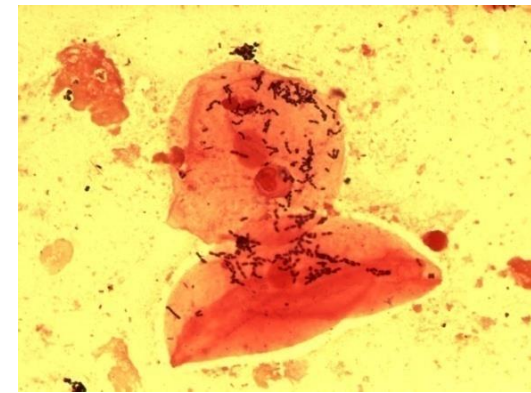
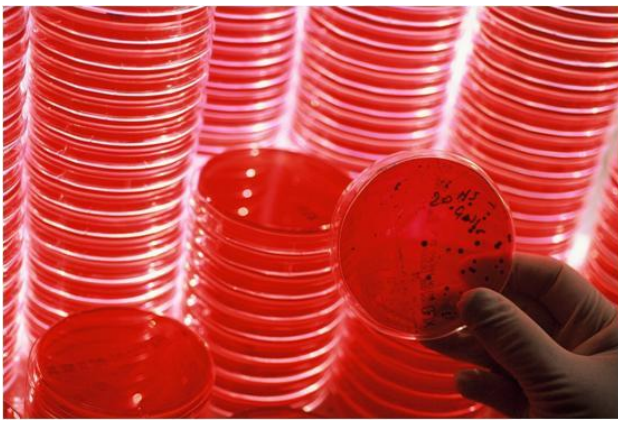


Molecular microbiology

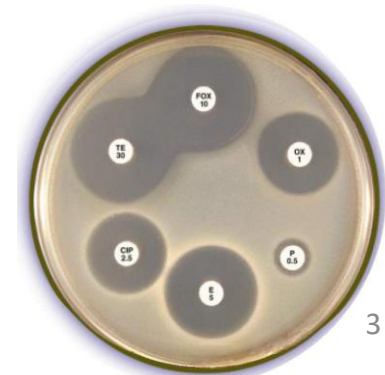


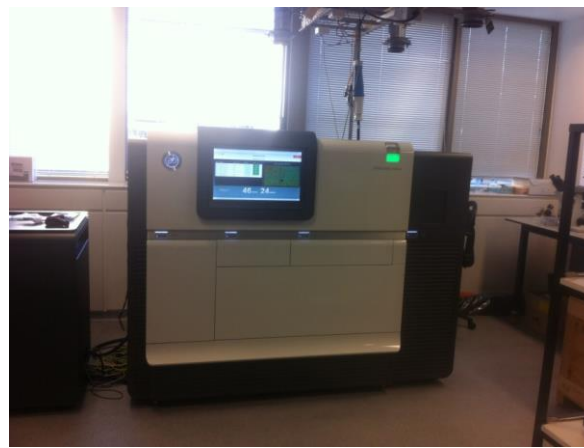
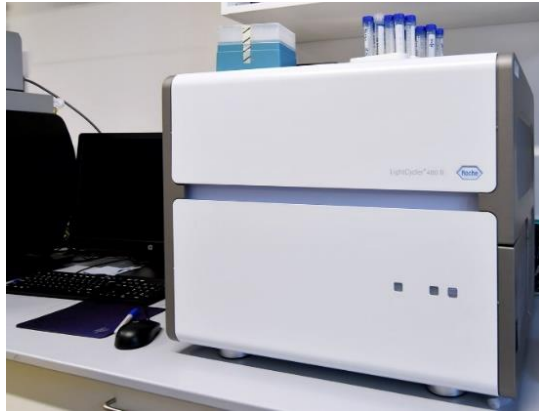
Pavel Drevinek



Traditional microbiology

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

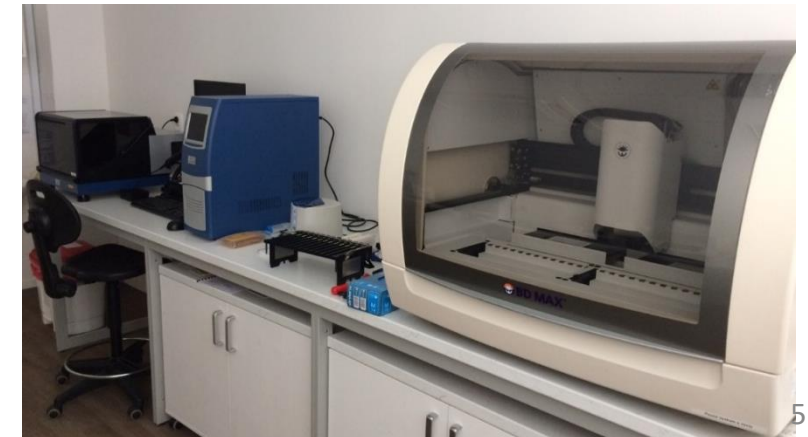




Molecular microbiology

DNA or RNA analysis for the purpose of:

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



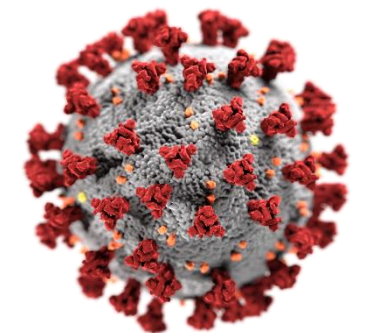
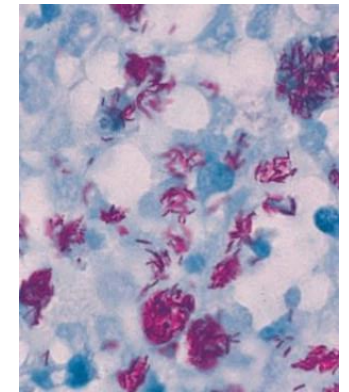
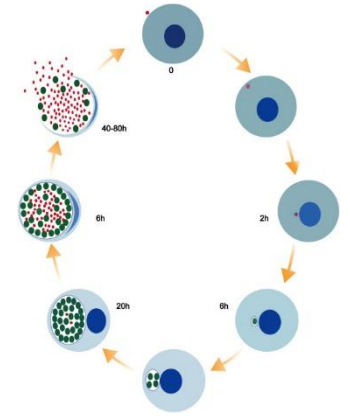
Why molecular diagnostics in microbiology

Diagnostics of culture negative cases

- non-culturable agents, slow growing, „fastidious“
- high sensitivity
- detection while on antibiotic therapy

Speed

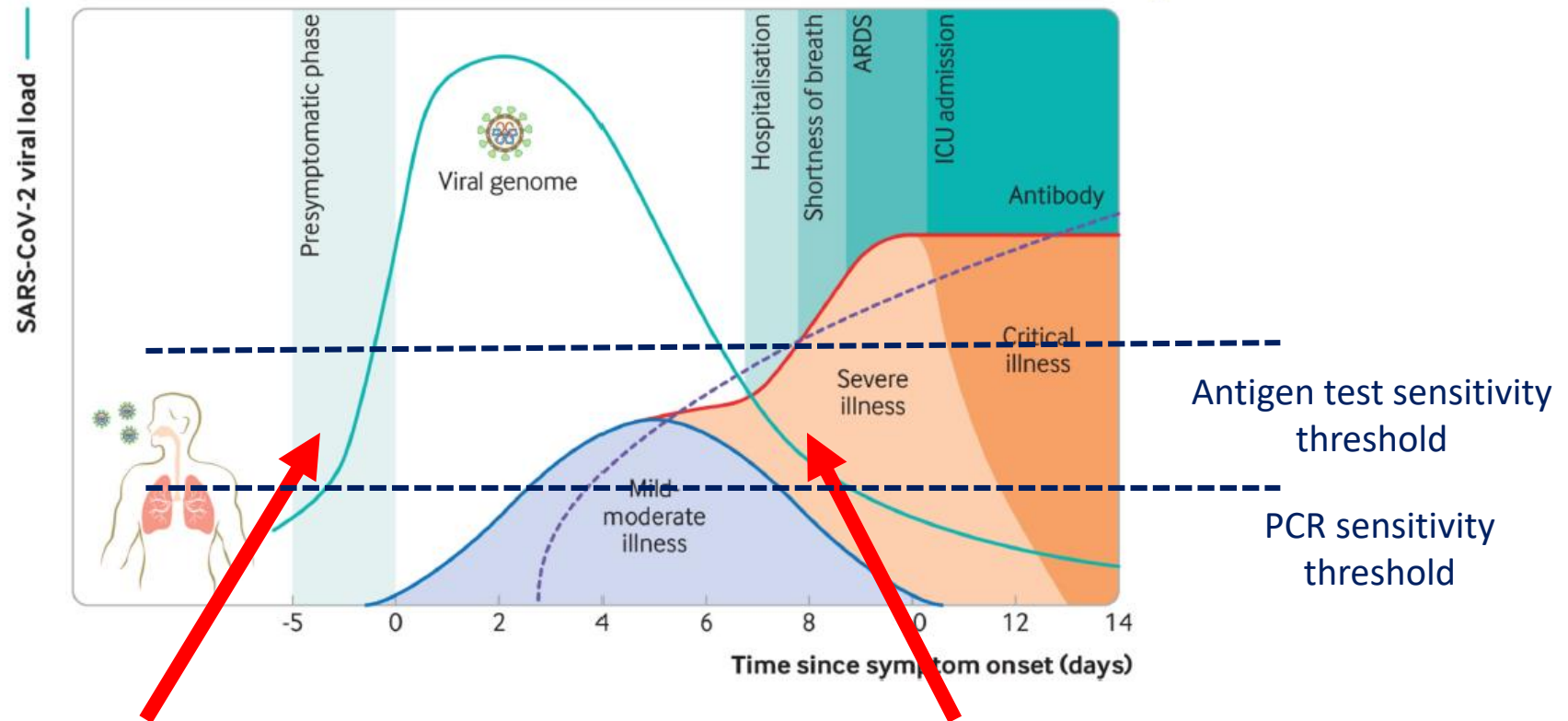
Quantification (viral load)



