ΕΘΝΙΚΟ ΚΑΠΟΔΙΣΤΡΙΑΚΟ
ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΠΓΝ ΑΤΤΙΚΟΝ

ΑΡΧΕΣ ΚΑΙ ΕΦΑΡΜΟΓΕΣ ΜΟΡΙΑΚΗΣ ΜΙΚΡΟΒΙΟΛΟΓΙΑΣ

Newer techniques for diagnosing infections

(το παράδειγμα των MALDI-TOF, FilmARRAY, Septifast, T2MR)

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Laboratory of Clinical Microbiology
ATTIKON 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

Evolution of Molecular Diagnostic Methodσ used
It was rapid in the COVID era…

FULL AUTOMATION:
RNA extraction
PCR preparation and set up
PCR run incorporated
Automated interpretation

NeuMoDx™ 96 Molecular System

SAMPLE-TO-RESULT

Molecular Techniques to the Diagnosis: Advantages

- High Sensitivity & Specificity
- Speed $\rightarrow$ 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 antimicrobial 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

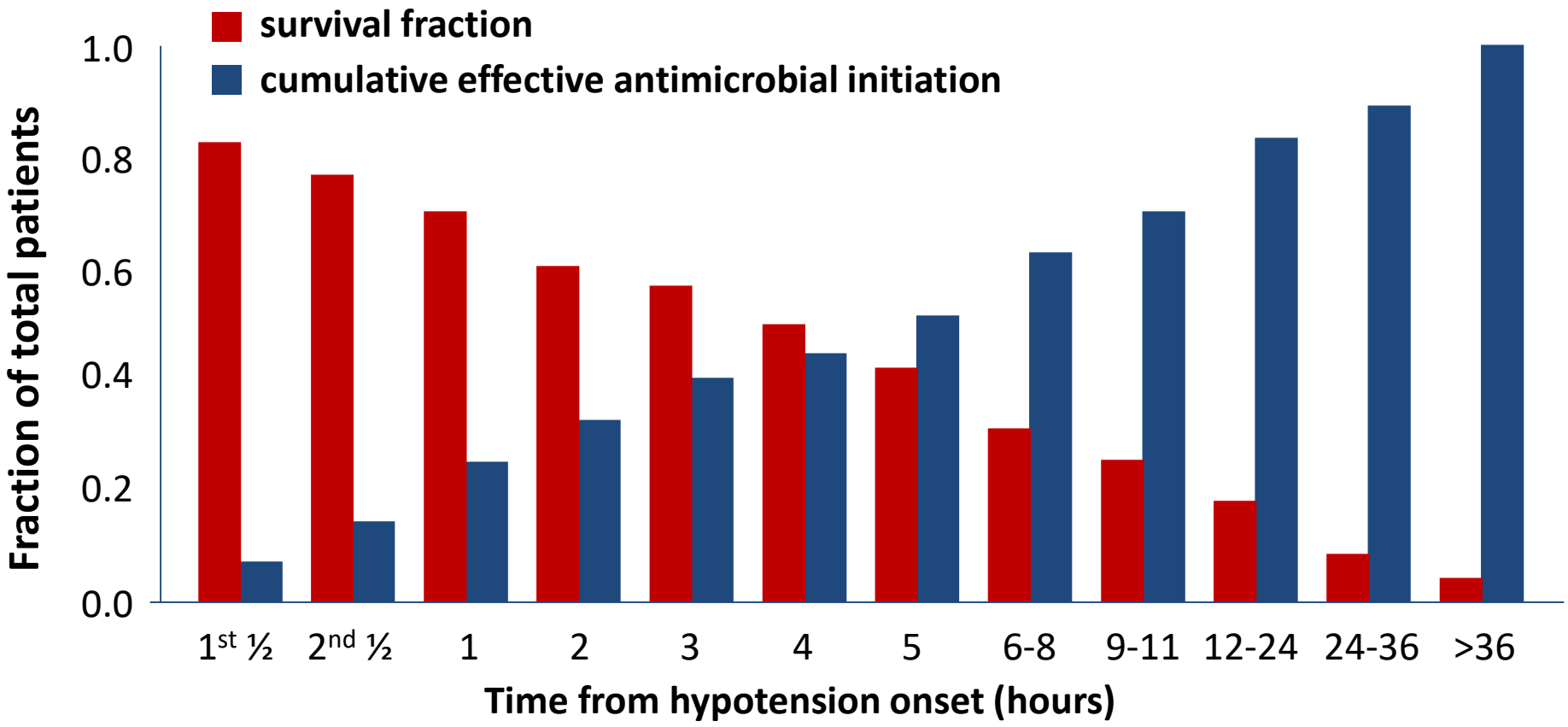
conventional microbiology possibly more robust?

- **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 LIVE AND DEAD BACTERIA

«In septic shock, time is life»

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

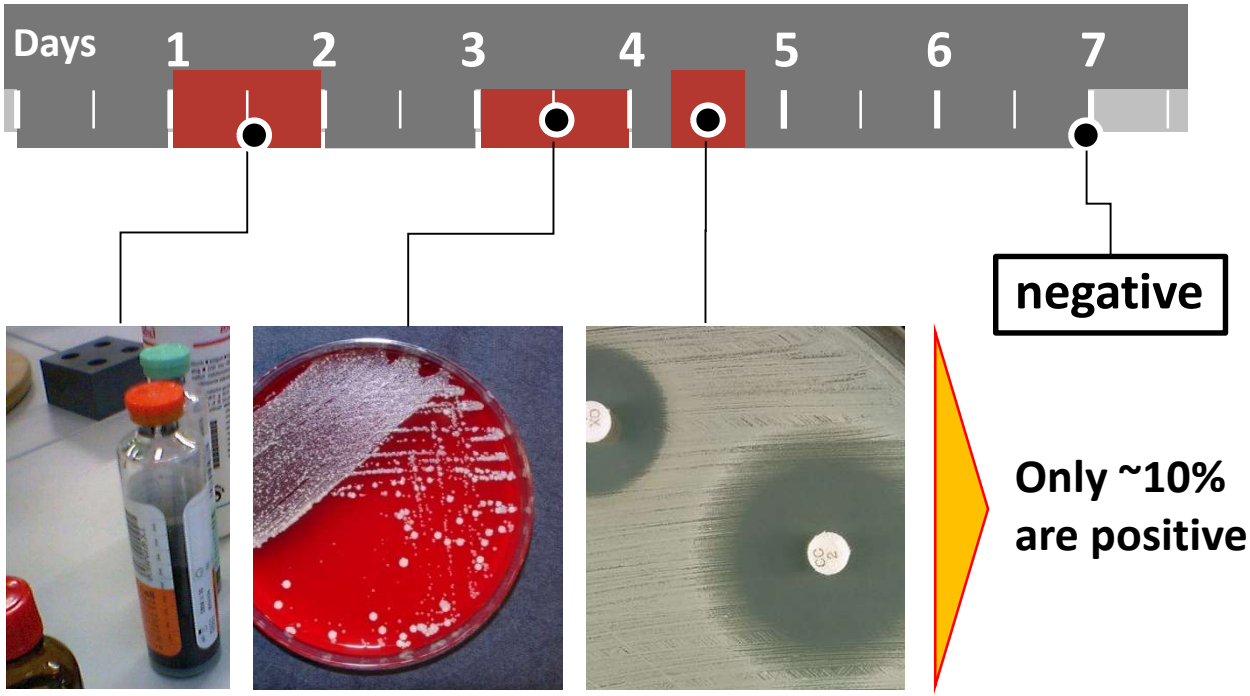


Each hour of delay (over the first 6 hours) resulting in a

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)

Book M et al. Best Pract Res Clin Anaesthesiol. 2013;27:279-88.

Clinical Samples for Molecular Testing

1. From pure culture → rapid identification

2. From positive blood culture

3. Directly from whole blood

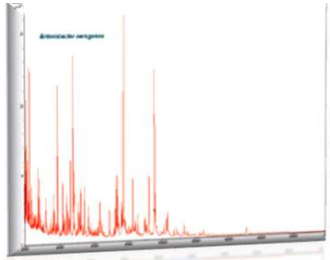
Detection or not of antibiotic resistance genes

Molecular Techniques: Applications

1. From pure culture → rapid identification

2. From positive blood culture

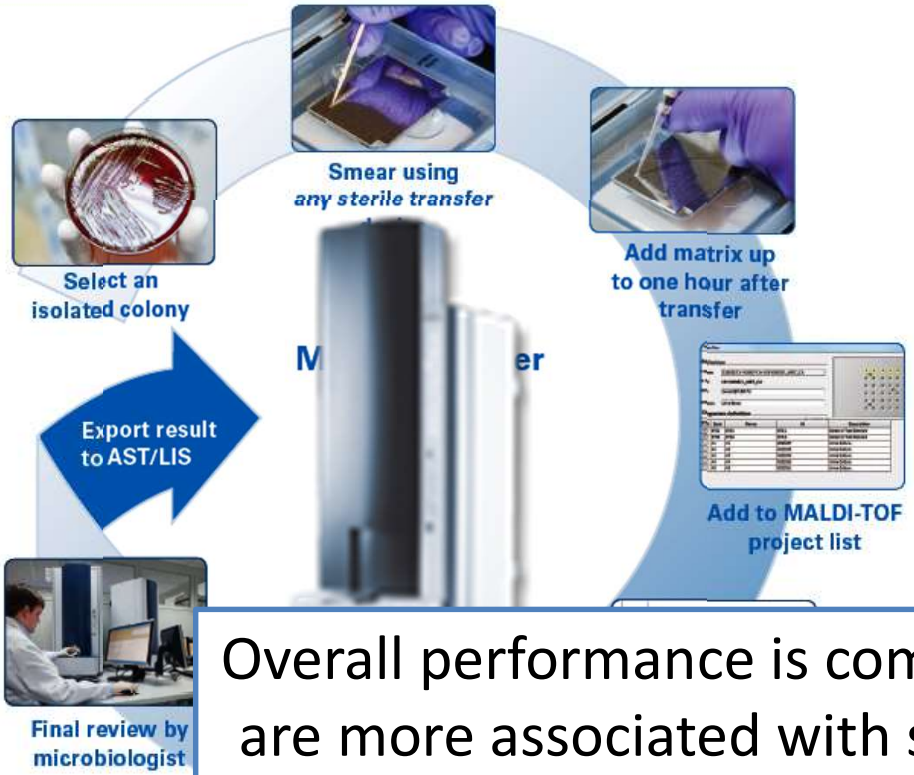
Mass spectrometry (MALDI-TOF MS)



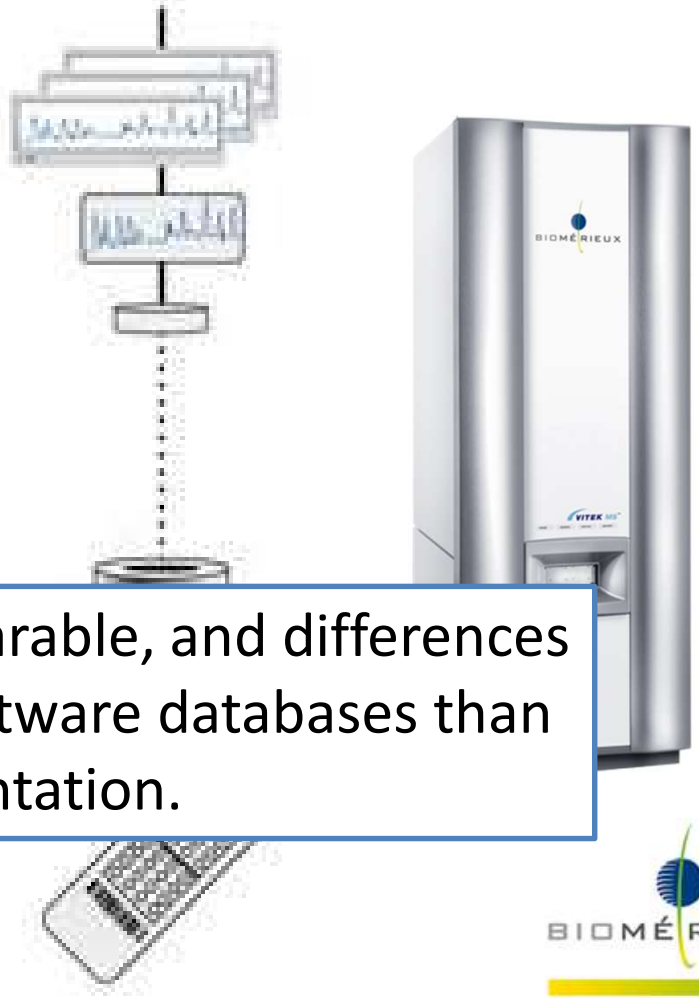
MALDI-TOF

"Identifying Microorganisms by Their Protein Fingerprint"

