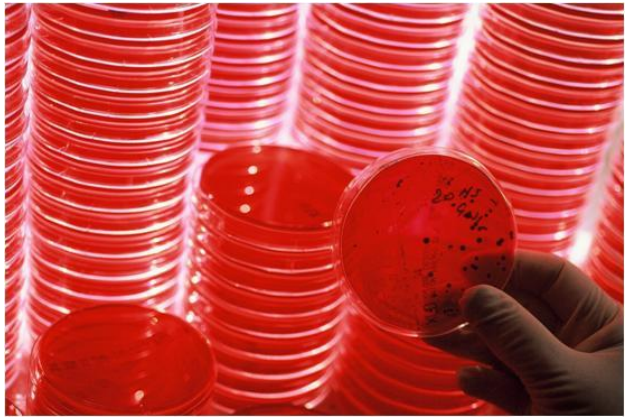
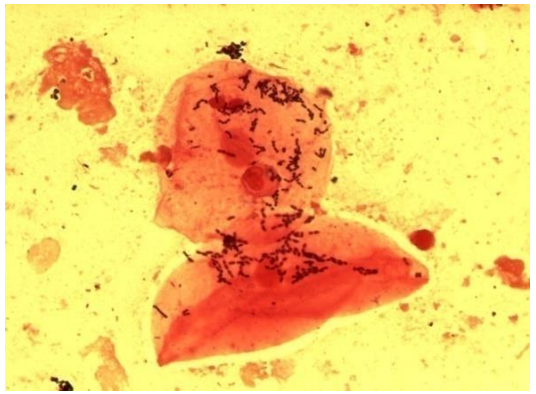
Molecular microbiology

Pavel Drevinek

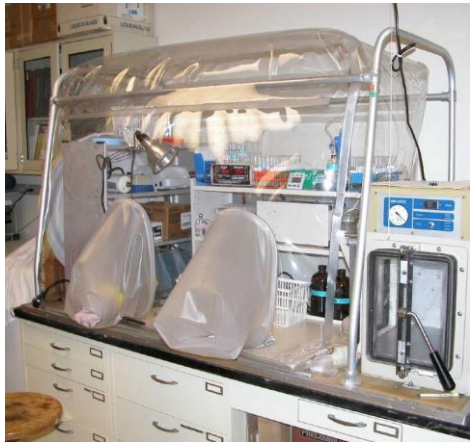


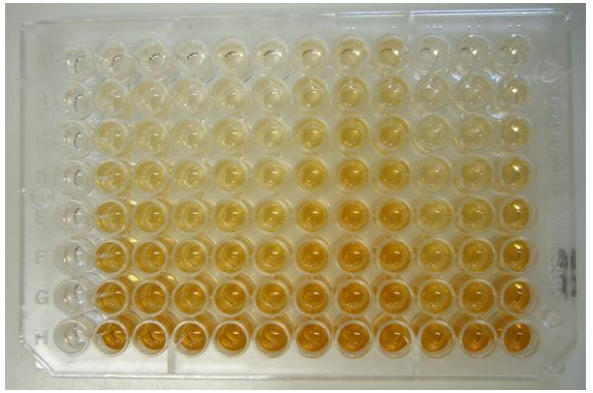


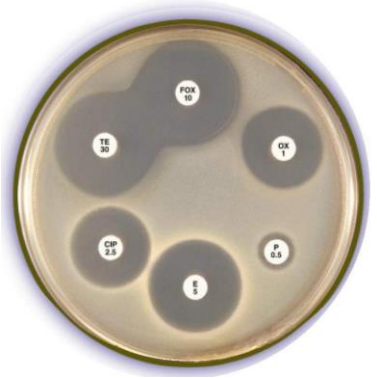
Traditional microbiology

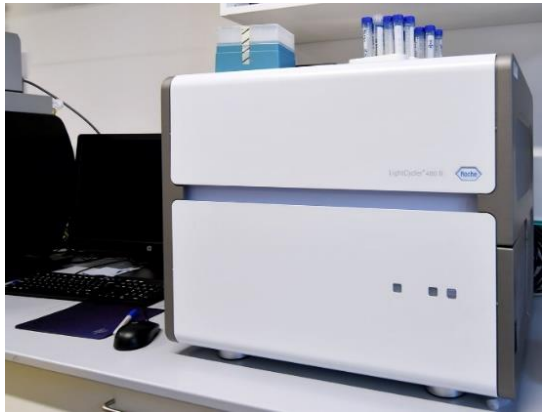
- microscopy
- culture, identification and antibiotic susceptibility tests
- antigen detection

