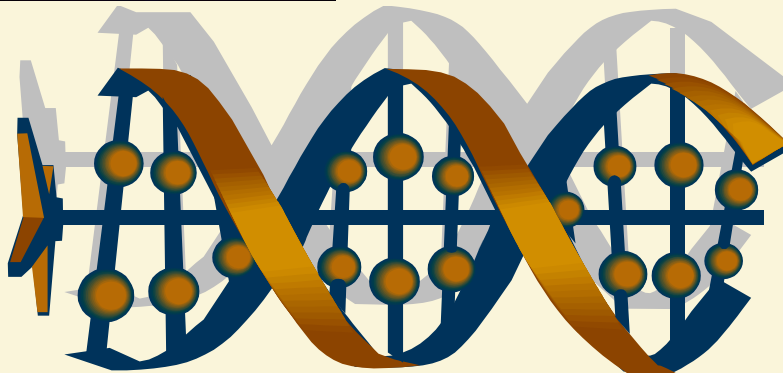
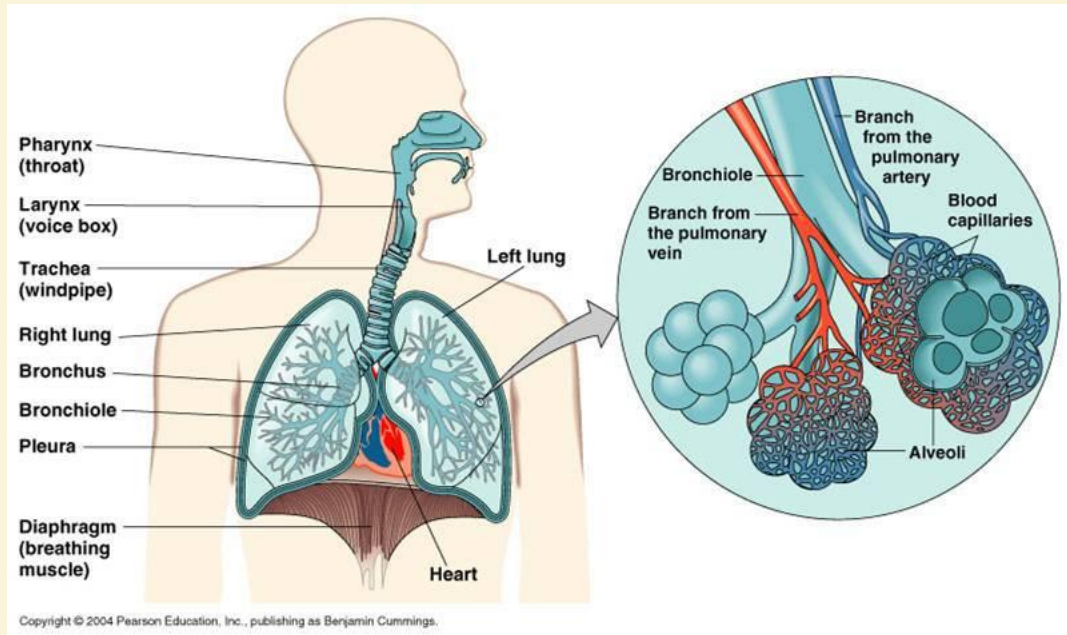
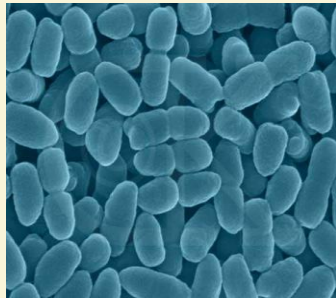


CF microbiology identification in the post genomic era



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Clinical Microbiology Laboratory

Rambam Health Care Campus

What have been changed in microbiology
diagnosis over the last century?

The classic bacteriology dogma

1. Direct Microscopy
2. Culturing
3. Biochemically bacterial identification
4. Antimicrobial susceptibility tests

Limitations

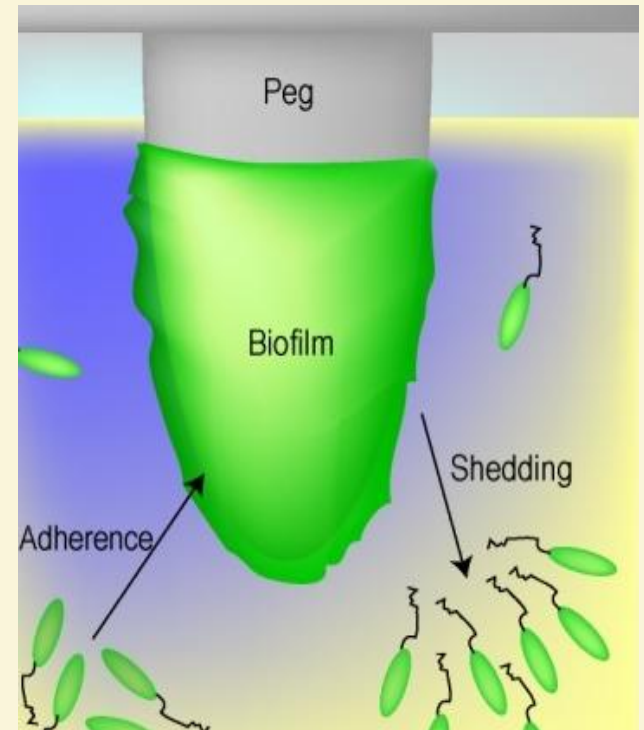
- Long turnaround time (days to weeks)
- Need for highly skill technicians
- Difficulty in detecting newly emerging pathogens
- Clinically relevance of bacterial isolates
- Accuracy of *in-vitro* antibacterial testing

Questions from (a desperate) clinician to bacteriologist

1. I gave IV antibiotics according to recent culture and there is no response. **Can you help with biofilm test?**
2. Patient does not fulfill ABPA criteria. **Can galctomannan or PCR help me** (sputum ? BAL?)
3. Patient is completely well but has positive culture for mycobacterium. **Is it really *M. abscessus* ?**
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5. MALDI-TOF MS , Next generation sequencing. **Is this the light at the end of the tunnel?**

bioFILM PA Kit

- Biofilm growth & inoculation system (MBEC- Minimum Biofilm Eradication Concentration Assay)
- Growth of biofilm from organisms recovered from sputum sample and inoculation of bacteria into wells



