



ΕΘΝΙΚΟ ΚΑΠΟΔΙΣΤΡΙΑΚΟ
ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΑΡΧΕΣ ΚΑΙ ΕΦΑΡΜΟΓΕΣ ΜΟΡΙΑΚΗΣ ΜΙΚΡΟΒΙΟΛΟΓΙΑΣ



ΠΓΝ ΑΤΤΙΚΟΝ

Newer techniques for the diagnosis of
infections

(MALDI-TOF, FilmARRAY, Septifast, T2MR)

Spyros Pournaras

Professor and Director
Laboratory of Clinical Microbiology
ΑΤΤΙΚΟΝ University Hospital
Medical School, University of Athens, Greece

Molecular or Conventional Techniques?

- For the majority of cases, both approaches are necessary and complement each other
- Disadvantages of molecular techniques:
 - relatively higher cost and
 - need of highly educated personnel
- Both gradually diminish due to the technology improvements

Molecular Techniques to the Diagnosis: Advantages

- High Sensitivity & Specificity
- Speed → facilitate effective treatment
- Detection of difficult to grow organisms & viruses
- Ability for simultaneous detection of multiple organisms + genes (multiplex PCR, arrays etc.)
- Future worldwide implementation of molecular antibiogram (**Whole-genome sequencing/Resistome..**)

Molecular Techniques to the Diagnosis: Disadvantages

- Detection only of already known genes
- Detection of antimicrobials resistance due to only already known genes
- Possibly lower sensitivity (?) for bacteria that grow easily and fast
- Samples' DNA/RNA Contamination
- Detection of DNA – not viable organism
→ insufficient correlation with clinical disease
- **Loss of personnel's expertise with conventional microbiological techniques!**

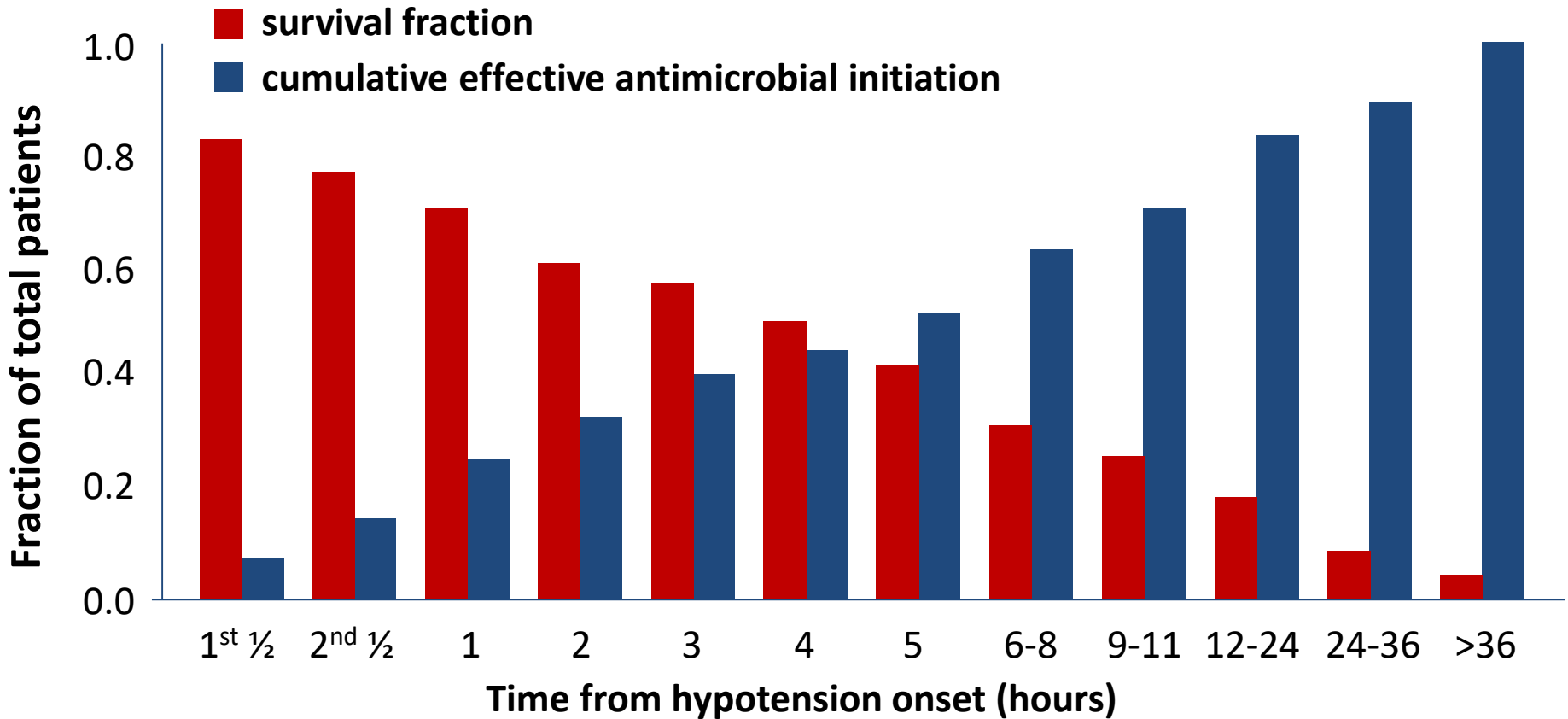
Severe infections due to MDR bacteria

conventional microbiology possibly more robust?

- **conventional diagnosis (culture-based):**
 - high sensitivity for common bacteria
 - potential for quantitative results
 - low cost
 - detection of viable bacteria
 - **allow susceptibility testing**
- **Molecular diagnosis**
 - high sensitivity and speed
 - simultaneous detection of resistance genes (e.g. *mecA*, *bla*_{VIM} ..), allowing appropriate antibiotic usage
 - not affected by antibiotic exposure
 - **BUT, CANNOT DISCRIMINATE BETWEEN ALIVE AND DEAD BACTERIA**

«In septic shock, time is life»

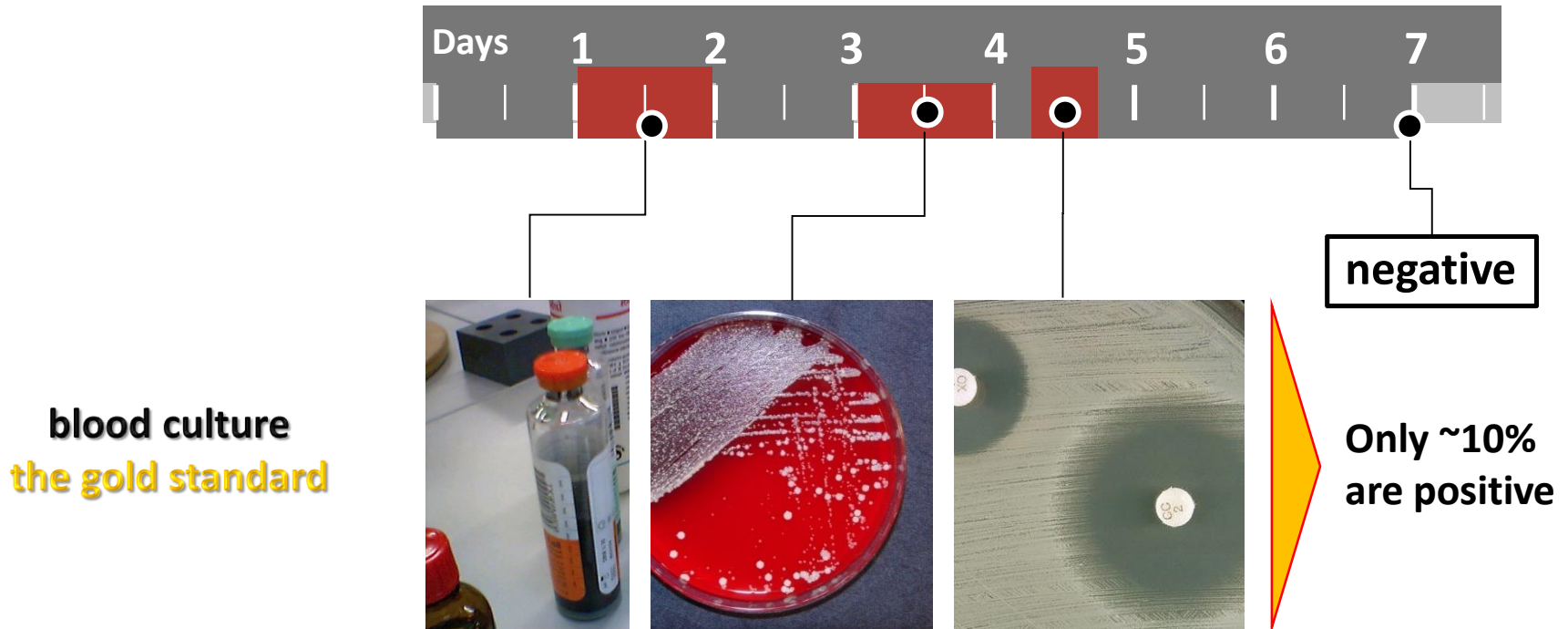
Kumar A, *Crit Care Med* 2006 34:1589–1596



Each hour of delay (over the first 6 hours) resulting in a **7.6% decrease in survival**

Clear medical need

Despite advances of blood culture techniques, better diagnostics are needed



1. **Poor specificity** (false positive rates: 5 to 50% depending on the methods of collection)
2. Despite optimization of the technique only **15–25% of positive results can be anticipated**
3. In up to **30% of the patients with fever clear results cannot be obtained at all** (blood culture sensitivity for slow growing and fastidious organisms can be poor)

Clinical Samples for Molecular Testing



1. From pure culture → rapid identification

2. From positive blood culture

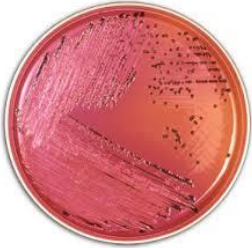


3. Directly from whole blood



Detection or not of antibiotic resistance genes

Molecular Techniques: Applications



1. From pure culture → rapid identification

2. From positive blood culture

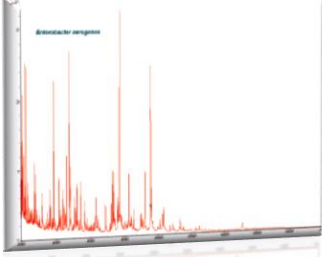


Mass spectrometry (**MALDI-TOF MS**)



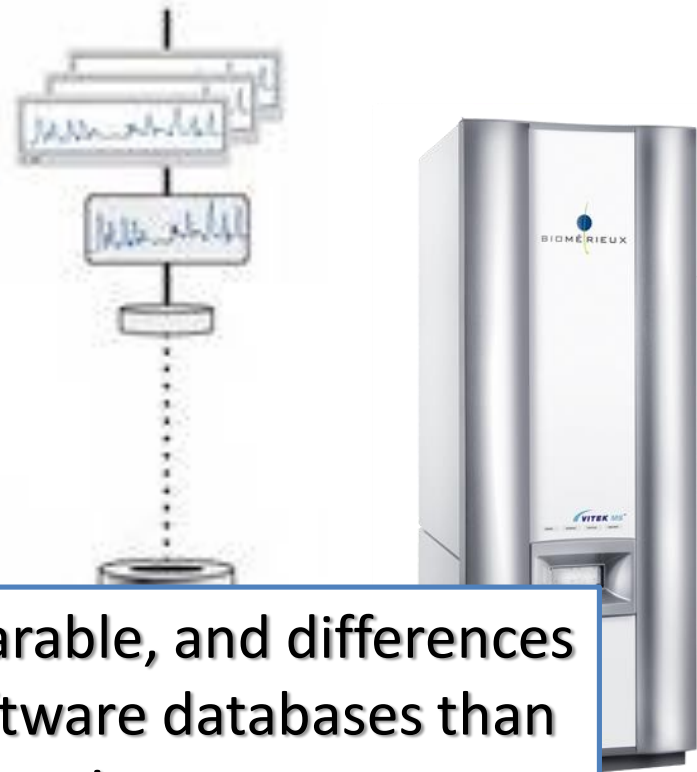
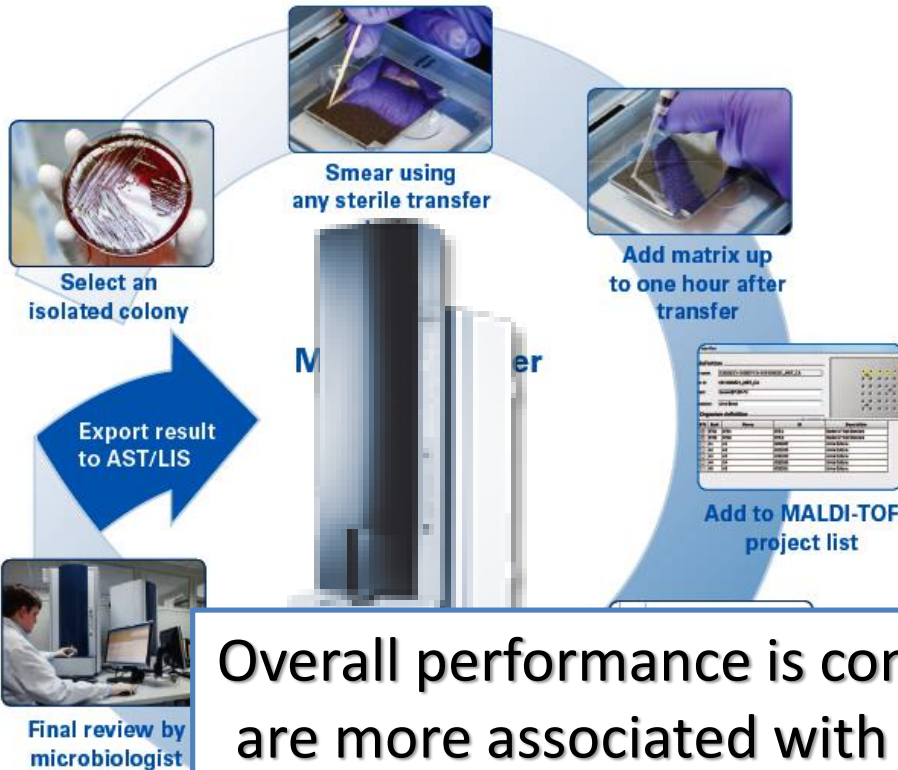
MALDI-TOF

