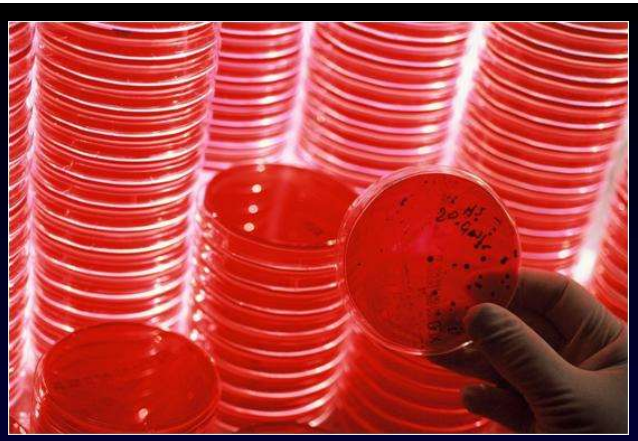
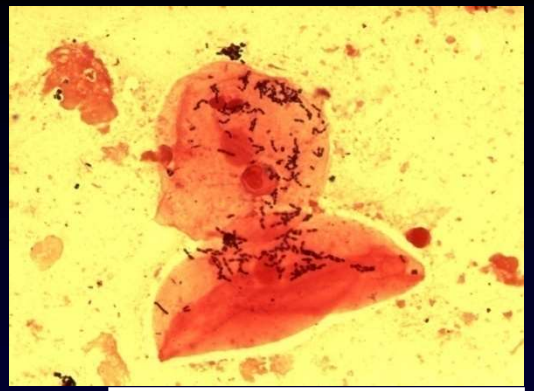
Molecular microbiology

Pavel Drevinek

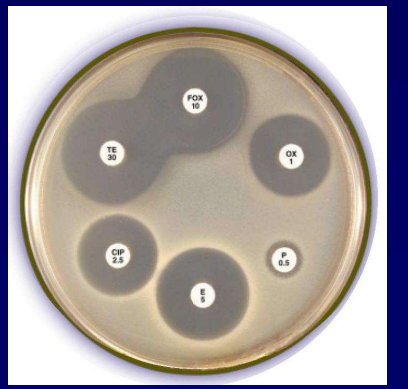




Traditional microbiology

microscopy
antigen detection
culture, ID a AST

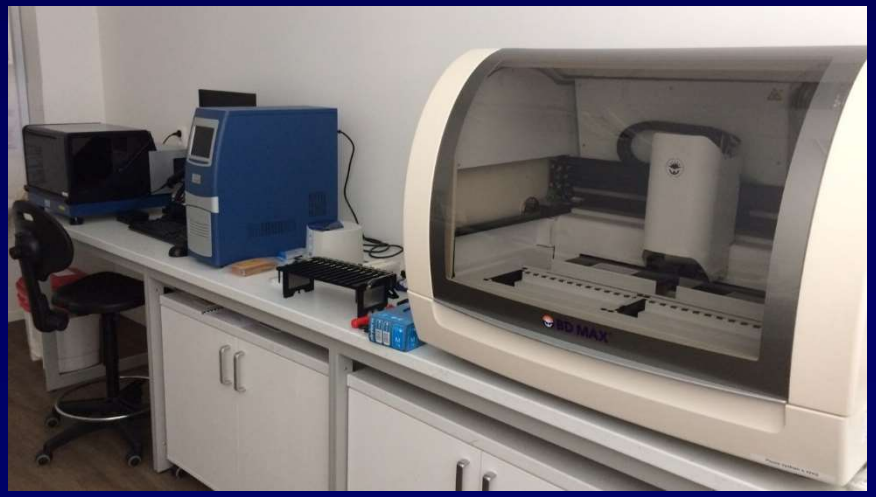
serology and antibodies



Molecular microbiology

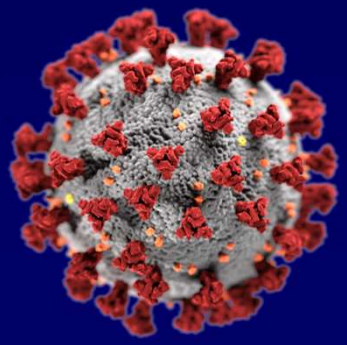
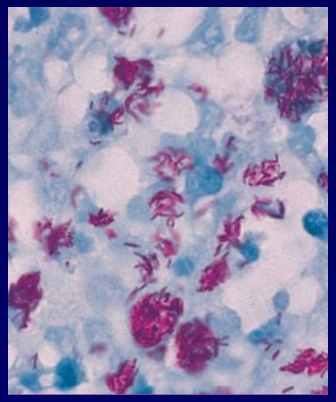
DNA or RNA analysis for the purpose of:

- diagnostics (ID)
- epidemiology
- virulence
- antimicrobial resistance



To detect DNA or RNA for diagnostic purposes

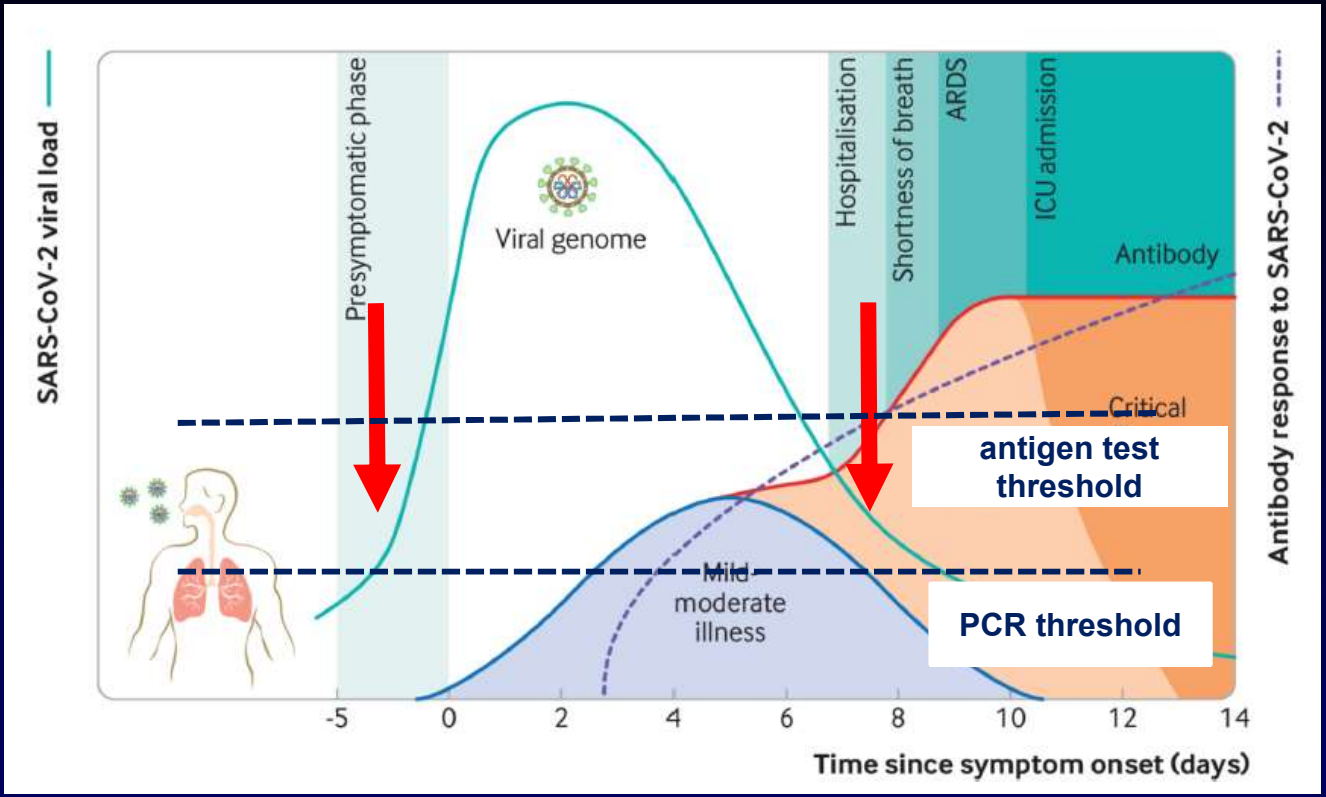
Advantages
• non-culturable agents, slow growing, „fastidious"
• detection even during antibiotic therapy
• fast
• high sensitivity
• quantification (viral load)

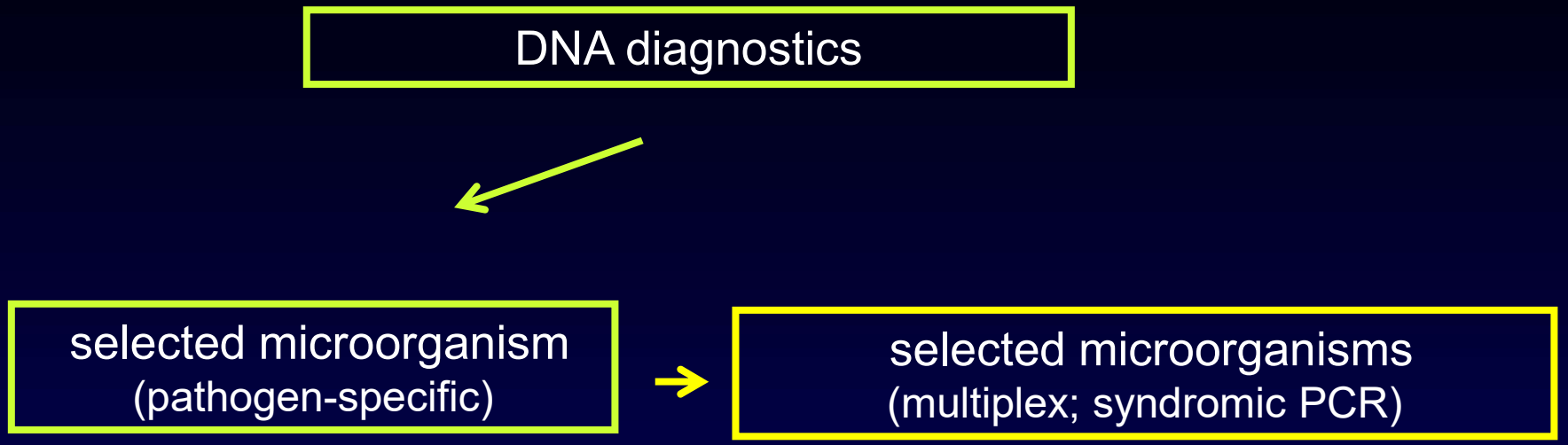
To detect DNA or RNA for diagnostic purposes

Drawbacks
pitfalls in interpretation, invisible at first sight:
• does the detected DNA originate from a viable agent?
• positive result: artifact, contaminant, bystander or pathogen?

PCR and "false positivity"



- early PCR detection
- persistent,
  not clinically relevant positivity



- open system
- closed system (useful as POCT; point-of-care test)

DNA diagnostics

selected microorganism
(pathogen-specific)

Open system:

Examples

- SARS-CoV-2
- *Bordetella pertussis*
- EBV (quantity)
- PVL

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DNA diagnostics

selected microorganism
(pathogen-specific)

