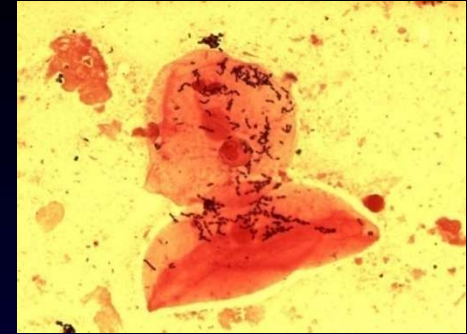


# Molecular microbiology

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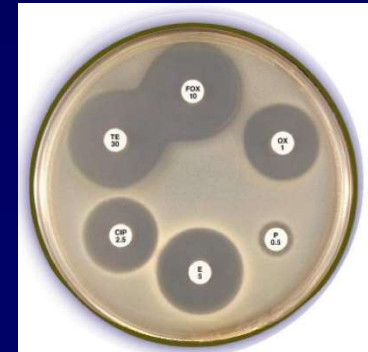
**Pavel Dřevínek**

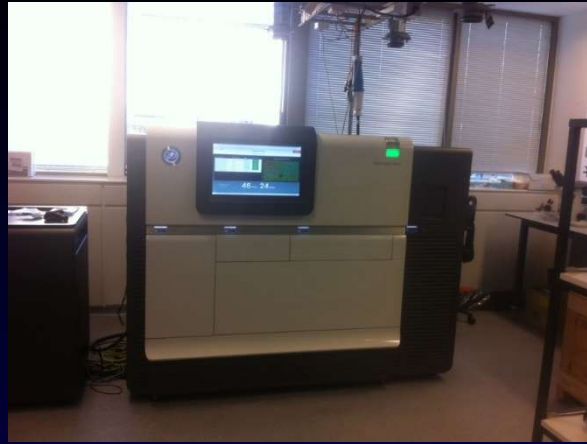
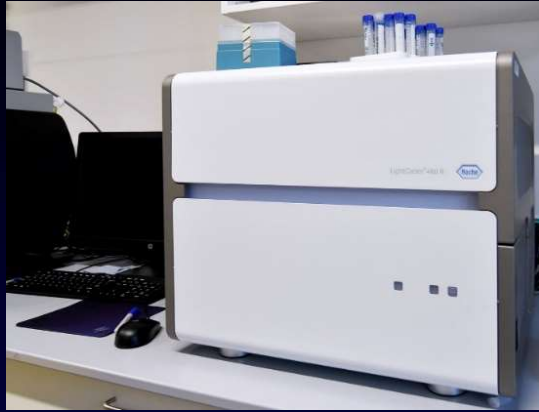


# Traditional microbiology

microscopy  
Antigen detection  
culture, ID a AST

Serology and antibodies

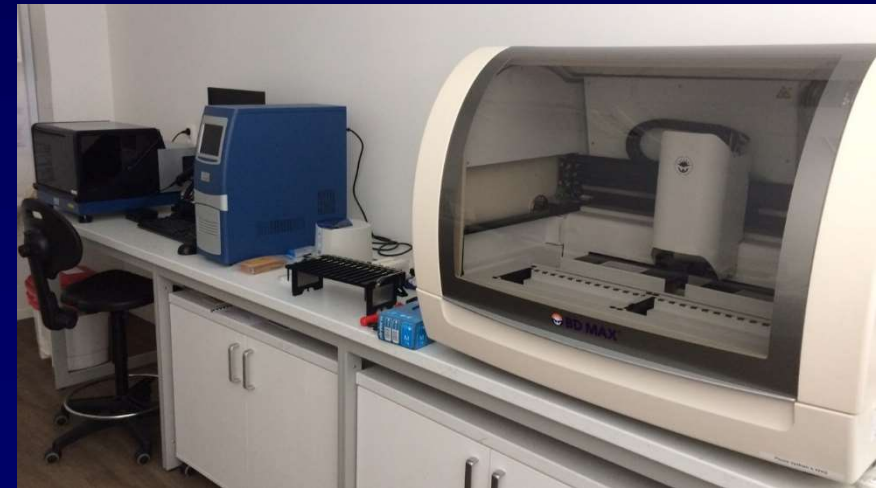




## New microbiology

DNA or RNA analysis for the purpose of:

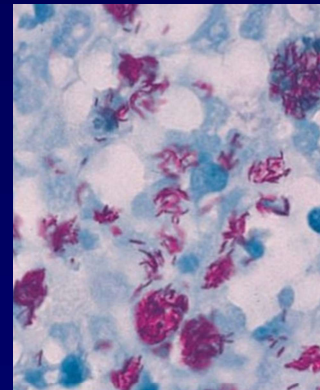
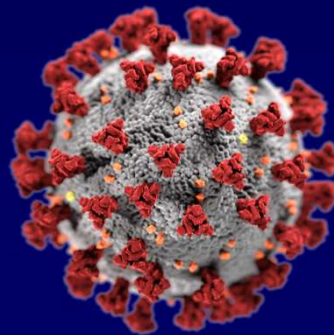
- diagnostics (ID)
- epidemiology
- virulence
- antimicrobial resistance



# Diagnostic reason to detect DNA or RNA

## Advantages

- non-culturable agents, slow growing, „fastidious“
- detection even during antibiotic therapy
- quickly performed
- high sensitivity
- quantification



## Diagnostic reason to detect DNA or RNA

### Limitations

**pitfalls in interpretation, invisible at first glance:**

- detected DNA originate from a viable agents?

DNA diagnostics



to a selected pathogen  
(pathogen-specific)



### Herpesvirus infections

(quantification)

HSV	HHV6
VZV	HHV7
EBV	HHV8
CMV	

### Other viral infections

*Parvovirus B19*  
BKV  
*adenoviruses A-G*  
*papillomaviruses*

### Bacterial infections

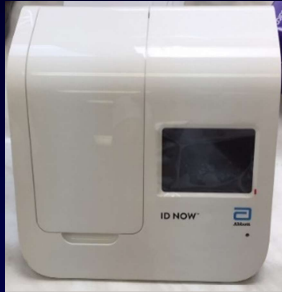
*Bordetella pertussis*  
*Pseudomonas aeruginosa*  
*Burkholderia cepacia complex*  
MRSA and PVL

### Fungal infections

*Mucorales*



## POCT (point-of-care testing)



15 mins

**Viral infections**  
(closed system)

SARS-CoV-2





## POCT (point-of-care testing)



80 mins

**Bacterial infections**  
(closed system)

*Mycobacterium tuberculosis*

DNA diagnostics



selected microorganism  
(pathogen-specific)



selected mikroorganisms  
(multiplex; syndromic PCR)



## 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 jiroveci*  
*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*



## meningitis

*Streptococcus pneumoniae*  
*Neisseria meningitidis*  
*Haemophilus influenzae*  
*Streptococcus agalactiae*  
*Escherichia coli*  
*Listeria monocytogenes*  
HSV  
VZV  
enteroviruses

## Dermatomycosis

*Trichophyton spp.*  
*Trichophyton rubrum*  
*Trichophyton mentagrophytes*  
*Microsporum*  
*Microsporum canis*  
*Epidermophyton floccosum*  
*Nannizzia gypsea*  
*Scopulariopsis*  
*Aspergillus spp.*  
*Candida spp.*  
*Candida albicans*  
*Candida guilliermondii*  
*Candida parapsilosis*  
*Candida glabrata*