Results in SIR format –

12 Individual Antibiotics + 35 Antibiotic combinations

Planktonic

Antibiotic	SIR	Antibiotic	SIR	Antibiotic	SIR	Antibiotic	SIR	Antibiotic	SIR	Antibiotic	SIR
GM/AZT	S	AK/AZT	S	TO/AZT	S	T/S/P/T	S	CP/P/T	S	TO	R
GM/CAZ	S	AK/CAZ	S	TO/CAZ	S	T/S/MER	S	C/MER	S	T/S	I
GM/P/T	S	AK/P/T	S	TO/P/T	S	T/S/AZT	S	C/CAZ	S	C	S
GM/CPE	S	AK/CPE	S	TO/CPE	S	T/S/CAZ	S	AK	I	CP	I
GM/MER	S	AK/MER	S	TO/MER	S	CP/AZT	S	P/T	S	CAZ	S
GM/CPE	S	AK/CPE	S	TO/CPE	S	CP/CT	S	AZT	S	CPE	S
GM/T/S	I	AK/T/S	I	TO/T/S	S	CP/T/S	S	CT	S	MER	S
GM/CT	S	AK/CT	S	TO/CT	S	CP/MER	S	GM	R		

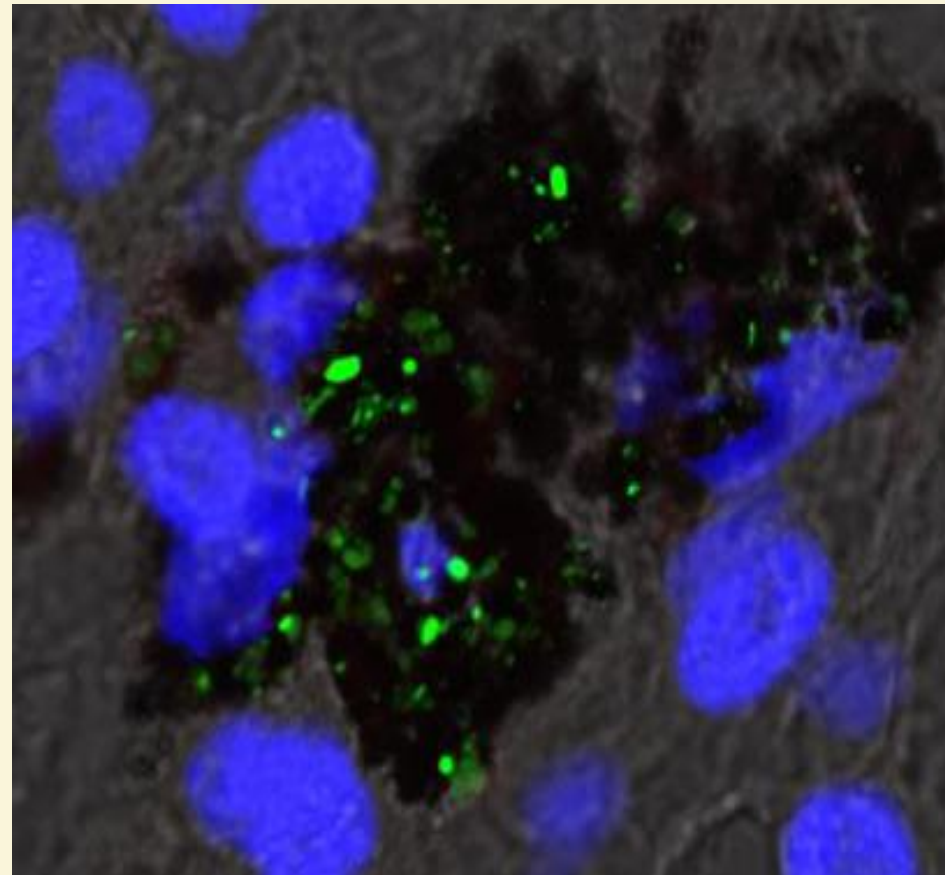
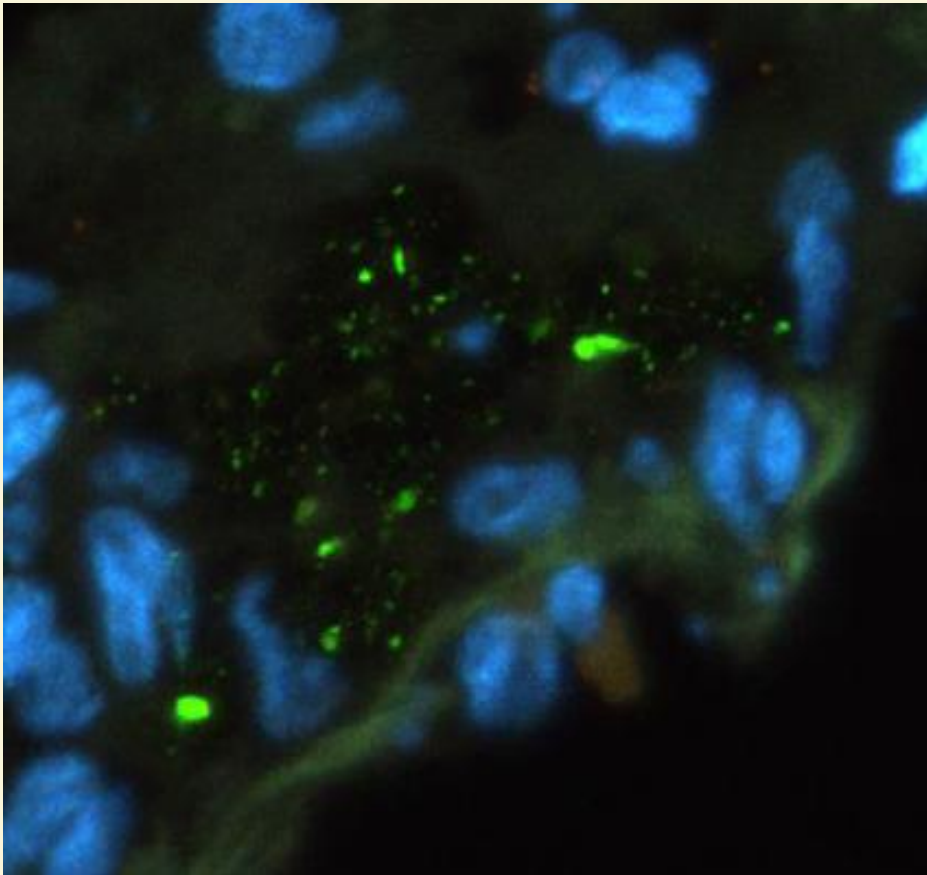
Biofilm

