarum*, and *Streptococcus mutans*, respectively. We conclude that the initial identifications of these three isolates were erroneous, may have misled clinicians, and potentially impacted patient care. 16S rRNA gene sequencing is a more objective identification tool, unaffected by phenotypic variation or technologist bias, and has the potential to reduce laboratory errors.

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 6073–6085 Vol. 43, N
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.12.6073–6085.2005

Use of Phylogenetic and Phenotypic Analyses To Identify Nonhemolytic Streptococci Isolated from Bacteremic Patients

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The aim of this study was to evaluate molecular and phenotypic methods for the identification of nonhemolytic streptococci. A collection of 148 strains consisting of 115 clinical isolates from cases of infective endocarditis, septicemia, and meningitis and 33 reference strains, including type strains of all relevant *Streptococcus* species, were examined. Identification was performed by phylogenetic analysis of nucleotide sequences of four housekeeping genes, *ddl*, *gdh*, *rpoB*, and *sodA*; by PCR analysis of the glucosyltransferase (*gtf*) gene; and by conventional phenotypic characterization and identification using two commercial kits, Rapid ID 32 STREP and STREPTOGRAM and the associated databases. A phylogenetic tree based on concatenated sequences of the four housekeeping genes allowed unequivocal differentiation of recognized species and was used as the reference. Analysis of single gene sequences revealed deviation clustering in eight strains (5.4%) due to homologous recombination with other species. This was particularly evident in *S. sanguinis* and in members of the anginosus group of streptococci. The rate of correct identification of the strains by both commercial identification kits was below 50% but varied significantly between species. The most significant problems were observed with *S. mitis* and *S. oralis* and 11 *Streptococcus* species described since 1991. Our data indicate that identification based on multilocus sequence analysis is optimal. As a more practical alternative we recommend identification based on *sodA* sequences with reference to a comprehensive set of sequences that is available for downloading from our server. An analysis of the species distribution of 107 nonhemolytic streptococci from bacteremic patients showed a predominance of *S. oralis* and *S. anginosus* with various underlying infections.

Identifikation af Streptokokker

- 24 IE cases med streptokokker
  (1 dyrkningsnegativ)
- 16 NHS fik mere præcis diagnose med PCR.
- NHS kunne være forurening
- 100 % match mellem blod-isolat og klap-PCR

Begrænsninger.

- Bakterielt DNA fjernes kun langsomt fra vævet efter bakterien er død.
- PCR kan ikke skelne behandlingssvigt.
  (dyrkning kan)
- PCR kan ikke skelne aktuelle fra tidligere infektioner.
  (dyrkning kan)
- PCR sensitivitet afhænger af AB-behandling.
  (PCR: uger / mdr. Dyrkning: dage)

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2005, p. 163–167 Vol. 43, No. 1
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.1.163–167.2005

PCR Detection of Bacteria on Cardiac Valves of Patients with Treated Bacterial Endocarditis

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We used broad-range PCR amplification and sequencing to detect and identify bacterial DNA in 156 valves of patients treated for infective endocarditis (IE). Bacterial DNA was found more frequently in patients who underwent valve replacement while on antibiotic treatment for IE (60%) than in patients who had completed antibiotic treatment for IE (37%; $P = 0.02$). We found specific bacterial DNA in valves removed from 11 of 30 patients who had completed antibiotic treatment for IE. Six had no histological evidence of IE. The presence of DNA was significantly correlated with the presence of histologic lesions ($P = 0.001$) and with the presence of bacteria detected by Gram staining ($P < 0.001$). *Bartonella* and streptococci were detected for much longer after antibiotic treatment by PCR than other species ($P = 0.047$ and 0.04, respectively), and coagulase-negative staphylococci were detected for much shorter periods ($P = 0.02$). The finding that bacterial DNA was more likely to be detected in valves of patients with active IE than in patients who had completed antibiotic treatment for IE shows that bacterial DNA is cleared slowly. There was no significant correlation between the duration of antibiotic therapy and the presence of bacterial DNA in valves. Since the persistence of bacterial DNA in valves does not necessarily indicate the persistence of viable bacteria, the detection of bacterial DNA in valves from IE patients should be interpreted with caution, in particular in those patients with a past history of treated IE.

PCR resultat og behandlingslængde

	Skejby Median (range)	Rovery Median (range)
alle	11 d	15 d
PCR pos	10 d (0-90)	14 d (1-79)
PCR neg	20 d (1-74)	20 d (1-150)

PCR af hjerteklap-prøver konklusioner

- 16S PCR er relevant til klapprøver fra patienter mistænkt for endocarditis.
- 16S PCR langt bedre end dyrkning.
- 16S PCR nødvendig hvis fuldt spektrum skal findes.
- 16S PCR, Dyrkning og histologi supplerer hinanden.