## POCT (point-of-care testing)



60 mins

ca 1,200 CZK/test

### Respiratory infections viral (closed system)

influenza A  
influenza B  
RSV  
SARS-CoV-2

Emergency department



## Pneumonia (closed system)

influenza A	<i>Klebsiella pneumoniae</i>
influenza B	<i>Klebsiella oxytoca</i>
RSV	<i>Klebsiella aerogenes</i>
rhinoviruses/enteroviruses	<i>Moraxella catarrhalis</i>
parainfluenza	<i>Serratia marcescens</i>
adenoviruses	<i>Staphylococcus aureus</i>
metapneumovirus	<i>Streptococcus pneumoniae</i>
seasonal coronavirus	<i>Streptococcus pyogenes</i>
SARS-CoV-2	<i>Streptococcus agalactiae</i>
<i>Mycoplasma pneumoniae</i>	
<i>Chlamydia pneumoniae</i>	
<i>Legionella pneumophila</i>	CTX-M
<i>Acinetobacter baumannii</i>	IMP
<i>Pseudomonas aeruginosa</i>	KPC
<i>Enterobacter cloacae</i>	NDM
<i>Proteus spp.</i>	VIM
<i>Escherichia coli</i>	OXA-45-like
<i>Haemophilus influenzae</i>	mecA/C a MREJ



Department of intensive care medicine

ca 3,500 CZK/test

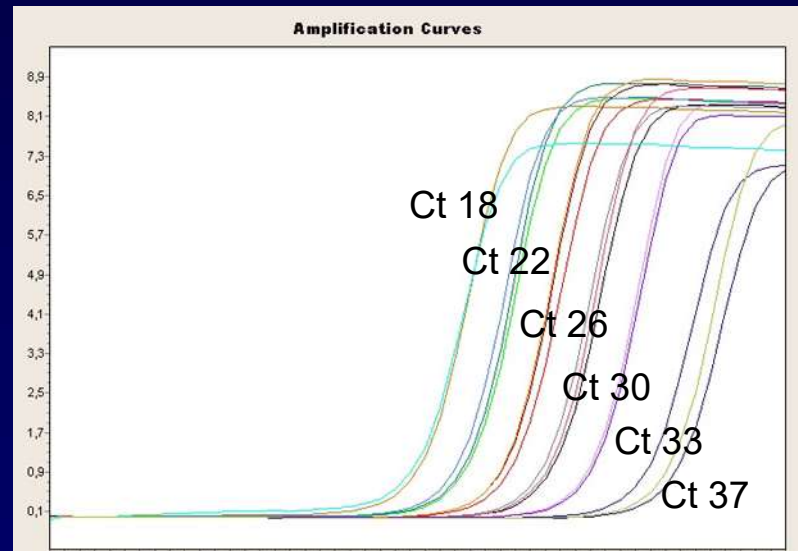
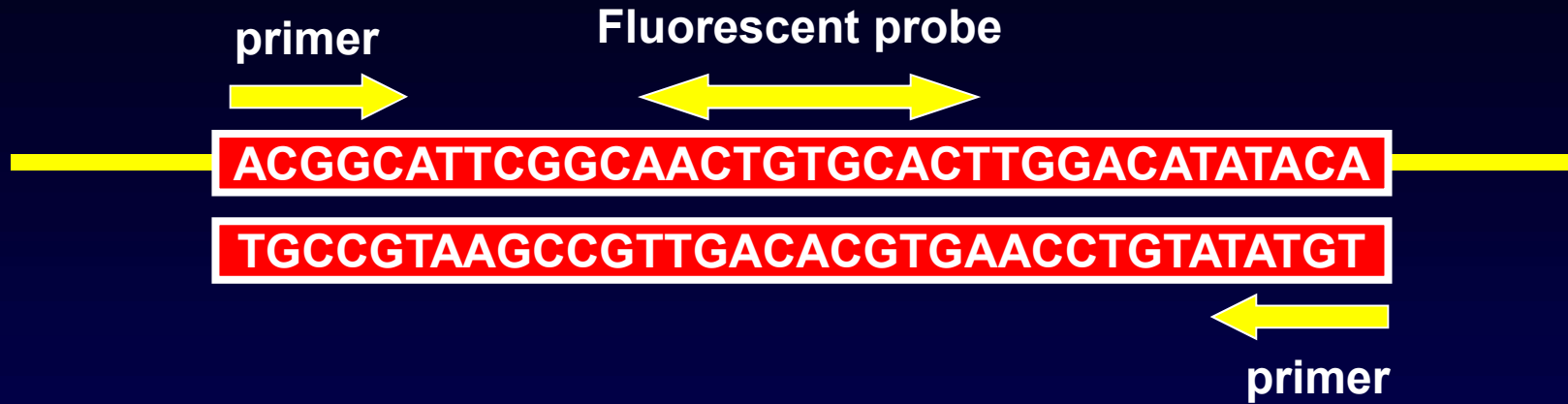
DNA diagnostics



```
graph TD; A[DNA diagnostics] --> B[any microorganism (broad-range)];
```