MALDI Biotyper



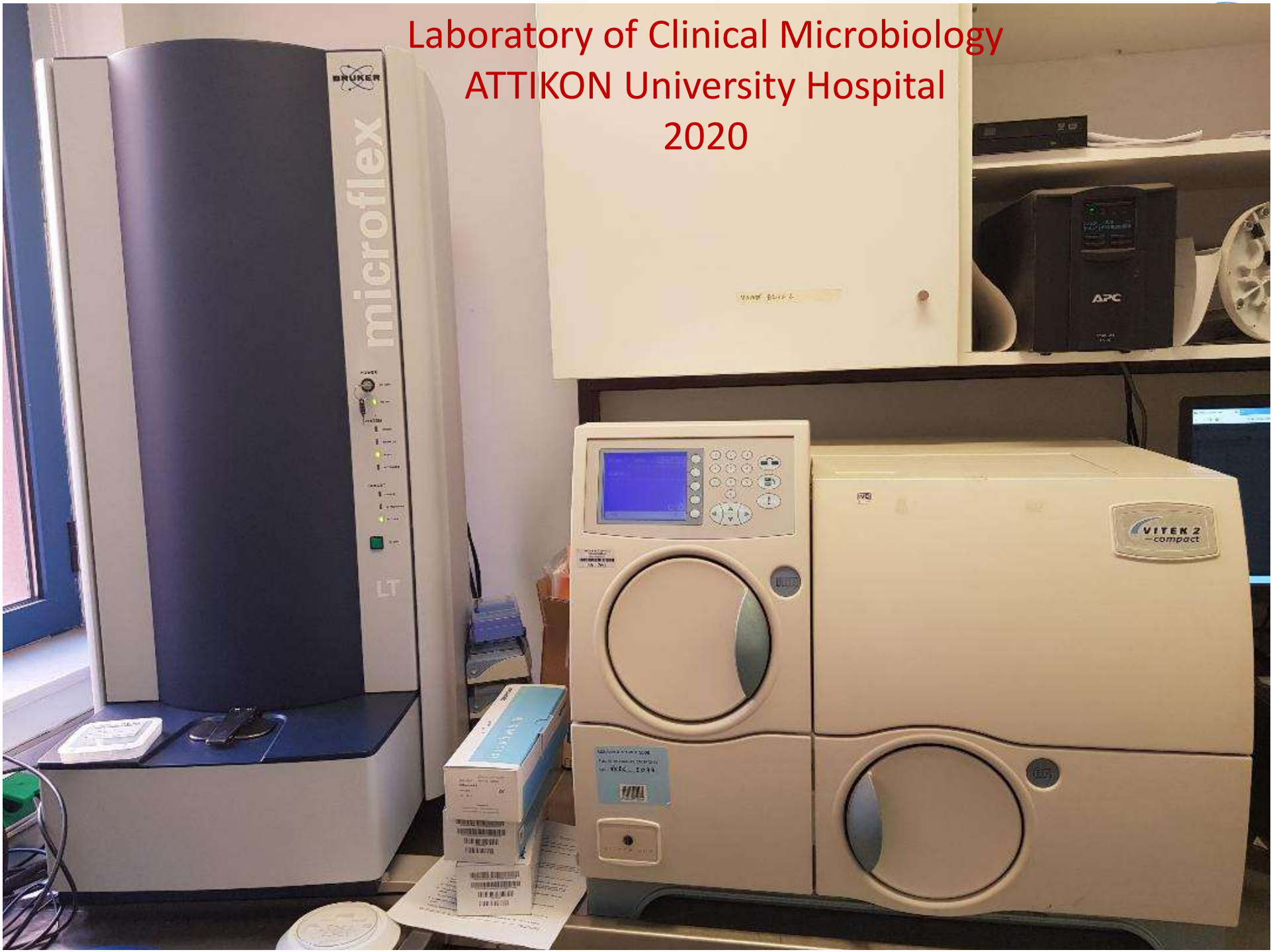
VITEK® MS



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

BRUKER

BIOMÉRIEUX


Laboratory of Clinical Microbiology
ATTIKON University Hospital
2020
BRUKER
microflex
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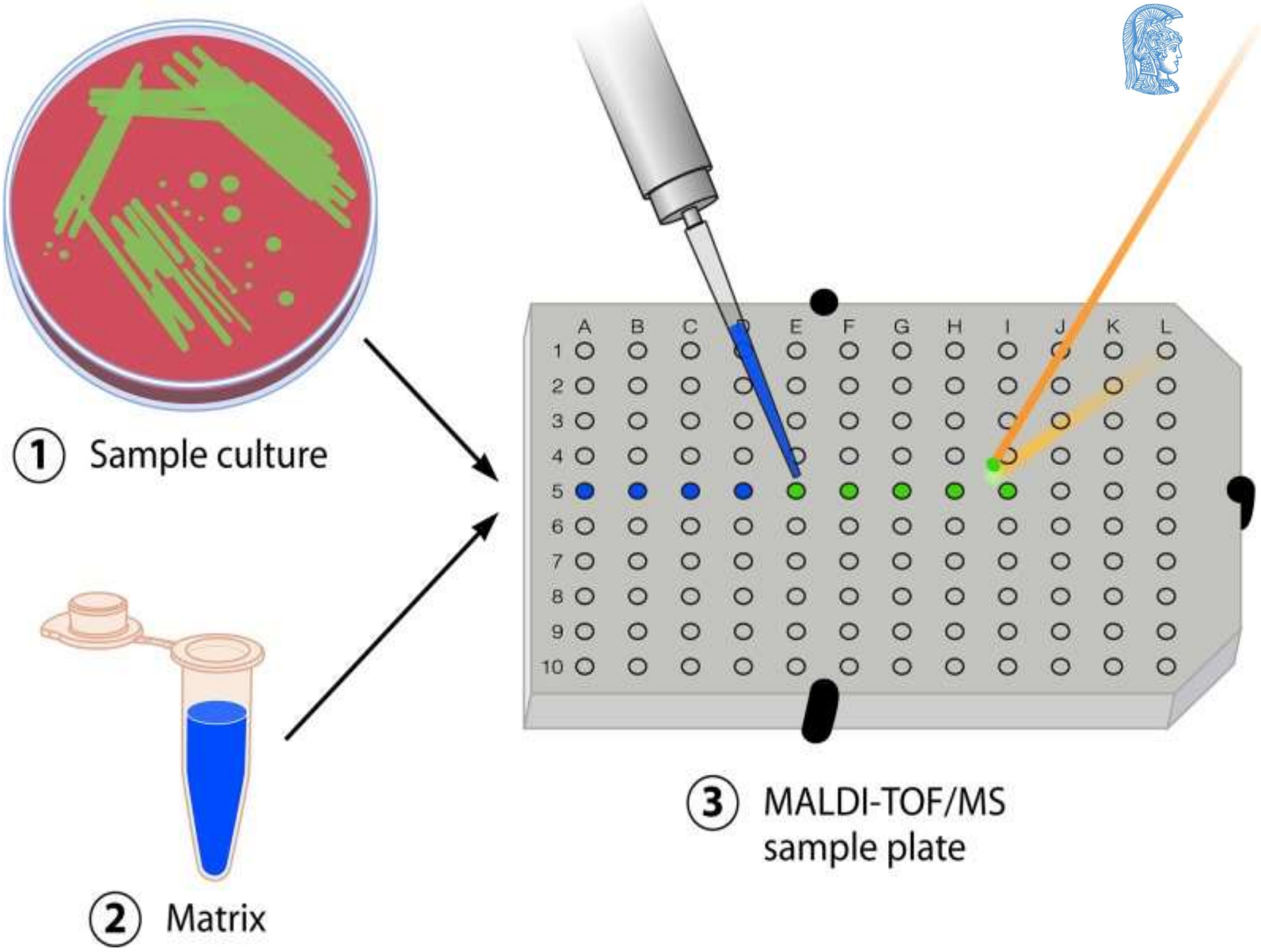
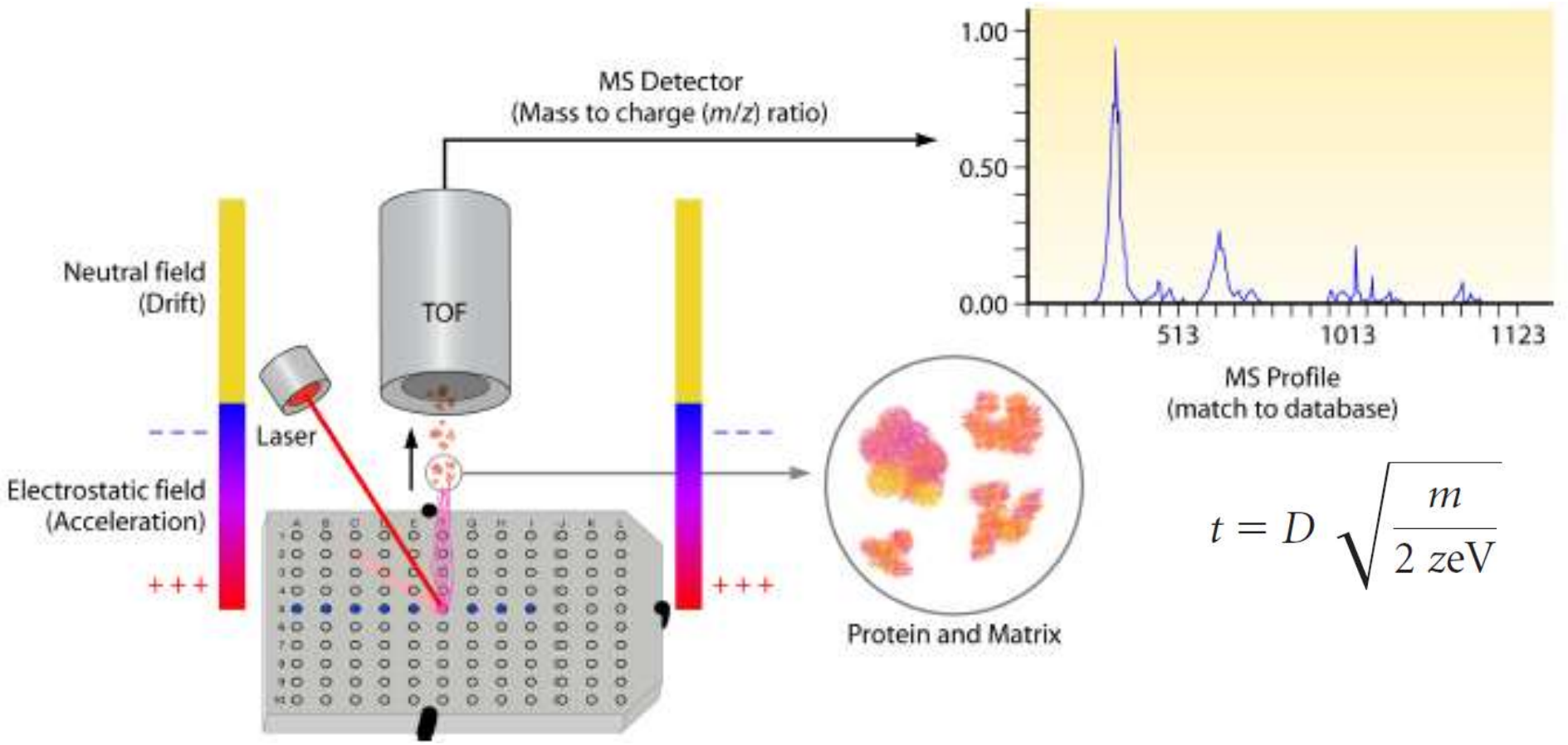