Why not always

Cost
Bacterial isolate not available (missing info on susceptibility)
Pitfalls in interpretation, invisible at first sight:
does the detected DNA originate from a viable agent?
is a positive finding medically relevant?

PCR and "false positivity"



- early detection (thanks to PCR)
- PCR remains positive at recovery

molecular diagnostics

```
graph TD; A[molecular diagnostics] --> B[preselected single microorganism]; A --> C[any single microorganism]; B --- D[pathogen specific PCR]; C --- E[universal, broad-range, panbacterial PCR]
```

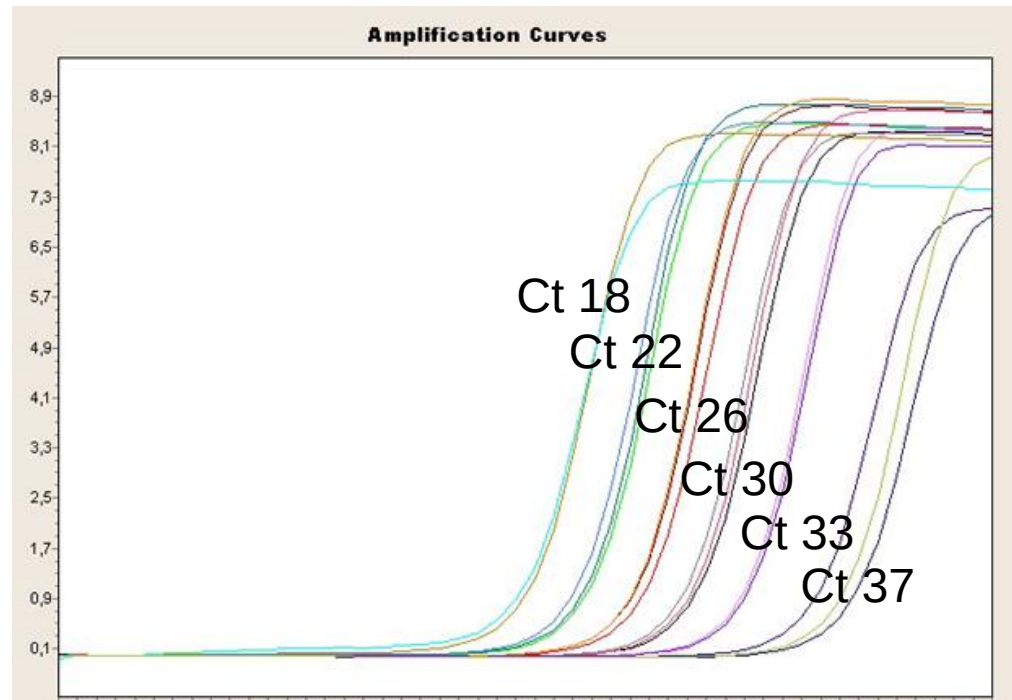
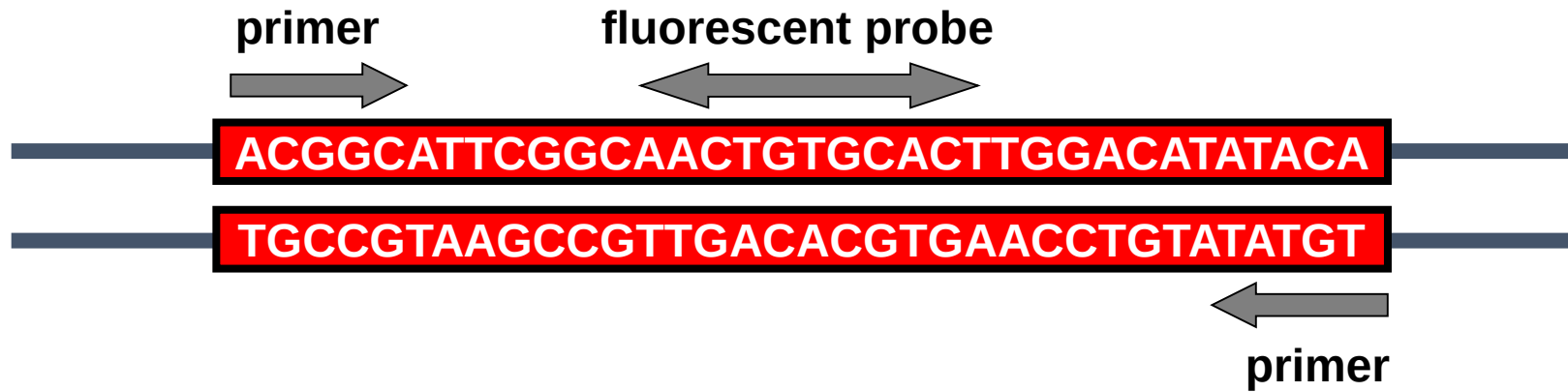
preselected single
microorganism