any microorganism  
(broad-range)

detection focused on the nucleotide sequence

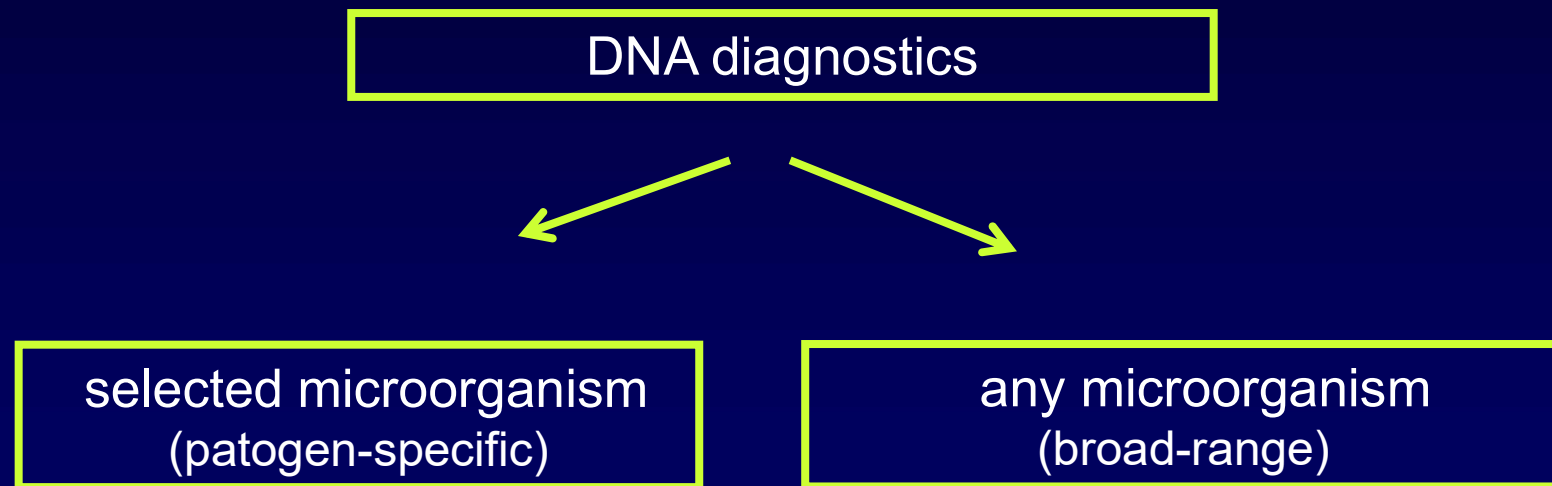


Ct treshold value = PCR cycle,  