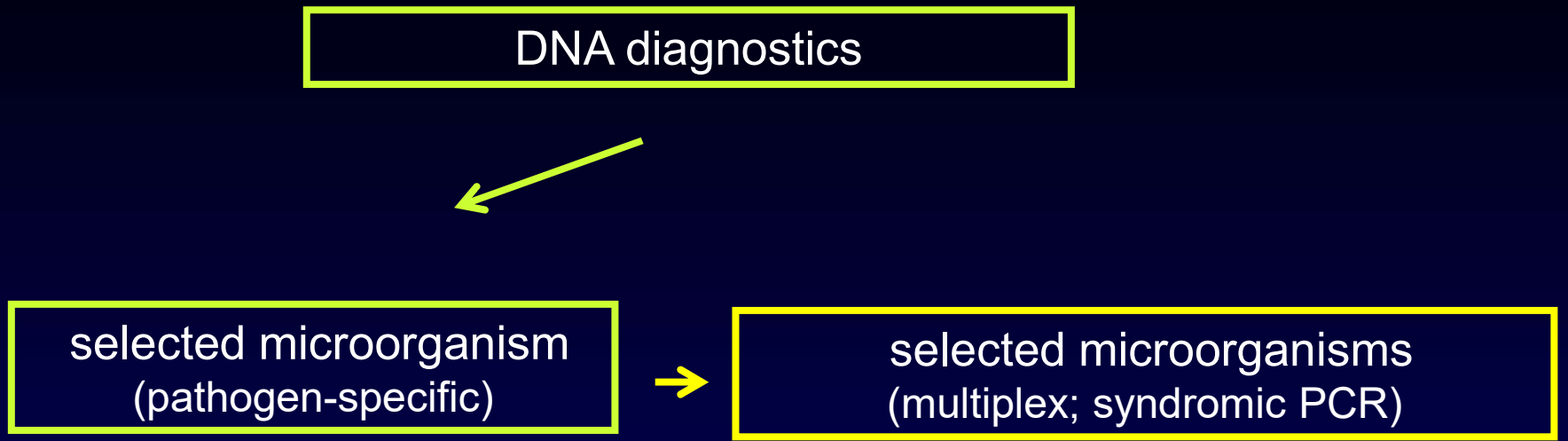
Closed system:

Examples

- SARS-CoV-2
- RSV
- flu
- *M. tuberculosis*

15 mins

80 mins


DNA diagnostics
selected microorganism
(pathogen-specific)
selected microorganisms
(multiplex; syndromic PCR)

Open system:

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Respiratory infections viral

influenza A
influenza B
RSV
rhinoviruses
enteroviruses
parainfluenza
adenoviruses
parechovirus
bocavirus
metapneumovirus
seasonal coronaviruses
SARS-CoV-2

Respiratory infections atypical

Mycoplasma pneumoniae
Chlamydia pneumoniae
Chlamydia psittaci
Legionella pneumophila
Pneumocystis jirovecii
Cryptococcus neoformans

Gut infections

Salmonella
Campylobacter
Shigella/ E. coli (EIEC)
Shiga toxin (EHEC)
Clostridium difficile (toxin A,B)
Aeromonas
Yersinia
Sapovirus
Rotavirus
Norovirus
Adenovirus
Astrovirus
Giardia intestinalis
Cryptosporidium spp.
Entamoeba histolytica

Sexually transmitted

Neisseria gonorrhoeae
Chlamydia trachomatis
Mycoplasma genitalium
Mycoplasma hominis
Ureaplasma urealyticum
Ureaplasma parvum
Trichomonas vaginalis

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DNA diagnostics

selected microorganism
(pathogen-specific)

→

selected microorganisms
(multiplex; syndromic PCR)

Closed system:

Respiratory infections
influenza A
influenza B
RSV
SARS-CoV-2

60 mins

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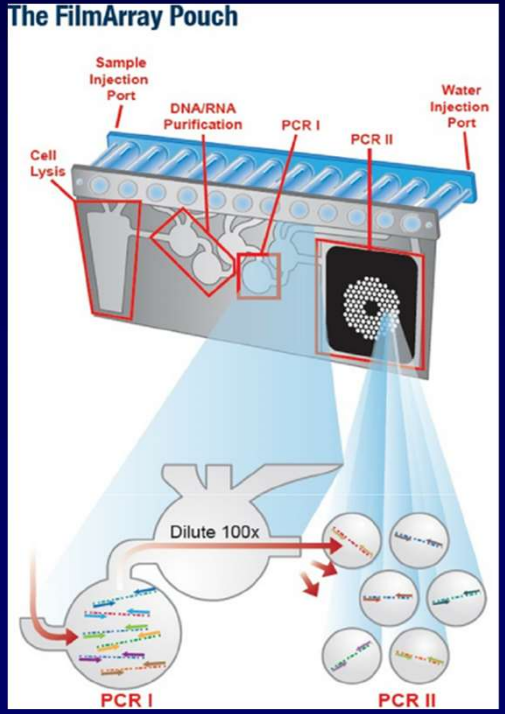
Meningitis

S. pneumoniae	enteroviry
N. meningitidis	HSV1
H. influenzae	HSV2
	VZV
S. agalactiae	CMV
E. coli	HHV6
L. monocytogenes	parechovirus
	Cryptococcus neoformans



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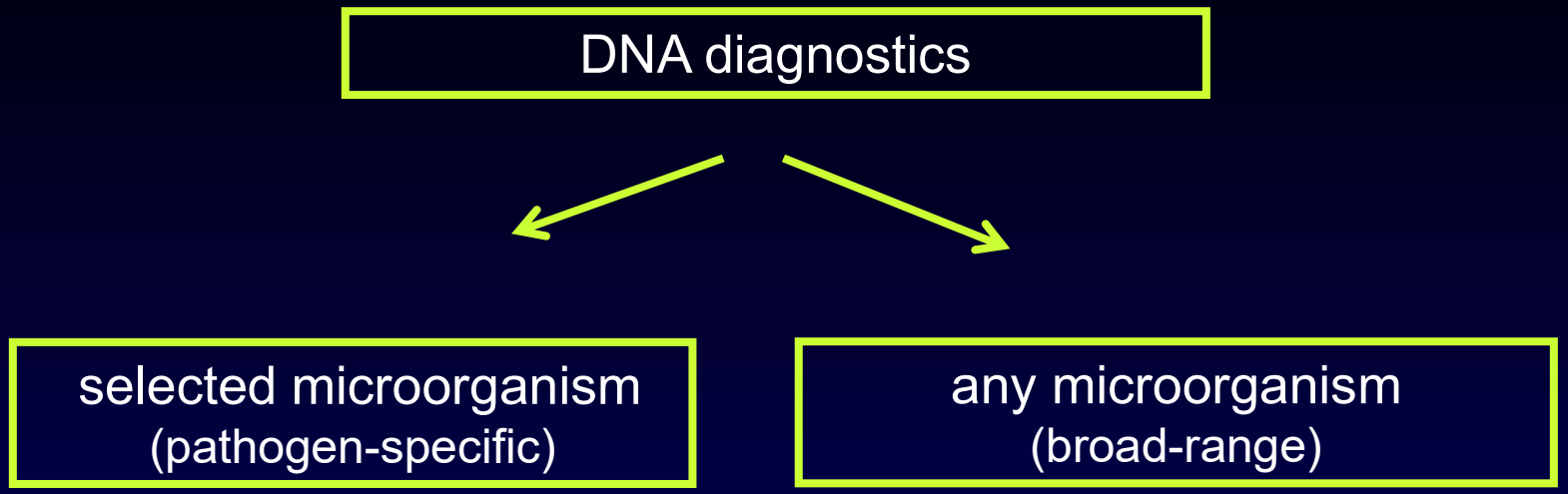
Pneumonia