“Identifying Microorganisms by Their Protein Fingerprint”



MALDI Biotyper

VITEK® MS



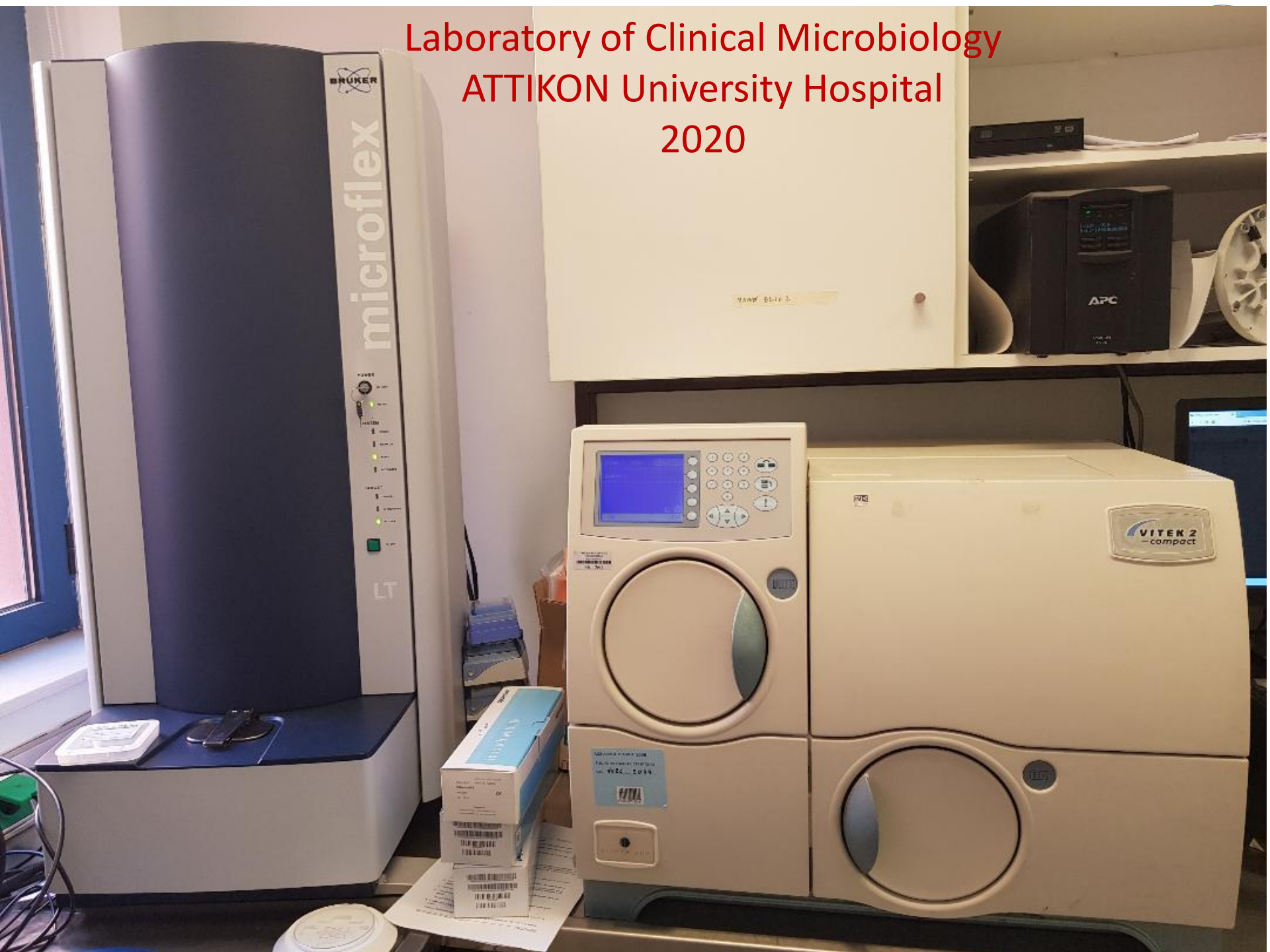
Overall performance is comparable, and differences are more associated with software databases than with instrumentation.



Spectrum instantly matched against reference database to give identification



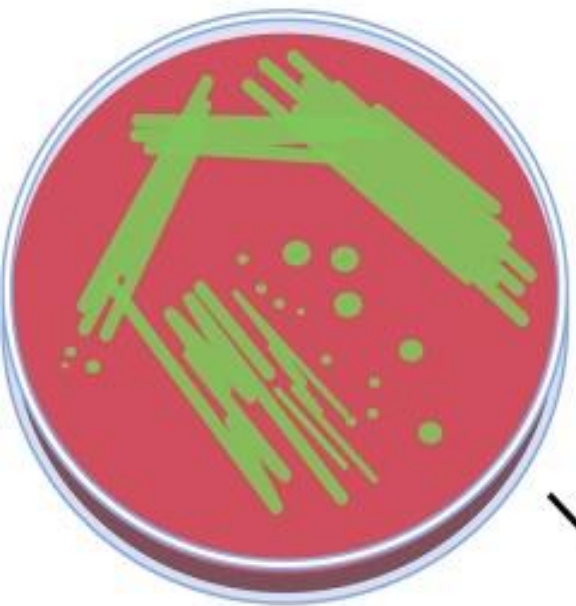
Laboratory of Clinical Microbiology
ATTIKON University Hospital
2020