when the PCR signal starts to grow

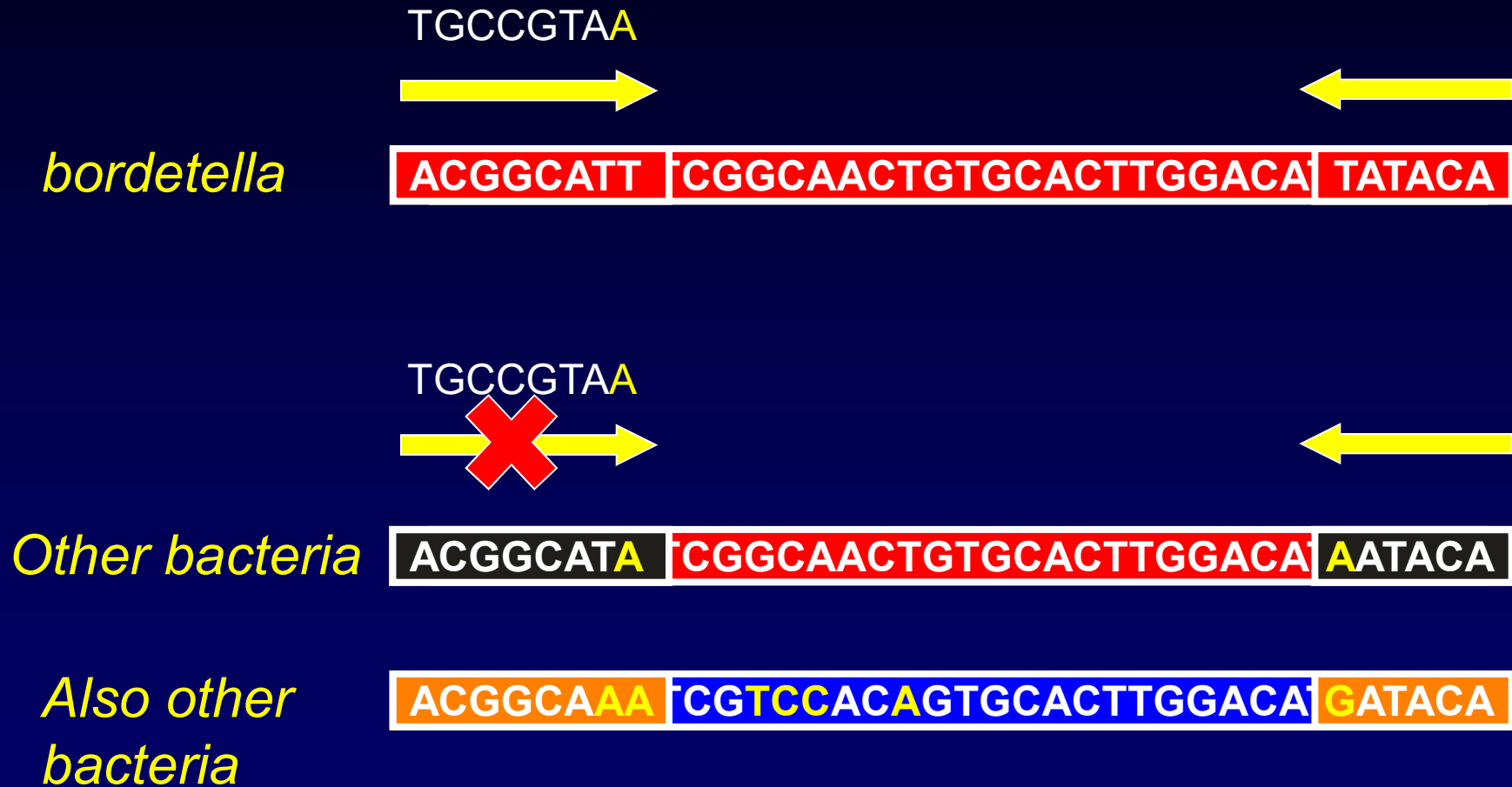
the lower the Ct value, the more agent is  
detected in the sample