Antibiotic	SIR	Antibiotic	SIR	Antibiotic	SIR	Antibiotic	SIR	Antibiotic	SIR	Antibiotic	SIR
GM/AZT	R	AK/AZT	S	TO/AZT	I	T/S/P/T	R	CP/P/T	R	TO	R
GM/CAZ	S	AK/CAZ	S	TO/CAZ	S	T/S/MER	R	C/MER	R	T/S	R
GM/P/T	I	AK/P/T	S	TO/P/T	S	T/S/AZT	S	C/CAZ	R	C	R
GM/CPE	R	AK/CPE	R	TO/CPE	S	T/S/CAZ	R	AK	R	CP	R
GM/MER	R	AK/MER	S	TO/MER	R	CP/AZT	R	P/T	R	CAZ	R
GM/CPE	R	AK/CPE	R	TO/CPE	S	CP/CT	S	AZT	R	CPE	R
GM/T/S	R	AK/T/S	R	TO/T/S	S	CP/T/S	R	CT	R	MER	R
GM/CT	R	AK/CT	I	TO/CT	I	CP/MER	R	GM	R		

bioFILM-PA Clinical significance

- Antibiotic regimens based on biofilm testing did not differ significantly from regimens based on conventional testing in terms of microbiological and clinical responses.
- Current evidence is insufficient to recommend choosing antibiotics based on biofilm antimicrobial susceptibility testing

Evidence of *M. abscessus* biofilm in an infected CF lung

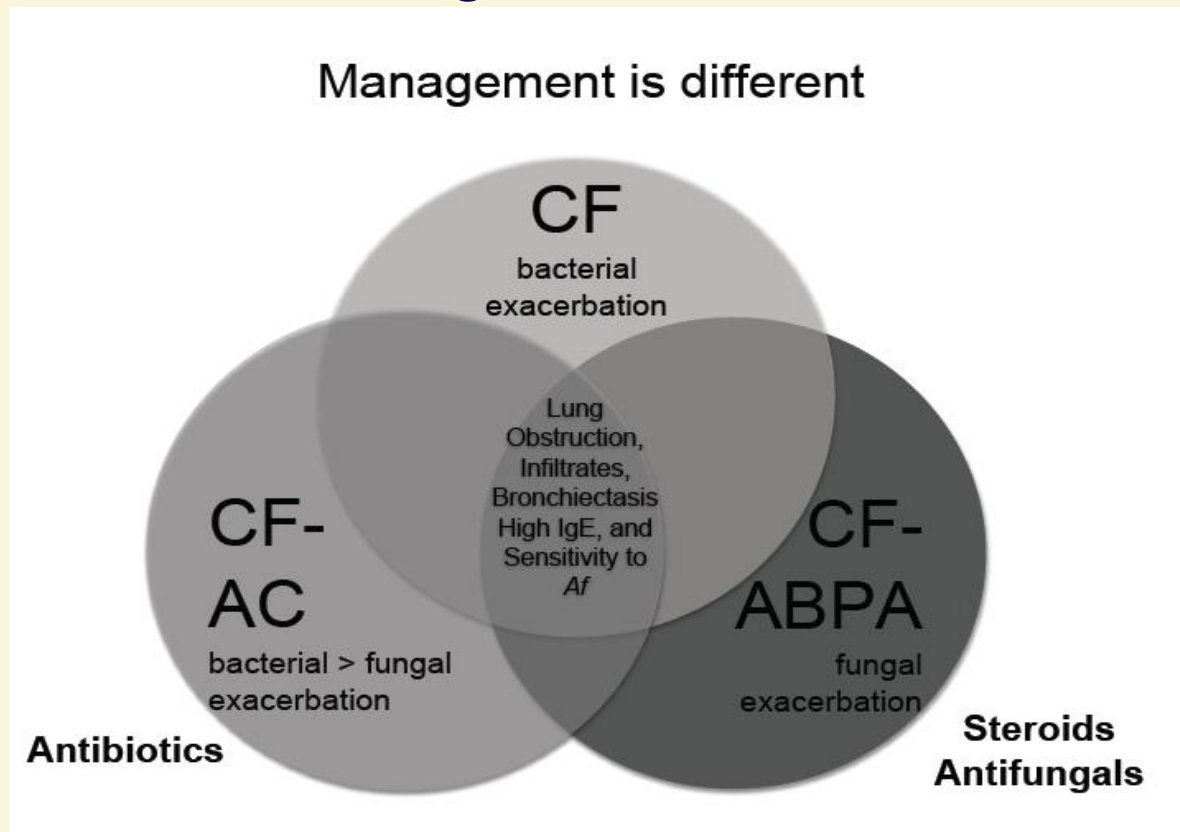


Questions from (a desperate) clinician to bacteriologist

1. I gave IV antibiotics according to recent culture and there is no response. **Can you help with biofilm test?**
2. Patient does not fulfill ABPA criteria. **Can serology, galctomannan or PCR help me (sputum ? BAL?)**
3. Patient is completely well but has positive culture for mycobacterium. **Is it really *M. abscessus* ?**
4. Last time we had to change antibiotics after 3 days due to lab results. **Can we get faster response?**
5. MALDI-TOF MS , Next generation sequencing. **Is this the light at the end of the tunnel?**