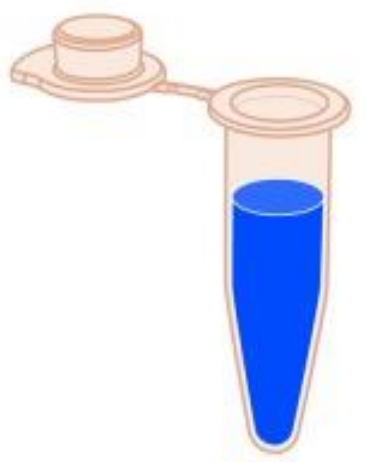


Laboratory of Clinical
Microbiology

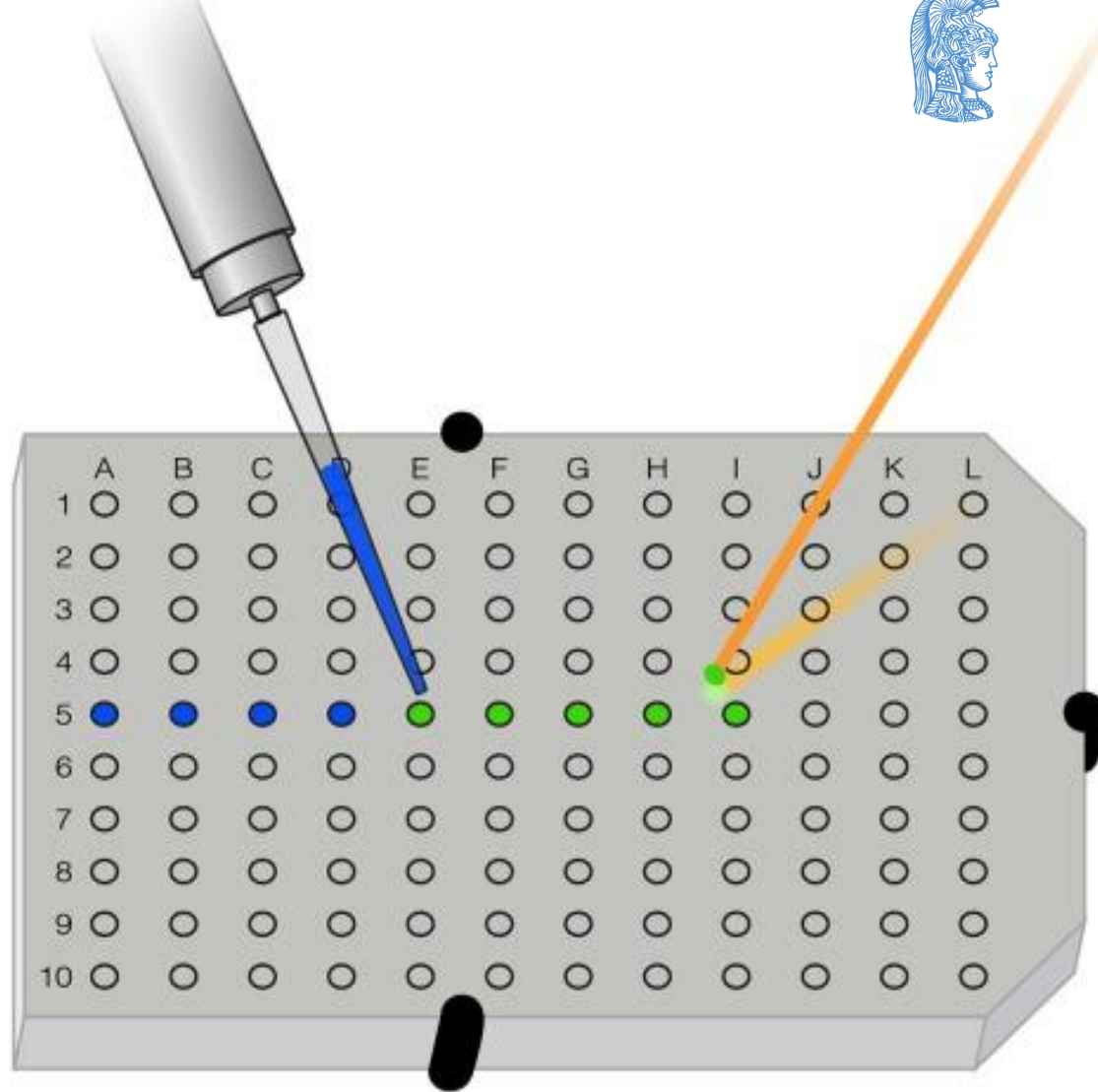
ATTIKON University
Hospital
2020



① Sample culture



② Matrix

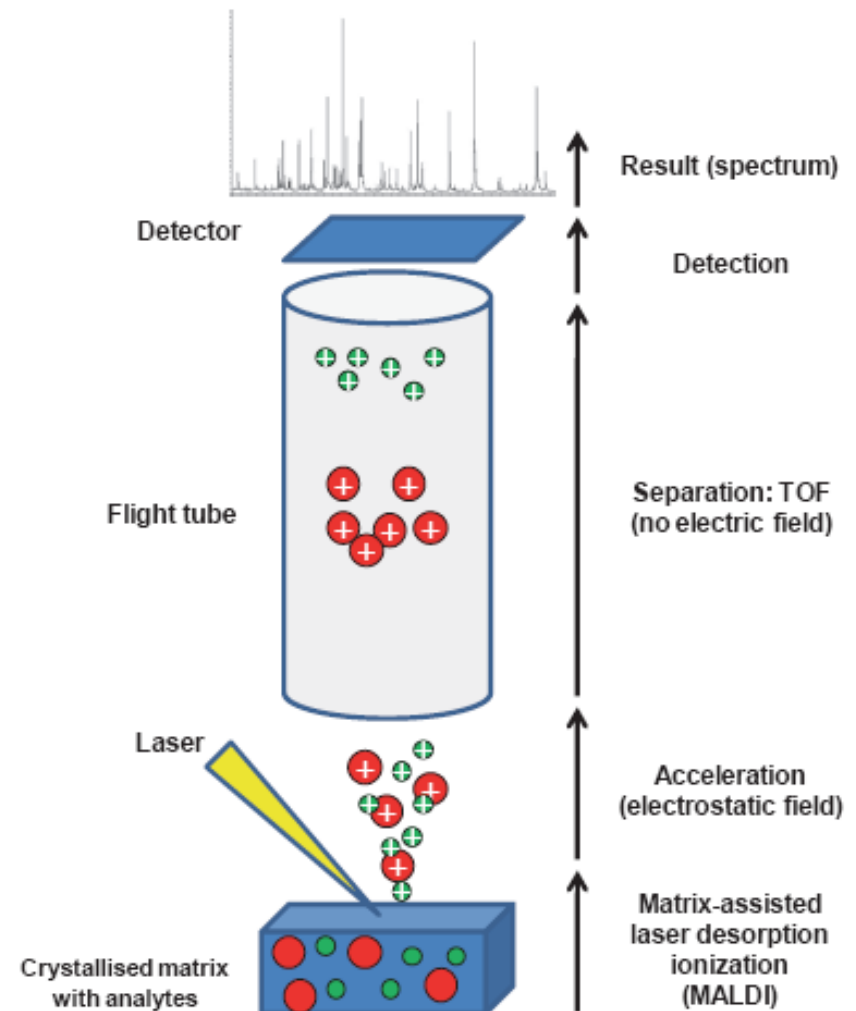


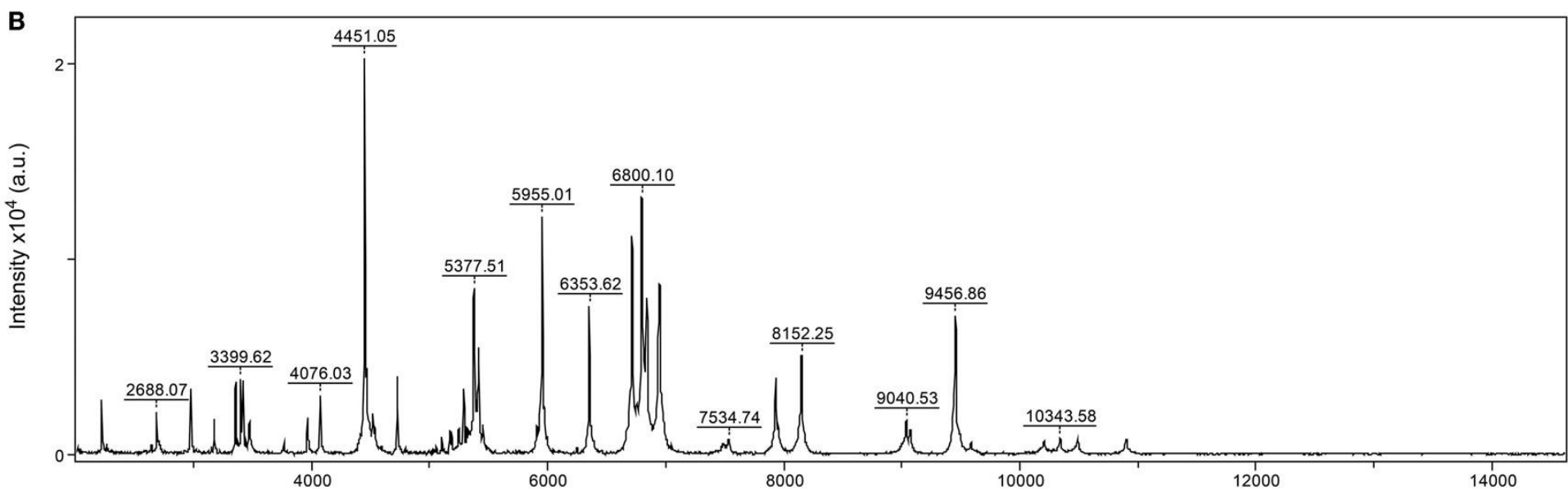
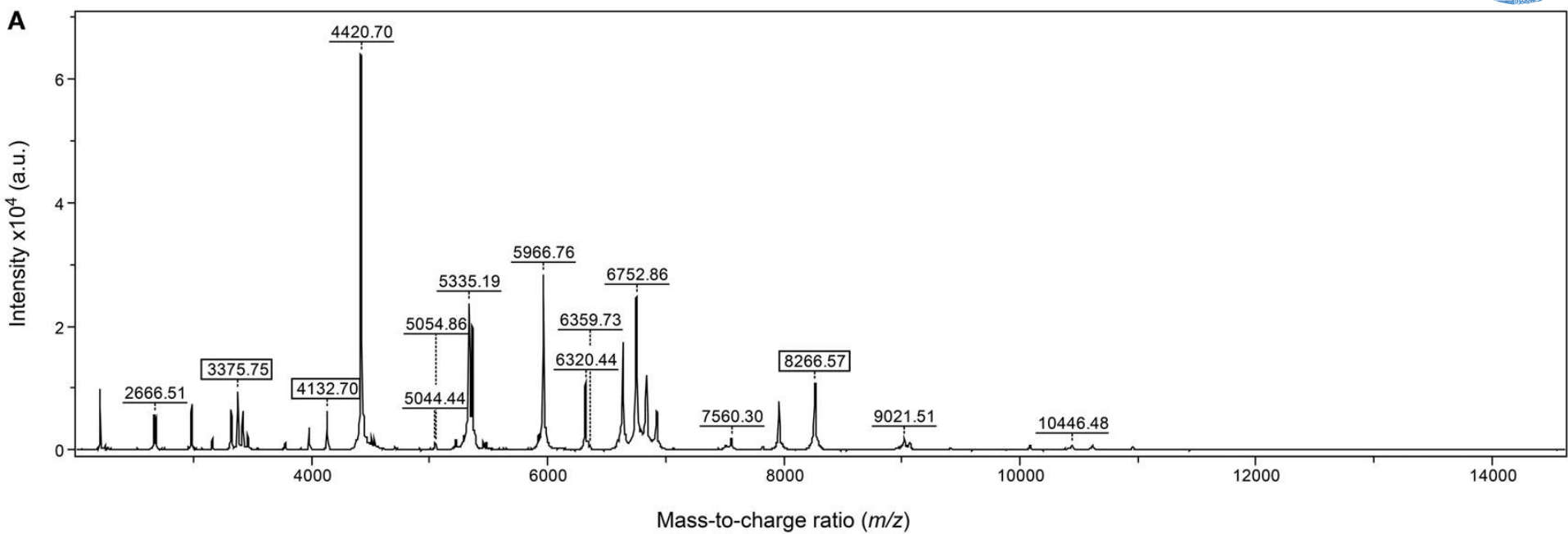
③ MALDI-TOF/MS sample plate



Matrix-assisted laser desorption ionization time-of-flight MALDI-TOF-MS

- The time to reach the detector (time of flight) varies according to the molecule's mass and charge
- The protein spectral profile is then compared with a database of spectra from reference strains
- Accurate identification depends, in part, on a sufficient sample to produce a spectral 'score' of high confidence





Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	<i>Pseudomonas aeruginosa</i> ATCC 27853 THL	2.237	287
2 (++)	<i>Pseudomonas aeruginosa</i> DSM 50071T HAM	2.191	287
3 (++)	<i>Pseudomonas aeruginosa</i> 8147_2 CHB	2.118	287
4 (++)	<i>Pseudomonas aeruginosa</i> DSM 1117 DSM	2.108	287
5 (+)	<i>Pseudomonas aeruginosa</i> 19955_1 CHB	1.908	287
6 (+)	<i>Pseudomonas aeruginosa</i> A07_08_Pudu FLR	1.901	287
7 (-)	<i>Pseudomonas jinjuensis</i> LMG 21316T HAM	1.619	198616
8 (-)	<i>Pseudomonas indica</i> DSM 14015T HAM	1.437	137658
9 (-)	<i>Pseudomonas citronellolis</i> DSM 50332T HAM	1.388	53408
10 (-)	<i>Pseudomonas taetrolens</i> LMG 2336T HAM	1.346	47884

Meaning of Score Values

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

MALDI Sepsityper Kit: positive blood culture bottles

MALDI Sepsityper product :

- **Identification of microorganisms from positive blood culture bottles in < 30 min.**
- Simple preparation protocols using just 1 ml sample material



Result Overview

Analyte/Value	Organism (best match)	Score/Value	Organism (second best match)	Score/Value
Sample 01 (+++)	<i>Acinetobacter baumannii</i>	2.05	<i>Acinetobacter baumannii</i>	2.03
Sample 02 (++)	<i>Escherichia coli</i>	2.05	<i>Escherichia coli</i>	2.03
Sample 03 (++)	<i>Staphylococcus aureus</i>	2.03	<i>Staphylococcus aureus</i>	2.01
Sample 04 (+++)	<i>Klebsiella pneumoniae</i>	2.01	<i>Klebsiella pneumoniae</i>	2.01
Sample 05 (+++)	<i>Candida albicans</i>	2.01	<i>Candida albicans</i>	2.01
Sample 06 (++)	<i>Enterobacter cloacae</i>	2.01	<i>Enterobacter cloacae</i>	2.01

- 1 Harvest 1 ml blood culture liquid in a test tube
- 2 Add Lysis Buffer and centrifuge
- 3 Add Washing Buffer and centrifuge
- 4 Suspend pellet in water
- 5 Standard Bruker extraction protocol for MALDI bacterial profiling
- 6 Spotting of 1 µl extract onto MALDI target, overlay with HCCA matrix
- 7 MALDI-TOF measurement
- 8 Receive result, ID

SE-MALDI accurately identified 332 (80.8%) of 411 blood cultures

Schieffer KM, et al. J Appl Microbiol. 2014;116:934-41. Jamal W, et al. Diagn Microbiol Infect Dis. 2013;76(4):404-8. Meex C, et al. J Med Microbiol. 2012;61(Pt 11):1511-6. Martiny D et al. Eur J Clin Microbiol Infect Dis. 2012;31:2269-81

Automated molecular systems for detection

2. From positive blood culture

TABLE 2 Comparison of FDA-cleared molecular methods for detection of microorganisms in positive blood culture broths

Test	Targets	Sensitivity (%)	Specificity (%)	Time to result (h)	Format and setup
Verigene BC-GP	12 Gram-positive genus or species targets and 3 resistance markers (<i>mecA</i> , <i>vanA</i> , <i>vanB</i>)	92–100	98–100	2.5	On-demand, microarray, automated sample processor, manual transfer of array to analyzer
Verigene BC-GN	8 Gram-negative genus or species targets and 6 resistance markers (KPC, NDM, CTX-M, VIM, IMP, OXA)	81–100	98–100	2	On-demand, microarray, automated sample processor, manual transfer of array to analyzer
FilmArray BCID	8 Gram-positive, 11 Gram-negative, and 5 yeast genus or species targets, 4 resistance markers (<i>mecA</i> , <i>vanA/B</i> , KPC, NDM)	88–100	94–100	1	On-demand, parallel miniaturized singleplex RT-PCR, full sample-to-result capability
GeneOHM StaphSR	<i>S. aureus</i> , MRSA	50–100	98–99	2	Batch, RT-PCR, offline manual sample lysis, extraction, and RT-PCR setup
Xpert MRSA/SA Blood Culture	<i>S. aureus</i> , MRSA	69–100	98–100	1	On-demand, RT-PCR, full sample-to-result capability
Septifast ^a	6 Gram-positive, 8 Gram-negative, and 5 yeast targets and <i>A. fumigatus</i>	42–79	67–97	6	Batch, 1.5–10 ml whole blood; offline extraction and setup of 3 parallel RT-PCRs

Emerging technologies for the clinical microbiology laboratory

Buchan BW, Ledebor NA. Clin Microbiol Rev. 2014,Oct;27:783-822

FilmArray, Biofire, BioMerieux

- **Rapid testing of multiple targets using microarrays**
- **Simultaneous detection of multiple pathogens in the same sample**
- **Result within 1-2 hours from sample receipt**



Rapid pathogens' identification **from positive blood cultures: Film Array**

BCID: Simultaneous Detection of 24 pathogens and 3 resistance markers

Gram-Positive Bacteria

Enterococcus

Listeria monocytogenes

Staphylococcus

Staphylococcus aureus

Streptococcus

Streptococcus agalactiae

Streptococcus pneumoniae

Streptococcus pyogenes

Gram-Negative Bacteria

Acinetobacter baumannii

Haemophilus influenzae

Neisseria meningitidis

Pseudomonas aeruginosa

Enterobacteriaceae

Enterobacter cloacae

Escherichia coli

Klebsiella oxytoca

Klebsiella pneumoniae

Proteus

Serratia marcescens

Yeast

Candida albicans

Candida glabrata

Candida krusei

Candida parapsilosis

Candida tropicalis

Antimicrobial Resistance Genes

mecA - methicillin resistance

vanA/B - vancomycin resistance

KPC - carbapenem resistance



Run Summary	
Sample ID:	SDY_9621_LED_50_6
Run Date:	29 May 2013 3:41 PM
Organisms Detected:	<i>Enterobacteriaceae</i> <i>Klebsiella pneumoniae</i>
Controls:	Passed
Applicable Antimicrobial Resistance Genes:	KPC - Detected

Result Summary - Interpretations

Antimicrobial Resistance Genes	
✓ Detected	KPC (carbapenem-resistance gene)
⊘ N/A	<i>mecA</i> (methicillin-resistance gene)
⊘ N/A	<i>vanA/B</i> (vancomycin-resistance genes)
<p>⚠ NOTE: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.</p>	

Gram Positive Bacteria

Not Detected	<i>Enterococcus</i>
Not Detected	<i>Listeria monocytogenes</i>
Not Detected	<i>Staphylococcus</i>
Not Detected	<i>Staphylococcus aureus</i>
Not Detected	<i>Streptococcus</i>
Not Detected	<i>Streptococcus agalactiae</i> (Group B)
Not Detected	<i>Streptococcus pneumoniae</i>
Not Detected	<i>Streptococcus pyogenes</i> (Group A)

Gram Negative Bacteria

Not Detected	<i>Acinetobacter baumannii</i>
✓ Detected	<i>Enterobacteriaceae</i>
Not Detected	<i>Enterobacter cloacae</i> complex
Not Detected	<i>Escherichia coli</i>
Not Detected	<i>Klebsiella oxytoca</i>
✓ Detected	<i>Klebsiella pneumoniae</i>
Not Detected	<i>Proteus</i>
Not Detected	<i>Serratia marcescens</i>
Not Detected	<i>Haemophilus influenzae</i>
Not Detected	<i>Neisseria meningitidis</i>
Not Detected	<i>Pseudomonas aeruginosa</i>

Yeast

Not Detected	<i>Candida albicans</i>
Not Detected	<i>Candida glabrata</i>
Not Detected	<i>Candida krusei</i>
Not Detected	<i>Candida parapsilosis</i>
Not Detected	<i>Candida tropicalis</i>

Run Details

Pouch:	BCID Panel	Protocol:	BCID
Run Status:	Completed	Operator:	R.Jones
Serial No.:	00631374	Instrument:	FA2075
Lot No.:	125313		

Rapid pathogens' identification **from positive blood cultures: Film Array BCID2**

BCID2: Simultaneous Detection of 33 pathogens and 9 resistance markers!