A B C D E F G H I J K L
1
2
3
4
5
6
7
8
9
10
1 Sample culture
2 Matrix
3 MALDI-TOF/MS
sample plate

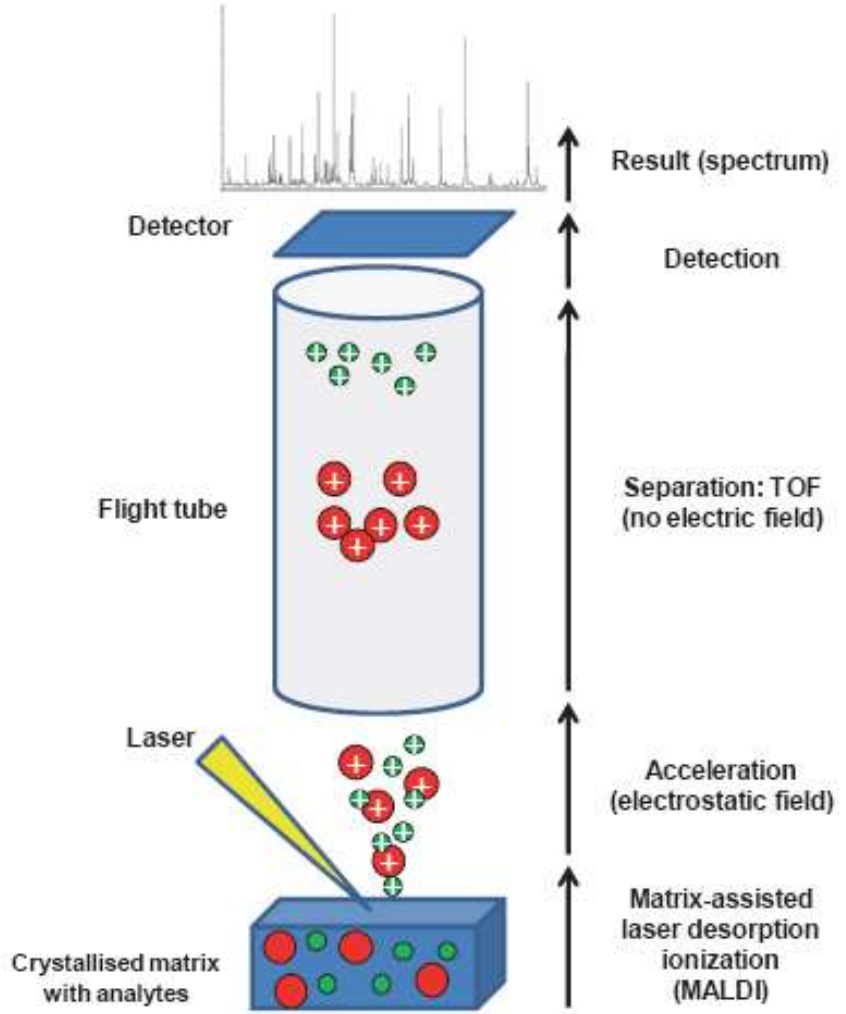


$$t = D\sqrt{\frac{m}{2\ zeV}}$$

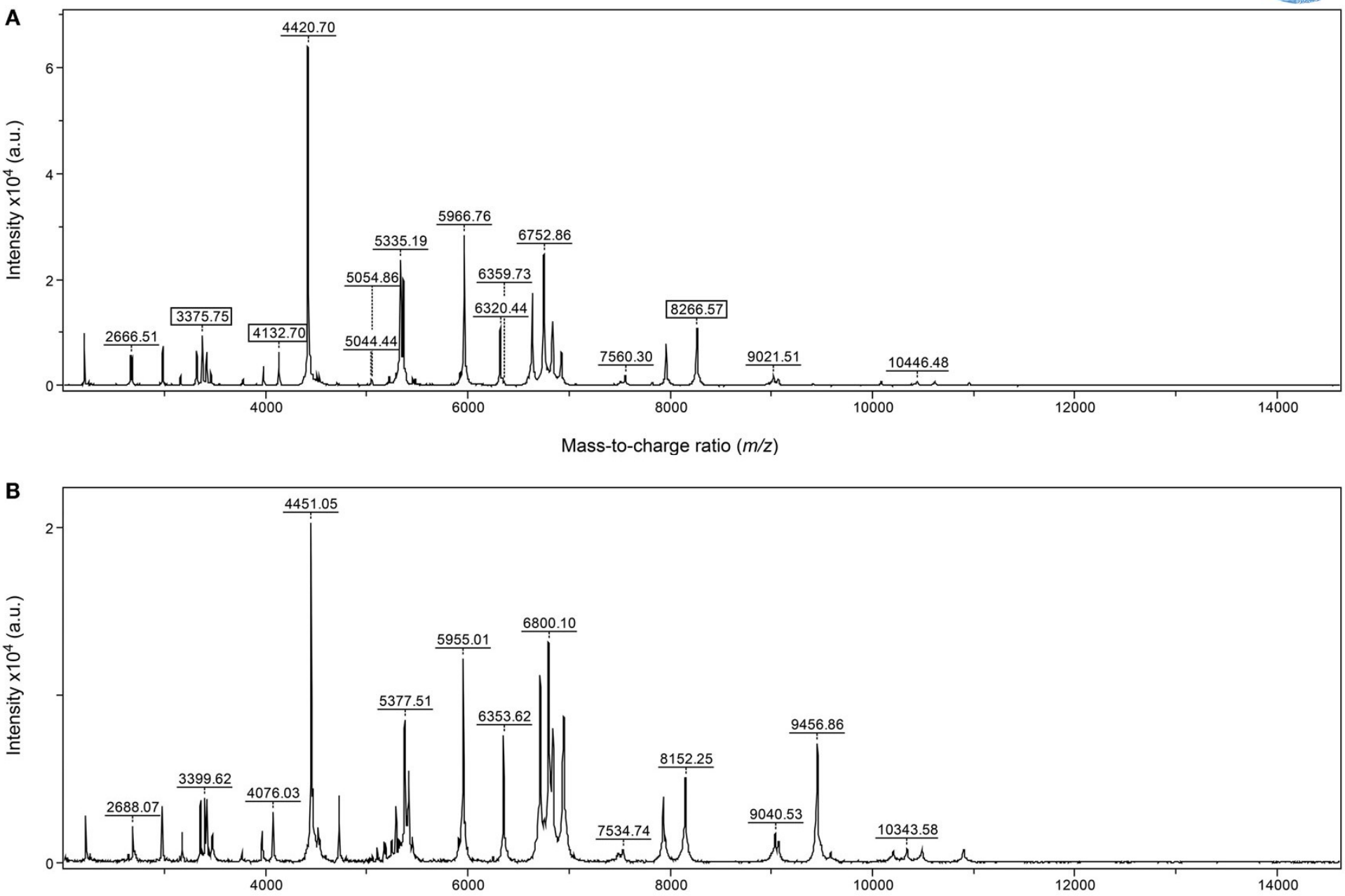
Matrix-assisted laser desorption ionization time-of-flight

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



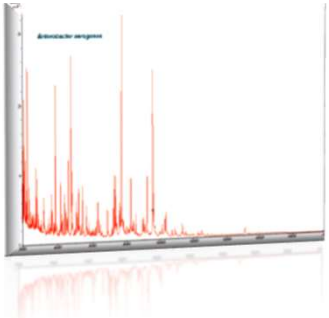
Clin Microbiol Infect 2010;16:1604


A
Intensity x10^4 (a.u.)
4420.70
5966.76
5335.19
6752.86
5054.86
6359.73
6320.44
3375.75
8266.57
2666.51
4132.70
5044.44
7560.30
9021.51
10446.48
Mass-to-charge ratio (m/z)
B
Intensity x10^4 (a.u.)
4451.05
6800.10
5955.01
5377.51
6353.62
9456.86
8152.25
3399.62
4076.03
2688.07
9040.53
7534.74
10343.58

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

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 (*mecA*, *vanA*, *vanB*)	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 (*mecA*, *vanA/B*, KPC, NDM)	88–100	94–100	1	On-demand, parallel miniaturized singleplex RT-PCR, full sample-to-result capability
GeneOHM StaphSR	*S. aureus*, MRSA	50–100	98–99	2	Batch, RT-PCR, offline manual sample lysis, extraction, and RT-PCR setup
Xpert MRSA/SA Blood Culture	*S. aureus*, 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 *A. fumigatus*	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, Ledeboer 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

FilmArray®
BCID Panel

BIOFIRE

www.BioFireDx.com

Run Summary

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

Result Summary - Interpretations

Antimicrobial Resistance Genes

✓ Detected	KPC (carbapenem-resistance gene)
⊘ N/A	*mecA* (methicillin-resistance gene)
⊘ N/A	*vanA/B* (vancomycin-resistance genes)

⚠ **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.

Gram Positive Bacteria

Not Detected	*Enterococcus*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus*
Not Detected	*Staphylococcus aureus*
Not Detected	*Streptococcus*
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)

Gram Negative Bacteria

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

Yeast

Not Detected	*Candida albicans*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*

Run Details

Pouch:	BCID Panel	**Protocol:**	BCID
Run Status:	Completed	**Operator:**	RJones
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
- $bla_{CTX\text{-}M}$
- bla_{IMP}
- bla_{KPC}
- *mcr-1*
- *mec*A/C and MREJ
- bla_{NDM}
- $bla_{OXA\text{-}48\text{-}like}$
- bla_{VIM}
- *van*A/B

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

J Clin Microbiol. 2016;54:687-98

TABLE 3 Performance summary of the BCID panel versus the comparator assays for bacteria in both clinical and seeded positive blood cultures combined

Organism	Isolates detected[a]: BCID/comparator		No. of results: BCID/comparator							
	Clinical arm	Seeded arm	TP +/+	FP +/−	FN −/+	TN −/−	Sensitivity or PPA[b]: TP/(TP + FN) (%)	95% CI	Specificity or NPA[b]: TN/(TN + FP) (%)	95% CI
Gram-positive bacteria										
Enterococcus	102/101	29/29	127	4	3	2,073	127/130 (97.7)	93.4–99.5	2,073/2,077 (99.8)	99.5–99.9
L. monocytogenes	0/0	36/36	36	0	0	2,171	36/36 (100)	90.3–100	2,171/2,171 (100)	99.8–100
Staphylococcus	780/797	2/1	770	12	28	1,397	770/798 (96.5)	95.0–97.7	1,397/1,409 (99.1)	98.5–99.6
S. aureus	257/257	0/0	253	4	4	1,946	253/257 (98.4)	96.1–99.6	1,946/1,950 (99.8)	99.5–99.9
Streptococcus	140/141	63/62	198	5	5	1,999	198/203 (97.5)	94.3–99.2	1,999/2,004 (99.8)	99.4–99.9
S. agalactiae (group B)	18/18	18/18	36	0	0	2,171	36/36 (100)	90.3–100	2,171/2,171 (100)	99.8–100
S. pneumoniae	26/25	12/12	36	2	1	2,168	36/37 (97.3)	85.8–99.9	2,168/2,170 (99.9)	99.7–100
S. pyogenes (group A)	8/7	31/31	38	1	0	2,168	38/38 (100)	90.7–100	2,168/2,169 (99.9)	99.7–100
Total	1,331/1,346	191/189	1,494	28	41	16,093	1,494/1,535 (97.3)	96.4–98.1	16,093/16,121 (99.8)	99.7–99.9
Gram-negative bacteria										
A. baumannii	16/14	40/37	51	5	0	2,151	51/51 (100)	93.0–100	2,151/2,156 (99.8)	99.5–99.9
Enterobacteriaceae	307/310	187/188	490	4	8	1,705	490/498 (98.4)	96.9–99.3	1,705/1,709 (99.8)	99.4–99.9
E. cloacae complex	24/22	17/17	38	3	1	2,165	38/39 (97.4)	86.5–99.9	2,165/2,168 (99.9)	99.6–100
E. coli	149/148	6/5	150	5	3	2,049	150/153 (98.0)	94.4–99.6	2,049/2,054 (99.8)	99.4–99.9
K. oxytoca	6/6	54/58	59	1	5	2,142	59/64 (92.2)[c]	82.7–97.4	2,142/2,143 (99.9)	99.7–100
K. pneumoniae	74/71	37/34	102	9	3	2,093	102/105 (97.1)	91.9–99.4	2,093/2,102 (99.6)	99.2–99.8
Proteus	22/22	17/17	39	0	0	2,168	39/39 (100)	91.0–100	2,168/2,168 (100)	99.8–100
S. marcescens	22/22	55/55	76	1	1	2,129	76/77 (98.7)	93.0–100	2,129/2,130 (99.9)	99.7–100
H. influenzae	8/8	35/35	43	0	0	2,164	43/43 (100)	91.8–100	2,164/2,164 (100)	99.8–100
N. meningitidis	1/1	35/35	36	0	0	2,171	36/36 (100)	90.3–100	2,171/2,171 (100)	99.8–100
P. aeruginosa	52/52	0/0	51	1	1	2,154	51/52 (98.1)	89.7–100	2,154/2,155 (99.9)	99.7–100
Total	681/676	483/481	1135	29	22	23,091	1,135/1,157 (98.1)	97.1–98.8	23,091/23,120 (99.9)	99.8–99.9