ABPA diagnosis

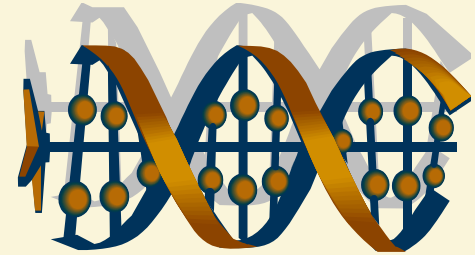
- Current techniques: skin test, total and specific IgE, sputum culture
- Signs and symptoms are non specific
- Cultures are often negative



Molecular diagnostics

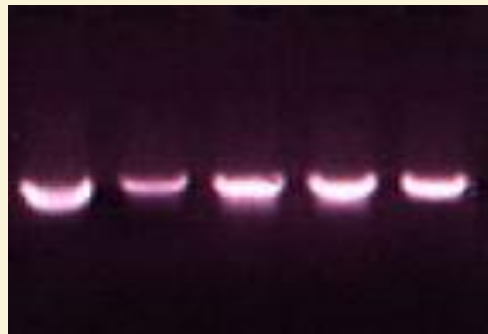


DNA extraction

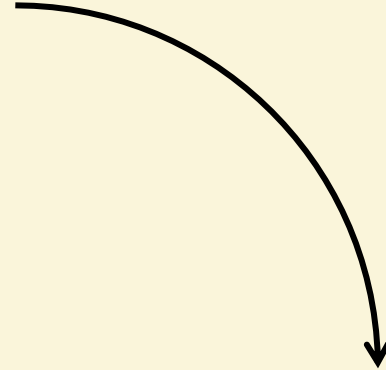
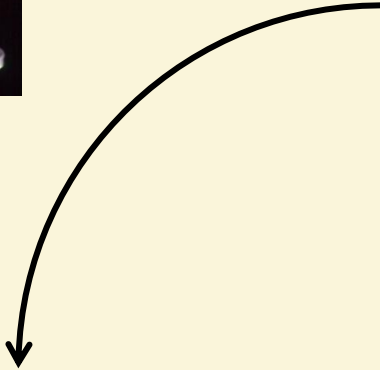
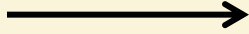


PCR

Identification



**Targeted
treatment**



**Organism-specific
PCR**

**Species-specific
PCR**

**Pan-species
PCR**

Aspergillus

**Actinomycetes/
Mycobacteria**

**Fungi
Bacteria**

Specific detection of Aspergillus by nested PCR

Sensitivity

100 fg/ μ l = 1 CFU

(fg = 10^{-15} g)

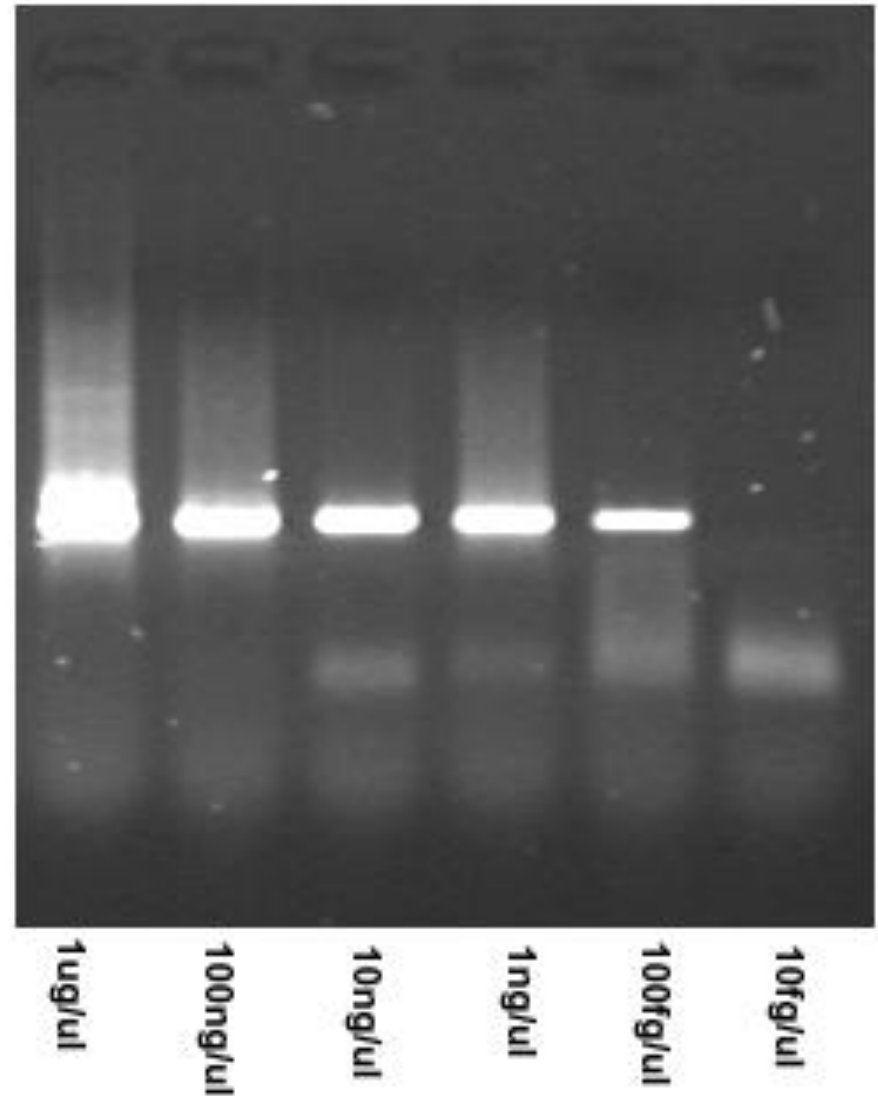
In BAL samples:

Sensitivity- 75%

Specificity- 100%

PPV- 100%

NPV- 87%



Galactomannan screening for ABPA

- Polysaccharides consisting of a mannose backbone with galactose side groups
- A component of the cell wall of *Aspergillus* and is released during growth
- Used to diagnose aspergillosis infections in serum, sputum and BAL
- GM sensitivity is lower in patients with non invasive manifestations of aspergillosis

Aspergillosis classification in CF patients

Combination of:

- Serologic tests (total IgE, *Af* specific IgE and IgG)
- Sputum real-time *Aspergillus* PCR
- Sputum GM

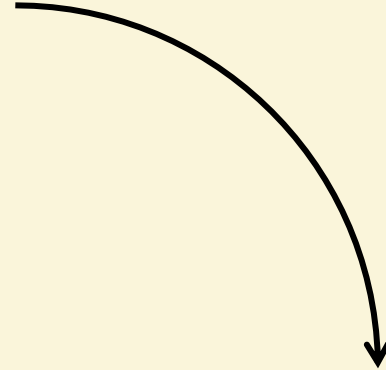
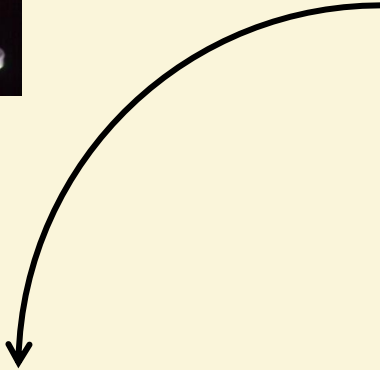
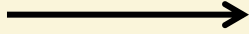
Three disease classes:

1. ABPA - IgE/IgG ↑, PCR(+), GM(+)
2. *Aspergillus* sensitized- IgE ↑, PCR(+/-), GM(-)
3. *Aspergillus* bronchitis- IgG ↑, PCR(+), GM(+)

Questions from (a desperate) clinician to bacteriologist



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**Organism-specific
PCR**

**Species-specific
PCR**

**Pan-species
PCR**

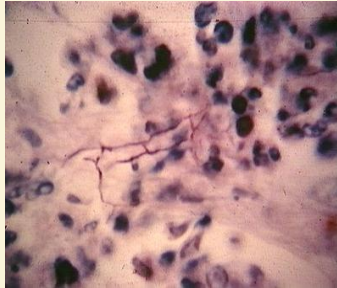
Aspergillus

**Actinomycetes/
Mycabacteria**

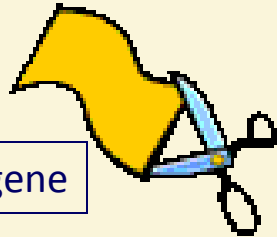
**Fungi
Bacteria**

Molecular diagnostics of NTM

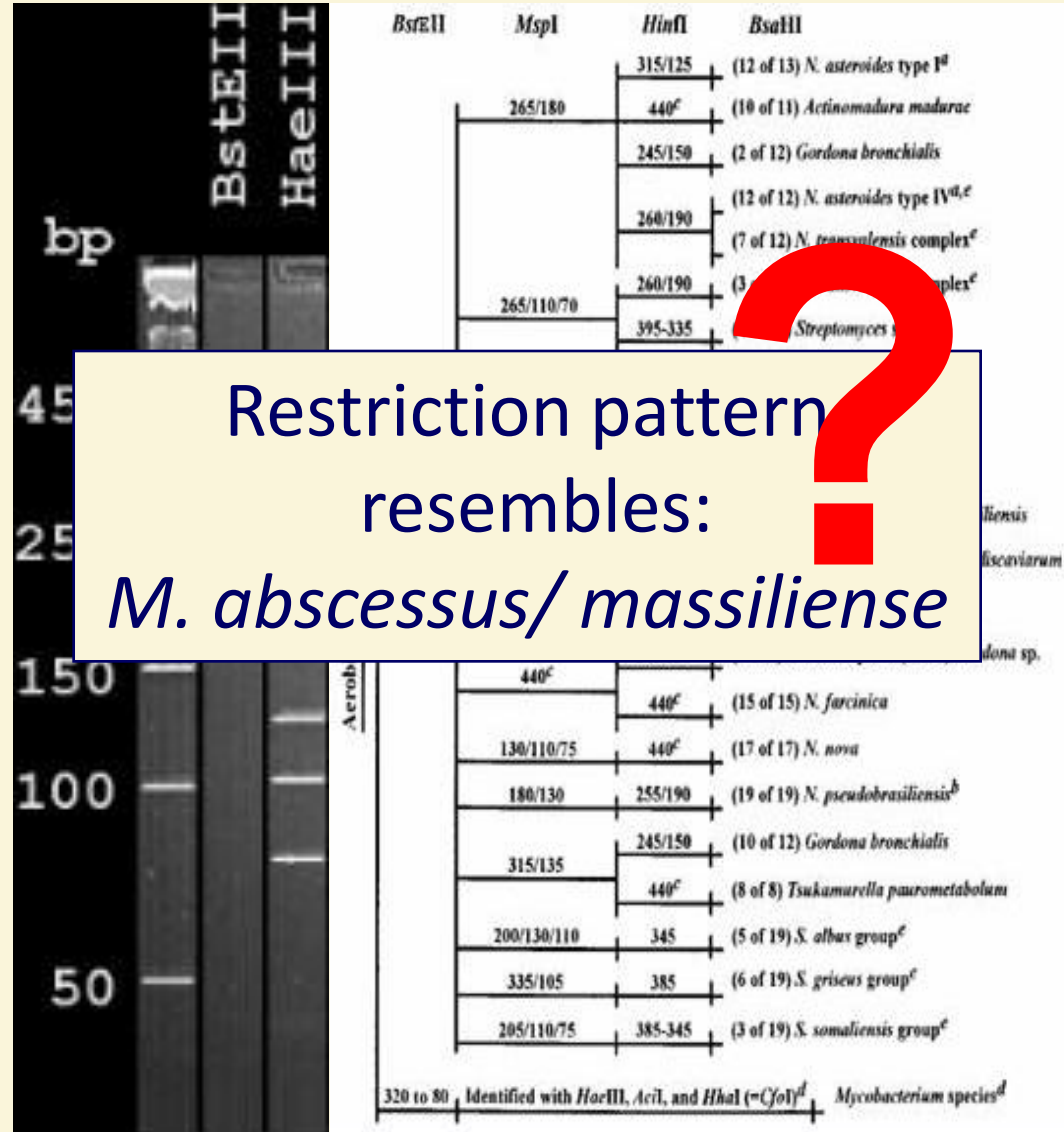
PCR-RFLP (restriction fragment length polymorphism) analysis of the 65-kDa heat shock protein gene