BioFire FilmArray Blood Culture Identification 2 (BCID2) Panel

Gram-negative Bacteria

Acinetobacter calcoaceticus-baumannii complex

Bacteroides fragilis

Enteric bacteria

Enterobacter cloacae complex

Escherichia coli

Klebsiella aerogenes

Klebsiella oxytoca

Klebsiella pneumoniae group

Proteus spp.

Serratia marcescens

Haemophilus influenzae

Neisseria meningitidis

Pseudomonas aeruginosa

Stenotrophomonas maltophilia

Yeast

Candida albicans

Candida auris

Candida glabrata

Candida krusei

Candida parapsilosis

Candida tropicalis

Cryptococcus neoformans/gattii

Gram-positive Bacteria

Enterococcus faecalis

Enterococcus faecium

Listeria monocytogenes

Staphylococcus spp.

Staphylococcus aureus

Staphylococcus epidermidis

Staphylococcus lugdunensis

Streptococcus spp.

Streptococcus agalactiae (Group B)

Streptococcus pneumoniae

Streptococcus pyogenes (Group A)

Antimicrobial Resistance Genes

*bla*_{CTX-M}

*bla*_{IMP}

*bla*_{KPC}

mcr-1

mecA/C and MREJ

*bla*_{NDM}

*bla*_{OXA-48-like}

*bla*_{VIM}

vanA/B

Film Array

Respiratory panel

20 targets: 17 viruses, 3 bacteria

BCID panel

27 targets: 19 bacteria, 5 fungi, 3 resistance genes

Gastro Intestinal panel

22 targets: 13 bacteria, 4 parasites, 5 viruses

Meningitis
Encephalitis panel

16 targets: 6 bacteria, 8 viruses, 2 fungi

Evaluation of the FilmArray Blood Culture Identification Panel: Results of a Multicenter Controlled Trial

J Clin Microbiol. 2016;54:687-698

TABLE 6 Comparison of FilmArray BCID resistance gene results to the prespecified comparator assay (PCR/sequencing directly from blood culture bottle)

Antimicrobial resistance gene(s)	Isolates detected: BCID/comparator		No. of results: BCID/comparator				Sensitivity or PPA ^a : TP/(TP + FN) (%)	95% CI	Specificity or NPA ^a : TN/(TN + FP) (%)	95% CI
	Clinical arm	Seeded arm	TP +/+	FP +/-	FN -/+	TN -/-				
<i>mecA</i> in association with:										
All <i>Staphylococcus</i> isolates detected ^b	491/494	2/2	488	5	8	281	488/496 (98.4)	96.8–99.3	281/286 (98.3)	96.0–99.4
<i>Staphylococcus</i> and <i>S. aureus</i> isolates detected	137/139	0/0	137	0	2	118	137/139 (98.6)	94.9–99.8	118/118 (100)	96.9–100
<i>vanA/B</i> in association with <i>Enterococcus</i> isolates detected	36/36	28/28	64	0	0	67	64/64 (100) ^c	94.4–100	67/67 (100)	94.6–100
<i>bla</i> _{KPC} in association with <i>Enterobacteriaceae</i> and/or <i>A. baumannii</i> and/or <i>P. aeruginosa</i> isolates detected	6/6 ^d	33/33	39	0	0	558	39/39 (100) ^e	91.0–100	558/558 (100)	99.3–100



Molecular Techniques: Applications (2)



3. Directly from whole blood



1. Multiplex real-time PCR

- LightCycler SeptiFast
- VYOO[®] - Multiplex PCR

2. Broad-range PCR

- Sepsitest

3. T2Direct Diagnostics™

- T2 Candida
- T2 Bacteria
- T2 Resistance

Detection of microorganisms directly from whole blood



LightCycler SeptiFast

Multiplex Real-time PCR

25 most common
pathogens

Detection limit: 3-30 CFU/mL

Time: **6 h**

- Rapid amplification with highly specific melting point analysis
- Semi-automated (manual: spin column based nucleic acid preparation)

SepsiTest

Degradation of human DNA and
free bacterial/fungal DNA

Broad-range PCR
(16S or 18S rRNA)

>345 bacterial and
fungal species

20-40 CFU/ml for *S. aureus*

Time: **8-12 h**

- Detection of viable pathogens
- Detection and identification of essentially any bacteria or yeast
- Open system that can be run on standard lab equipment

VYOO[®] - Multiplex PCR

Isolation of methylated
bacterial DNA

Multiplex Real-time PCR

34 bacterial and
6 fungal species

3-10 CFU/mL

Time: **8 h**

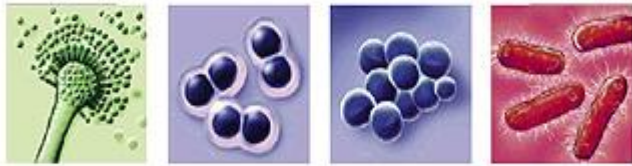
- Rapid amplification with highly specific melting point analysis
- Enrichment of pathogen DNA

SeptiFast test

- Molecular test based on Real Time PCR
- Detects and identifies fast 25 different pathogens [(Gram(+) & Gram(-) bugs, fungi),
→ causative agents of 90% of septicemia cases

SeptiFast – Advantages

- High sensitivity (few DNA copies)
- Speed: > 6 hours
- Low sample volume (1.5 or 0.5 ml blood)
- Prior antibiotic exposure does not affect results



SeptiFast panel: ~ 25 pathogens

Detection and identification

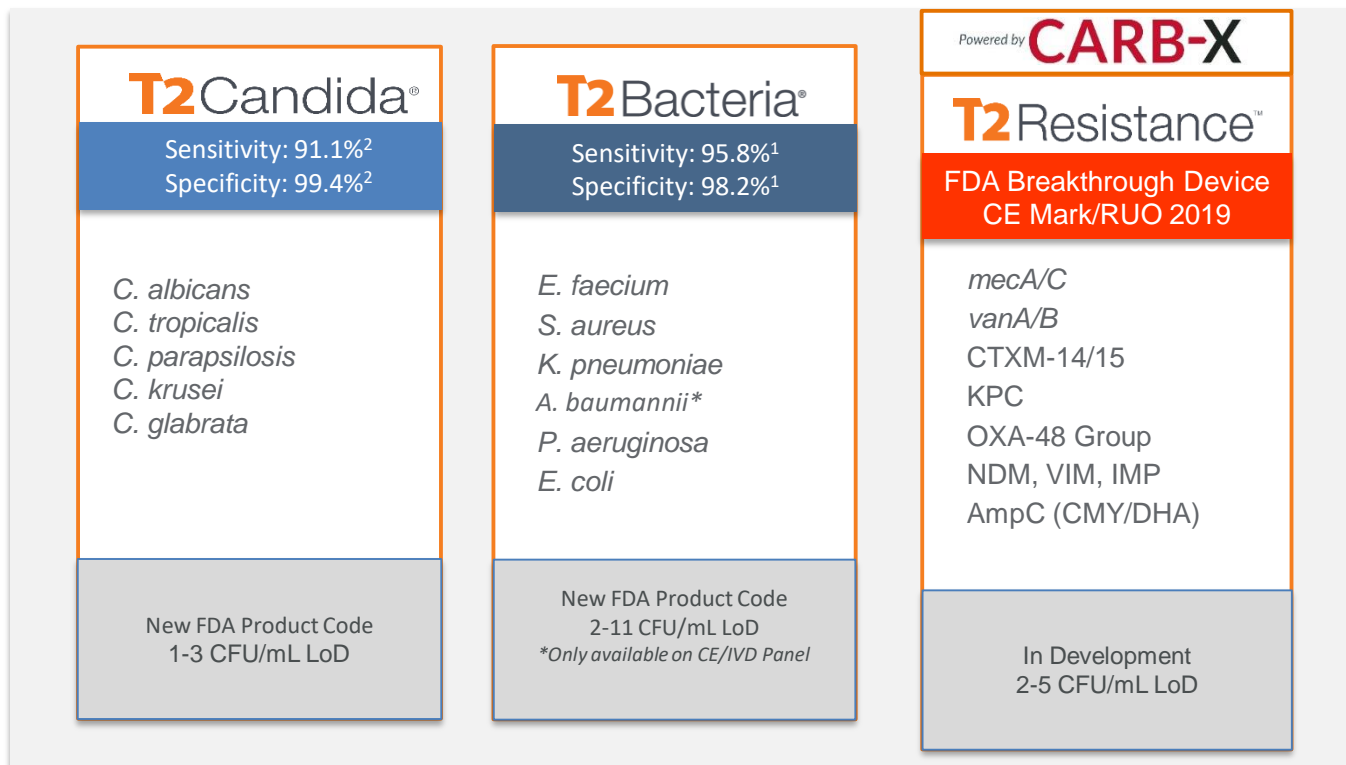
Gram-negative bacteria	Gram-positive bacteria	Fungi
<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
<i>Klebsiella (pneumoniae / oxytoca)</i>	CoNS (Coagulase negative Staphylococci)	<i>Candida tropicalis</i>
<i>Serratia marcescens</i>	<i>Streptococcus pneumoniae</i>	<i>Candida parapsilosis</i>
<i>Enterobacter (cloacae / aerogenes)</i>	<i>Streptococcus</i> spp.	<i>Candida krusei</i>
<i>Proteus mirabilis</i>	<i>Enterococcus faecium</i>	<i>Candida glabrata</i>
<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Asperigillus fumigatus</i>
<i>Acinetobacter baumannii</i>	<i>mecA</i> (MRSA)	
<i>Stenotrophomonas maltophilia</i>		

CoNS: *S. epidermidis*, *S. haemolyticus*, *S. xylosus*, *S. hominis*, *S. cohnii*, *S. lugdunensis*, *S. saprophyticus*, *S. saprophyticus*, *S. capitis*, *S. pasteurii*, *S. warneri*.

Streptococci: *S. pyogenes*, *S. agalactiae*, *S. mitis*, *S. mutans*, *S. oralis*, *S. anginosus*, *S. bovis*, *S. constellatus*, *S. cristatus*, *S. vestibularis.*, *S. gordonii*, *S. intermedius*, *S. milleri*, *S. salivarius*, *S. sanguinis*, *S. thermophilus*, *S. parasanguinis*.

T2Direct Diagnostics™

FDA-Cleared and CE Marked Products Plus Pipeline of New Targets



1. T2Bacteria Pivotal Clinical Study. This is a combination of samples run in both prospective and contrived arms of study. T2Bacteria showed an overall average sensitivity of 90% in the prospective arm of the study and the contrived arm an overall average PPA of 97%.

2. Mylonakis, E., Clancy, C.J., Ostrosky-Zeichner, L., et al. (2015). *Clinical Infectious Diseases*

3. Research reported in this presentation is supported by the Cooperative Agreement Number IDSEP160030 from ASPR/BARDA and by an award from Wellcome Trust, as administered by CARB-X. The content is solely the responsibility of T2 Biosystems and does not necessarily represent the official views of the Department of Health and Human Services Office of the Assistant Secretary for Preparedness and Response, other funders, or CARB-X.

T2Direct Diagnostics

Detection of Significantly More True Infections than Blood Culture

T2Bacteria Panel	T2 Panel Positive	Blood Culture Positive
Paired +	35	39
Add'l Proven/Probable +	63	0
Total +	98/102 (96%)	39/102 (38%)

T2Candida Panel	T2 Panel Positive	Blood Culture Positive
Paired +	31	33
Add'l Proven/Probable +	22	0
Total +	53/55 (96%)	33/55 (60%)

* Paired blood culture

T2Direct Diagnostics enable, early targeted therapy by providing:

- Detection of significantly more true infections than blood culture
- No interference by antimicrobial therapy
- Accurate, actionable results in 3-5 hours
 - Up to 10x faster than blood-culture dependent testing¹

Review of Rapid Diagnostic Tests Used by Antimicrobial Stewardship Programs

CID 2014;59(S3):S134–45

Karri A. Bauer,¹ Katherine K. Perez,^{2,3,4} Graeme N. Forrest,⁵ and Debra A. Goff¹

Rapid - innovative microbiological methods

- provide opportunities for **antimicrobial stewardship** programs to improve antimicrobial use and clinical and economic outcomes
- are considered **“game changers”** and represent a significant advancement in the management of infectious diseases

Rapid methods used for **antimicrobial stewardship**:

CID 2014;59(S3):S134–45

- Multiplex PCR assays:
 - **FilmArray BCID**, tests for 24 organisms:
- *Staphylococcus species, Enterococcus species, Listeria monocytogenes, Streptococcus species, Acinetobacter baumannii, Haemophilus influenzae, Neisseria meningitidis, Pseudomonas aeruginosa, Enterobacter cloacae complex, Escherichia coli, Klebsiella species, Proteus species, Serratia marcescens, Candida*
- Antimicrobial resistance genes [mecA, vanA/B, carbapenem resistance]

Effect of FilmArray BCID application to antibiotic policies

Clinical and economic impact of antimicrobial stewardship interventions with the FilmArray blood culture identification panel

Joe Pardo ^{a,*}, Kenneth P. Klinker ^a, Samuel J. Borgert ^a, Brittany M. Butler ^b,
Patricia G. Giglio ^c, Kenneth H. Rand ^b

Diagn Microbiol Infect Dis. 2016 Feb;84(2):159-64

The BCID led **to shorter duration of empirical vancomycin for patients with contaminated blood cultures (P=0.005) and methicillin-susceptible *Staphylococcus aureus* bacteremia (P<0.001)**. Patients with vancomycin-resistant enterococcal bacteremia received active therapy earlier than historical controls (P=0.047)

The BCID, coupled with antimicrobial stewardship intervention, was a cost-effective tool to improve patient care

Effect of FilmArray BCID application to antibiotic policies (2)

Rapid Identification of Microorganisms by FilmArray Blood Culture Identification Panel Improves Clinical Management in Children

*Stephen T. J. Ray, MPhil, *† Richard J. Drew, MD, ‡§ Fiona Hardiman, BSc, ¶ Barry Pizer, PhD, || and Andrew Riordan, MD*†*

The Pediatric Infectious Disease Journal • Volume 35, Number 5, May 2016

Conclusions: Rapid identification of microorganisms in pediatric blood cultures by FA-BCIP, led to changes in clinical management for half of the episodes. **This improved antimicrobial stewardship and allowed early discharge from hospital for 10% of children**

Future studies should focus on how to use best this technology in a cost-effective manner

Effect of FilmArray BCID application to antibiotic policies (3)

Edina Avdic, Karen C. Carroll, Infect Dis Clin N Am 28 (2014) 215–235

Clinical Impact and Provider Acceptability of Real-Time Antimicrobial Stewardship Decision Support for Rapid Diagnostics in Children With Positive Blood Culture Results

Journal of the Pediatric Infectious Diseases Society pp. 1–9, 2016.