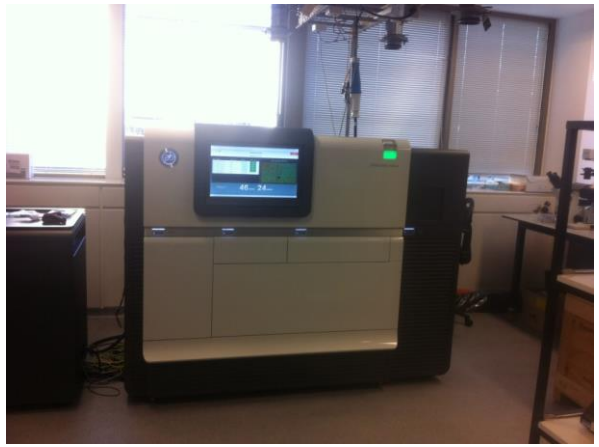
- serology and antibodies







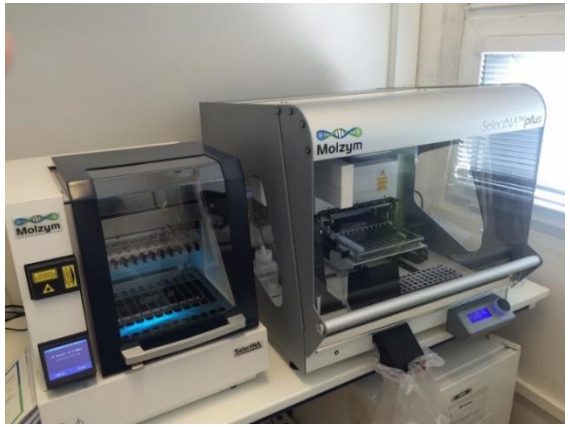


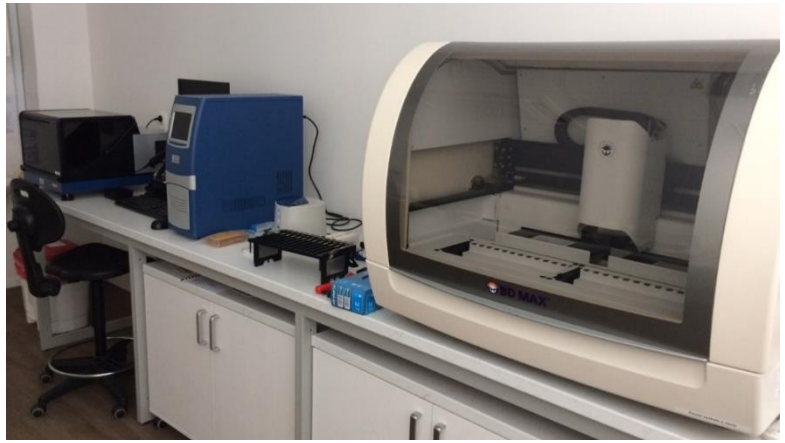


Molecular microbiology

DNA or RNA analysis for the purpose of:

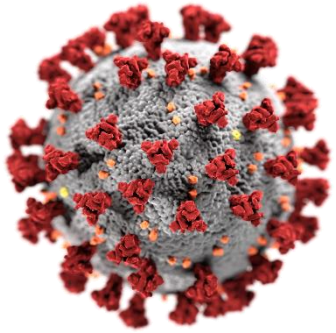
- diagnostics
- detection of virulence genes
- detection of antimicrobial resistance genes
- epidemiology

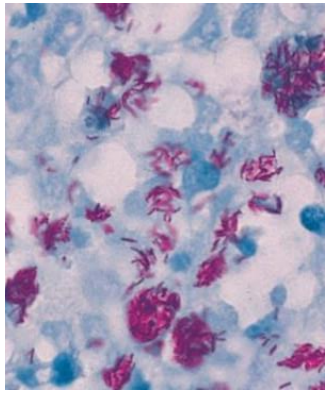




To detect DNA or RNA for diagnostic purposes

Advantages
Diagnostics of culture negative infections
• non-culturable agents, slow growing, „fastidious"
• detection while on antibiotic therapy
• high sensitivity
Speed
Quantification (viral load)

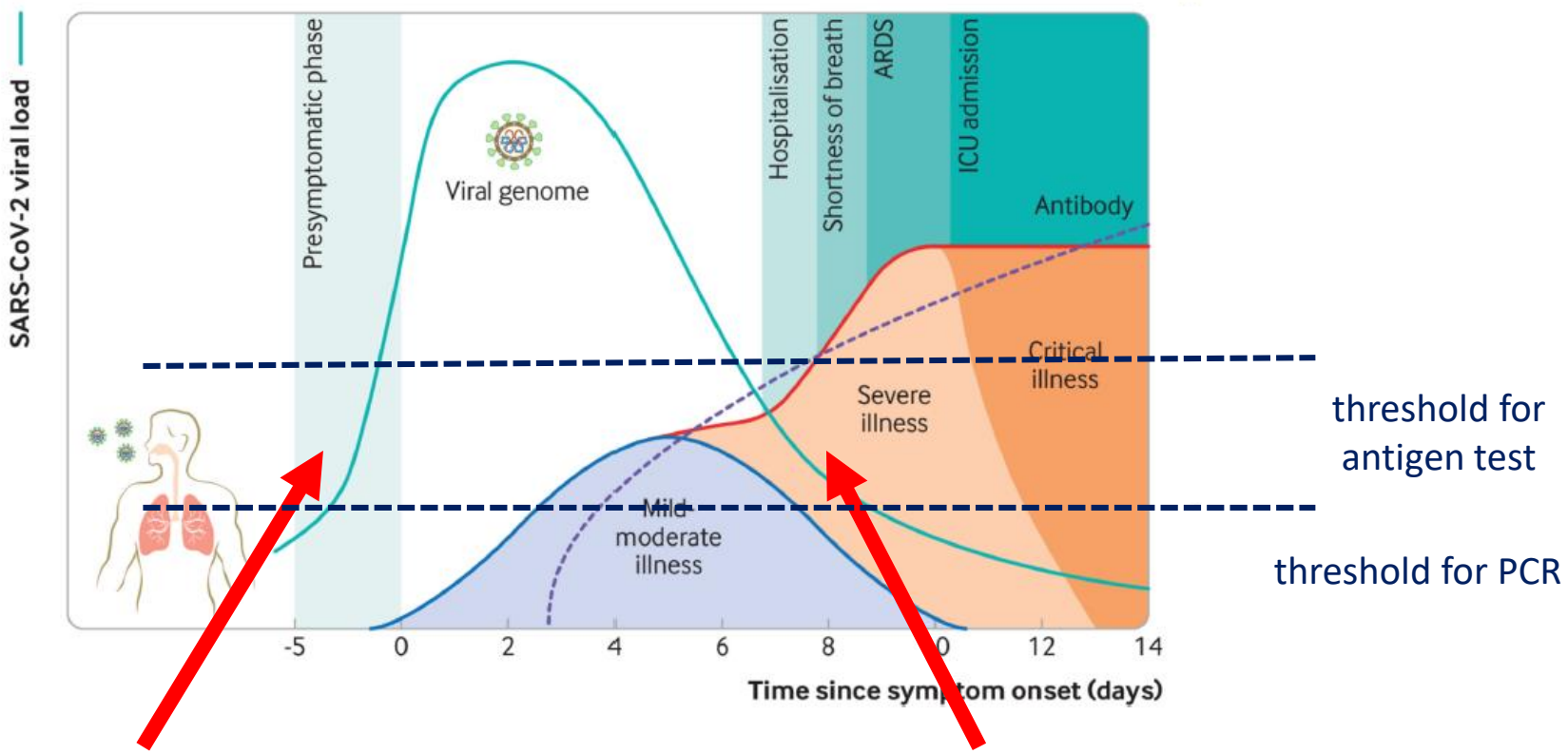




To detect DNA or RNA for diagnostic purposes

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

PCR and "false positivity"