Amplification of Hsp65 gene



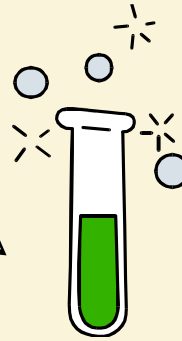
Mycobacterium spp.



Confirmation



Tissue sample



DNA extraction



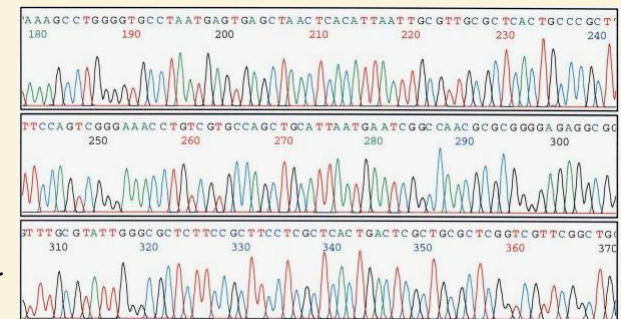
PCR amplification with Panbacterial rRNA primers

M. massiliense

(Only 5 bp difference !)

GC GGCCCGTTCGCGGGCAGCTCTGCGTAGTAATATCCACTCGCACCGGGACCCGGGCGCGGCCACGCCGT
TAAACACCCCCACCTTCCGAAT

Direct sequencing



Database mining/BLAST analysis

Questions from (a desperate) clinician to bacteriologist

1. I gave IV antibiotics according to recent culture and there is no response. **Can you help with biofilm test?**
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Fully automated molecular detection systems - GeneXpert

Clinical Test Menu

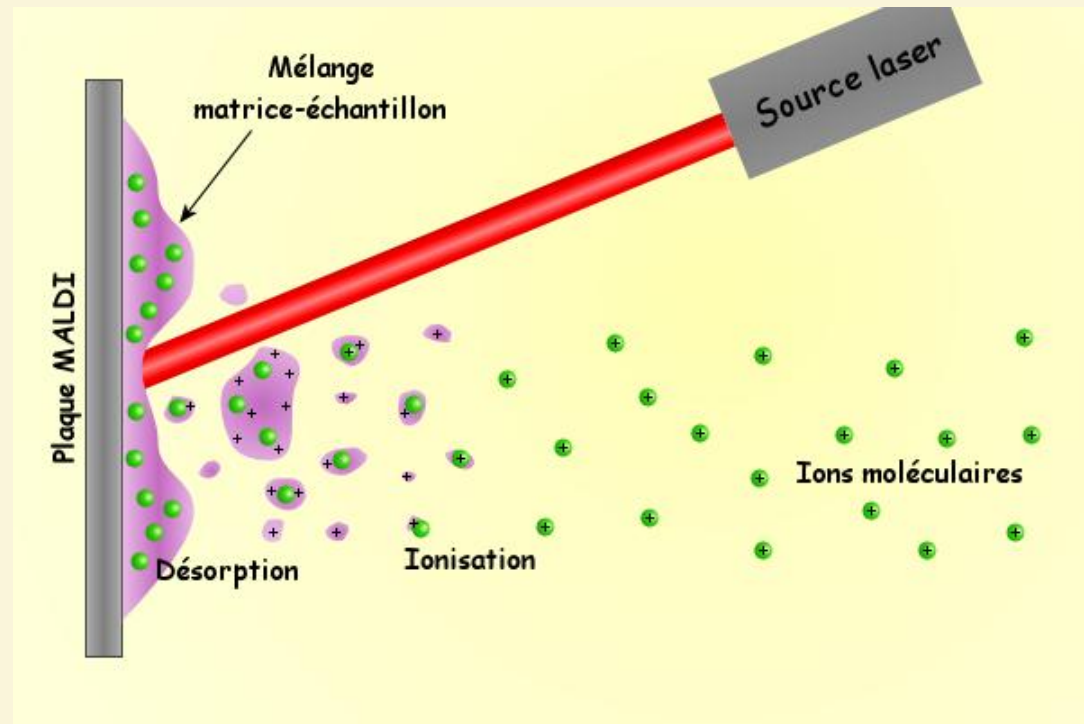
- Two hour detection of MTB and resistance to rifampin
- Influenza viruses A&B
- MRSA
- Group B Streptococcus
- Detection of *C. difficile* toxin