Kevin Messacar,^{1,2} Amanda L. Hurst,³ Jason Child,³ Kristen Campbell,⁴ Claire Palmer,⁵ Stacey Hamilton,⁶
Elaine Dowell,⁶ Christine C. Robinson,⁶ Sarah K. Parker,¹ and Samuel R. Dominguez^{1,7}

The median time to optimal therapy decreased from 60.2 hours before intervention to 26.7 hours after intervention (P = .001).

Unnecessary antibiotic initiation for children with a culture that contained organisms considered to be contaminants decreased from 76% to 26% (P= .001). Providers reported a change in management as a result of BCID results in 73% of the cases and a mean overall satisfaction rating of 4.8 on a 5-point Likert scale.

Conclusions. Real-time AS decision support for rapid diagnostics is associated with improved antimicrobial use and high satisfaction ratings by providers

T2Bacteria Impacts Patient Care and Provides Opportunity for Antimicrobial Stewardship Optimization

Growing number of success stories



- 1200-bed tertiary-care teaching hospital¹
- Emergency Department, Infectious Diseases Unit, and ICU (n=140 samples)
- T2 Detected 20 Positive Cases Missed By Blood Culture, MALDI and BioFire.
- T2B+/BCx- results significantly more likely in patients receiving antibiotics, p<0.001
- 66.7% of Infected Patients Missed by BC and Detected By T2 Were Being Inappropriately Treated at Time of T2B Result



- 1423-bed not-for-profit community hospital²
- Patients with sepsis in the Emergency Department (n=25)
- Identified organisms 20 hours sooner than blood cultures
- Negative results provided 122 hours sooner than blood cultures
- Numerous opportunities for stewardship intervention identified, including 36 opportunities for de-escalation of coverage for *S. aureus* and *P. aeruginosa*



- 2600-bed academic medical center³
- Hematologic Malignancy/ HSCT (n=94)
- T2Bacteria assay showed significantly faster time to species ID than a culture-dependent rapid diagnostic method
- T2Bacteria could have potentially influenced care and provided an opportunity to place (T2+/BC-) patients on effective therapy faster than with culture dependent methods.



- Subset of ED patients included in the Pivotal Trial (n=137)⁴
- Detection of more pathogens in 11 patients where blood cultures remained negative
- 70% (16/23) of patients with a positive T2 result could have experienced at least some clinical benefit from the T2Bacteria result
- T2Bacteria assay could have reduced time to effective therapy by an average of 28.0 hours

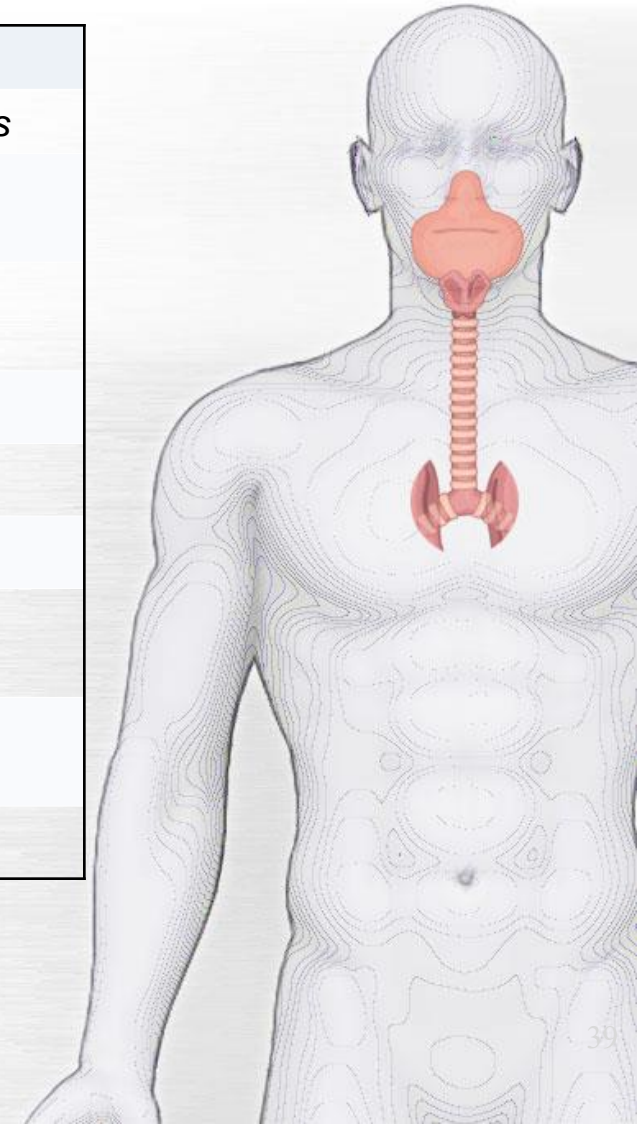
(1) De Angelis G et al. T2Bacteria magnetic resonance assay for the rapid detection of ESKAPEC pathogens directly in whole blood. J Antimicrob Chemother. 2018 Mar 1;73(suppl_4):iv20-iv26.

(2) Weisz E et al. MAD-ID 2018. (3) Walsh T et al. ECCMID-EIM 2019. (4) Voight C et al. ECCMID-EIM 2019

FilmArray, other applications: Detection of respiratory pathogens

FilmArray Respiratory Panel: 20 Pathogens

Ioί	Βακτήρια
Adenovirus	<i>Bordetella pertussis</i>
Coronavirus HKU1	<i>Chlamydophila pneumoniae</i>
Coronavirus NL63	<i>Mycoplasma pneumoniae</i>
Coronavirus 229E	
Coronavirus OC43	
Human Metapneumovirus	
Human Rhinovirus/Enterovirus	
Influenza A	
Influenza A/H1	
Influenza A/H3	
Influenza A/H1-2009	
Influenza B	
Parainfluenza Virus 1	
Parainfluenza Virus 2	
Parainfluenza Virus 3	
Parainfluenza Virus 4	
Respiratory Syncytial Virus	



Nasopharyngeal sample



Run Summary

Sample ID: RYNAND0232	Run Date: 21 Jun 2013 7:34 PM
Detected: Influenza A H1-2009 Respiratory Syncytial Virus	Controls: Passed
Equivocal: None	

Result Summary

Not Detected	Adenovirus
Not Detected	Coronavirus 229E
Not Detected	Coronavirus HKU1
Not Detected	Coronavirus NL63
Not Detected	Coronavirus OC43
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
✓ Detected	Influenza A H1-2009
Not Detected	Influenza B
Not Detected	Parainfluenza Virus 1
Not Detected	Parainfluenza Virus 2
Not Detected	Parainfluenza Virus 3
Not Detected	Parainfluenza Virus 4
✓ Detected	Respiratory Syncytial Virus
Not Detected	<i>Bordetella pertussis</i>
Not Detected	<i>Chlamydophila pneumoniae</i>
Not Detected	<i>Mycoplasma pneumoniae</i>

Run Details

Pouch: Respiratory Panel v1.7	Protocol: NPS v2.0
Run Status: Completed	Operator: KMRAP
Serial No.: 00345787z	Instrument: ITI FA "FA2004"
Lot No.: 114612	

Meningitis / Encephalitis

Clinical Sample: CSF

Bacteria:

E. coli

H. influenzae

L. monocytogenes

N. meningitidis

S. agalactiae

S. pneumoniae

Fungi:

Cryptococcus

neoformans / gattii

Viruses:

Cytomegalovirus (CMV)

Enterovirus

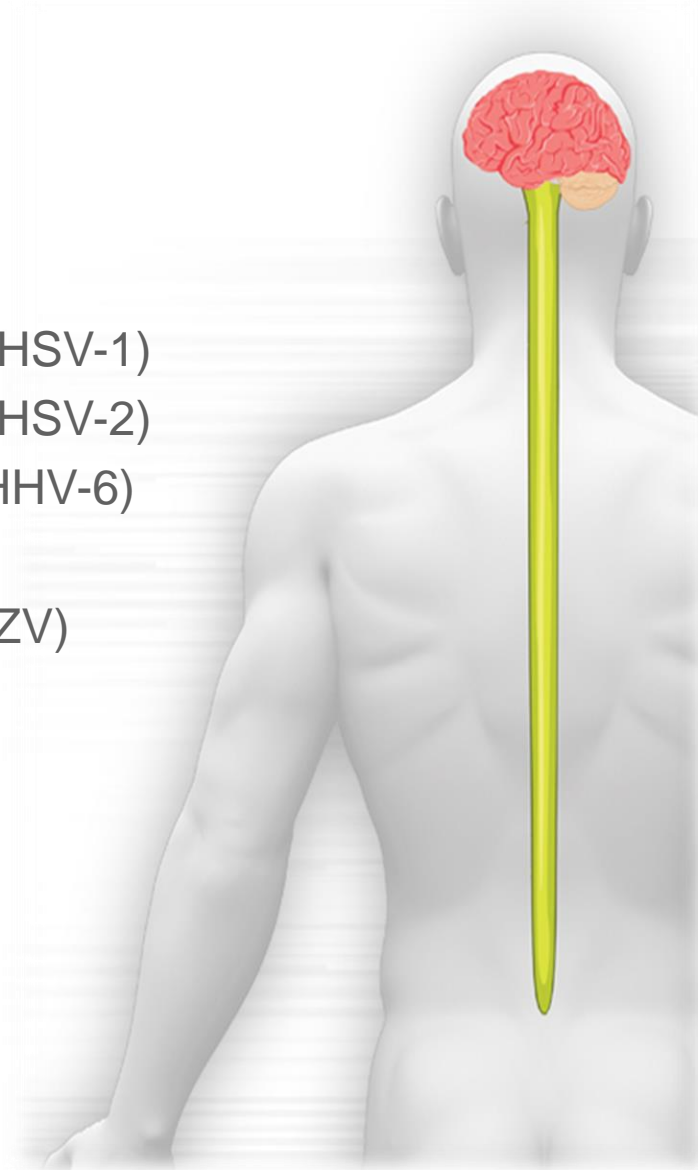
Herpes simplex type 1 (HSV-1)

Herpes simplex type 2 (HSV-2)

Human herpesvirus 6 (HHV-6)

Parechovirus

Varicella zoster virus (VZV)



Pneumonia Film Array Panel: 30 Pathogens, 7 ABR Markers

Clinical Sample: BAL, sputum

Typical Bacteria (semi-quantitative)

Acinetobacter calcoaceticus-baumannii complex

Serratia marcescens

Proteus spp.

Klebsiella pneumoniae

Enterobacter aerogenes /

Enterobacter cloacae complex

Escherichia coli

Haemophilus influenzae

Moraxella catarrhalis

Pseudomonas aeruginosa

Staphylococcus aureus

Stenotrophomonas maltophilia

Streptococcus pneumoniae

Klebsiella oxytoca

Streptococcus pyogenes

Streptococcus agalactiae

Atypical Bacteria (qualitative)

Legionella pneumophila

(removed *Legionella* spp. assay)

Mycoplasma pneumoniae

Chlamydomphila pneumoniae

Viruses

Influenza A

Influenza B

Respiratory Syncytial virus

Human Rhinovirus/Enterovirus

Human Metapneumovirus

Parainfluenza virus

Adenovirus

Coronavirus

MERS-CoV

Fungi

Pneumocystis jirovecii

Aspergillus spp.

Cryptococcus spp.