influenza A
influenza B
RSV
rhinoviruses/enteroviruses
parainfluenza
adenoviruses
metapneumovirus
seasonal coronoaviruses
SARS-CoV-2
Mycoplasma pneumoniae
Chlamydia pneumoniae
Legionella pneumophila
Acinetobacter baumannii
Pseudomonas aeruginosa
Enterobacter cloacae
Proteus spp.
Escherichia coli
Haemophilus influenzae

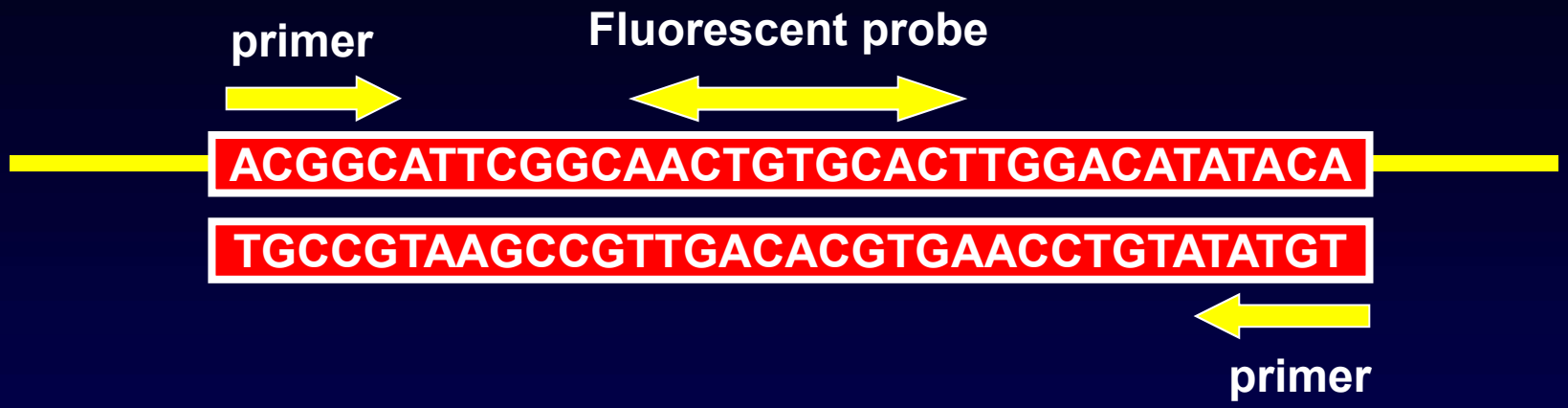
Klebsiella pneumoniae
Klebsiella oxytoca
Klebsiella aerogenes
Moraxella catarrhalis
Serratia marcescens
Staphylococcus aureus
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus agalactiae

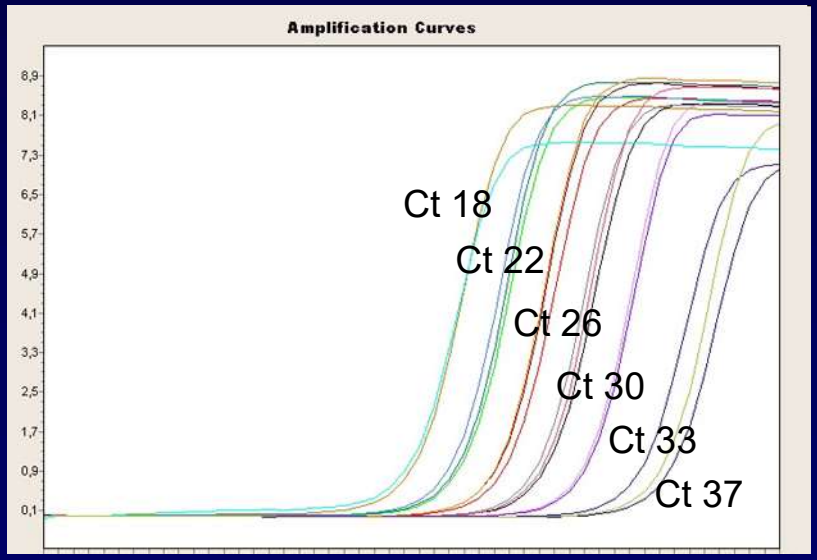
CTX-M
IMP
KPC
NDM
VIM
OXA-45-like
mecA/C a MREJ


DNA diagnostics
selected microorganism
(pathogen-specific)
any microorganism
(broad-range)