- early (and desirable) detection (only with PCR)
- persistent, artificial, not clinically relevant positivity

FN MOTOL

DNA diagnostics

preselected single microorganism

pathogen specific PCR

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

FN MOTOL

DNA diagnostics

preselected single microorganism → preselected multiple microorganisms

multiplex, syndromic, panel PCR

Respiratory infections atypical
Mycoplasma pneumoniae
Chlamydia pneumoniae
Chlamydia psittaci
Legionella pneumophila
Pneumocystis jirovecii
Cryptococcus neoformans

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

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

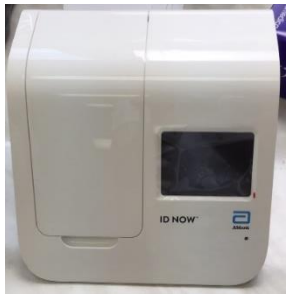
POCT

point-of-care tests or bedside or near-patient testing

- instant availability of results
- run by personnel not qualified for laboratory work

Single agents:

- SARS-CoV-2
- RSV
- influenza



by 15 mins

600 Kc

- *M. tuberculosis*
- MRSA
- *C. difficile*

45 to 80 mins

1500 Kc

POCT

point-of-care tests or bedside or near-patient testing

- instant availability of results
- run by personnel not qualified for laboratory work

Multiple agents:

influenza A
influenza B
RSV
SARS-CoV-2

60 mins

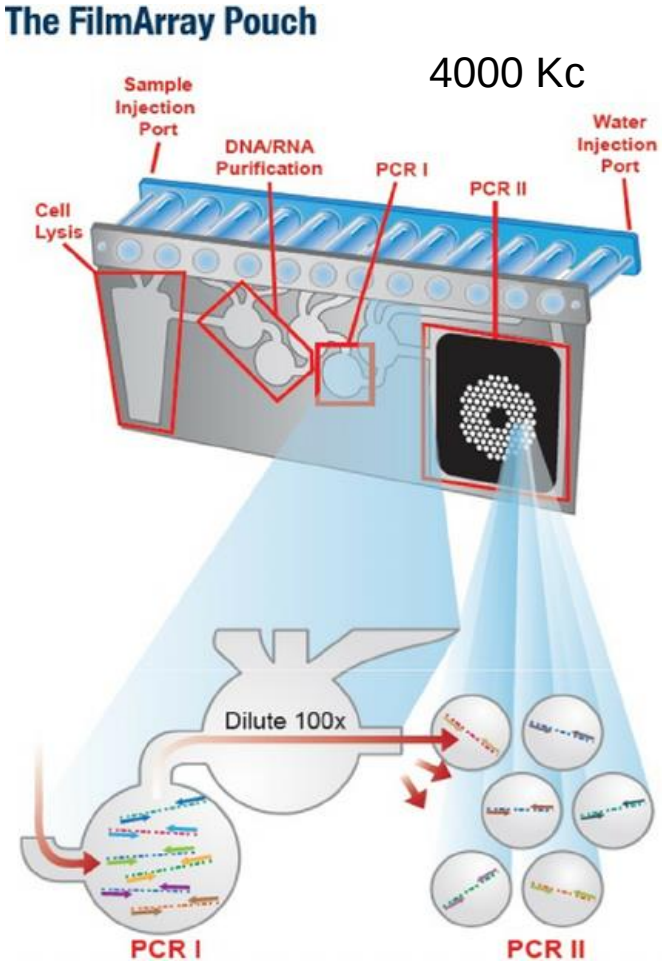
POCT

point-of-care tests or bedside or near-patient testing

- instant availability of results
- run by personnel not qualified for laboratory work

60 mins

4000 Kc



Multiple agents:

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

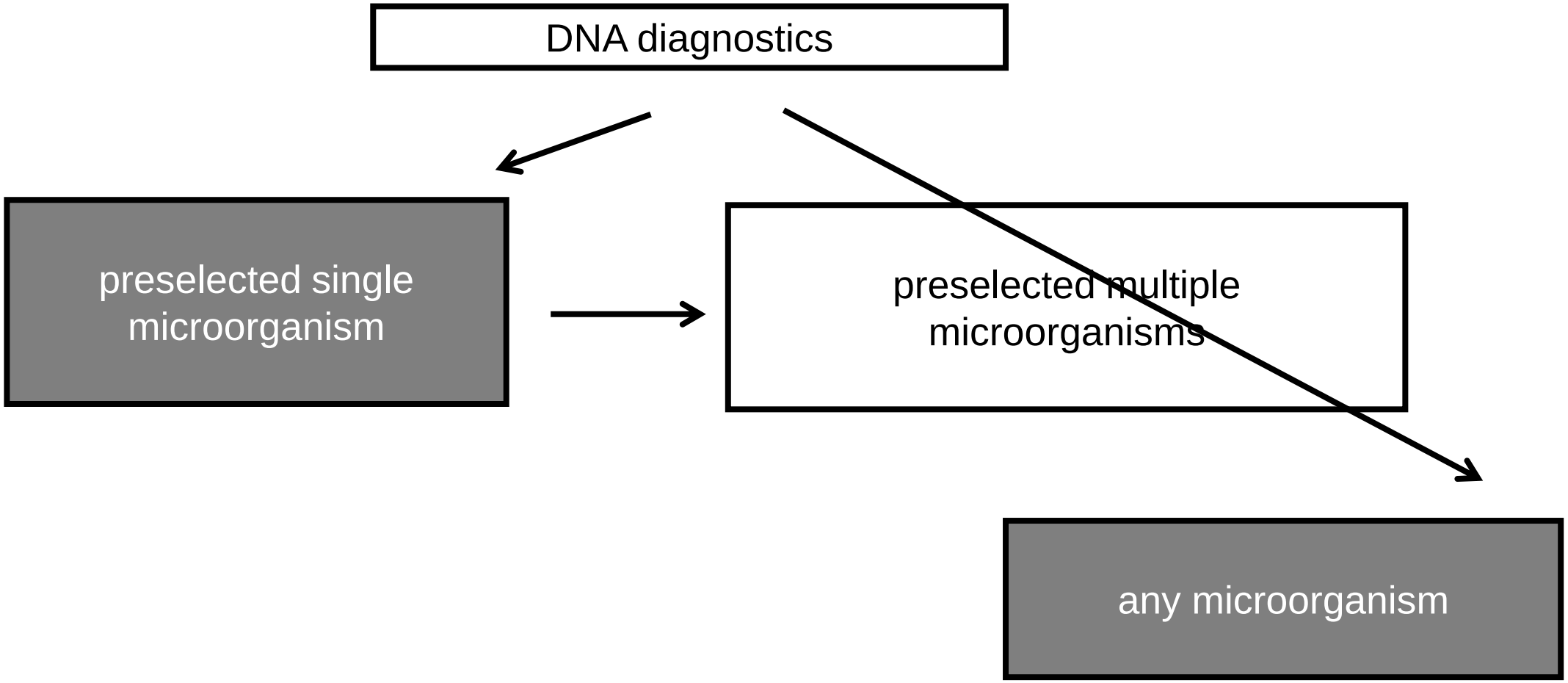
Cryptococcus neoformans

Pneumonia panel

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

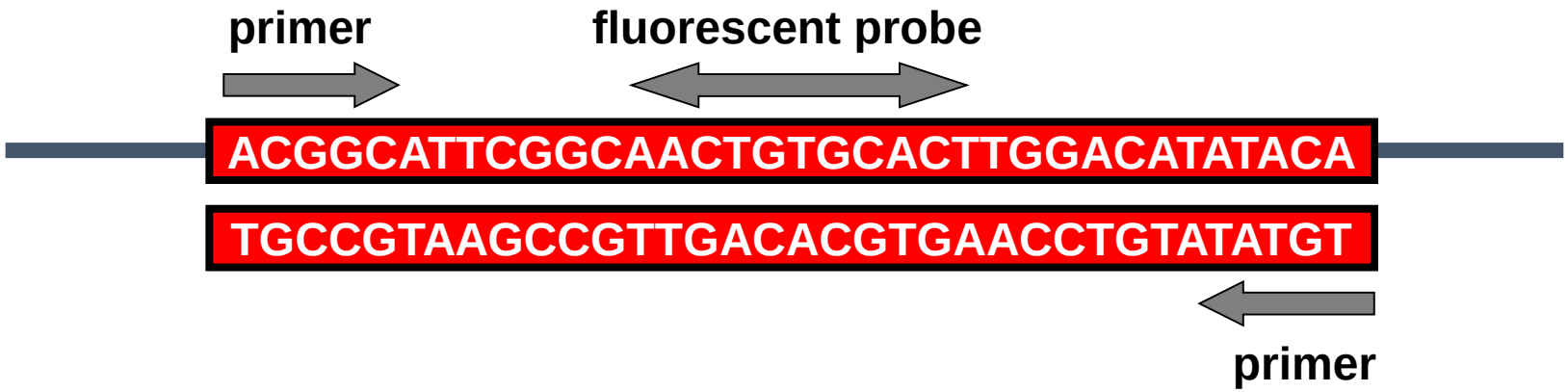
Klebsiella pneumoniae
Klebsiella oxytoca
Klebsiella aerogenes
Moraxella catarrhalis
Serratia marcescens
Staphylococcus aureus
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus agalactiae

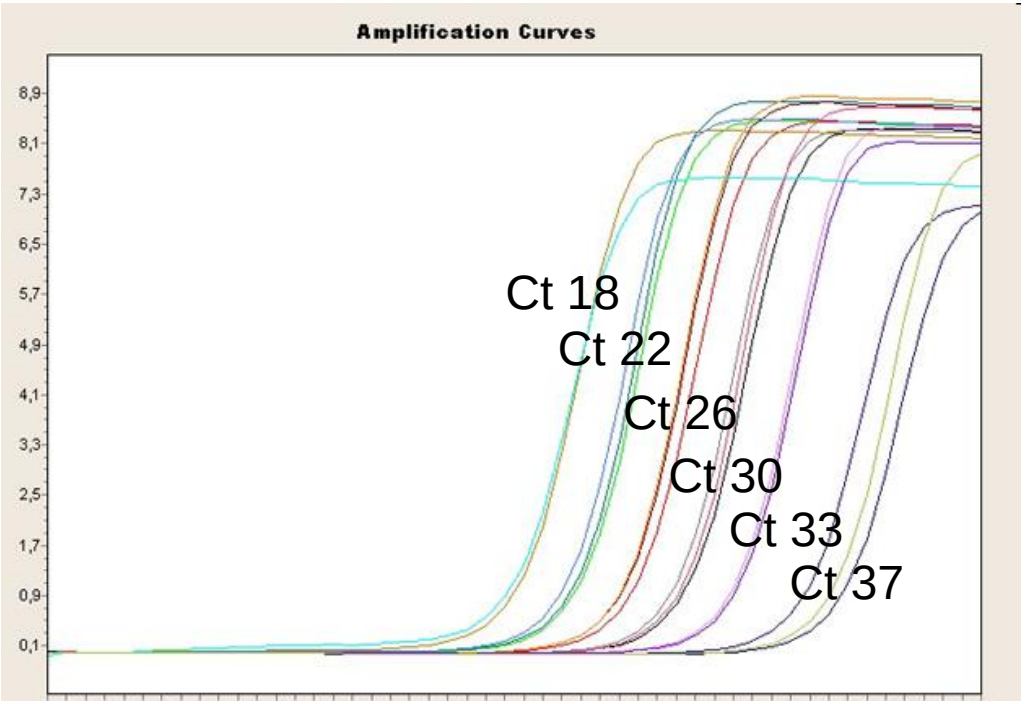
CTX-M
IMP
KPC
NDM
VIM
OXA-45-like
mecA/C and MREJ


DNA diagnostics
preselected single microorganism
preselected multiple microorganisms
any microorganism