Next-Generation Sequencing for Diagnosis of Complex Bacterial Infections

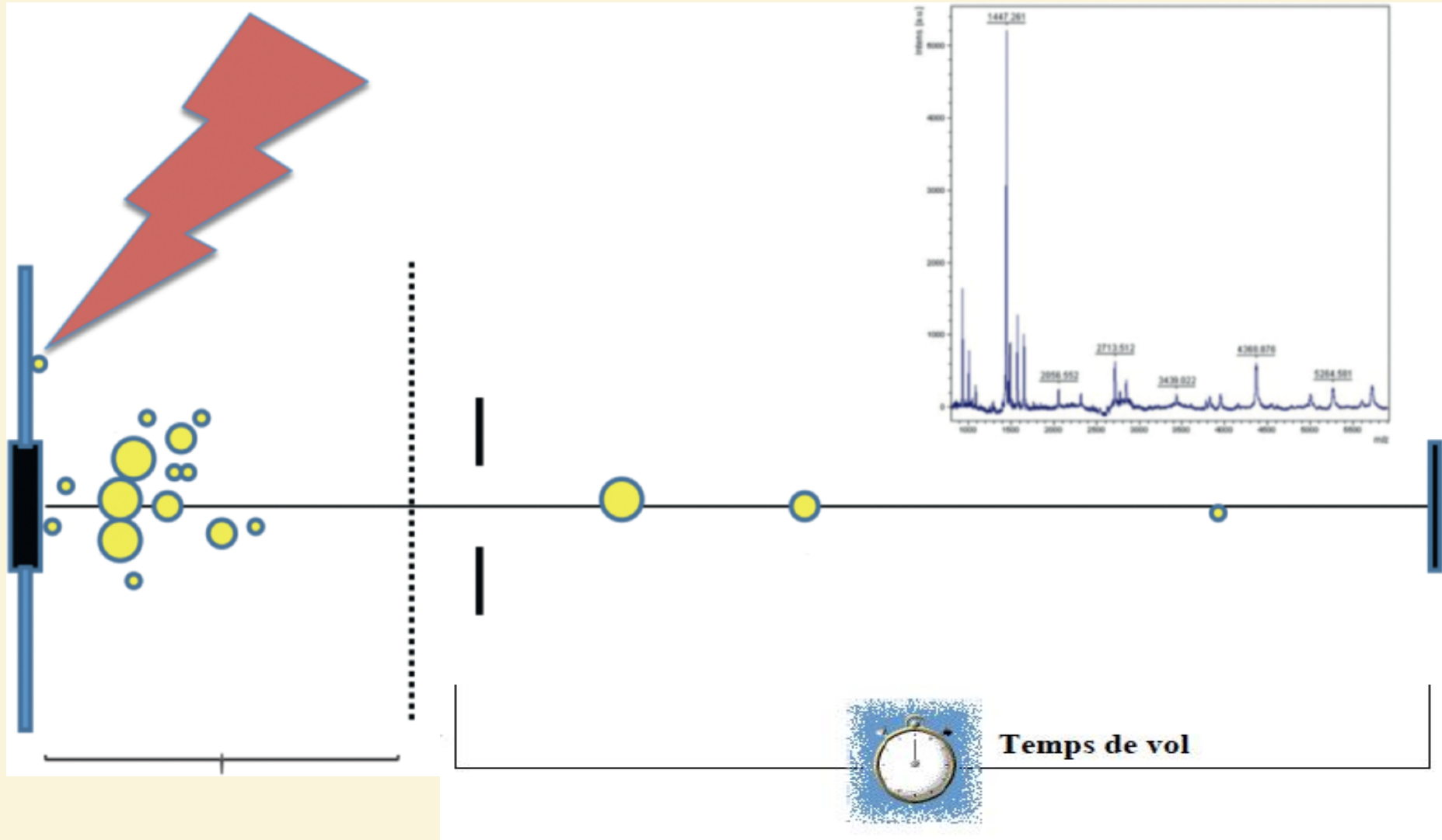
- Polymicrobial specimens remains a challenge for both culture-based and molecular techniques
- Next-Generation Sequencing = High-throughput sequencing
- Accelerate the sequencing process by producing thousands of sequences concurrently.
- Lower the cost of DNA sequencing
- Same day turnaround times
- Analyzing sputum samples from CF patients reveals well-described CF pathogens in specimens
- High sensitivity for low-prevalence or fastidious bacteria