pathogen specific PCR

any single
microorganism

universal, broad-range,
panbacterial PCR

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



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?

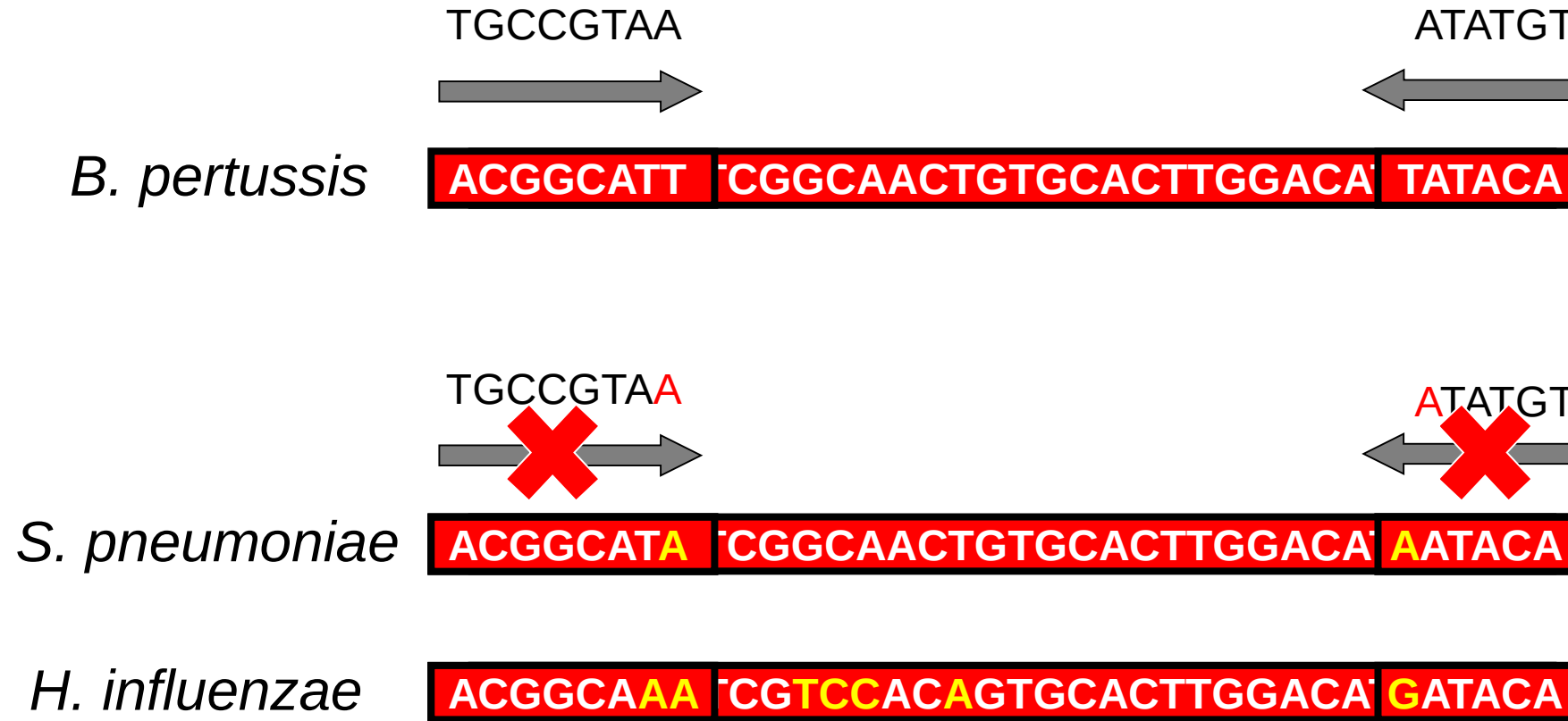
(you need to detect only *Bordetella pertussis*; ignore others)