PCR af spondylit prøver

- **I alt**: 132 prøver heraf 26 positive (20 %)
- **Analyseret**: 38 prøver fra 38 pt.

Kurt Fuursted og Lisbeth Nørum Pedersen

PCR af spondylit prøver
Resultater

20 kontroller:

Dyrkning: 9 positive PCR: 0 positive

18 cases:

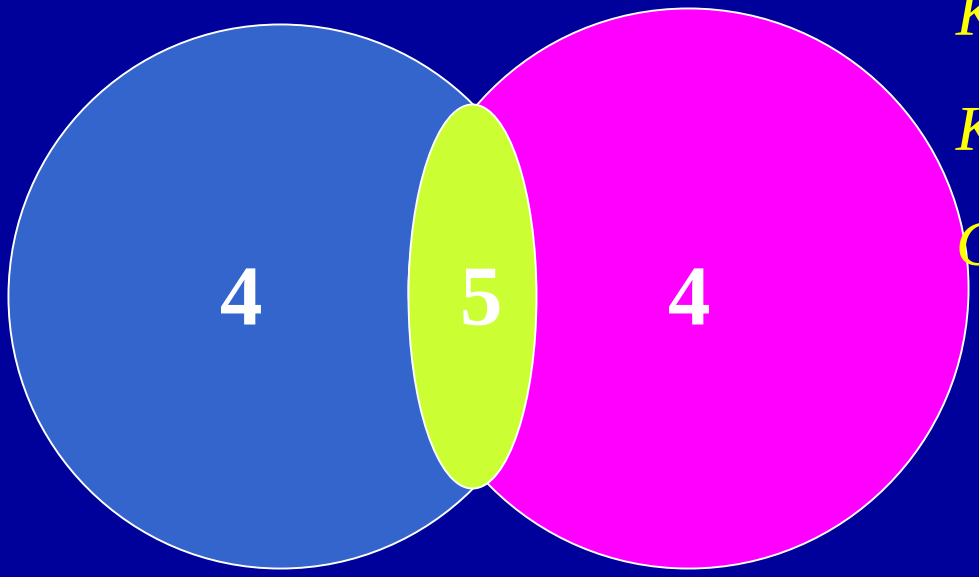
Dyrkning: 9 positive PCR: 9 positive

Spondylit prøver

Dyrkning / PCR

3 *S. aureus*

P. aeruginosa



NH Streptokok

K. pneumoniae

Kingella kingae

C. histolyticum

3 *S. aureus*

Salmonella sp.