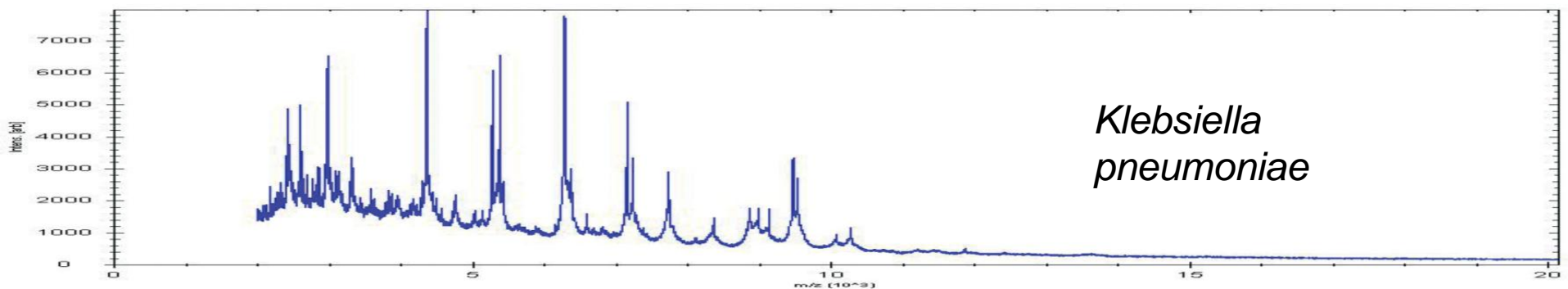
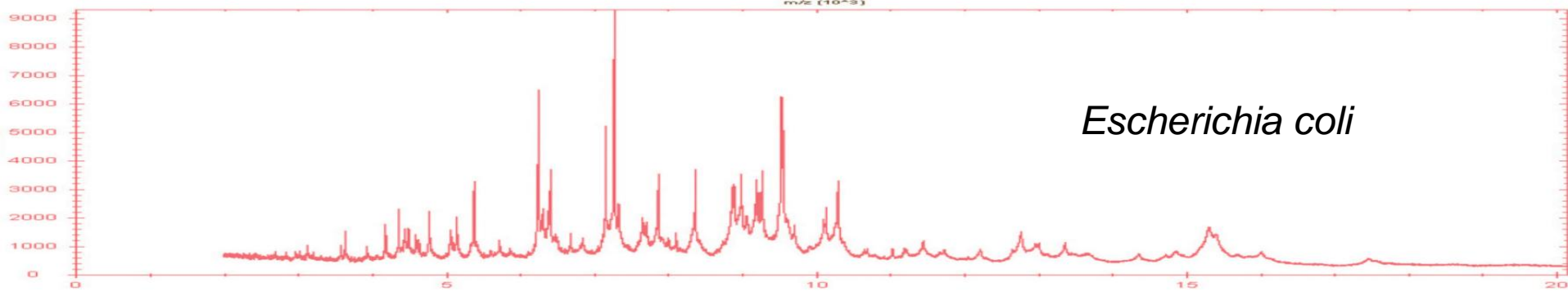
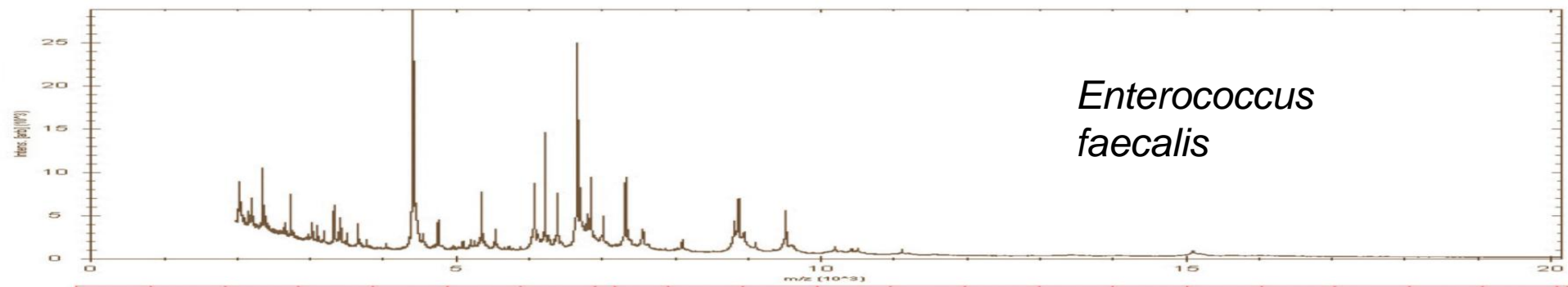
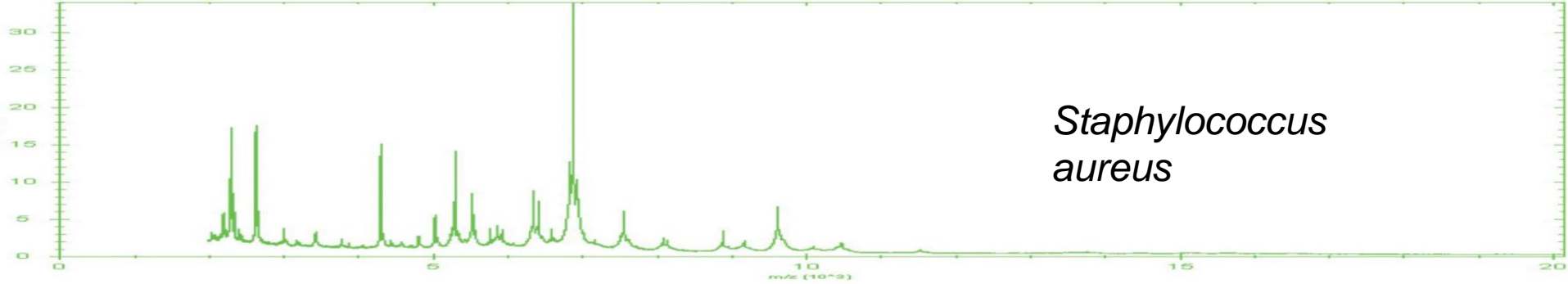
MALDI-TOF MS :

Matrix Assisted Laser Desorption Ionization time-of-flight mass spectrometry

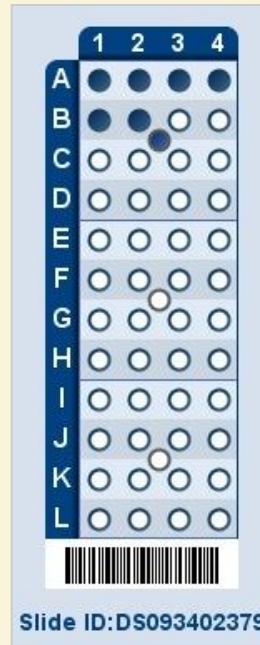


TOF=Time of Flight





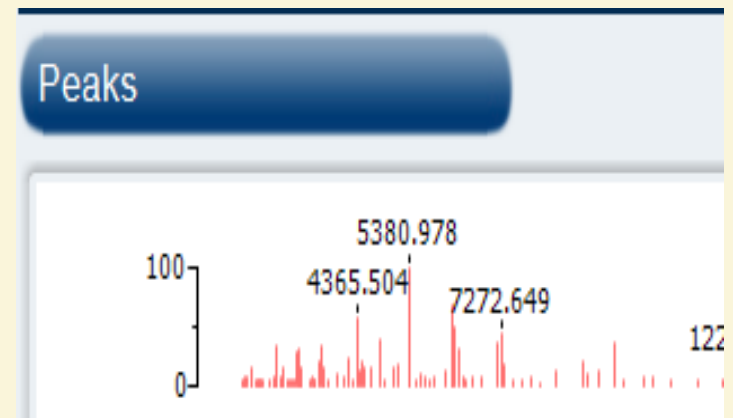
MLDI-TOF workflow



**Identification
in <1 min !!!**



Data base



Summary

- Current CF diagnostic microbiology relies mostly on culture-dependent methods
- Results are significantly delays and biases towards the most numerous and easily cultured organisms
- Requires highly specialized personnel
- Culture-independent molecular diagnostics for direct detection of multiple pathogens in sputum could improve CF patient care. But, no comprehensive molecular test for CF samples.
- AST still lack good culture-independent solution.

Where do we go from here?

- Routine high throughput/ metagenomics analysis of clinical samples.
- Mass spectrometry incorporates multiplex RT-PCR step for target amplification.
- Mass spectrometry and molecular detection of specific resistance genes and resistant mechanisms