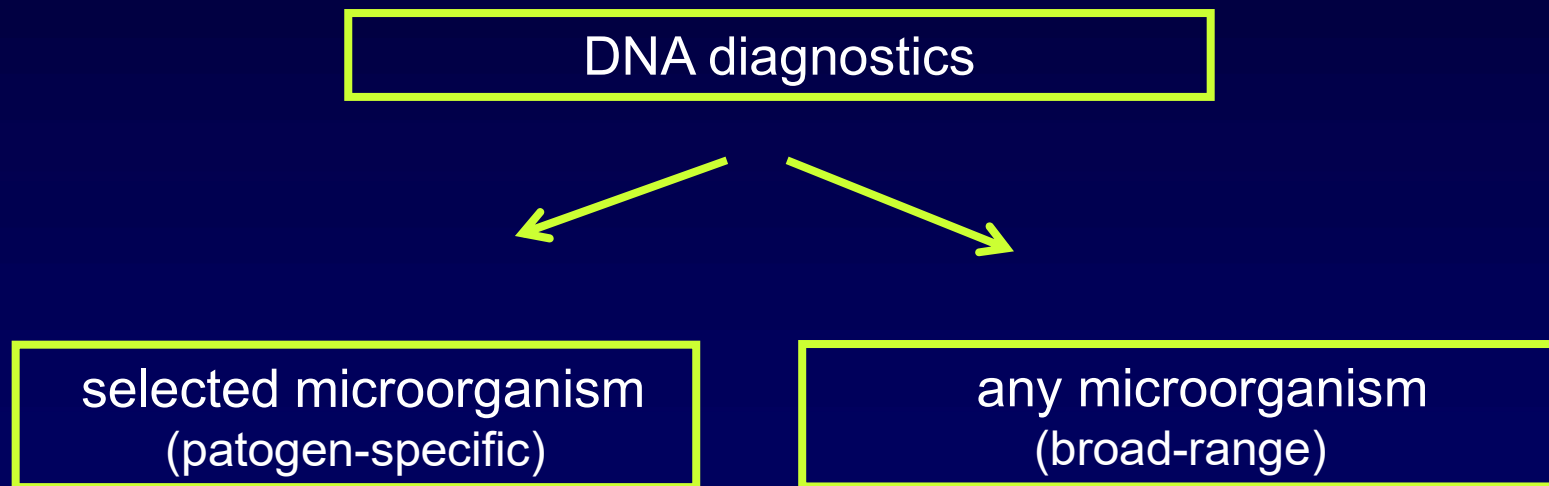
Does the patient have a whooping cough?  
(need to detect only *Bordetella pertussis*)



the target sequence for the primers must be unique to bordetella



Fever. What is the cause?  
(need to detect any bacteria,  
because any bacteria can cause sepsis)



the target sequence for the primers is present in all bacteria

TGCCGTAA



Staphylococcus

ACGGCATT | CGGCAACTGTGCACTTGGACA | TATACA

Streptococcus

ACGGCATT | CGATTACTGTACACTTGCC | TATACA

Enterococcus

ACGGCATT | CGTCCACAGTGCCTTGGACA | TATACA

PCR positivity

+ sequencing

DETECTION OF BACTERIA,  
but which one?

IDENTIFICATION  
on species level

the target sequence for the primers is present in all bacteria

primers



Stahylococcus

ACGGCATT | CGGCAACTGTGCACTTGGACA | TATACA

Streptococcus

ACGGCATT | TCGATTACTGTACACTTGCCAT | TATACA

Enterococcus

ACGGCATT | CGTCCACAGTGCCTTGGACA | TATACA

16S rRNA gene



DNA diagnostics



```
graph TD; A[DNA diagnostics] --> B[any microorganism (broad-range)];
```

any microorganism  
(broad-range)

- from primary sterile material

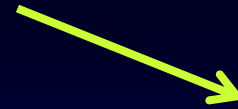
# Broad-range “16S PCR”

– samples from cardiovascular surgery:

ID	materiál	16S sekvenace	kultivace
1	trikuspidální chlopeň	<i>Staphylococcus aureus</i>	negativní
2	aortální chlopeň	<i>Bartonella quintana</i>	negativní
3	aortální chlopeň	<i>Enterococcus faecalis</i>	negativní
4	trikuspidální chlopeň	<i>Staphylococcus aureus</i>	negativní
5	mitrální chlopeň	<i>Streptococcus anginosus</i>	negativní
6	mitrální chlopeň	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>
7	trikuspidální chlopeň	negativní	negativní
8	trikuspidální chlopeň	<i>Staphylococcus aureus</i>	negativní
9	aortální chlopeň	<i>Bartonella quintana</i>	negativní
10	mitrální chlopeň	<i>Enterococcus faecalis</i>	<i>Enterococcus faecalis</i>
11	aortální chlopeň	<i>Enterococcus faecium</i>	negativní
12	trikuspidální chlopeň	<i>Enterococcus faecium</i>	negativní
14	aortální chlopeň	<i>Enterococcus faecalis</i>	negativní
15	mitrální chlopeň	<i>Staphylococcus aureus</i>	negativní
16	chlopeň	<i>Bartonella quintana</i>	negativní



## DNA diagnostics



All microorganisms  
(metagenomics)

Any microorganisms  
(širokospektré vyšetření)