S. aureus/*S. mutans*

PCR af spondylit prøver
Resultater

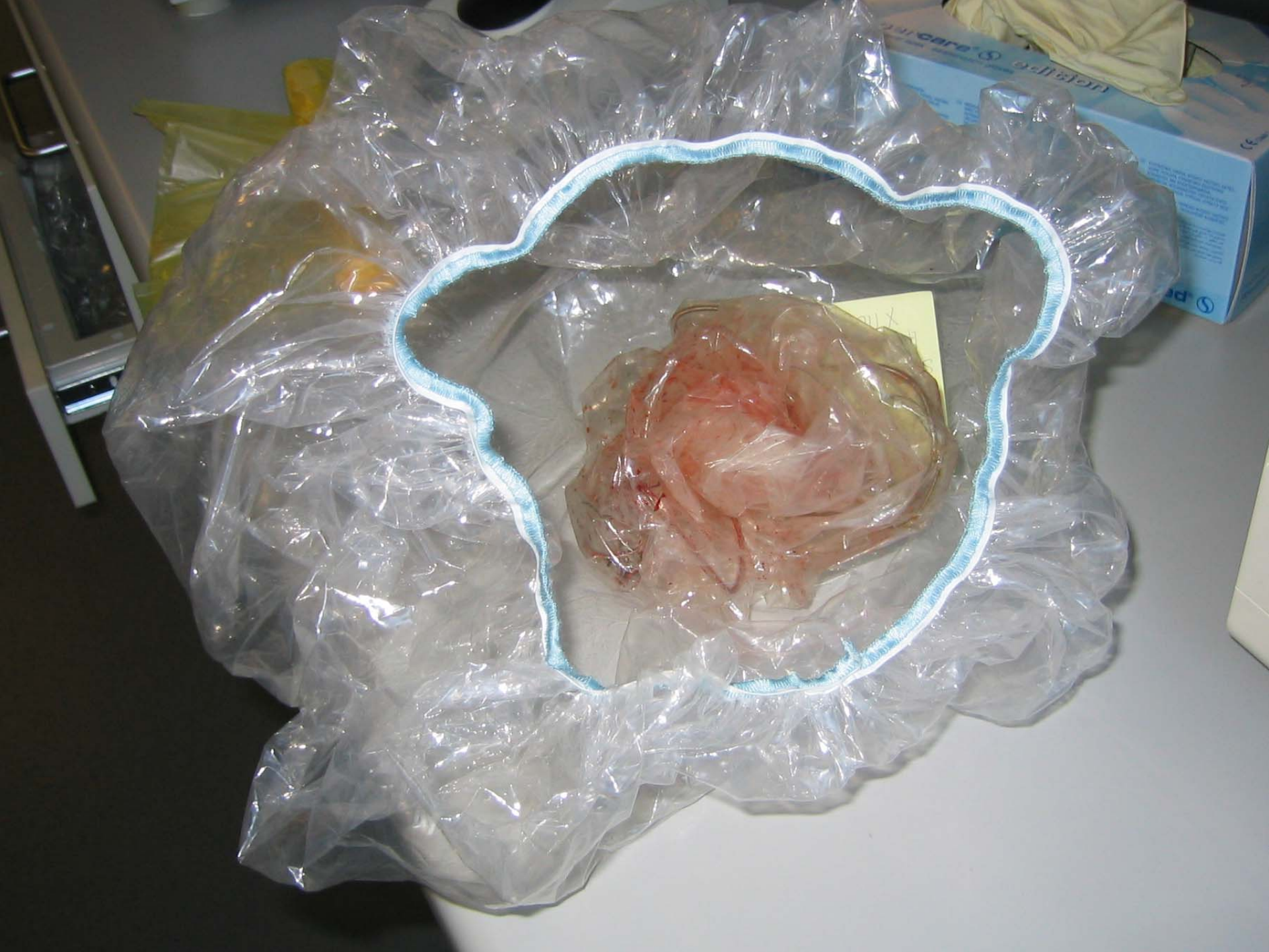
- **Dyrkning**
  Sensitivitet: 50 % Specificitet: 55 %
- **PCR**
  Sensitivitet: 50 % Specificitet: 100 %
- **Dyrkning + PCR**
  Sensitivitet: 72 %

PCR af spondylitis prøver konklusioner

- Både konventionel dyrkning
  og 16S PCR har relativ lav sensitivitet,
  men de supplerer hinanden godt.
- PCR har høj specificitet.
- Med PCR detekteres også de kræsne bakterier.

PCR af pacemaker prøver

- **I alt**: 33 prøver heraf 14 positive (42 %)



PCR af div ortopæd kir. prøver

- **I alt**: 178 prøver fra 140 pt, heraf 18 positive (10 %)

- Kun PCR prøve: 68 patienter
  PCR: 11 pos (16%)

- Både PCR og dyrkning: 72 patienter
  PCR: 7 pos (10%)
  Dyrkning: 19 pos (26%)

  (per prøve: 7% pos PCR og 8% pos Dyrkning)

”Kamme”-agtige biopsier underextremitet

20 patienter

	Prøver pr patient	Positivt resultat pr patient
PCR	1	0
Dyrkning	5	3

PCR af div ortopæd kir. prøver
Konklusioner

- Selve PCR analysen fungerer fint
  - relevante fund.
- Ingen forurening.
- Er der tale om desperate prøver?
  – sandt negative?
- Umiddelbart ikke de store resultater.
- Der mangler systematiske studier.

PCR af prøver fra div. lokalisationer

- 50 prøver : 9 positive (18 %)

Patienter	prøver	positive	Per pt
16	19 PCR	7	44 %
19	30 PCR 35 dyrkn	2 0	10 %
I alt 35		9	26 %

PCR af prøver fra div. lokalisationer

- Det er sandsynligt at 16SPCR er relevant til vævsprøver i specielle situationer,
  - vi ved bare ikke hvornår!