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

Molecular microbiology: targetting nucleotide sequence of a microbe



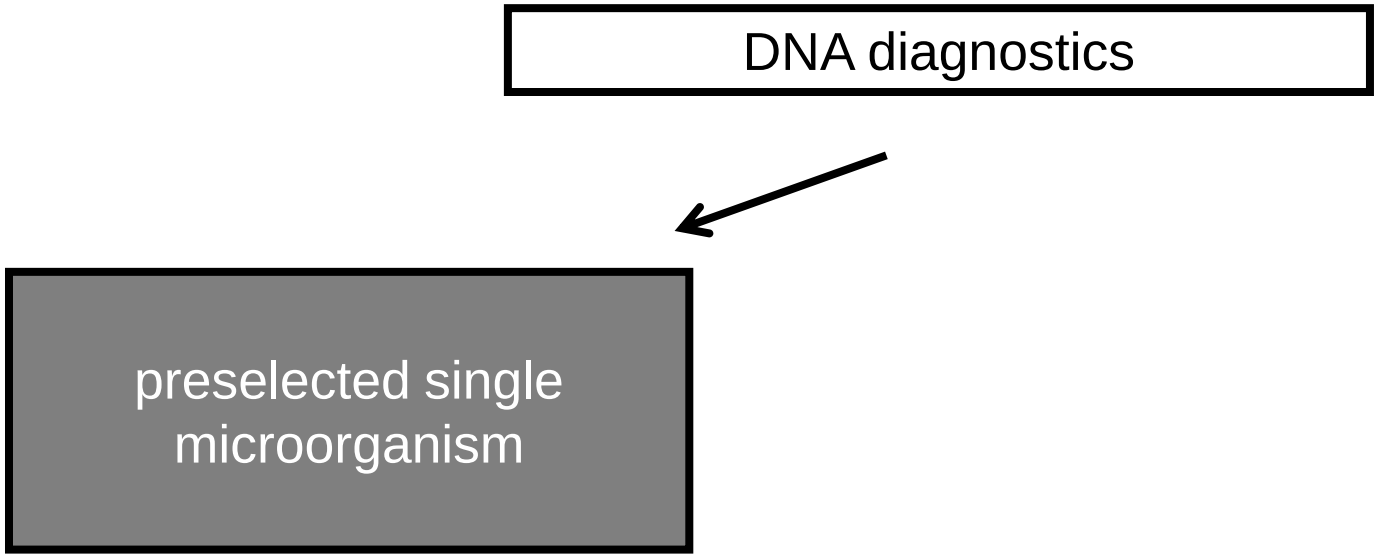


Ct value = PCR cycle
when the signal starts to grow

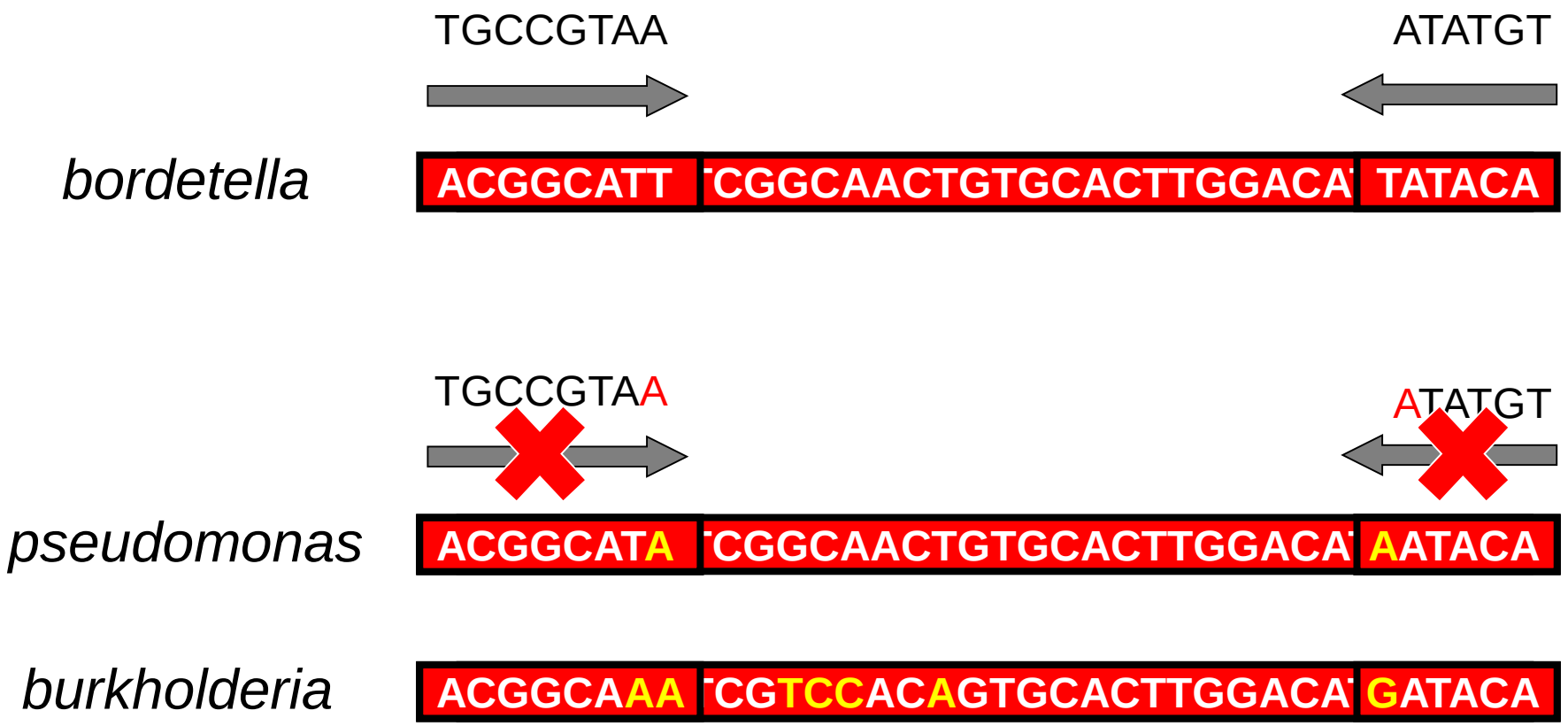
the higher the Ct value, the less agent is detected in the sample

Does the patient have a whooping cough?