ABR Markers

mecA/C – MREJ (MRSA)

KPC (Carbapenem resistance)

NDM (Carbapenem resistance)

Oxa48-like (Carbapenem resistance)

CTX-M (ESBL resistance)

VIM (Carbapenem resistance)

IMP (Carbapenem resistance)

GI Panel

Clinical Sample: feces

Bacteria:

Aeromonas

Campylobacter

Clostridium difficile (Toxin A/B)

Plesiomonas shigelloides

Salmonella

Vibrio

Vibrio cholerae

Yersinia enterocolitica

Diarrheagenic *E. coli* / *Shigella*

E. coli O157

Enteroaggregative *E. coli* (EAEC)

Enteropathogenic *E. coli* (EPEC)

Enterotoxigenic *E. coli* (ETEC)

Shiga-like toxin-producing *E. coli* (STEC)

Shigella/Enteroinvasive *E. coli* (EIEC)

Protozoa:

Cryptosporidium

Cyclospora cayetanensis

Entamoeba histolytica

Giardia lamblia

Viruses:

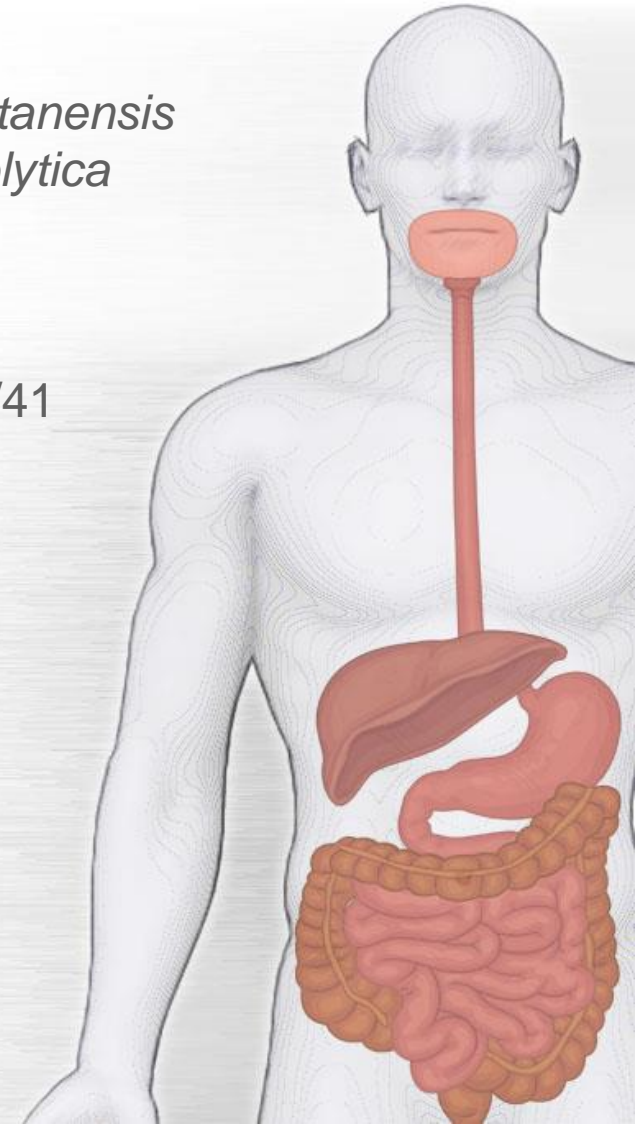
Adenovirus F 40/41

Astrovirus

Norovirus GI/GII

Rotavirus A

Sapovirus





ΠΑΝΕΛΛΗΝΙΟ ΣΥΝΕΔΡΙΟ
ΚΛΙΝΙΚΗΣ ΜΙΚΡΟΒΙΟΛΟΓΙΑΣ

Αθήνα, Royal Olympic Hotel, 21-23 Φεβρουαρίου 2019



FilmArray® Pneumonia Syndromic Testing & Λοιμώξεις Κατώτερου Αναπνευστικού: το 1ο Crash Test στην Ελλάδα

**FilmArray® Pneumonia:
Πολυκεντρική Μικροβιολογική
Αξιολόγηση του FilmArray®
Pneumonia panel**



PNEUMONIA PANEL_{PLUS} - 34 TARGETS



15 Common Bacteria with bins results

Bacteria

Semi - Quantitative

- Acinetobacter calcoaceticus-baumannii* complex
- Enterobacter aerogenes*
- Enterobacter cloacae*
- Escherichia coli*
- Haemophilus influenzae*
- Klebsiella oxytoca*
- Klebsiella pneumoniae* group
- Moraxella catarrhalis*
- Proteus* spp.
- Pseudomonas aeruginosa*
- Serratia marcescens*
- Staphylococcus aureus*
- Streptococcus pneumoniae*
- Streptococcus pyogenes*
- Streptococcus agalactiae*

Atypical Bacteria

Qualitative

- Legionella pneumophila*
- Mycoplasma pneumoniae*
- Chlamydia pneumoniae*

3 Qualitative Bacteria that cause Atypical Pneumonia

Viruses

Qualitative

- Influenza A
- Influenza B
- Adenovirus
- Coronavirus
- Parainfluenza virus
- Respiratory Syncytial virus
- Human Rhinovirus/Enterovirus
- Human Metapneumovirus
- Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

9 Viruses
• Same as in RP2_{plus}
• No Sub-typing

Antibiotic Resistance Genes

Methicillin Resistance

mecA/mecC and MREJ

7 antimicrobial resistance markers

ESBL

CTX-M

Carbapenemases

- KPC
- NDM
- Oxa48-like
- VIM
- IMP



TWO APPROVED SAMPLE TYPES



Sputum like

Expectorated sputum
& Induced sputum:
No Pretreatment



Bronchoalveolar lavage (BAL) like



EndoTracheal Aspirates (ETA)



Note : Pneumonia Panel is FDA cleared and CE-IVD on non pre-treated low respiratory samples



SAMPLES NOT VALIDATED



- Pleural Fluids
- Bronchial brushes (PSB) → strongly not recommended : very diluted
- Bronchial wash
- NP swab



SEMI-QUANTITATIVE REPORTING



- **Negative assays** (no measurable amplification or value less than $10^{3.5}$ copies/mL) are reported as **Not Detected**
- **Positive assays** are reported as **Detected** and a **Bin Result** is assigned based on the assay value
- Each bin is defined by discrete upper and lower limits spanning a 1-log range of values such that the Bin Result reflects the assay value within the nearest ± 0.5 -log

Assay Result		Reported Result and Bin Result	
Negative OR	$<10^{3.5}$ copies/mL	Not Detected	
Positive AND	$\geq 10^{3.5} - <10^{4.5}$ copies/mL	Detected	10^4 copies/mL
Positive AND	$\geq 10^{4.5} - <10^{5.5}$ copies/mL	Detected	10^5 copies/mL
Positive AND	$\geq 10^{5.5} - <10^{6.5}$ copies/mL	Detected	10^6 copies/mL
Positive AND	$\geq 10^{6.5}$ copies/mL	Detected	$\geq 10^7$ copies/mL



ANTIBIOTIC RESISTANCE GENES



- A resistance marker(s) is only reported if a microorganism is detected that could potentially contain that resistance gene

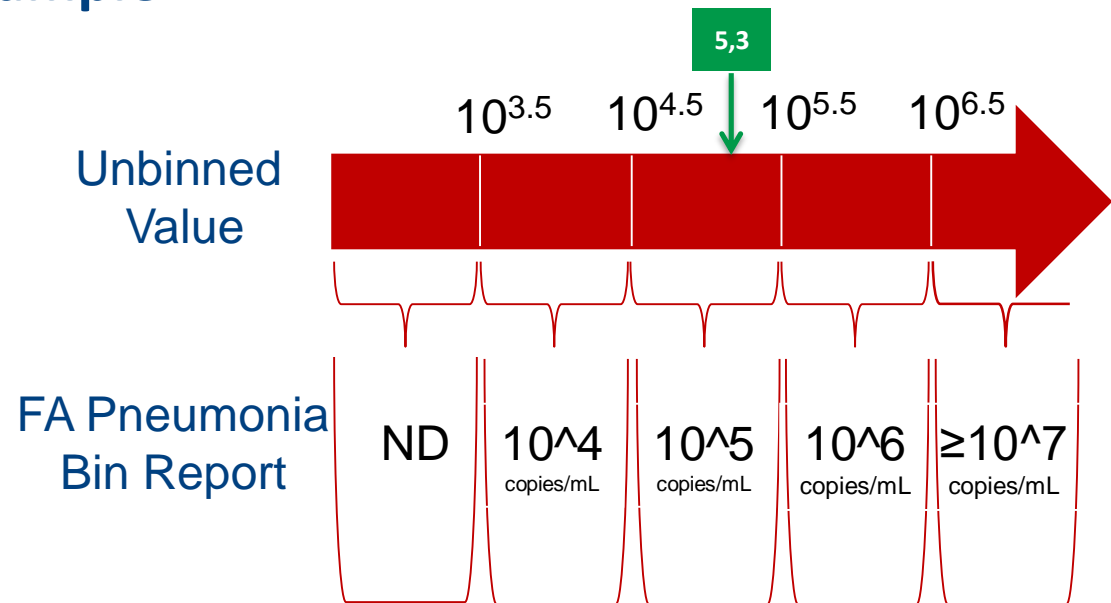
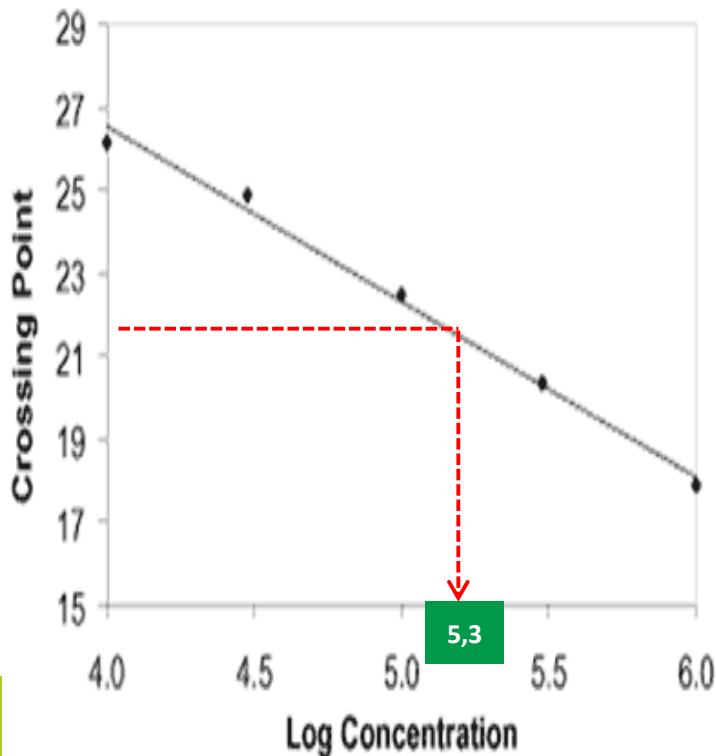
Applicable Bacteria Detected	Antibiotic Resistance Genes	Resistance Class
<i>Acinetobacter calcoaceticus-baumannii</i> complex <i>Pseudomonas aeruginosa</i>	CTX-M KPC VIM IMP NDM	ESBLs Carbapenemases
<i>Klebsiella aerogenes</i> <i>Enterobacter cloacae</i> complex <i>Escherichia coli</i> <i>Klebsiella oxytoca</i> <i>Klebsiella pneumoniae</i> <i>Proteus spp.</i> <i>Serratia marcescens</i>	CTX-M KPC VIM IMP NDM OXA-48-like	ESBLs Carbapenemases



SEMI-QUANTITATIVE MEASURES



- Common bacteria single copy genes are targeted
- Bacterial DNA is quantified in copies/ml using a quantified internal nucleic acid standard that is processed with the sample





2 PAGES REPORT



- Page 2 : Semi quantitative analytes with graphic representation and qualitative detected/non detected reporting

Antimicrobial Resistance Genes	
✓ Detected	CTX-M
Not Detected	IMP
Not Detected	KPC
⊗ N/A	mecA/C and MREJ
Not Detected	NDM
Not Detected	OXA-48-like
Not Detected	VIM
<p> Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility to the resistance drug class. Culture is required for species identification and susceptibility testing of isolates.</p>	
Atypical Bacteria	
Not Detected	<i>Chlamydia pneumoniae</i>
Not Detected	<i>Legionella pneumophila</i>
Not Detected	<i>Mycoplasma pneumoniae</i>
Viruses	
Not Detected	Adenovirus
Not Detected	Coronavirus
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
✓ Detected	Influenza A
Not Detected	Influenza B
Not Detected	Parainfluenza Virus
Not Detected	Respiratory Syncytial Virus

Result Summary					
Bacteria					
	Bin (copies/mL)	Bin (copies/mL)			
		10 ⁴	10 ⁵	10 ⁶	≥10 ⁷
Not Detected	<i>Acinetobacter calcoaceticus-baumannii</i> complex				
Not Detected	<i>Enterobacter aerogenes</i>				
Not Detected	<i>Enterobacter cloacae</i> complex				
Not Detected	<i>Escherichia coli</i>				
✓ Detected	10 ⁴ <i>Haemophilus influenzae</i>	██████			
Not Detected	<i>Klebsiella oxytoca</i>				
✓ Detected	≥10 ⁷ <i>Klebsiella pneumoniae</i> group	██████	██████	██████	██████
Not Detected	<i>Moraxella catarrhalis</i>				
Not Detected	<i>Proteus</i> spp.				
Not Detected	<i>Pseudomonas aeruginosa</i>				
Not Detected	<i>Serratia marcescens</i>				
Not Detected	<i>Staphylococcus aureus</i>				
Not Detected	<i>Streptococcus agalactiae</i>				
Not Detected	<i>Streptococcus pneumoniae</i>				
✓ Detected	10 ⁶ <i>Streptococcus pyogenes</i>	██████	██████	██████	

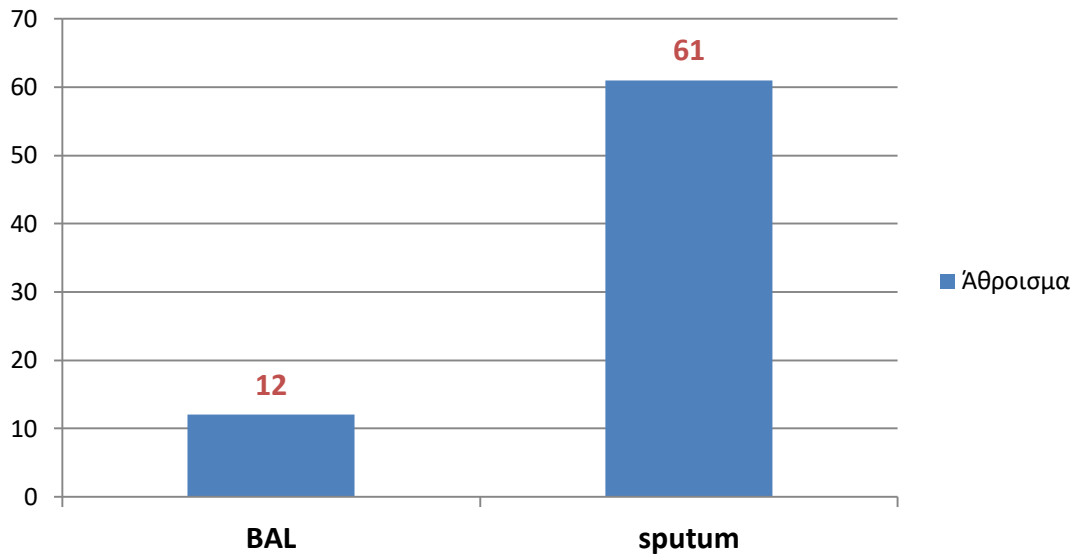
For not detected target it will not be possible to view the melting curves as for the other panels.