molecular diagnostics

preselected single
microorganism



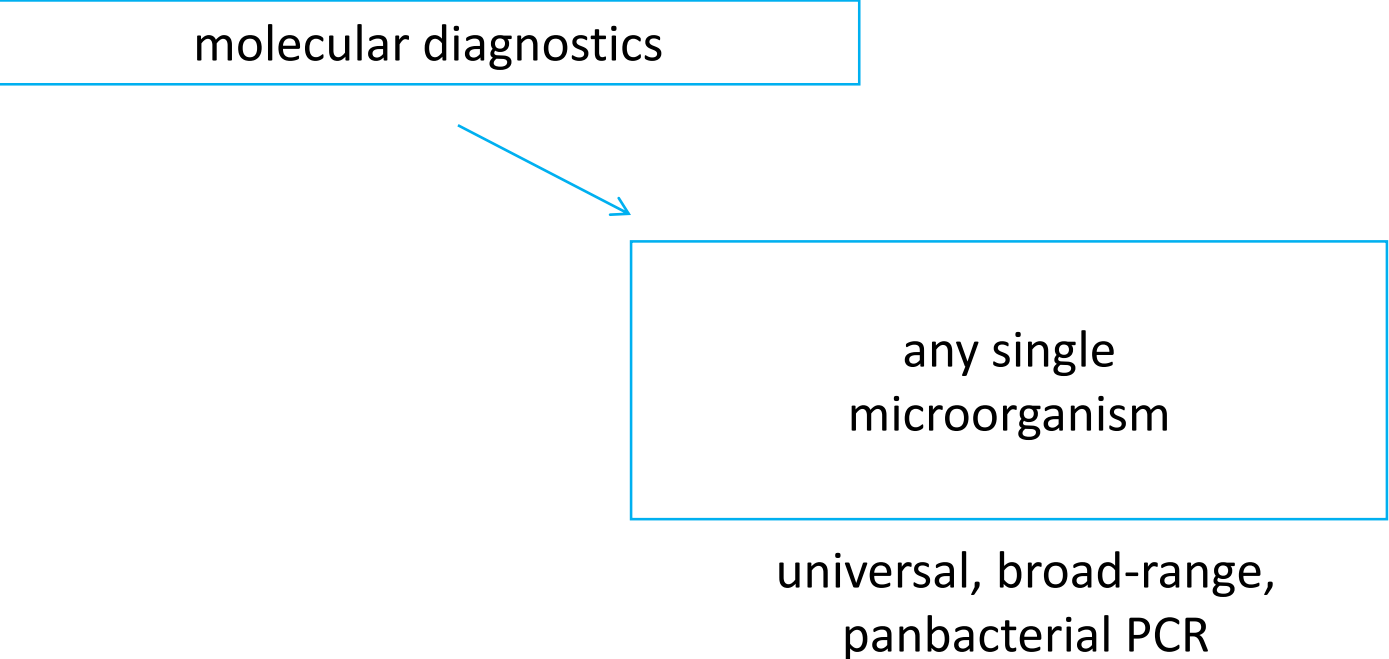
the target sequence for the primers must be unique to *Bordetella pertussis*



Joint infection. What is the cause?