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

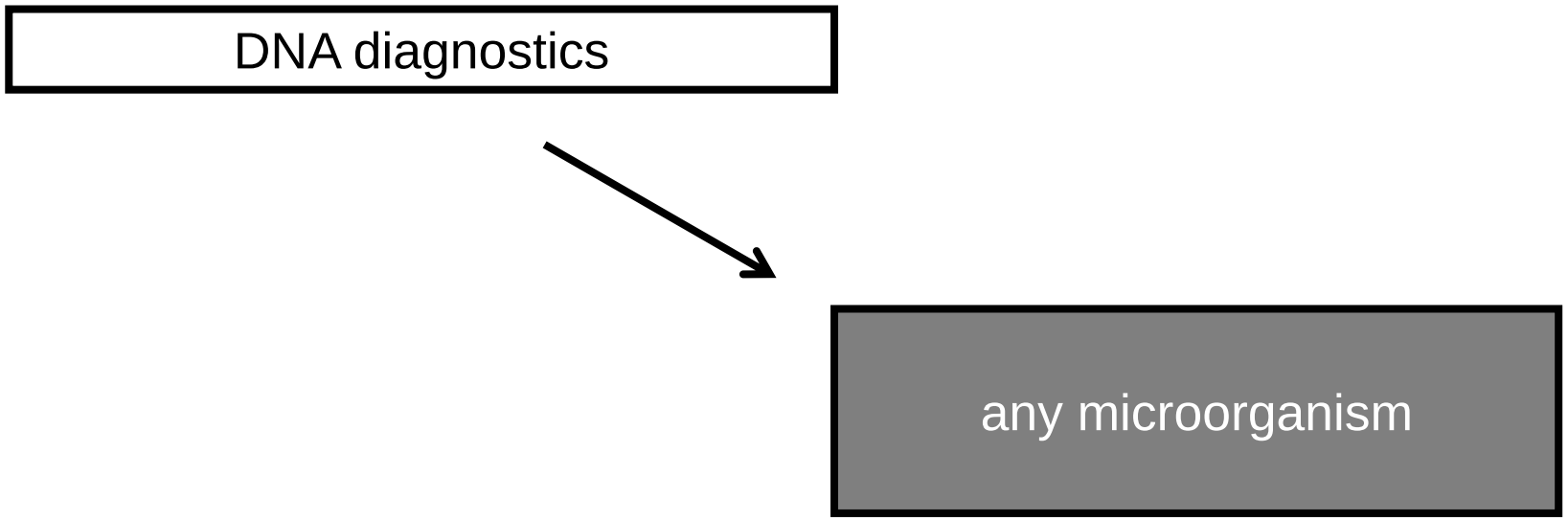


the target sequence for the primers must be unique to bordetella

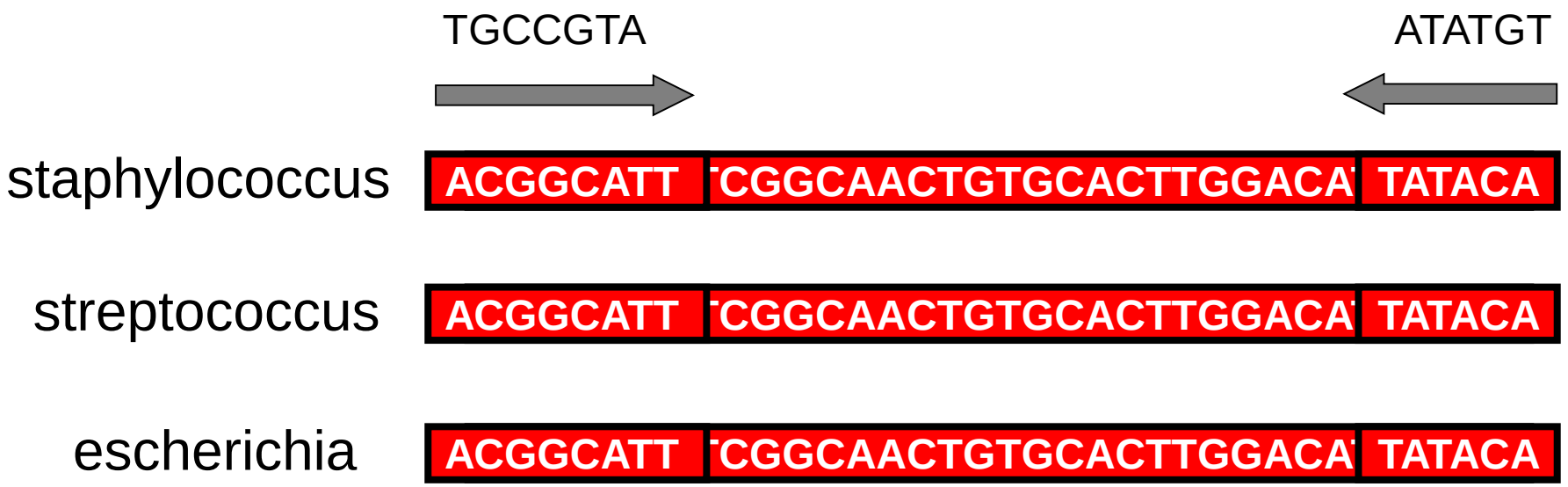


Joint infection. What is the cause?