Πολυκεντρική Μικροβιολογική Αξιολόγηση του FilmArray® Pneumonia panel

Σύγκριση ημιποσοτικής καλλιέργειας
με *FilmArray pneumo plus* σε ETA ή BAL σε
διασωληνωμένους ασθενείς 3 Νοσοκομείων της Αθήνας:

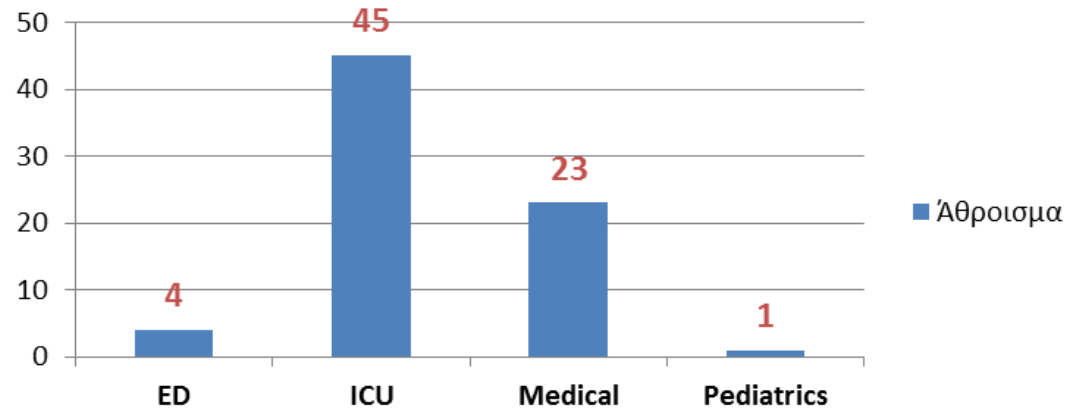
- ΠΓΝ ΑΤΤΙΚΟ
- ΓΝ ΤΖΑΝΕΙΟ
- ΕΥΡΩΚΛΙΝΙΚΗ

Sample Type



Count of Setting where sample was taken : ER/ICU/other Medical Ward

Wards



Setting where sample was taken : ER/ICU/other Medical Ward

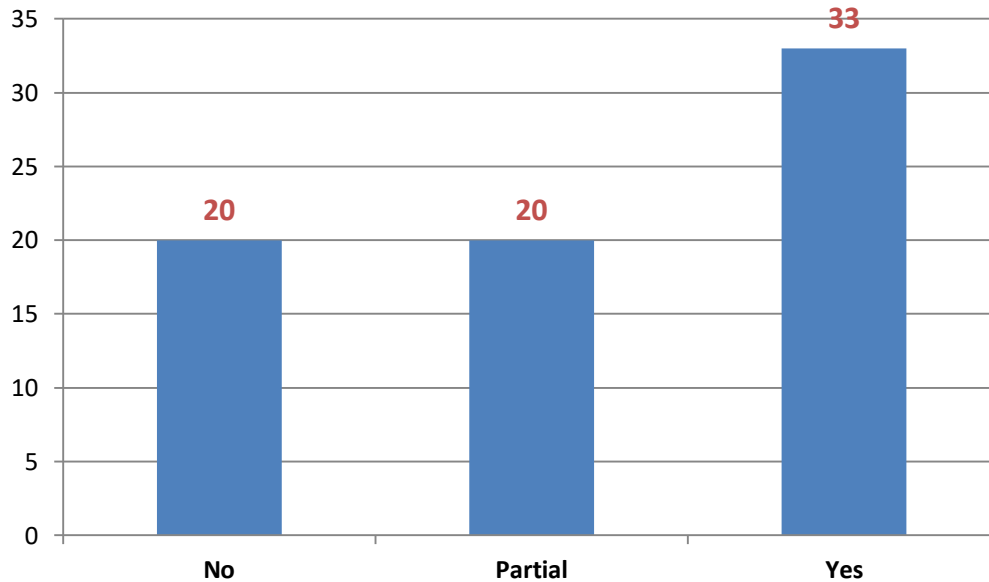
Quantification Culture	Quantification FilmArray
A. baumannii 3+	A. baumannii $\geq 10^7$ copies/ml
A. baumannii 3+	A. baumannii $\geq 10^7$ copies/ml
P. mirabilis 2+	P. mirabilis 10^5 copies/ml
K. oxytoca 2+	K. oxytoca 10^4 copies/ml
P. aeruginosa 3+	P. aeruginosa 10^6 copies/ml
A. baumannii 3+	A. baumannii 10^6 copies/ml
S. marcescens 2+	S. marcescens 10^5 copies/ml
A. baumannii 2+	A. baumannii 10^6 copies/ml
E. aerogenes 2+	E. aerogenes $\geq 10^7$ copies/ml
P. aeruginosa 3+	P. aeruginosa $\geq 10^7$ copies/ml
A. baumannii 3+	A. baumannii 10^6 copies/ml
K. pneumoniae 2+	K. pneumoniae 10^5 copies/ml
P. aeruginosa 1+	P. aeruginosa $\geq 10^7$ copies/ml
P. aeruginosa 1+	P. aeruginosa 10^4 copies/ml
P. aeruginosa 2+	P. aeruginosa 10^6 copies/ml
A. baumannii 2+	A. baumannii $\geq 10^7$ copies/ml
A. baumannii 3+	A. baumannii $\geq 10^7$ copies/ml
P. aeruginosa 1+	P. aeruginosa 10^4 copies/ml
A. baumannii 3+	A. baumannii $\geq 10^7$ copies/ml
A. baumannii 2+	A. baumannii 10^5 copies/ml
A. baumannii 2+	A. baumannii $\geq 10^7$ copies/ml
P. aeruginosa 1+	P. aeruginosa 10^5 copies/ml
A. baumannii 2+	A. baumannii 10^6 copies/ml
A. baumannii 3+	A. baumannii 10^6 copies/ml
K. pneumoniae 1+	K. pneumoniae 10^5 copies/ml
A. baumannii 1+	A. baumannii 10^6 copies/ml
A. baumannii 1+	A. baumannii 10^6 copies/ml
P. aeruginosa 2+	P. aeruginosa 10^5 copies/ml
A. baumannii 2+	A. baumannii 10^5 copies/ml

comparison contains
only detections made in
both methods

<i>A. baumannii</i>			<i>P. aeruginosa</i>			Others		
No strains	Culture result	FilmArray result (copies/ml)	No strains	Culture result	FilmArray result	No strains	Culture result	FilmArray result
2	<i>A. baumannii</i> 1+	<i>A. baumannii</i> 10 ⁶			<i>P. aeruginosa</i> ≥ 10 ⁷	1	<i>E. aerogenes</i> 2+	<i>E. aerogenes</i> ≥ 10 ⁷
6	<i>A. baumannii</i> 2+	<i>A. baumannii</i> 10 ⁶	4	<i>P. aeruginosa</i> 1+	<i>P. aeruginosa</i> 10 ⁴	1	<i>K. pneumoniae</i> 1+	<i>K. pneumoniae</i> 10 ⁵
		<i>A. baumannii</i> ≥10 ⁷			<i>P. aeruginosa</i> 10 ⁴	1	<i>K. pneumoniae</i> 2+	<i>K. pneumoniae</i> 10 ⁵
		<i>A. baumannii</i> 10 ⁵			<i>P. aeruginosa</i> 10 ⁵	1	<i>K. oxytoca</i> 2+	<i>K. oxytoca</i> 10 ⁴
		<i>A. baumannii</i> ≥10 ⁷			<i>P. aeruginosa</i> 10 ⁶	1	<i>P. mirabilis</i> 2+	<i>P. mirabilis</i> 10 ⁵
		<i>A. baumannii</i> 10 ⁶	2	<i>P. aeruginosa</i> 2+	<i>P. aeruginosa</i> 10 ⁵	1	<i>S. marcescens</i> 2+	<i>S. marcescens</i> 10 ⁵ c
		<i>A. baumannii</i> 10 ⁵	2	<i>P. aeruginosa</i> 3+	<i>P. aeruginosa</i> 10 ⁶			
7	<i>A. baumannii</i> 3+	<i>A. baumannii</i> ≥10 ⁷			<i>P. aeruginosa</i> ≥ 10 ⁷			
		<i>A. baumannii</i> ≥10 ⁷						
		<i>A. baumannii</i> 10 ⁶						
		<i>A. baumannii</i> 10 ⁶						
		<i>A. baumannii</i> ≥10 ⁷						
		<i>A. baumannii</i> ≥10 ⁷						
		<i>A. baumannii</i> 10 ⁶						

When Culture result= 3+ , FilmArray ≥ 10⁶ copies/ml

Agreement with Culture



Clinical Microbiological Criteria of Agreement (2 blinded evaluators)	
23 Disagreements	
17 in favor of FA	6 in favor of Culture

Important disagreement

Site	Sample type	Setting: ER/ICU/other Medical Ward	Standard of care diagnostics results (Gram stain, culture, urinary atigens [if performed])	Result Culture	FilmArray Results (indicate if any detection would not be ported by the lab)	Result FilmArray	Agree- ment	Final Result
A	sputum	Pathology	<i>S. marcescens</i>	1 Detection	<i>Influenzae A</i>	1 Detection	No	FilmArray correct
A	sputum	Pathology	Negative	No Growth	<i>S. aureus</i>	1 Detection	No	FilmArray correct
A	sputum	ICU	Negative	No Growth	<i>Legionella pneumophila</i>	1 Detection	No	FilmArray correct
A	sputum	Pathology	Negative	No Growth	<i>Mycoplasma pneumoniae</i>	1 Detection	No	FilmArray correct
A	BAL	Pathology	Negative	No Growth	<i>Mycoplasma pneumoniae</i>	1 Detection	No	FilmArray correct
A	BAL	Pathology	Negative	No Growth	<i>Mycoplasma pneumoniae</i>	1 Detection	No	FilmArray correct
A	sputum	ICU	<i>S. maltophilia</i>	1 Detection	None detected	Negative	No	Culture Correct
A	sputum	Pathology	Negative	No Growth	<i>S. aureus</i>	1 Detection	No	FilmArray correct
A	sputum	Pathology	Negative	No Growth	<i>M. Catarrhalis</i>	2 Detections	No	FilmArray correct
A	sputum	Pathology	Negative	No Growth	<i>S. marcescens</i>	1 Detection	No	FilmArray correct
B	sputum	ICU	Negative	No Growth	<i>A. baumannii</i>	1 Detection	No	FilmArray correct
B	sputum	ICU	Negative	No Growth	<i>A. baumannii</i>	1 Detection	No	FilmArray correct
B	sputum	ICU	<i>A. baumannii</i>	1 Detection	None detected	Negative	No	Culture Correct
C	Sputum	ED	Normal oral flora	No Growth	<i>H. Infuenzae</i>	2 Detection	No	FilmArray correct
C	Sputum	Pathology	Normal oral flora	No Growth	<i>E. aerogenes</i>	2 Detection	No	FilmArray correct
C	Sputum	Pathology	Normal oral flora	No Growth	<i>H. infuenzae , S. aureus</i>	2 Detections	No	FilmArray correct