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

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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							
	Clinical arm	Seeded arm	TP +/+	FP +/−	FN −/+	TN −/−	Sensitivity or PPA[a]: TP/(TP + FN) (%)	95% CI	Specificity or NPA[a]: TN/(TN + FP) (%)	95% CI
mecA in association with:										
All *Staphylococcus* 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
Staphylococcus and *S. aureus* isolates detected	137/139	0/0	137	0	2	118	137/139 (98.6)	94.9–99.8	118/118 (100)	96.9–100
vanA/B in association with *Enterococcus* isolates detected	36/36	28/28	64	0	0	67	64/64 (100)[c]	94.4–100	67/67 (100)	94.6–100
bla_{KPC} in association with *Enterobacteriaceae* and/or *A. baumannii* and/or *P. aeruginosa* 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	SepsiTest	VYOO ®- Multiplex PCR
		Degradation of human DNA and free bacterial/fungal DNA	Isolation of methylated bacterial DNA
	Multiplex Real-time PCR	Broad-range PCR (16S or 18S rRNA)	Multiplex Real-time PCR
	25 most common pathogens	>345 bacterial and fungal species	34 bacterial and 6 fungal species
Detection limit:	**3-30 CFU/mL**	**20-40 CFU/ml for *S. aureus***	**3-10 CFU/mL**
Time:	**6 h**	**8-12 h**	**8 h**
	– Rapid amplification with highly specific melting point analysis – Semi-automated (manual: spin column based nucleic acid preparation)	– Detection of viable pathogens – Detection and identification of essentially any bacteria or yeast – Open system that can be run on standard lab equipment	– Rapid amplification with highly specific melting point analysis – Enrichment of pathogen DNA

Skvarc M Eur J Microbiol Immunol (Bp). 2013 Jun;3(2):97-104; Paulocci M Intern J Antimicrob Agents 2010;36S:S6

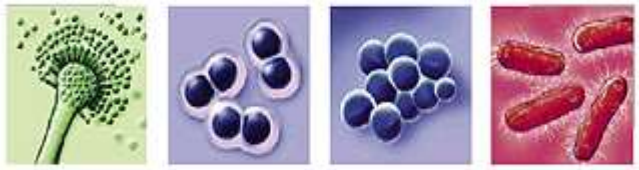
Septi*Fast* test

- 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

Septi*Fast* – 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

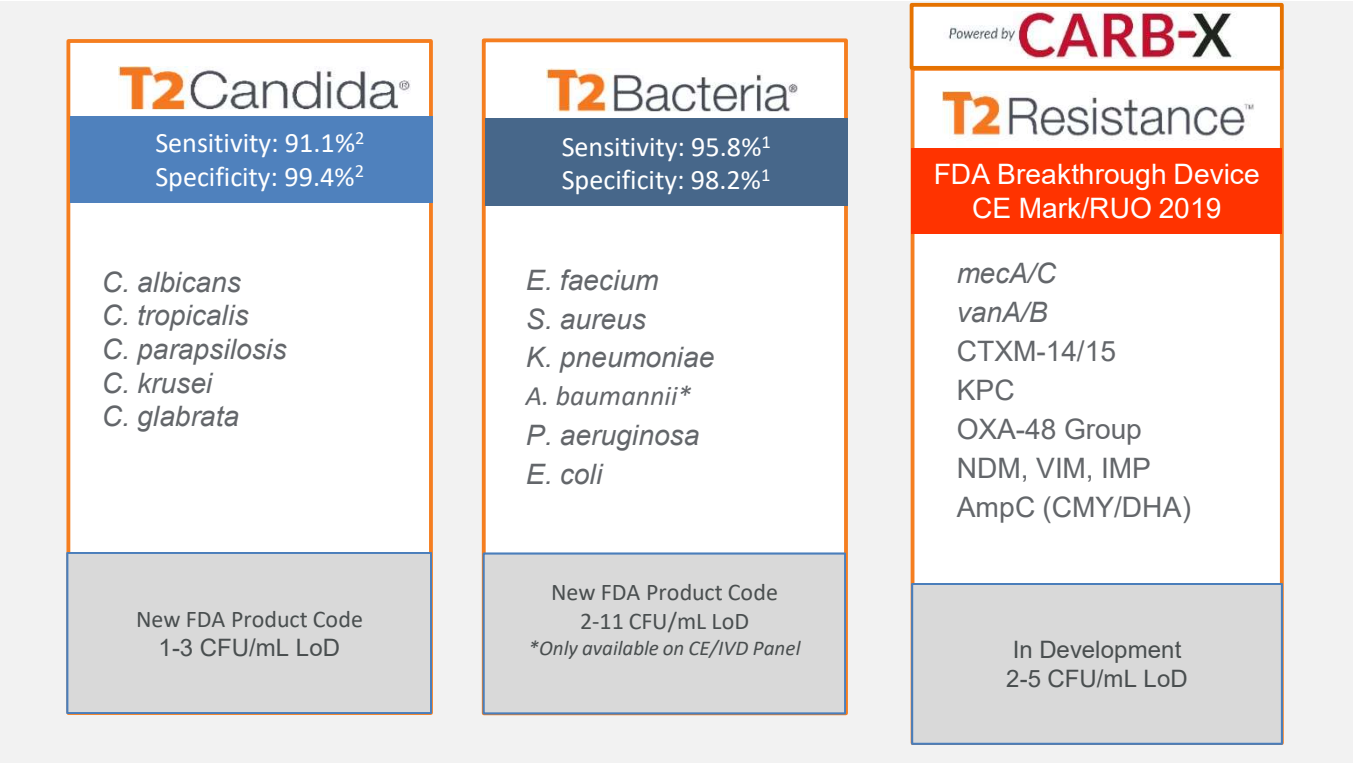
Gram-negative bacteria	Gram-positive bacteria	Fungi
Escherichia coli	*Staphyloccus aureus*	*Candida albicans*
Klebsiella (pneumoniae / oxytoca)	CoNS (Coagulase negative Staphylococci)	*Candida tropicalis*
Serratia marcescens	*Streptococcus pneumoniae*	*Candida parapsilosis*
Enterobacter (cloacae / aerogenes)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

CoNS: *S. epidermidis, S. haemolyticus, S. xylosus, S. hominis, S. cohnii, S. lugdunensis, S. saprophyticus, S. saprophyticus, S. capitis, S. pasteuri, 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 administrated 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
Addt'l Proven/Probable +	63	0
Total +	98/102 (96%)	39/102 (38%)

T2Candida Panel	T2 Panel Positive	Blood Culture Positive
Paired +	31	33
Addt'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 testing[1]

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. Goff[1]

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,*,1], 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

Gemelli
Fondazione Policlinico Universitario Agostino Gemelli IRCCS
Università Cattolica del Sacro Cuore

- 1200-bed tertiary-care teaching hospital[1]
- 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

LEE HEALTH
Caring People. Inspiring Health.

- 1423-bed not-for-profit community hospital[2]
- 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*

NewYork-Presbyterian

- 2600-bed academic medical center[3]
- 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.

Ochsner TGH Tampa General Hospital

- 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

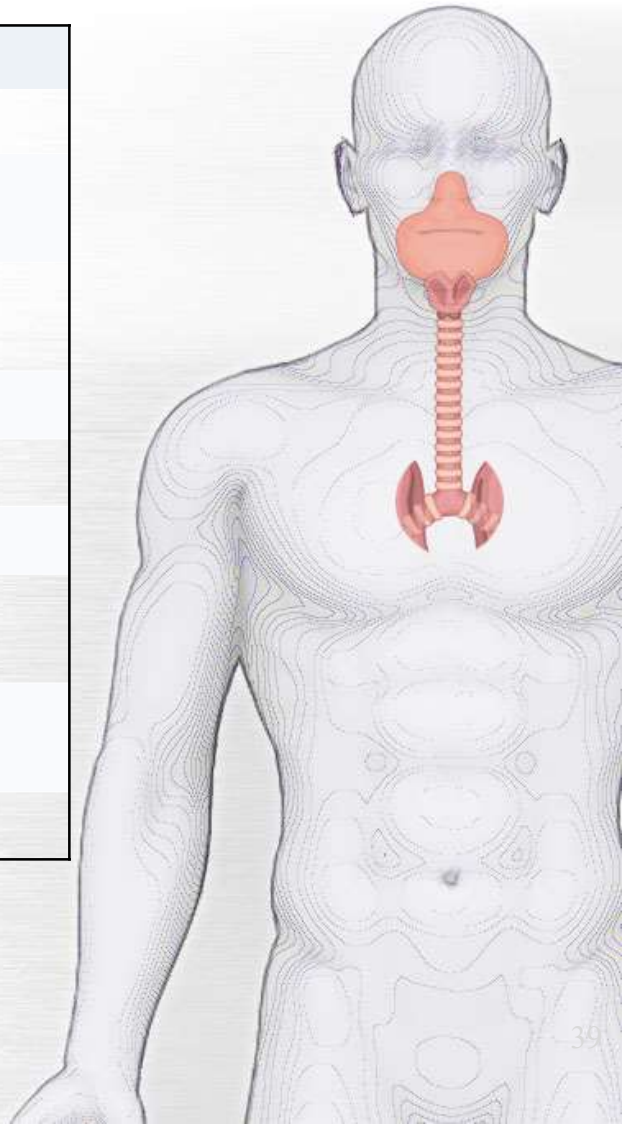
(1) De Angelis G et al. T2Bacteria magnetic resonance assay for the rapid detection of ESKAPEc pathogens directly in whole blood. J Antimicrob Chemother. 2018 Mar 1;73(suppl_4):iv20-iv26.
(2) Weisz E et al. MAD-ID 2018. (3) Walsh T et al. ECCMID-EIM 2019. (4) Voight C et al. ECCMID-EIM 2019

FilmArray, other applications: Detection of respiratory pathogens

FilmArray Respiratory Panel: 20 Pathogens

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

Nasopharyngeal sample



FilmArray®
Respiratory Panel

BIOFIRE

www.BioFireDx.com

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	*Bordetella pertussis*
Not Detected	*Chlamydophila pneumoniae*
Not Detected	*Mycoplasma pneumoniae*

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:

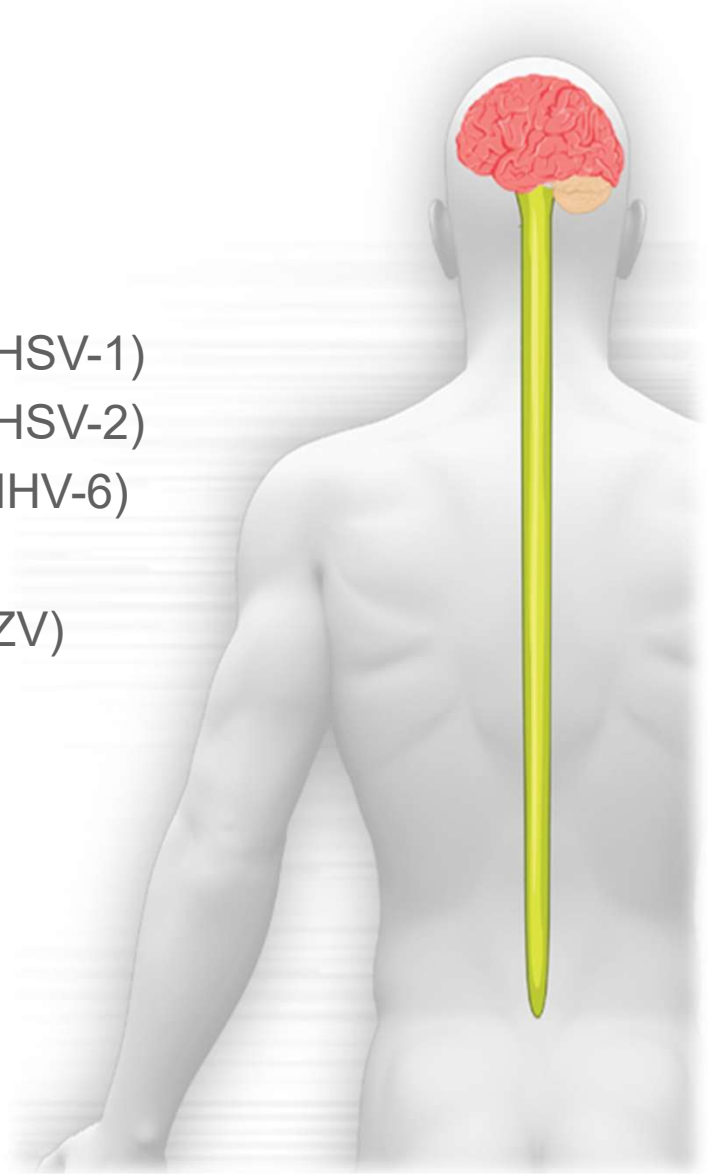
E. coli
H. influenzae
L. monocytogenes
N. meningitidis
S. agalactiae
S. pneumoniae

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)	**Atypical Bacteria (qualitative)**	**Fungi**
Acinetobacter calcoaceticus-baumannii complex	*Legionella pneumophila* (removed *Legionella* spp. assay)	*Pneumocystis jirovecii*
Serratia marcescens	*Mycoplasma pneumoniae*	*Aspergillus* spp.
Proteus spp.	*Chlamydophila pneumoniae*	*Cryptococcus* spp.
Klebsiella pneumoniae	**Viruses**	**ABR Markers**
Enterobacter aerogenes / Enterobacter cloacae complex	Influenza A	mecA/C – MREJ (MRSA)
Escherichia coli	Influenza B	KPC (Carbapenem resistance)
Haemophilus influenzae	Respiratory Syncytial virus	NDM (Carbapenem resistance)
Moraxella catarrhalis	Human Rhinovirus/Enterovirus	Oxa48-like (Carbapenem resistance)
Pseudomonas aeruginosa	Human Metapneumovirus	CTX-M (ESBL resistance)
Staphylococcus aureus	Parainfluenza virus	VIM (Carbapenem resistance)
Stenotrophomonas maltophilia	Adenovirus	IMP (Carbapenem resistance)
Streptococcus pneumoniae	Coronavirus	
Klebsiella oxytoca	MERS-CoV	
Streptococcus pyogenes		
Streptococcus agalactiae		

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*

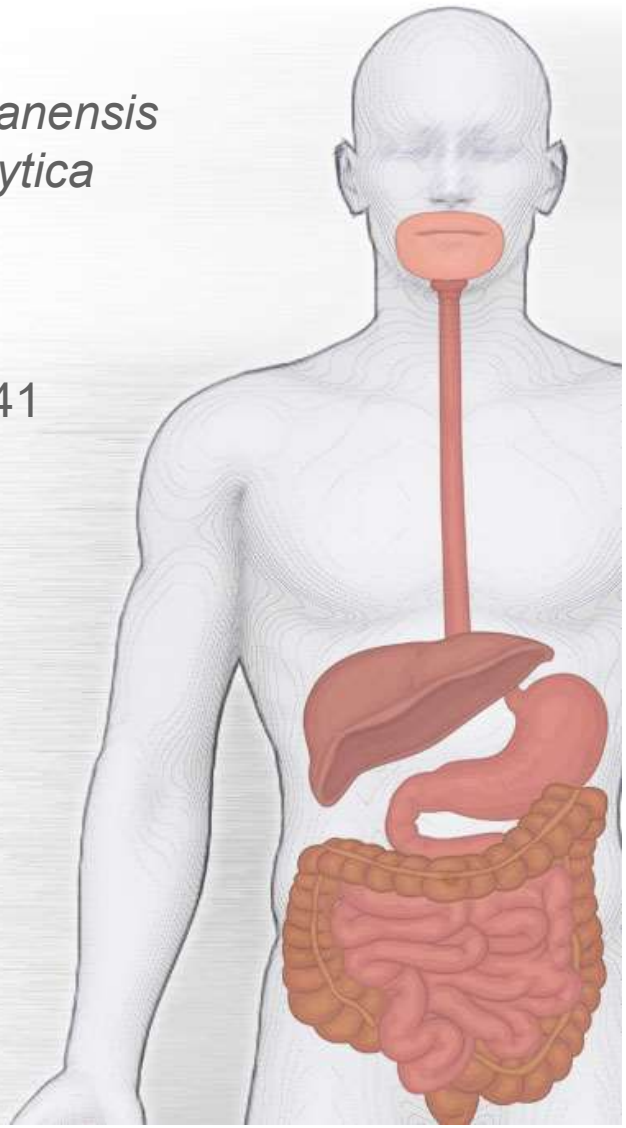
E. coli O157
Enteroaggregative *E. coli* (EAEC)
Enteropathogenic *E. coli* (EPEC)
Enterotoxigenic *E. coli* (ETEC)
Shiga-like toxin-producing *E. coli* (STEC)
Shigella/Enteroinvasive *E. coli* (EIEC)

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

ΛΟΙΜΩΞΕΙΣ ΚΑΤΩΤΕΡΟΥ ΑΝΑΠΝΕΥΣΤΙΚΟΥ:
ΤΑ ΠΑΘΟΓΟΝΑ ΑΙΤΙΑ ΠΟΙΚΙΛΟΥΝ

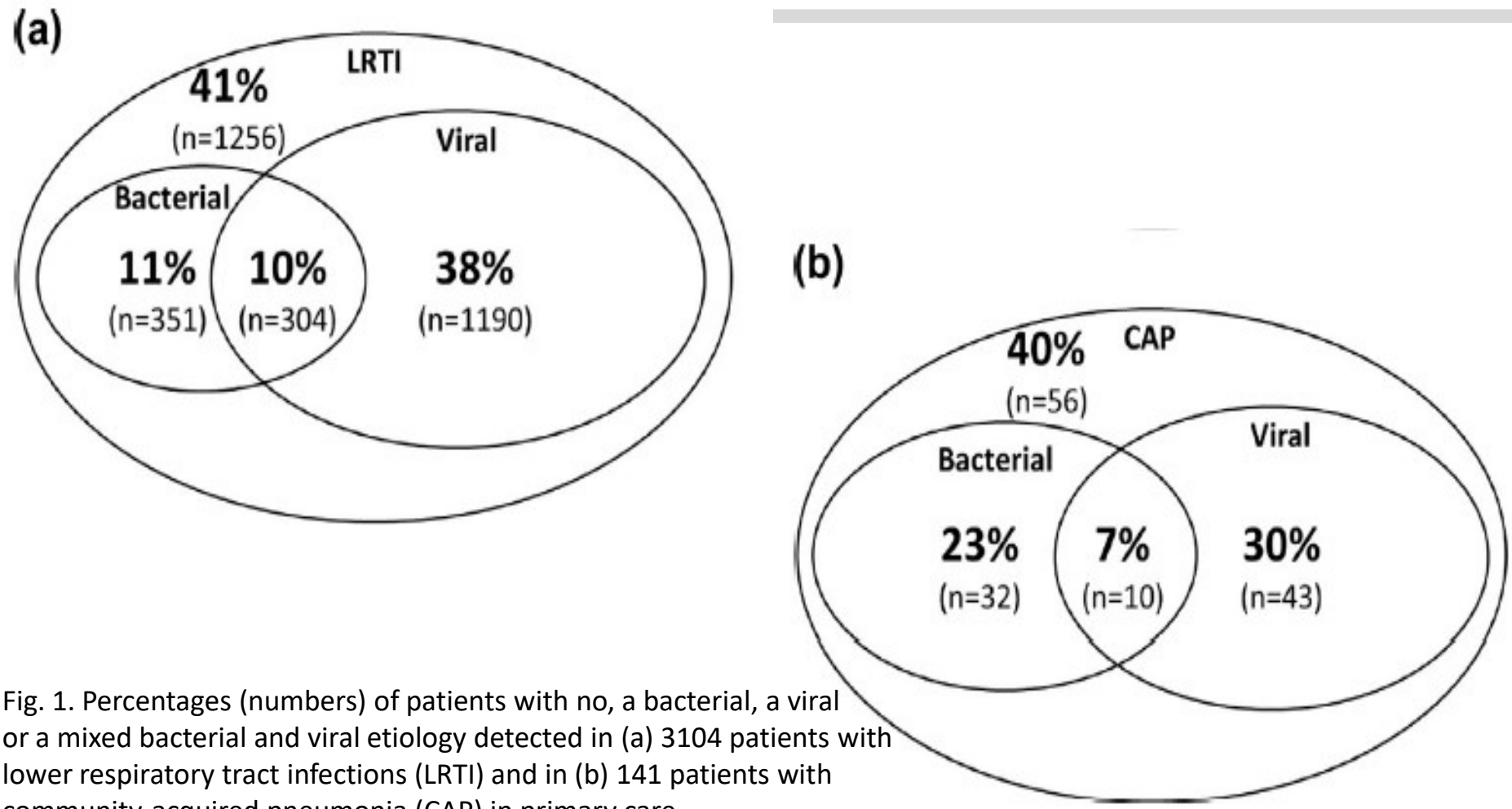


Fig. 1. Percentages (numbers) of patients with no, a bacterial, a viral or a mixed bacterial and viral etiology detected in (a) 3104 patients with lower respiratory tract infections (LRTI) and in (b) 141 patients with community-acquired pneumonia (CAP) in primary care.