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



the target sequence for the primers is present in all bacteria

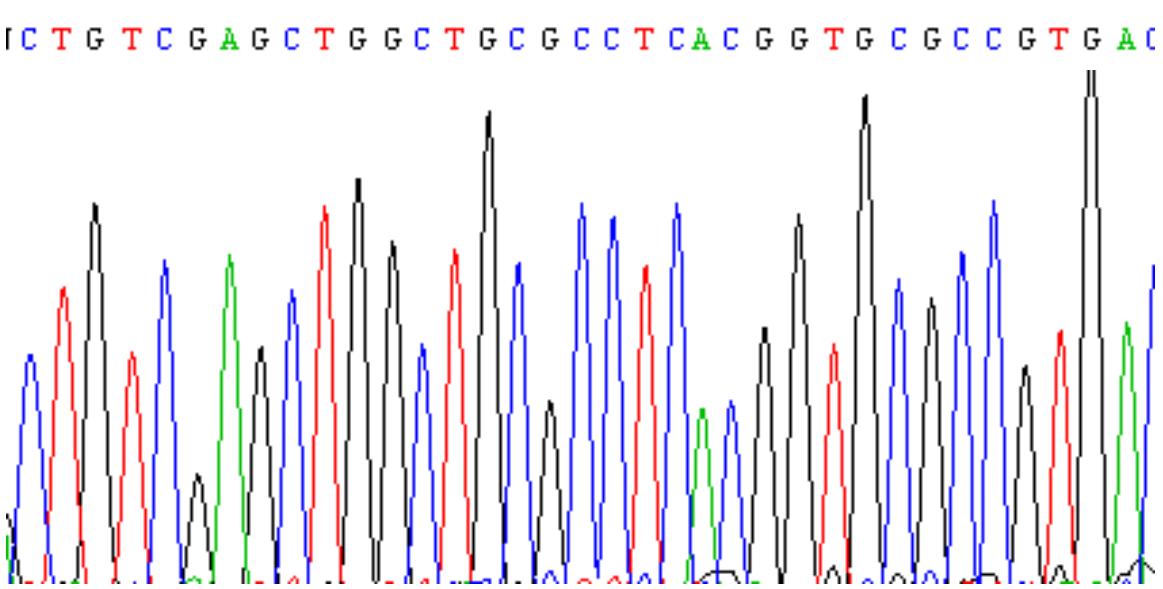


PCR positivity + sequencing

DETECTION OF BACTERIA,
but which one?

IDENTIFICATION
on a species level

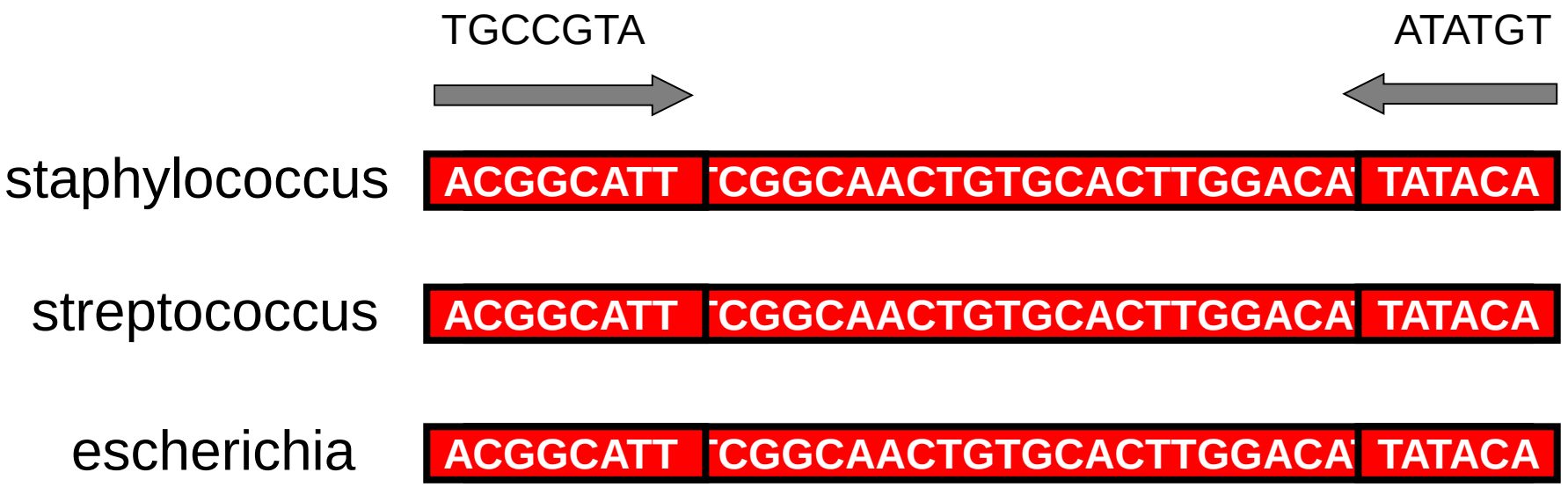
PCR product sequencing:



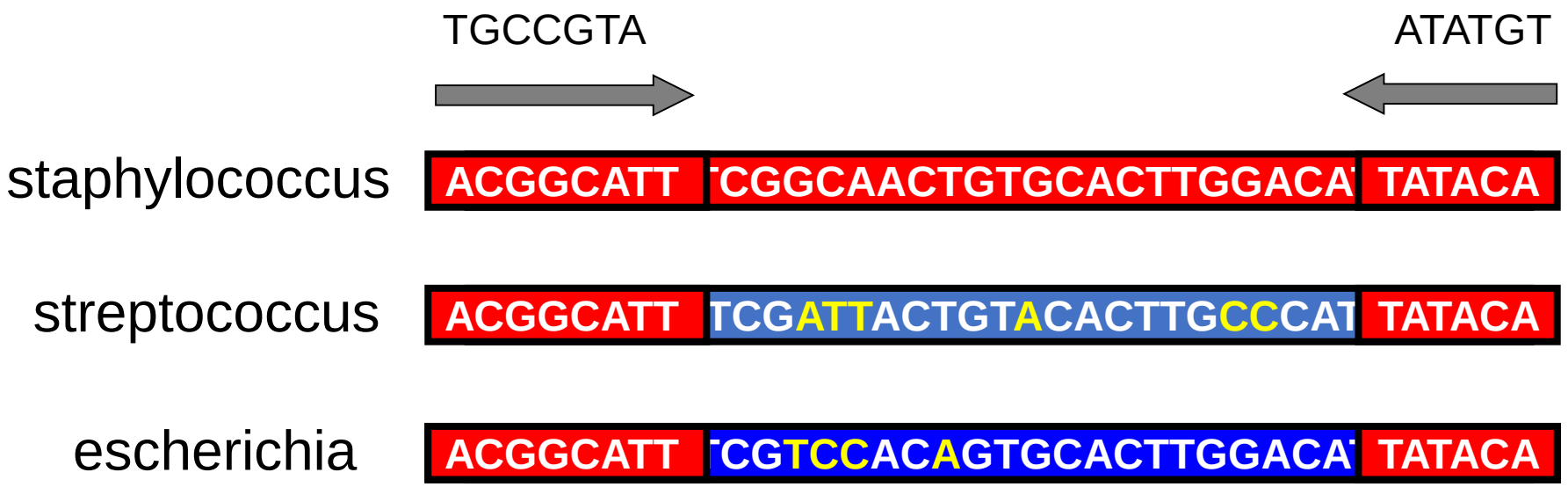
→ *S. aureus*

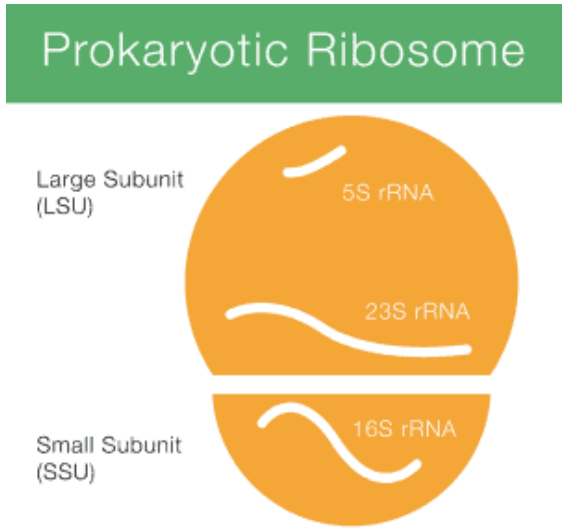
C T G A

the target sequence for the primers is present in all bacteria

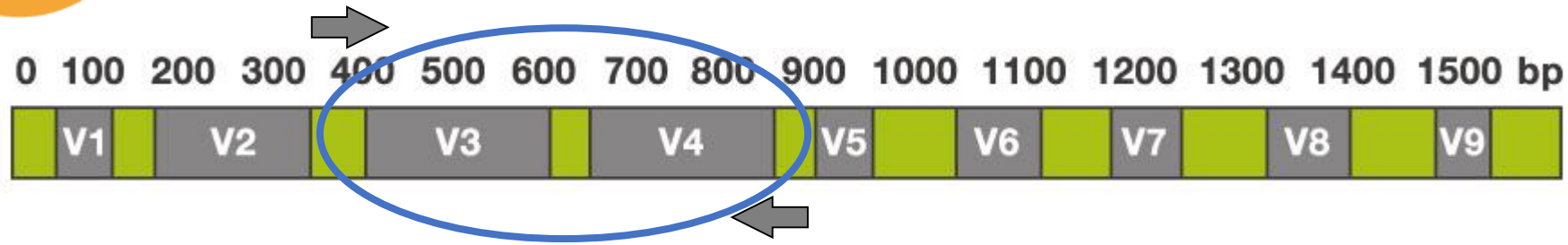


the target sequence for the primers is present in all bacteria





16S rRNA gene



Note:
panbacterial PCR to be done from primary sterile material only

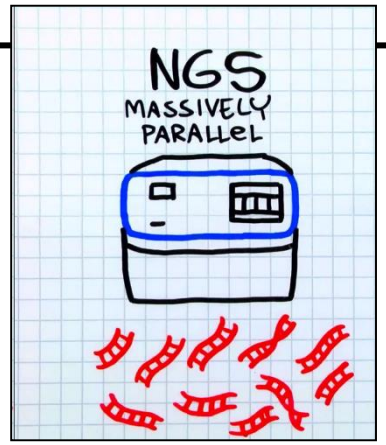
DNA diagnostics

all microorganisms
(metagenome)

any microorganism

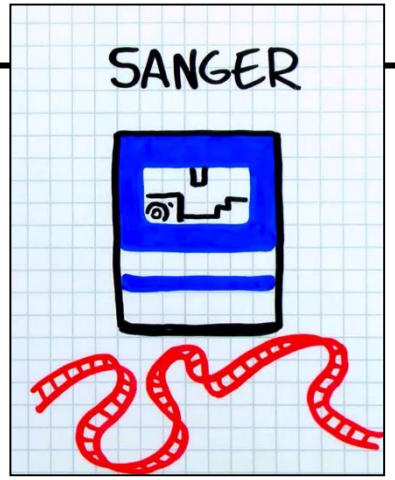
Method

Massive parallel sequencing
(NGS)



Method

PCR 16S rRNA
and Sanger sequencing



Conclusions

- Reasons for the use of molecular approach in diagnostics:

    - speed (extreme as POCT)
    - sensitivity, specificity
    - detection of non-culturable agents
    - detection while on antibiotics

- In principle, two types of tests:

    - pathogen specific PCR (single agent or multiplex)
    - panbacterial PCR (metagenomics in future)

When to use which?

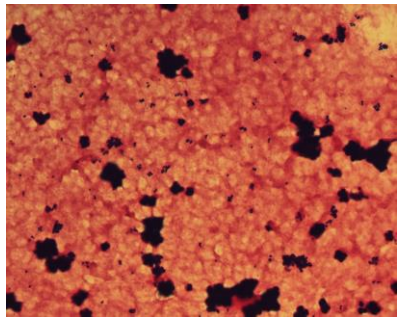
Examples: diagnostics of bloodstream infections

1- 3 days

positive

negative

5 days



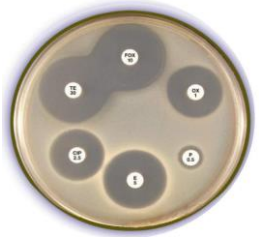
Gram staining

1 day

culture on solid media

identification and AST

1 day



Examples: diagnostics of bloodstream infections

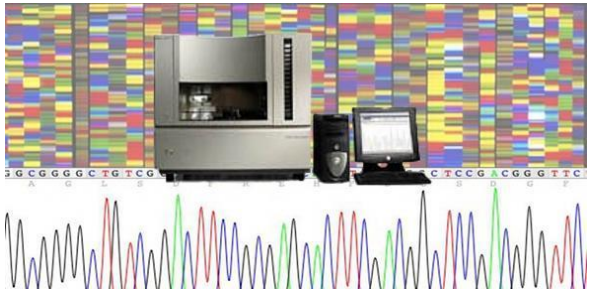
DNA diagnostics

preselected multiple microorganisms

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

3.5 hours

any microorganism



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, ...

dozens of hours

Advantages of DNA dx
• non-culturable organisms
• detection while on antibiotics
• high sensitivity
• speed

Disadvantages of DNA dx
• cost
• bacterial isolate not available
• missing info on AST
• not always available