Partial Agreement

Site	Sample type	Setting: ER/ICU/other Medical Ward	Standard of care diagnostics results (Gram stain, culture, urinary antigens)	FilmArray Results (indicate if any detection would not be ported by the lab)	Agreement
A	sputum	Pathology	<i>E. Coli</i>	<i>A.baumannii</i> , <i>K.pneumoniae</i> , <i>E.cloacae</i> , <i>E.Coli</i>	Partial
A	sputum	ICU	<i>E. Coli</i>	<i>E.Coli</i> , <i>P.aeruginosa</i>	Partial
A	sputum	ED	<i>S. marcescens</i>	<i>A.baumannii</i> , <i>S.marcescens</i> , <i>H.influenzae</i>	Partial
B	sputum	ICU	<i>A. baumannii</i> , <i>P. mirabilis</i> , <i>K. oxytoca</i> ,	<i>A. baumannii</i> , <i>P.mirabilis</i> , <i>K.o xytoca</i> , <i>P. aeruginosa</i>	Partial
B	sputum	ICU	<i>K. pneumoniae</i> , <i>S. marcescens</i> <i>A. baumannii</i>	<i>S. marscenscens</i> , <i>A.baumannii</i>	Partial
B	sputum	ICU	<i>P. Aeruginosa</i>	<i>P. aeruginosa</i> , <i>A. baumannii</i>	Partial
B	sputum	ICU	<i>K. pneumoniae</i> , <i>S. marcescens</i> <i>A. baumannii</i>	<i>A. baumannii</i> , <i>K. pneumoniae</i> KPC	Partial
B	sputum	ICU	<i>A. Baumannii</i>	<i>S. marscenscens</i> , <i>A. baumannii</i> ,	Partial
B	sputum	ICU	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> , <i>K. pneumoniae</i>	Partial
B	sputum	ICU	<i>A. baumannii</i>	<i>A. baumannii</i> , <i>H. influenzae</i>	Partial
C	Sputum	Pathology	<i>P. aeruginosa</i> , <i>S. marcescens</i> , <i>Enterobacter spp</i>	<i>P. aeruginosa</i>	Partial
C	BAL	ICU	<i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>S. maltophilia</i>	<i>A. baumannii</i> , <i>K. pneumoniae</i> group	Partial
C	BAL	ICU	<i>A. baumannii</i> , <i>K. pneumoniae</i> ,	<i>A. baumannii</i> , <i>K. pneumoniae</i> group , <i>P. aeruginosa</i>	Partial
C	Sputum	Card	<i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>S. aureus</i> , <i>A. xylooxidans</i>	<i>A. baumannii</i> , <i>K. pneumoniae</i> group , <i>S. aureus</i>	Partial
C	BAL	ICU	<i>P. Aeruginosa</i>	<i>A .baumannii</i> , <i>P. aeruginosa</i>	Partial
C	BAL	ICU	<i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>E. coli</i>	<i>A. baumannii</i> , <i>K. pneumoniae</i> group , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>H.influenzae</i>	Partial

Resistance Genes Detected

Sample type	Setting: ER/ICU/other Medical Ward	Standard of care diagnostics results (Gram stain, culture, urinary atigens [if performed])	FilmArray Results (indicate if any detection would not be ported by the lab) (copies/ml)	Resistance Genes
Sputum	ICU	<i>K. pneumoniae</i> , <i>S. marcescens</i> , <i>A. baumannii</i>	<i>A. baumannii</i> , <i>K. pneumoniae</i>	KPC
Sputum	ICU	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> , <i>K. pneumoniae</i>	CTX-M, KPC, NDM, OXA-48
Sputum	ICU	<i>K. pneumoniae</i> <i>A. baumannii</i>	<i>A. baumannii</i> <i>K. pneumoniae</i>	NDM
BAL	ICU	<i>K. pneumoniae</i> , <i>A. baumannii</i>	<i>A. baumannii</i> , <i>K. pneumoniae</i>	KPC
Sputum	Pathology	<i>K. pneumoniae</i> , <i>P. aeruginosa</i> <i>S. aureus</i>	<i>K. pneumoniae</i> group , <i>P. aeruginosa</i> <i>S. aureus</i>	mecA/C and MREJ
BAL	ICU	<i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>Stenotrophomonas maltophilia</i>	<i>A. baumannii</i> , <i>Klebsiella pneumoniae</i> group	KPC, CTX-M
BAL	ICU	<i>A. baumannii</i> , <i>K. pneumoniae</i>	<i>A. baumannii</i> , <i>Klebsiella pneumoniae</i> group , <i>P. aeruginosa</i>	KPC
BAL	ICU	<i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>P. Aeruginosa</i>	<i>A. baumannii</i> , <i>K. pneumoniae</i> <i>P. Aeruginosa</i>	KPC
BAL	ICU	<i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i>	<i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i>	KPC

Σύγκριση ποσοτικής καλλιέργειας με *FilmArray pneumo plus* σε δείγμα BAL έναντι Βρογχικών εκκρίσεων σε διασωληνωμένους ασθενείς

Υλικό Ιανουάριος 2019: 15 διασωληνωμένοι ασθενείς στη ΜΕΘ του ΑΤΤΙΚΟΥ, με υποψία λοίμωξης κατώτερου αναπνευστικού

Σκοπός Η αξιολόγηση της δυνατότητας χρήσης δείγματος βρογχικών εκκρίσεων έναντι BAL σε ασθενείς με αναπνευστήρα, για τη διάγνωση λοιμώξεων αναπνευστικού

Μέθοδος

- Λήψη **BAL και βρογχικών εκκρίσεων** κατά την πρωινή βάρδια για ποσοτικές καλλιέργειες και *FilmArray pneumo plus*. Στους ίδιους ασθενείς λήφθηκε για *FilmArray pneumo plus* και ποσοτική καλλιέργεια.
- Τα αποτελέσματα *FilmArray pneumo plus* ήταν διαθέσιμα σε **περίπου 1 - 2 ώρες, την ημέρα παραλαβής** των δειγμάτων.
- Ημέρα 2: αναφέρονται οι αρνητικές καλλιέργειες
- Ημέρα 3: Οι αποικίες που έχουν αναπτυχθεί ταυτοποιούνται και γίνεται MIC (VITEK 2, Phoenix). Τα αποτελέσματα αναφέρονται στην ΜΕΘ (**συνήθως 48** ή 72 ώρες μετά την παραλαβή του δείγματος)

Αποτελέσματα/Ευρήματα

- ✓ Συμφωνία αποτελέσματος: *FilmArray Pneumo Plus* στο **BAL** **συγκριτικά με βρογχικές** των ίδιων ασθενών παρατηρήθηκε σε 11 από τους 15 ασθενείς
- ✓ Αρνητικά δείγματα: (N=9) Απόλυτη συσχέτιση αποτελεσμάτων της ποσοτικής καλλιέργειας με αποτελέσματα από δείγμα BAL και βρογχικές επί βακτηρίων
- ✓ Το *FilmArray Pneumo plus*, ταυτοποίησε **11 ιούς** σε 10 ασθενείς εκ των οποίων οι **5** ήταν **Influenza A**, που δεν είναι δυνατόν να ταυτοποιηθούν με SOC και έπρεπε να έχει σταλεί δείγμα για PCR (πληροφορία διαθέσιμη σε 1 ώρα από την αποστολή του δείγματος)

Αποτελέσματα/Ευρήματα

- ✓ **Συν-λοιμώξεις:** Στο *FilmArray pneumo plus* κατεγράφησαν σε δείγματα BAL :
1 ασθενής με 5 μικροοργανισμούς και 2 ασθενείς με 2 μικροοργανισμούς.
Στα αντίστοιχα δείγματα βρογχικών: 1 ασθενής με 5 μικροοργανισμούς και 5 ασθενείς με 2 μικροοργανισμούς
- ✓ **Ευαισθησία:** Σε 5 περιπτώσεις απομονώθηκε *Candida spp* στην ποσοτική καλλιέργεια (έχει εξαιρεθεί από το FilmArray)
- ✓ Σε 1 περίπτωση απομονώθηκε *S. maltophilia* στην ποσοτική καλλιέργεια (έχει εξαιρεθεί από το FilmArray)
- ✓ **Ποσοτικοποίηση με FilmArray:** Η «θετικοποίηση» του *FilmArray pneumo plus* στα 4 δείγματα βρογχικών (που ήταν αρνητικά σε BAL), ήταν **ΌΛΑ** στη κατώτερη ποσοτική τιμή του **FilmArray** (**10^4 copies/ml**, cut-off θετικού αποτελέσματος)
- ✓ Με τα πρώτα στοιχεία φαίνεται μία τάση που υποδηλώνει ότι οι βρογχικές εκκρίσεις σε διασωληνωμένους ασθενείς με μία τιμή ίση ή πάνω από **10^5 copies/ml** στο *FilmArray pneumo plus* είναι απόλυτα συγκρίσιμες με την ποσοτική καλλιέργεια του BAL, εντός μόνο 1 ώρας

Ανάλυση των θετικών ποσοτικών Κ/Α σε BAL σε σχέση με βρογχικές εκκρίσεις, ΠΓΝ ΑΤΤΙΚΟ

	Δείγμα	Ποσοτική καλλιέργεια	FilmArray pneumonia	Συμφωνία FA BAL / Βρογχικών
Nb 2.	BAL	<i>A. baumannii</i> , 10 ⁵ cfu/ml	<i>A. baumannii</i> , 10 ⁶ copies/ml	Yes
	Βρογχικές	<i>A. baumannii</i> , >10 ⁶ cfu/ml	<i>A. baumannii</i> , ≥10 ⁷ copies/ml	
Nb 3.	BAL	<i>S. maltophilia</i> *, 10 ⁴ cfu/ml	Human Rhinovirus/enterovirus	Yes
	Βρογχικές	<i>S. maltophilia</i> *, 10 ⁴ cfu/ml	Human Rhinovirus/enterovirus	
Nb 5 10,	BAL	1. <i>A. baumannii</i> , >10 ⁵ cfu/ml	1. <i>A. baumannii</i> , ≥10 ⁷ copies/ml	Yes
		2. <i>P. aeruginosa</i> , >10 ⁵ cfu/ml	2. <i>P. aeruginosa</i> , ≥10 ⁷ copies/ml	
			3. <i>S. aureus</i> , 10 ⁶ copies/ml	
			4. <i>E. cloacae</i> , 10 ⁴ copies/ml	
			5. Parainfluenza virus	
	Βρογχικές	1. <i>A. baumannii</i> , >10 ⁶ cfu/ml	1. <i>A. baumannii</i> , ≥10 ⁷ copies/ml	
	BAL	2. <i>P. aeruginosa</i> , >10 ⁶ cfu/ml	2. <i>P. aeruginosa</i> , ≥10 ⁷ copies/ml	
			3. <i>S. aureus</i> , ≥10 ⁷ copies/ml	
			4. <i>E. cloacae</i> , 10 ⁵ copies/ml	
			5. Parainfluenza virus	
	Βρογχικές			
Nb 7. 11/1	BAL	<i>A. baumannii</i> , >10 ⁵ cfu/ml	1. <i>A. baumannii</i> , 10 ⁶ copies/ml	Yes
	Βρογχικές	<i>A. baumannii</i> , >10 ⁶ cfu/ml	1. <i>A. baumannii</i> , ≥10 ⁷ copies/ml	
Nb 9 16/1	BAL	<i>Candida spp.*</i>	Influenza A	Yes
	Βρογχικές	<i>Candida spp.*</i>	Influenza A	
Nb 12 21/1	BAL	<i>A. baumannii</i> , 10 ² cfu/ml	<i>A. baumannii</i> , 10 ⁵ copies/ml	Yes
			CTX-M	
	Βρογχικές	1. <i>A. baumannii</i> , 10 ⁵ cfu/ml	<i>A. baumannii</i> , 10 ⁶ copies/ml	
	2. <i>K. pneumoniae</i> , 10 ² cfu/ml	OXA-48-like		

Ανάλυση των θετικών ποσοτικών Κ/Α σε BAL σε σχέση με βρογχικές εκκρίσεις, ΠΓΝ ΑΤΤΙΚΟ

	Δείγμα	Ποσοτική καλλιέργεια	FilmArray pneumonia	Συμφωνία FA BAL / Βρογχικών
Nb 13 24/1	BAL	Αρνητική	None detected	Yes
	Βρογχικές	Αρνητική	None detected	
Nb 10 16/1	BAL	Αρνητική	None detected	Yes
	Βρογχικές	Αρνητική	None detected	
Nb 1121/1	BAL	Αρνητική	None detected	Yes
	Βρογχικές	Αρνητική	None detected	
Nb 8. 11/1	BAL	Αρνητική	1. Influenza A 2. Parainfluenza virus	Yes
	Βρογχικές	Αρνητική	1. Influenza A 2. Parainfluenza virus	
Nb 6. 10/1/2019	BAL	Αρνητική	Influenza A	No
	Βρογχικές	<i>Candida spp.*</i>	1. <i>S. aureus</i> MRSA, 10 ⁴ copies/ml 2. Influenza A	
Nb 4. 3/1/2019	BAL	Αρνητική	None detected	No
	Βρογχικές	<i>Candida spp.*</i>	1. <i>Serratia marcescens</i> , 10 ⁴ copies/ml 2. Human Rhinovirus/enterovirus	
Nb 14 30/1	BAL	Αρνητική	Influenza A	Yes
	Βρογχικές	<i>Candida spp.*</i>	Influenza A	
Nb 15 24/1	BAL	Αρνητική	None detected	No
	Βρογχικές	<i>A. baumannii</i> , 10 ² cfu/ml (2 αποικίες μόνο)	Influenza A	
Nb 1.	BAL	Αρνητική	None detected	No
	Βρογχικές	<i>A. baumannii</i> , 10 ⁵ cfu/ml	<i>A. baumannii</i> , 10 ⁴ copies/ml	





**THANK YOU FOR YOUR
ATTENTION!**