How to actually „design“ PCR
to make it working according to our wishes

detection thanks to the existence of nucleotide sequences





Ct threshold value = PCR cycle,
when the PCR signal starts to grow

the lower the Ct value, the more
agent is detected in the sample

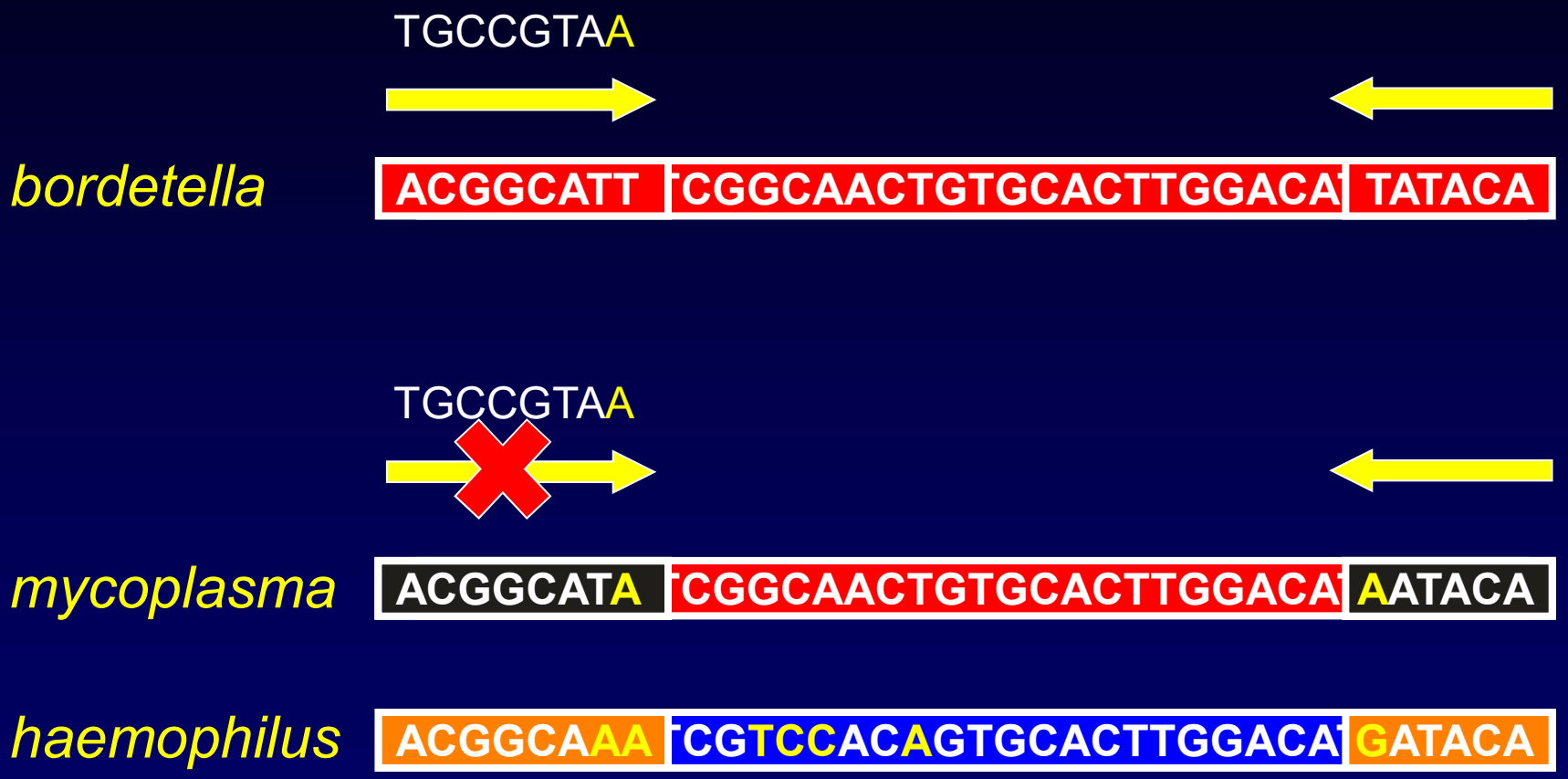
Does the patient have a whooping cough?

(you need to detect only *Bordetella pertussis;* ignore others)

DNA diagnostics

selected microorganism
(pathogen-specific)

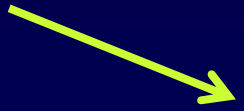
the target sequence for the primers must be unique to bordetella



Infective endocarditis. What is the cause?

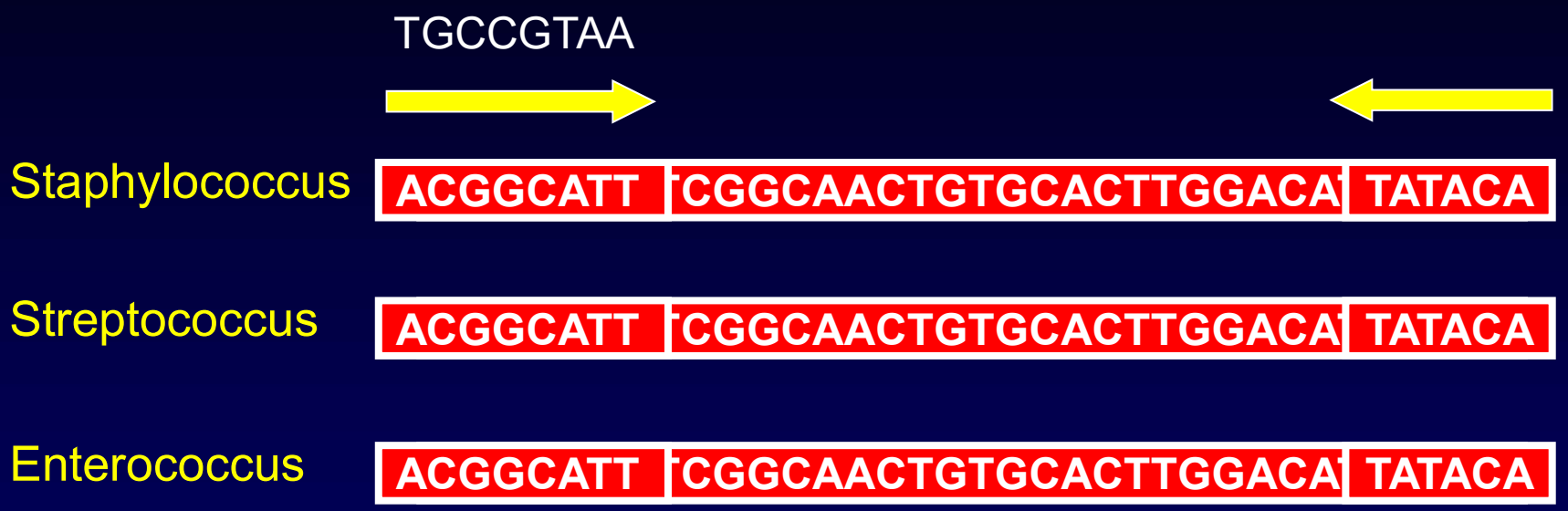
(you need a tool that enables to detect any bacteria)