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

molecular diagnostics

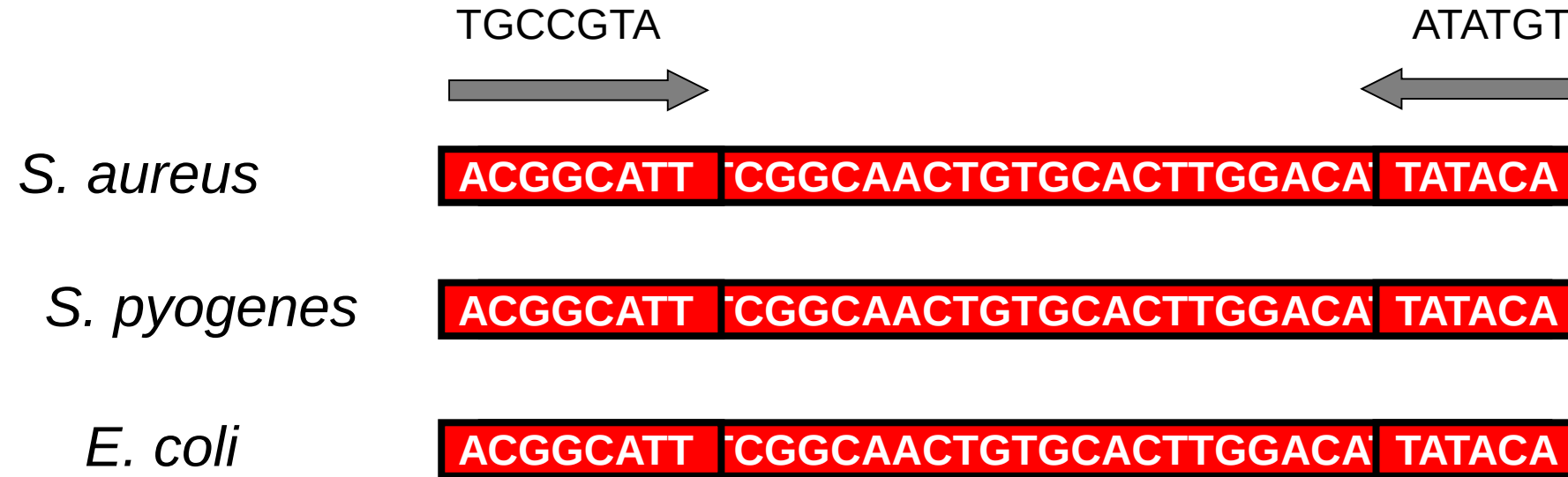


```
graph TD; A[molecular diagnostics] --> B[any single microorganism];
```

any single
microorganism

universal, broad-range,
panbacterial PCR

the target sequence for the primers is present in all bacteria



PCR positivity

+

sequencing

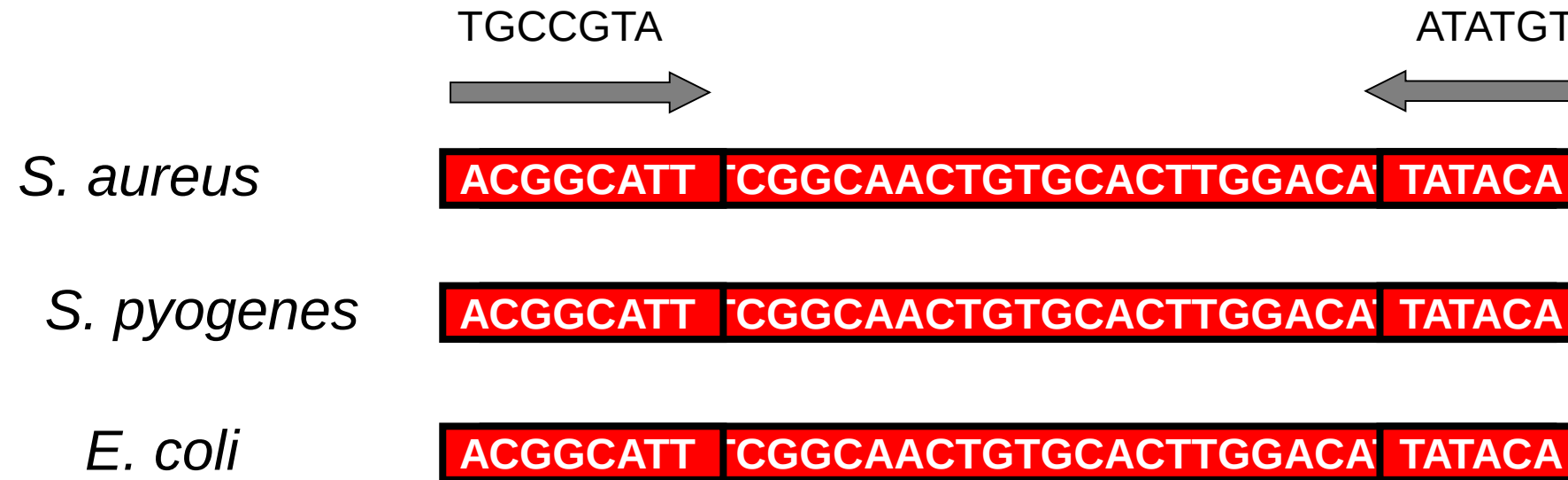


DETECTION OF BACTERIA,
but which one?