### Method

Massive parallel sequencing  
(NGS)

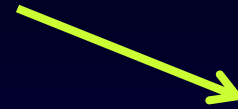
### Metoda

PCR 16S rRNA gene and  
Sanger sequencing

Useful (in future) for:

- diagnosis of unexplained diagnosis (directly from clinical material)
- microbiome

## DNA diagnostics



All microorganisms  
(metagenomics)

Any microorganisms  
(širokospektré vyšetření)



### Method

Massive parallel sequencing  
(NGS)

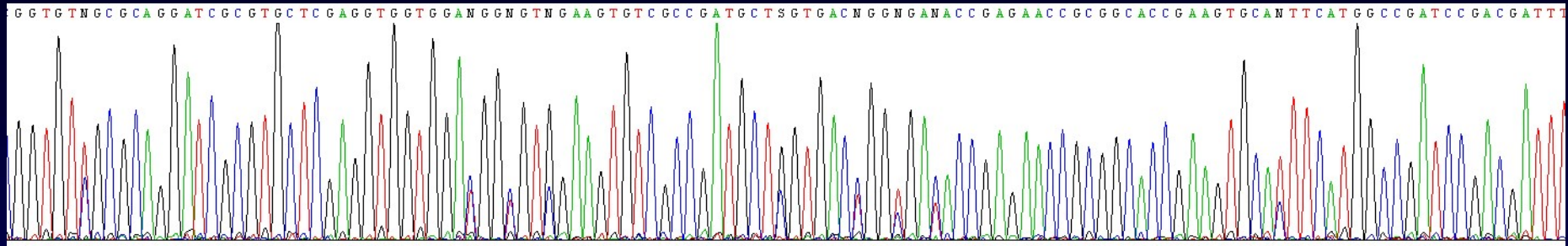
### Metoda

PCR 16S rRNA gene and  
Sanger sequencing

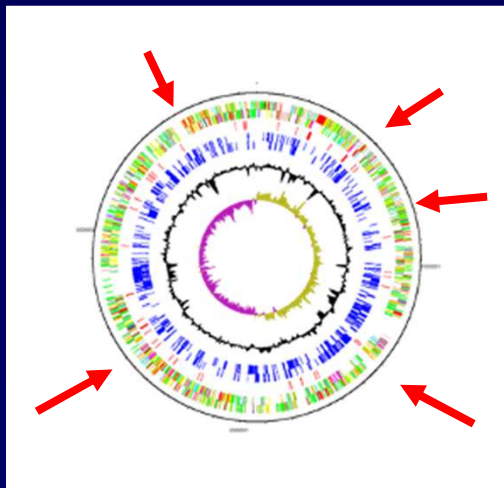
Useful (already today) for:

- whole genome sequencing (WGS) and genotyping

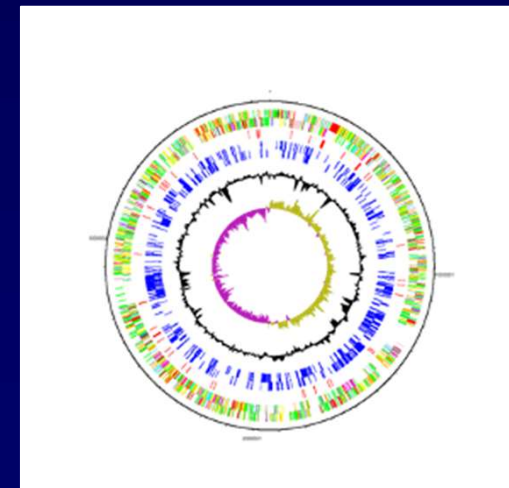
# Genotyping



Selected genes  
= multilocus sequence  
typing (MLST)



All genes  
= whole genome  
sequencing (WGS)



## Conclusions

Molecular methods create another pillar of microbiological diagnostics

(but for comprehensive diagnostics we need more than one pillar)

Standard methods in practice:

- multiplex (extensive) panels (in central laboratories)
- fast tests POCT
- panbacterial tests (in central laboratories)

Near future – NGS and metagenomics