DNA diagnostics



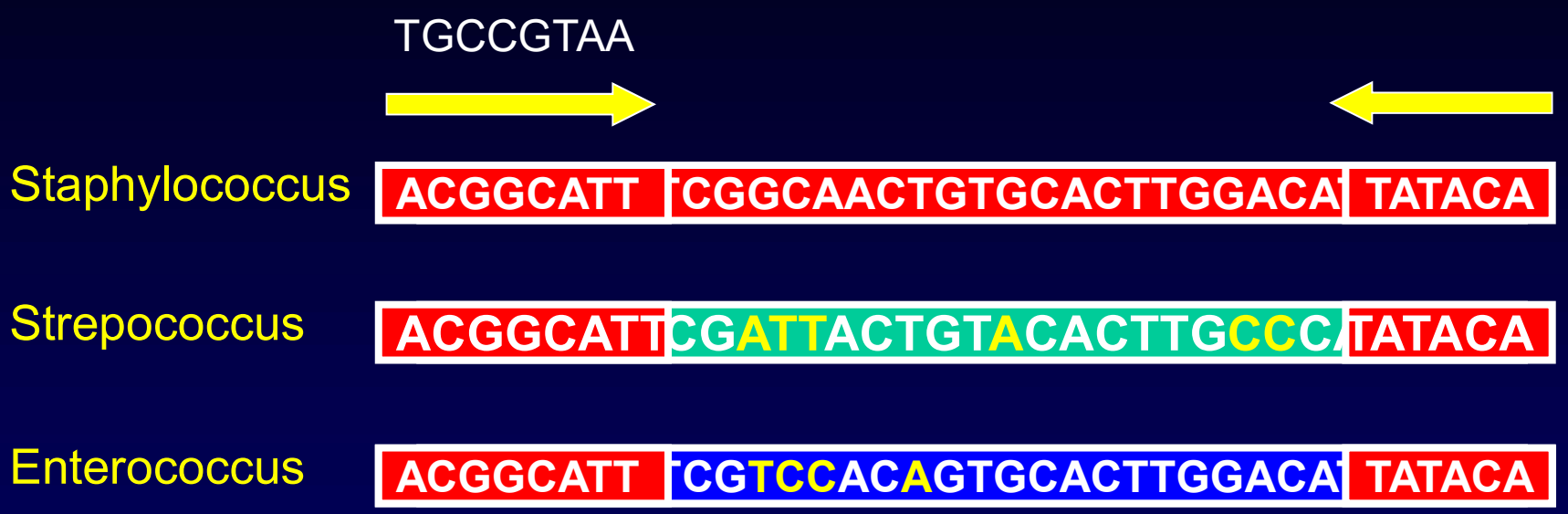
any microorganism
(broad-range)

the target sequence for the primers is present in all bacteria



Situation A

the target sequence for the primers is present in all bacteria



Situation B

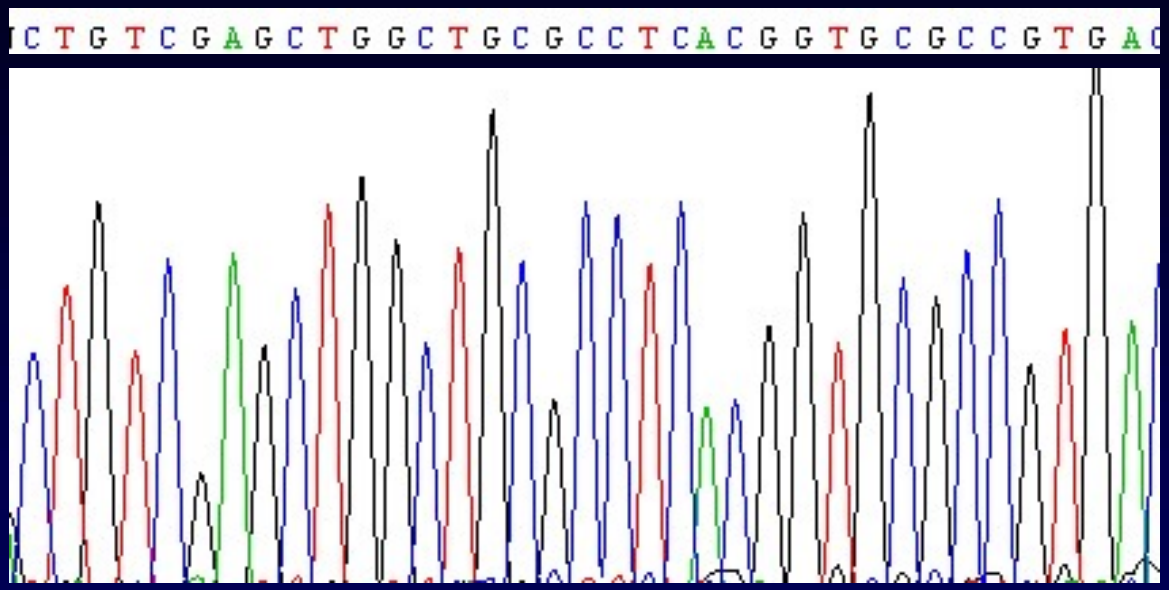
PCR positivity

DETECTION OF BACTERIA,
but which one?