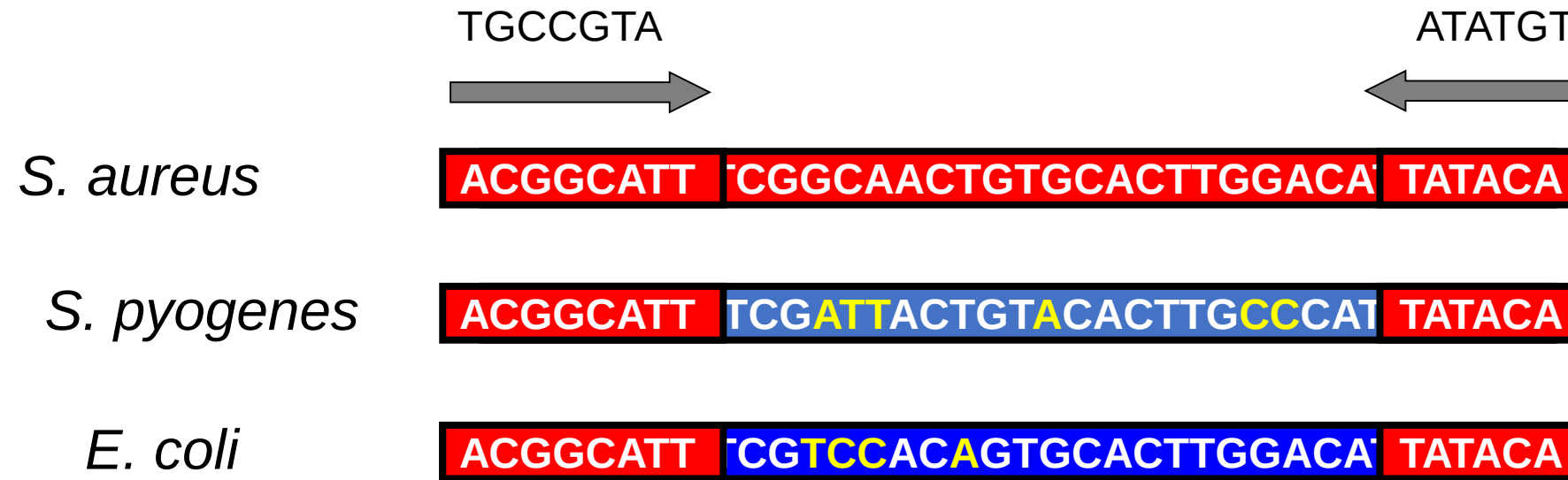


IDENTIFICATION
on a species level

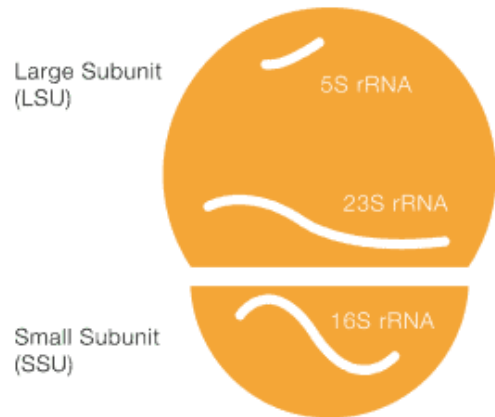
the target sequence for the primers is present in all bacteria



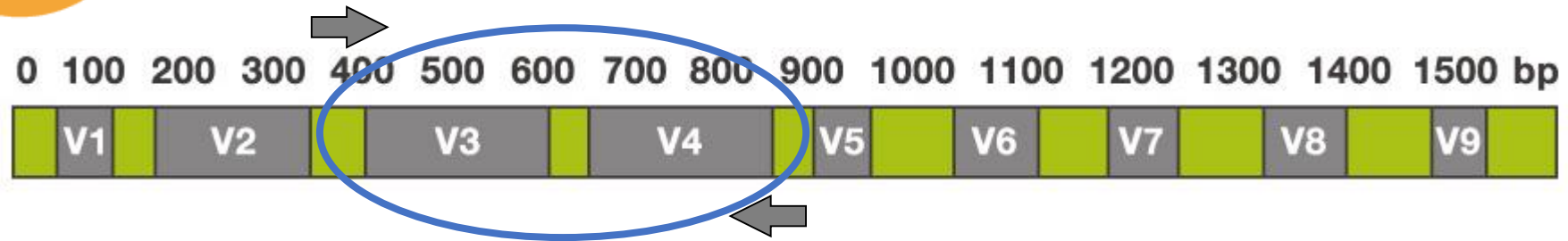
the target sequence for the primers is present in all bacteria