PCR til ID af isolater fra rutinen

- **I alt**: 52 prøver heraf 52 positive (100 %)

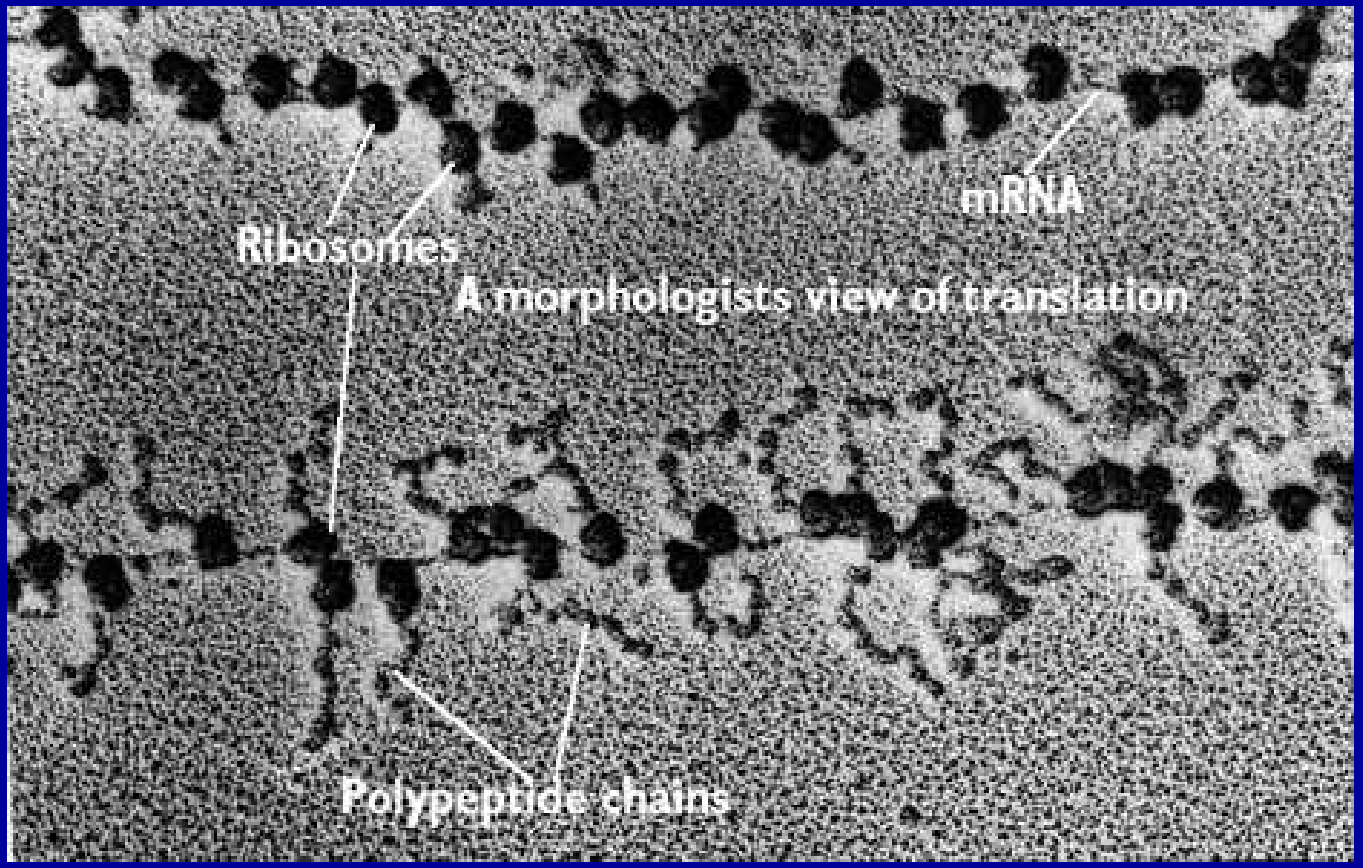
- Fungerer fint!!
- Stigende antal prøver de sidste år
- Hvilket identifikationsnivaeu er relevant for de forskellige kliniske prøver?
- Afhængighed af databaserne
- Fremtiden for ekspertisen i klassisk mikrobiologi

Samlet konklusion

- Fungerer fint til kliniske prøver.
- Giver præcise diagnoser.
- Bidrager væsentligt til udredning af endocarditis og spondylitis patienter.
- Den endelige plads i rutine diagnostikken kendes ikke.
- Der mangler studier for hver enkel prøve-kategori.

- Lone Pødenphant
- Solvej Henriksen
- Ortopædkirugisk afdeling, AKH
- Thoraxkirurgisk afdeling, Skejby
- Cardiologisk Afdeling , Skejby

Ribosomer


mRNA
Ribosomes
A morphologists view of translation
Polypeptide chains

Ribosomer