+ sequencing

IDENTIFICATION
on species level

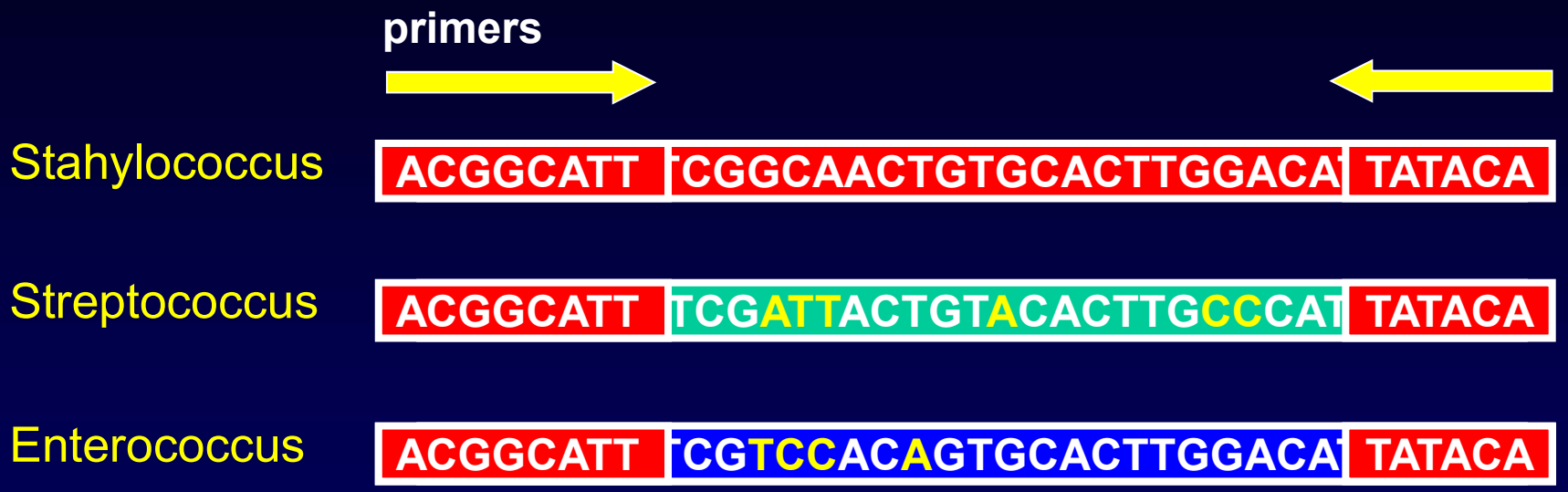
PCR product sequencing



→ *S. aureus*

C T G A

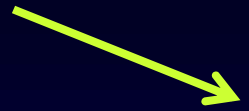
the target sequence for the primers is present in all bacteria



Situation B !