Leven M, et al., Clin Microbiol and Infect (2018), https://doi.org/10.1016/j.cmi.2018.02.004

PNEUMONIA PANEL*PLUS* - 34 TARGETS

Bacteria

15 Common Bacteria with bins results

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

Methicilin Resistance

mecA/*mecC* and MREJ

7 antimicrobial resistance markers

ESBL

CTX-M

Carbapenemases

KPC
NDM
Oxa48-like
VIM
IMP

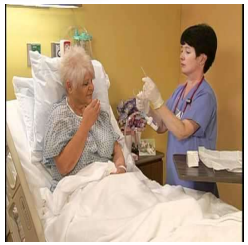
TWO APPROVED SAMPLE TYPES

Sputum like

Expectorated sputum

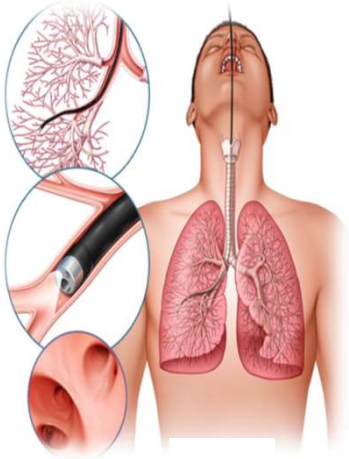
& Induced sputum:

No Pretreatment



EndoTracheal Aspirates (ETA)

Bronchoalveolar lavage (BAL) like



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

SAMPLES NOT VALIDATED

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

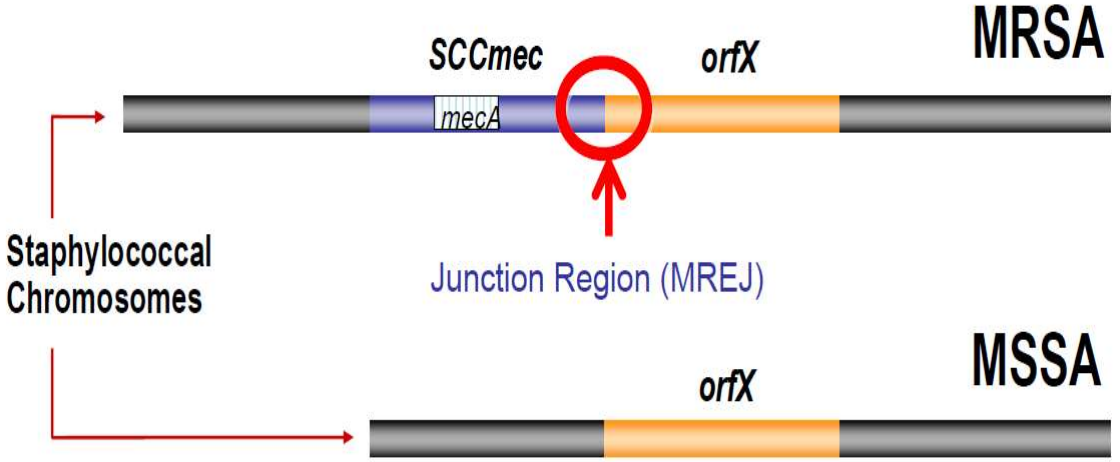
SEMI-QUANTITATIVE REPORTING

- 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	≥10^3.5 – <10^4.5 copies/mL	Detected	10^4 copies/mL
Positive AND	≥10^4.5 – <10^5.5 copies/mL	Detected	10^5 copies/mL
Positive AND	≥10^5.5 – <10^6.5 copies/mL	Detected	10^6 copies/mL
Positive AND	≥10^6.5 copies/mL	Detected	≥10^7 copies/mL

MRSA & MSSA

- MRSA are resistant to the whole class of β-lactams (including Penicillins and Cephalosporins)



- **The resistance is due to the presence of the mecA/C gene that is acquired by *S. aureus* and is included in the region named SCCmec cassette** (staphylococcal cassette chromosome mec). **The presence of this cassette can be confirmed by detecting its junction in *S. aureus* genome « MREJ »**
- **The SSCmec cassette can be found in other Staphylococcus species.**
- **Therefore, only a test detecting a specific *S. aureus* gene + mecA/C + the cassette junction can reliably answer for a MRSA positive result**

mecA/C and MREJ

- Both *mecA/C* & MREJ must be detected to be taken into account :

mecA/C assay	MREJ assay	mecA/C and MREJ result
Positive	Positive	Detected
Positive	Negative	Not detected
Negative	Positive	Not detected
Negative	Negative	Not detected

- Resistance marker is only reported if *S. aureus* is reported :

mecA/C and MREJ Result	*Staphylococcus aureus*	INTERPRETATION
Detected	Detected	MRSA positive
Not Detected	Detected	MSSA
N/A	Not Detected	MSSA/MRSA negative

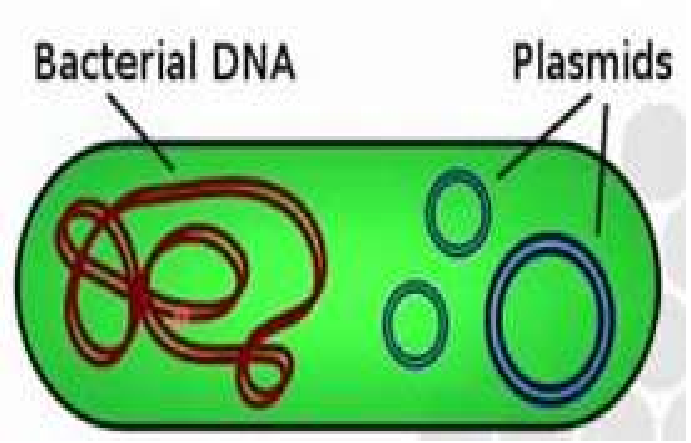
ANTIBIOTIC RESISTANCE GENES

- 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
Acinetobacter calcoaceticus-baumannii **complex** ***Pseudomonas aeruginosa***	**CTX-M** **KPC** **VIM** **IMP** **NDM**	**ESBLs** **Carbapenemases**
Klebsiella aerogenes ***Enterobacter cloacae*** **complex** ***Escherichia coli*** ***Klebsiella oxytoca*** ***Klebsiella pneumoniae*** ***Proteus spp.*** ***Serratia marcescens***	**CTX-M** **KPC** **VIM** **IMP** **NDM** **OXA-48-like**	**ESBLs** **Carbapenemases**

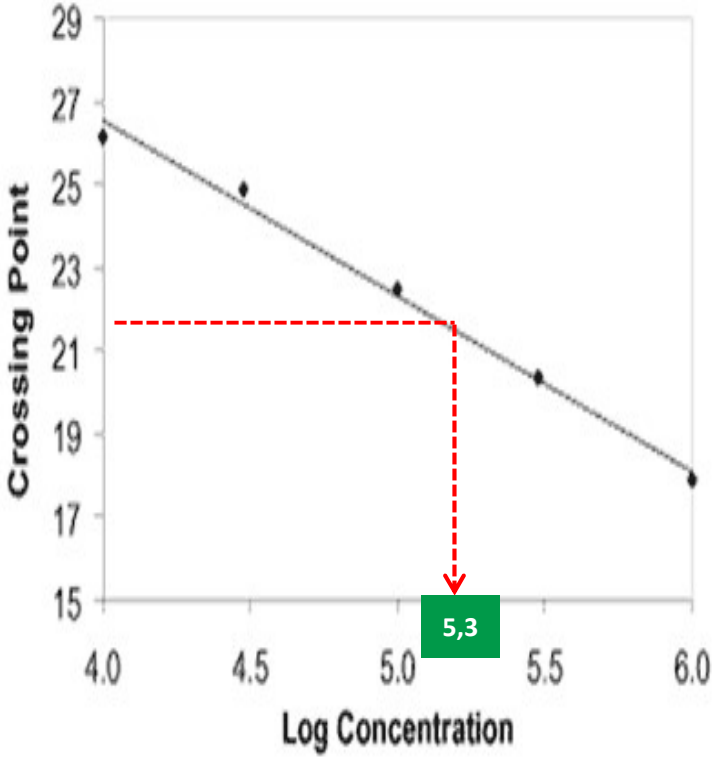
THE PNEUMONIA PANEL ESBL/CARBAPENEMASES AMR GENES: **ΠΕΡΙΟΡΙΣΜΟΊ**

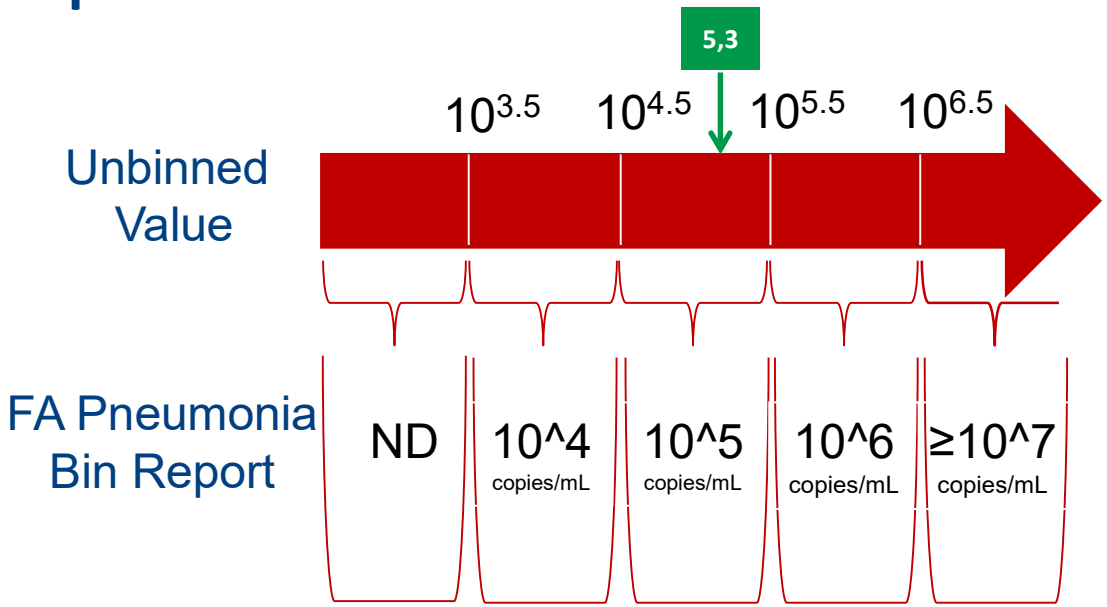
- Resistance genes are carried on plasmids or mobile elements
- They are frequently not integrated into the bacterial genome
- They can “jump” from one organism to another
- It is possible to detect an AMR gene from an organism that is not detected by the panel, if another applicable organism is detected by the panel



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 1 : “detection summary” showing only the detected analytes

FilmArray
Pneumonia Panel *plus* - IVD

BIOFIRE
www.BioFireDx.com

Run Information			
Sample ID	Example Report	Run Date	12 Jul 2016 12:00 AM
Protocol	Sputum v3.3	Serial No.	01234567
Pouch Type	Pneumoplus v2.0	Lot No.	012345
Controls	Passed	Operator	Anonymous
Run Status	Completed	Instrument	FA0000

Detection Summary

Bacteria

		Bin (copies/mL)		Bin (copies/mL)			
				10^4	10^5	10^6	≥10^7
Detected:	✓	≥10^7	*Klebsiella pneumoniae* group				
	✓	10^6	*Streptococcus pyogenes*				
	✓	10^4	*Haemophilus influenzae*				

Note: Detection of bacterial nucleic acid may be indicative of colonizing or normal respiratory flora and may not indicate the causative agent of pneumonia. Semi-quantitative BIN (copies/mL) results generated by the FilmArray Pneumonia Panel *plus* are not equivalent to CFU/mL and do not consistently correlate with the quantity of bacterial analytes compared to CFU/mL. For specimens with multiple bacteria detected, the relative abundance of nucleic acids (copies/mL) may not correlate with the relative abundance of bacteria as determined by culture (CFU/mL). Clinical correlation is advised to determine significance of semi-quantitative BIN [copies/mL] for clinical management.

Antimicrobial Resistance Genes

Detected: ✓ CTX-M

Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for a genetic marker of antimicrobial resistance does not indicate susceptibility to associated antimicrobial drugs or drug classes. A Detected result for a genetic marker of antimicrobial resistance cannot be definitively linked to the microorganism(s) detected. Culture is required to obtain isolates for antimicrobial susceptibility testing, and FilmArray Pneumonia Panel *plus* results should be used in conjunction with culture results for the determination of susceptibility or resistance.

Atypical Bacteria

Detected: None

Viruses

Detected: ✓ Influenza A

Equivocal: ⇹ Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

2 PAGES REPORT

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

Result Summary

Bacteria

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

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

⚠ **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.