Prokaryotic Ribosome



16S rRNA gene

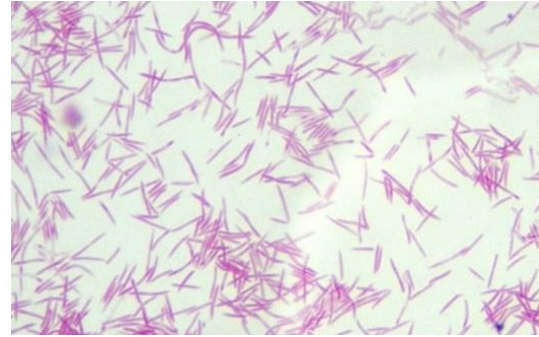


Note:

panbacterial PCR to be done from primary sterile material only („not dirty“)

Fusobacterium necrophorum

- strict anaerobic G- rod
- part of mouth microbiota



- can cause
 - abscesses and phlegmons (peri-, retro-tonsillar)
 - thrombosis of the v. jugularis int. (Lemierre's syndrome)
 - septic condition after angina
 - otitis and meningitis

molecular diagnostics

preselected single
microorganism

- ***Bordetella pertussis***
- ***Mycobacterium tuberculosis***
- ***Helicobacter pylori***
- SARS-CoV-2
- influenza A and B
- RSV
- HHV1 to HHV7
- parvovirus B19
- HBV, HCV, HEV
- measles virus
- monkey pox virus
- BKV, JCV
- ***Burkholderia cepacia* complex (in house)**
- ***Pseudomonas aeruginosa* (in house)**
- *S. aureus* PVL
- *S. aureus mecA*
- *S. aureus* TSST-1

molecular diagnostics

preselected single
microorganism

pathogen specific PCR

preselected multiple
microorganisms

multiplex, syndromic, panel PCR

- Meningitis
- **Meningoencefalitis**
- Respiratory infections: viral
- **Respiratory infections: atypical**
- Pneumonia
- GIT infections
- **Sexually transmitted**

- Dermatophytes
- Aspergilli
- Malaria
- Tropical fever
- Exanthemic

How commercial PCRs differ from each other

Aspect No. 1: panel width (number of targets detected)

- only one microorganism
- basic panel
- expanded panel

flu A

flu A

flu B

RSV

SARS-CoV-2

flu A

flu B

RSV

SARS-CoV-2

adenovirus

metapneumovirus

viry parainfluenza

other coronaviruses

rhinovirus

enterovirus

bocavirus

...

Aspect No. 2: speed to result, user friendly or not that friendly

- conventional (manual)
- **point-of-care test** (POCT), performed at the site of care
 - the advantage of being able to be used outside normal working hours
 - immediate availability of the result
 - performed by staff who are not qualified for laboratory work

POCT



15 minutes
600 CZK per single test

SARS-CoV-2



60 minutes
1500 CZK

SARS-CoV-2
flu A
flu B
RSV



15 minutes
4000 CZK

SARS-CoV-2
flu A
flu B
RSV
other coronaviruses
metapneumovirus
rhinovirus
adenovirus

B. pertussis
B. parapertussis
C. pneumoniae
M. pneumoniae

Aspect No. 3: test sensitivity



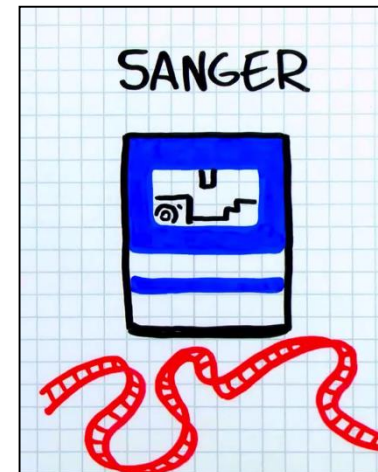
		conventional PCR			92 %
		pozitivní	negativní	celkem	
test POCT	pozitivní	33	3	36	
	negativní	10	251	261	
	celkem	43	254	297	

77 %

molecular diagnostics

all microorganisms
(metagenome)

any single
microorganism



Conclusions

- Reasons to use molecular methods in microbiology:
 - speed (also as POCT)
 - sensitivity
 - detection of non-cultivable agents
 - detection while on antibiotics
- In principle, two types of tests:
 - pathogen-specific (single or multiplex)
 - panbacterial (16S based on metagenomic in future)