16S rRNA gene

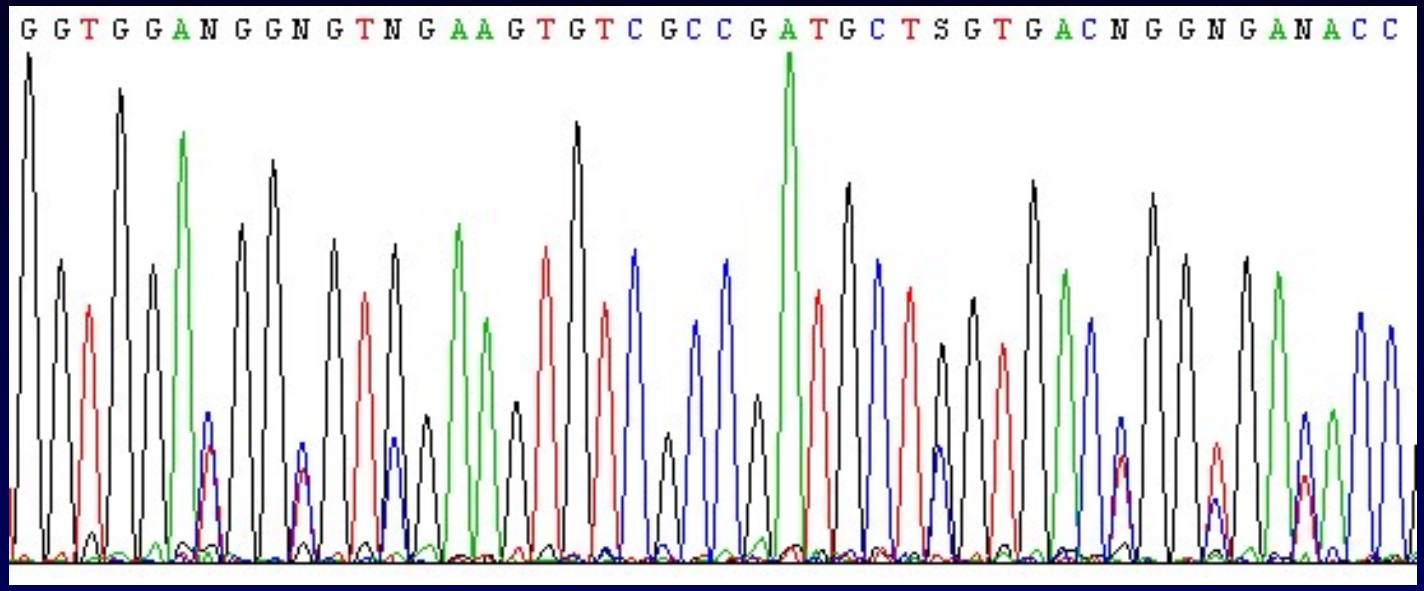
DNA diagnostics

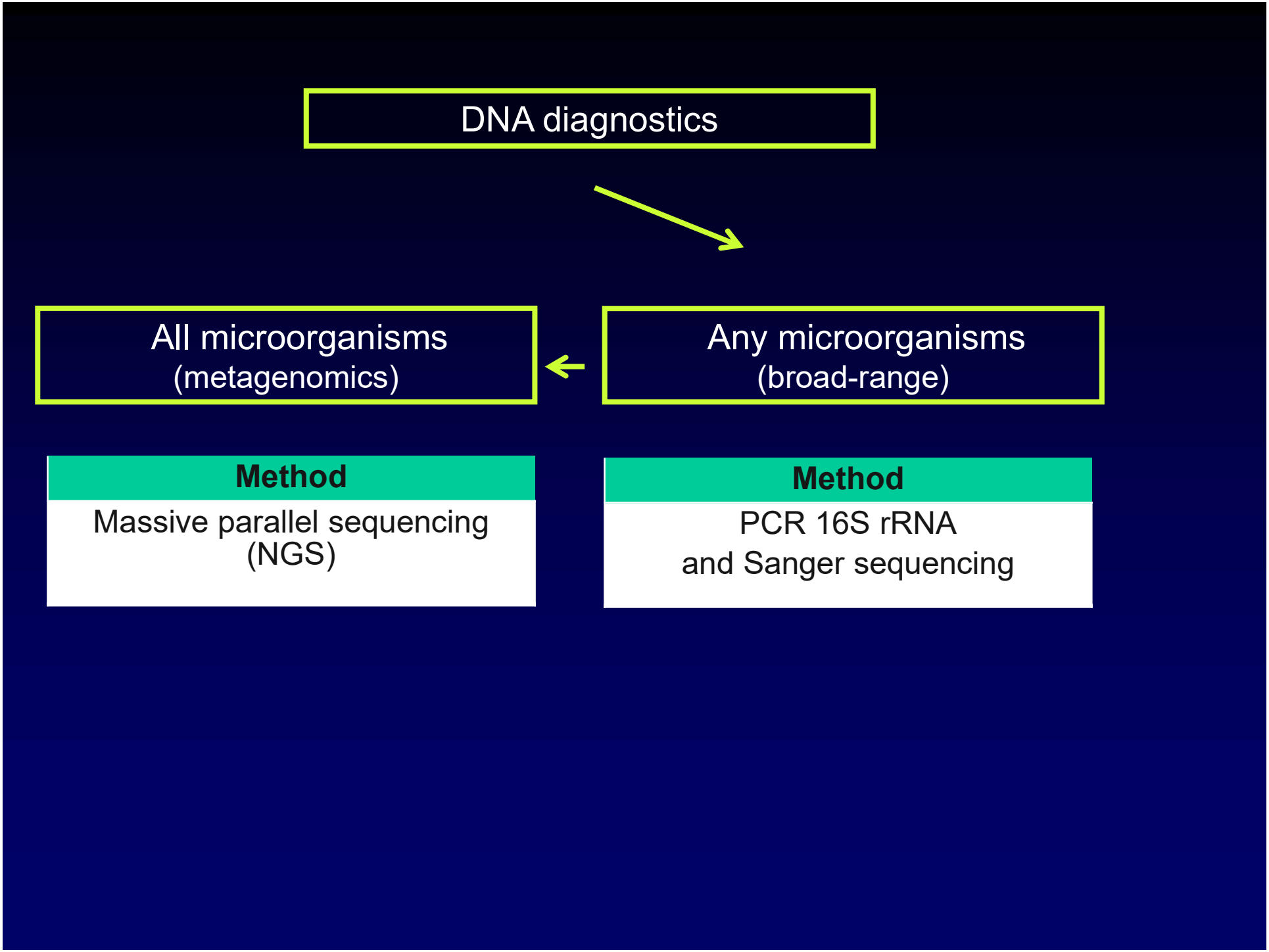


any microorganism
(broad-range)

- from primary sterile material

.... otherwise this will happen :


G G T G G A N G G N G T N G A A G T G T C G C C G A T G C T S G T G A C N G G N G A N A C C


DNA diagnostics
All microorganisms
(metagenomics)
Any microorganisms
(broad-range)
Method
Massive parallel sequencing
(NGS)
Method
PCR 16S rRNA
and Sanger sequencing

Conclusions

- Molecular methods create another pillar of microbiological diagnostics (but for comprehensive diagnostics we need more than one pillar)

Standard molecular microbiology approaches:

- multiplex (even extensive) panels (in central laboratories)
- fast tests (can be used as POCT)
- panbacterial tests (in central laboratories)

blood sample

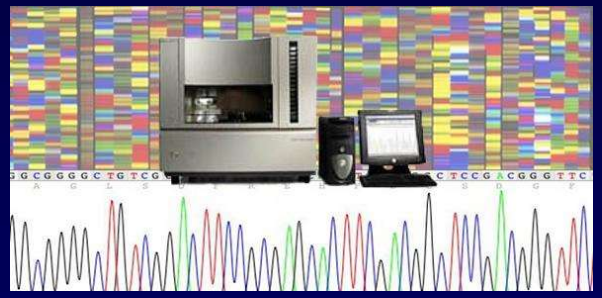
DNA diagnostics

selected microorganisms (pathogen-specific)

Bloodstream infections

Escherichia coli
Staphylococcus aureus
Klebsiella pneumoniae
Acinetobacter baumannii
Pseudomonas aeruginosa
Enterococcus faecium

Any microorganisms (broad-range)



PCR 16S rRNA and Sanger sequencing

S. aureus, S. lugdunensis, S. epidermidis, S. hominis, S. haemolyticus, H. influenzae, S. pneumoniae, S. pyogenes, S. intermedius, S. mitis, L. monocytogenes, E. faecalis, E. faecium, E. coli, S. enterica, E. cloacae, P. stuartii, M. morganii, P. mirabilis, P. vulgaris, C. jejuni, C. foetus, N. meningitidis, B. fragilis, P. gingivalis, F. necrophorum, P. micros, F. magna, ...

Conclusions

- Molecular methods create another pillar of microbiological diagnostics (but for comprehensive diagnostics we need more than one pillar)

Standard molecular microbiology approaches:

- multiplex (even extensive) panels (in central laboratories)
- fast tests (can be used as POCT)
- panbacterial tests (in central laboratories)

- Interpretation of results is crucial; based on the knowledge of microbiology and the technologies used

Automation (of culture-based diagnostics)