Atypical Bacteria	
Not Detected	*Chlamydia pneumoniae*
Not Detected	*Legionella pneumophila*
Not Detected	*Mycoplasma pneumoniae*

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

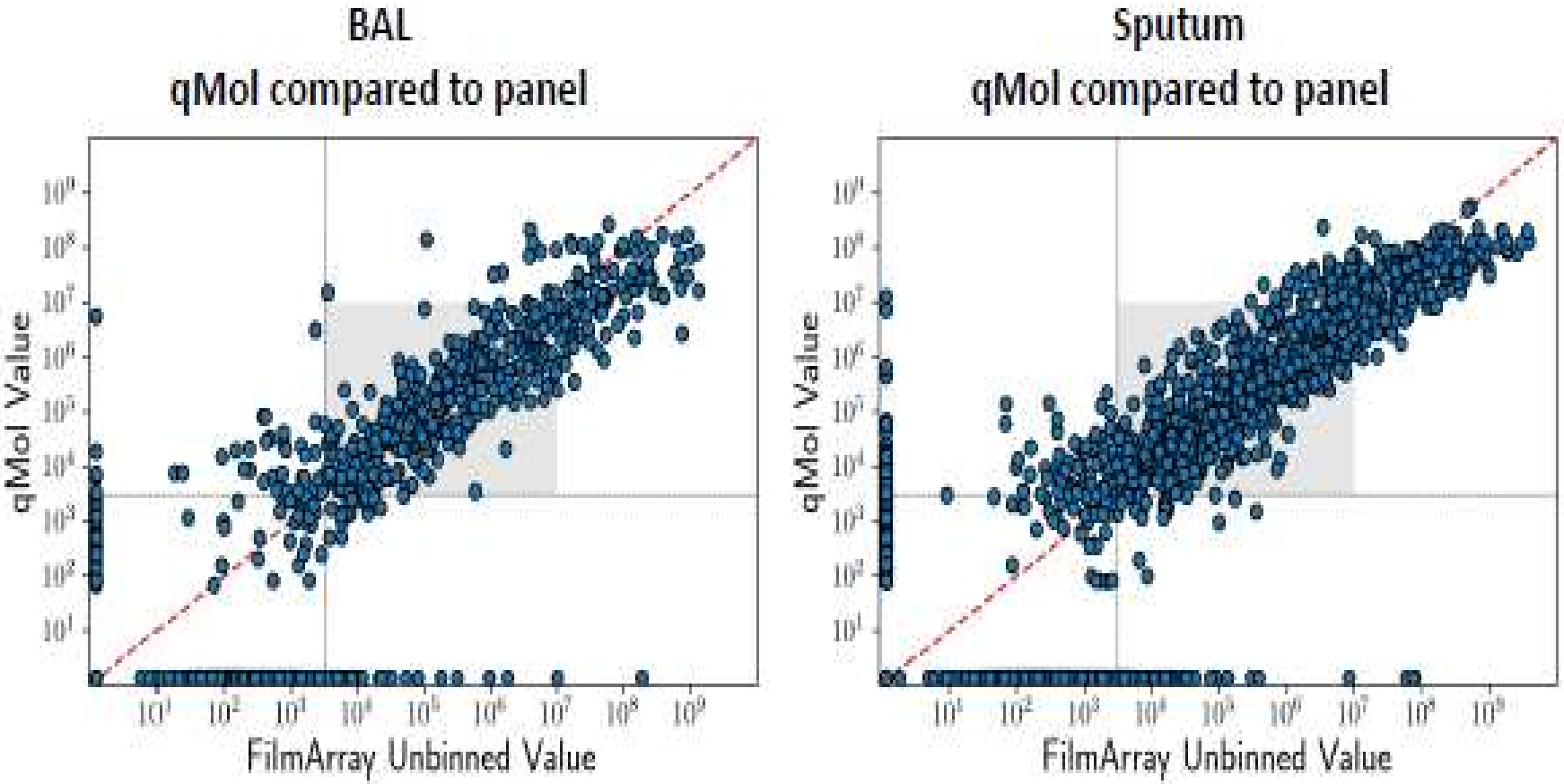
For not detected target it will not be possible to view the melting curves as for the other panels.

COMPARISON OF PNEUMONIA PANEL TO SOC

- FilmArray® Pneumonia is measuring genome copies/ml and culture is measuring CFU (colony forming units)
- There will not be a 1:1 relationship between those 2 results
- Generally, the FilmArray® Pneumonia will over quantify relative to SOC cultures

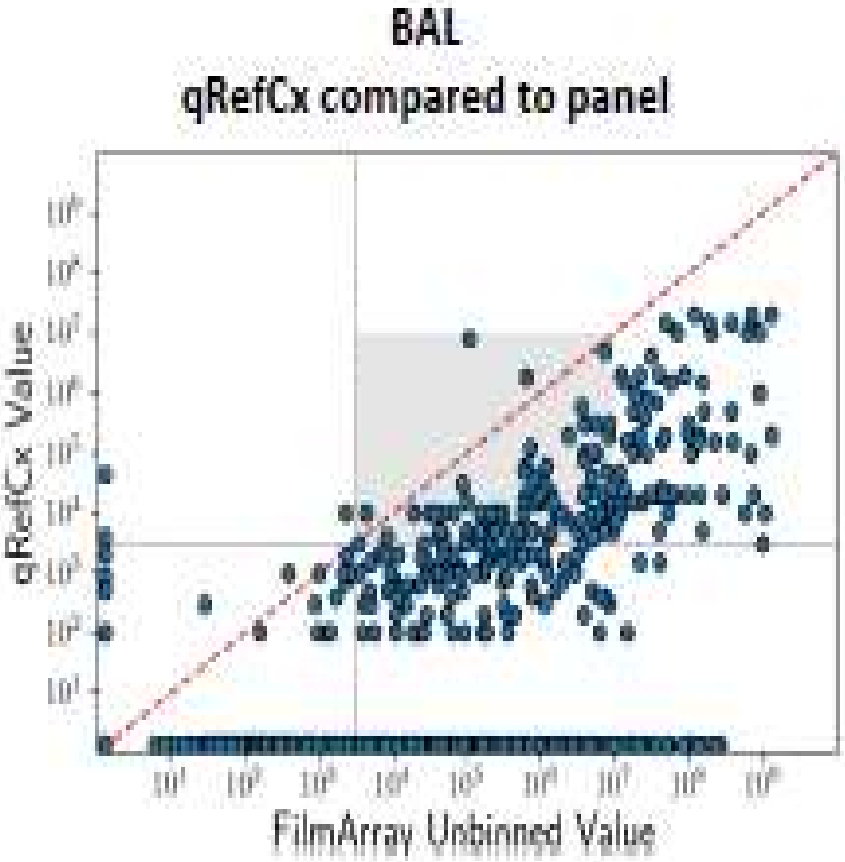
REPRODUCIBILITY OF FILMARRAY RESULTS VS Q-PCR

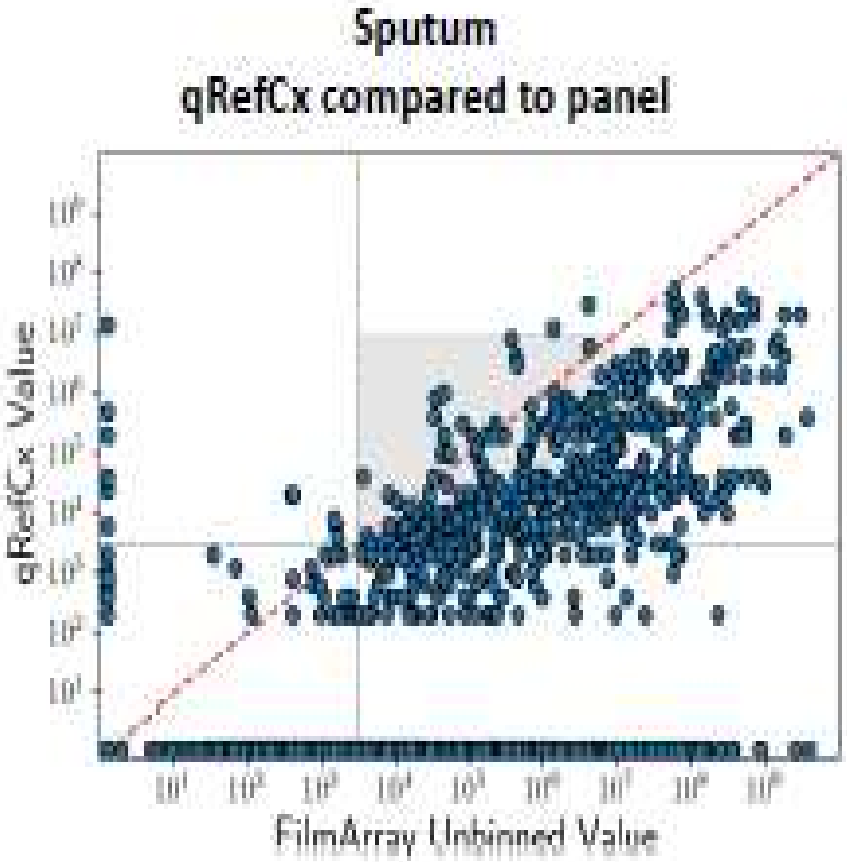
- This figure shows how the Pneumonia Panel plus unbinned (raw) values on the *x* axis compares with the qMol (quantitative reference molecular method) on *y* axis. The correlation is very high across all values.



COMPARISON OF FILMARRAY RESULTS VS CULTURE

- This figure shows how the Pneumonia Panel plus unbinned (raw) values on the *x* axis compares with the qRefCx (reference culture based method) on *y* axis. The Pneumonia panel is over quantifying over culture methods.

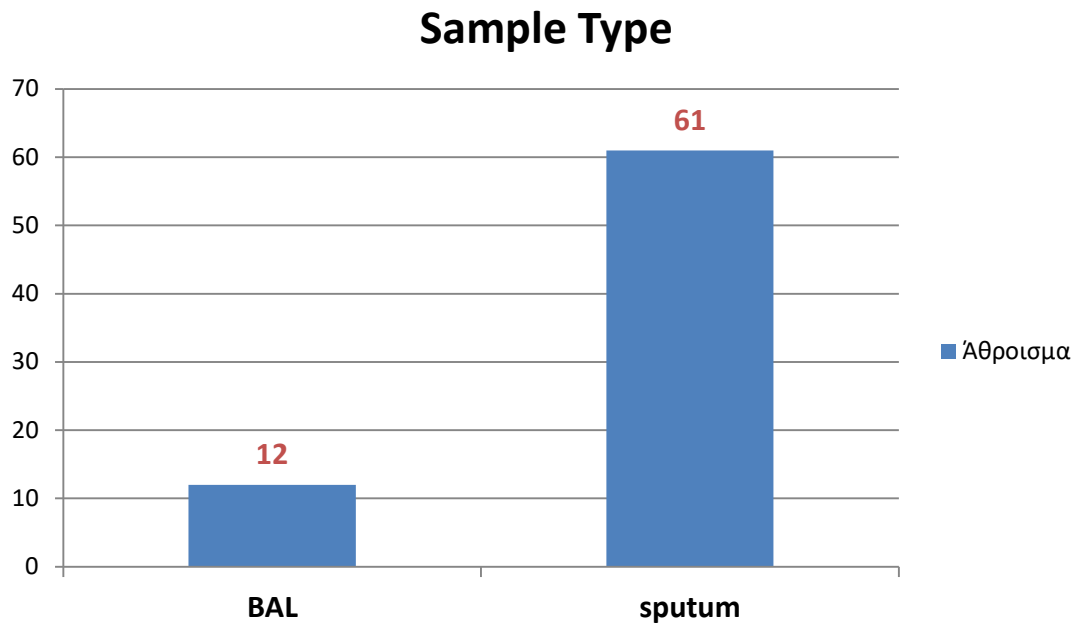


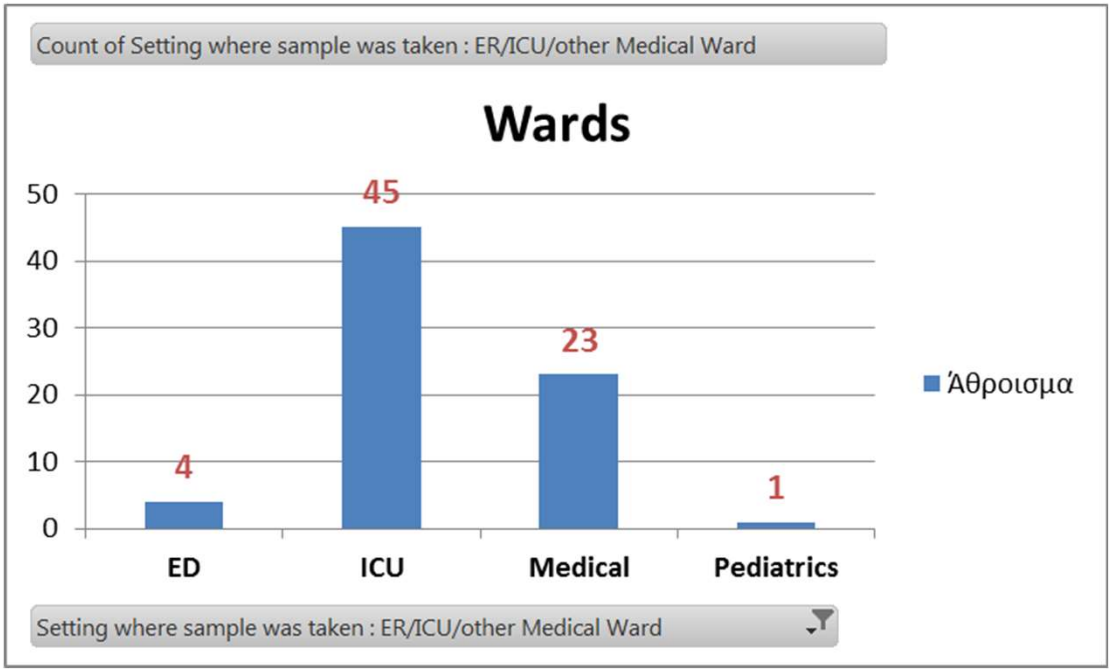


Πολυκεντρική Μικροβιολογική Αξιολόγηση του FilmArray® Pneumonia panel

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

- ΠΓΝ ΑΤΤΙΚΟ
- ΓΝ ΤΖΑΝΕΙΟ
- ΕΥΡΩΚΛΙΝΙΚΗ


Sample Type
70
60
50
40
30
20
10
0
61
12
BAL
sputum
Άθροισμα

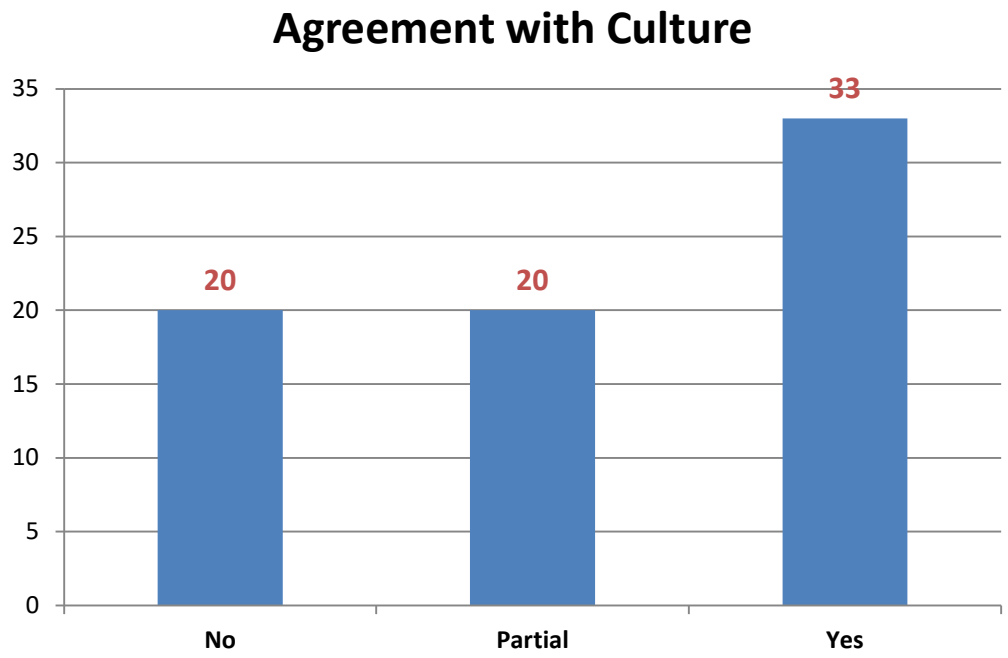

Count of Setting where sample was taken : ER/ICU/other Medical Ward
Wards
50
40
30
20
10
0
45
23
4
1
ED
ICU
Medical
Pediatrics
Άθροισμα
Setting where sample was taken : ER/ICU/other Medical Ward

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. marscescens 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

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

When Culture result= 3+ , FilmArray ≥ 10^6 copies/ml



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	*S. marcesens*	1 Detection	Influenzae A	1 Detection	No	FilmArray correct
A	sputum	Pathology	Negative	No Growth	*S. aureus*	1 Detection	No	FilmArray correct
A	sputum	ICU	Negative	No Growth	*Legionella pneumophila*	1 Detection	No	FilmArray correct
A	sputum	Pathology	Negative	No Growth	*Mycoplasma pneumoniae*	1 Detection	No	FilmArray correct
A	BAL	Pathology	Negative	No Growth	*Mycoplasma pneumoniae*	1 Detection	No	FilmArray correct
A	BAL	Pathology	Negative	No Growth	*Mycoplasma pneumoniae*	1 Detection	No	FilmArray correct
A	sputum	ICU	*S. maltophilia*	1 Detection	None detected	Negative	No	Culture Correct
A	sputum	Pathology	Negative	No Growth	*S. aureus*	1 Detection	No	FilmArray correct
A	sputum	Pathology	Negative	No Growth	*M. Catarrhalis*	2 Detections	No	FilmArray correct
A	sputum	Pathology	Negative	No Growth	*S. marcescens*	1 Detection	No	FilmArray correct
B	sputum	ICU	Negative	No Growth	*A. baumannii*	1 Detection	No	FilmArray correct
B	sputum	ICU	Negative	No Growth	*A. baumannii*	1 Detection	No	FilmArray correct
B	sputum	ICU	*A. baumannii*	1 Detection	None detected	Negative	No	Culture Correct
C	Sputum	ED	Normal oral flora	No Growth	*H. Infuenzae*	2 Detection	No	FilmArray correct
C	Sputum	Pathology	Normal oral flora	No Growth	*E. aerogenes*	2 Detection	No	FilmArray correct
C	Sputum	Pathology	Normal oral flora	No Growth	*H. infuenzae , S. aureus*	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	*E. Coli*	*A.baumanii , K.pneumoniae, E.cloacae, E.Coli*	Partial
A	sputum	ICU	*E. Coli*	*E.Coli, P.aeruginosa*	Partial
A	sputum	ED	*S. marcesens*	*A.baumanii ,S.marcescens, H.influenzae*	Partial
B	sputum	ICU	*A. baumannii, P. mirabilis, K. oxytoca,*	*A. baumannii, P.mirabilis, K.o xytoca, P. aeruginosa*	Partial
B	sputum	ICU	*K. pneumoniae, S. marcescens A. baumannii*	*S. marscescens, A.baumannii*	Partial
B	sputum	ICU	*P. Aeruginosa*	*P. aeruginosa, A. baumannii*	Partial
B	sputum	ICU	*K. pneumoniae, S. marcescens A. baumannii*	*A. baumannii, K. pneumoniae KPC*	Partial
B	sputum	ICU	*A. Baumannii*	*S. marscescens, A. baumannii,*	Partial
B	sputum	ICU	*P. aeruginosa*	*P. aeruginosa, K. pneumoniae*	Partial
B	sputum	ICU	*A. baumannii*	*A. baumannii, H. influenzae*	Partial
C	Sputum	Pathology	*P. aeruginosa, S. marcescens, Enterobacter spp*	*P. aeruginosa*	Partial
C	BAL	ICU	*A. baumannii, K. pneumoniae, S. maltophilia*	*A. baumanii, K. pneumoniae group*	Partial
C	BAL	ICU	*A. baumannii, K. pneumoniae,*	*A. baumanii, K. pneumoniae group , P. aeruginosa*	Partial
C	Sputum	Card	*A. baumannii, K. pneumoniae, S. aureus, A. xylosoxidans*	*A. baumanii, K. pneumoniae group , S. aureus*	Partial
C	BAL	ICU	*P. Aeruginosa*	*A .baumanii, P. aeruginosa*	Partial
C	BAL	ICU	*A. baumannii, K. pneumoniae, P. aeruginosa, E. coli*	*A. baumanii, K. pneumoniae group , P. aeruginosa , E. coli , H.influenzae*	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	*K. pneumoniae*, S. marcescens, A. baumannii	*A. baumannii* , K. pneumoniae	KPC
Sputum	ICU	P. aeruginosa	*P. aeruginosa , K. pneumoniae*	CTX-M, KPC, NDM, OXA-48
Sputum	ICU	K. pneumoniae A. baumannii	*A. baumannii* K. pneumoniae	NDM
BAL	ICU	K. pneumoniae, A. baumannii	*A. baumannii* , K. pneumoniae	KPC
Sputum	Pathology	K. pneumoniae, P. aeruginosa S. aureus	K. pneumoniae group , P. aeruginosa S. aureus	mecA/C and MREJ
BAL	ICU	*A. baumannii, K. pneumoniae, Stenotrophomonas maltophilia*	A. baumannii , Klebsiella pneumoniae group	KPC, CTX-M
BAL	ICU	*A. baumannii, K. pneumoniae*	A. baumannii, Klebsiella pneumoniae group , P. aeruginosa	KPC
BAL	ICU	*A. baumannii, K. pneumoniae, P. Aeruginosa*	*A. baumannii*, K. pneumoniae P. Aeruginosa	KPC
BAL	ICU	*A. baumannii, K. pneumoniae, P. aeruginosa*	A. baumannii , K. pneumoniae ,P. aeruginosa	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

- ✓ **<u>Συμφωνία αποτελέσματος:</u>** *FilmArray Pneumo Plus* στο **BAL συγκριτικά με βρογχικές** των ίδιων ασθενών παρατηρήθηκε σε 11 από τους 15 ασθενείς

- ✓ **<u>Αρνητικά δείγματα: (N=9)</u>** 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**	*A. baumannii*, 10^5 cfu/ml	*A. baumannii*, 10^6 copies/ml	**Yes**
	Βρογχικές	*A. baumannii*, $>10^6$ cfu/ml	*A. baumannii*, $\geq 10^7$ copies/ml	
Nb 3.	**BAL**	*S. maltophilia**, 10^4 cfu/ml	Human Rhinovirus/enterovirus	**Yes**
	Βρογχικές	*S. maltophilia**, 10^4 cfu/ml	Human Rhinovirus/enterovirus	
Nb 5 10/	**BAL**	1. *A. baumannii*, $>10^5$ cfu/ml	1. *A. baumannii*, $\geq 10^7$ copies/ml	**Yes**
		2. *P. aeruginosa*, $>10^5$ cfu/ml	2. *P. aeruginosa*, $\geq 10^7$ copies/ml	
			3. *S. aureus*, 10^6 copies/ml	
			4. *E. cloacae*, 10^4 copies/ml	
			5. Parainfluenza virus	
	Βρογχικές	1. *A. baumannii*, $>10^6$ cfu/ml	1. *A. baumannii*, $\geq 10^7$ copies/ml	
		2. *P. aeruginosa*, $>10^6$ cfu/ml	2. *P. aeruginosa*, $\geq 10^7$ copies/ml	
			3. *S. aureus*, $\geq 10^7$ copies/ml	
			4. *E. cloacae*, 10^5 copies/ml	
			5. Parainfluenza virus	
Nb 7. 11/1	**BAL**	*A. baumannii*, $>10^5$ cfu/ml	1. *A. baumannii*, 10^6 copies/ml	**Yes**
			2. *Serratia marcescens*, 10^5 copies/ml	
	Βρογχικές	*A. baumannii*, $>10^6$ cfu/ml	1. *A baumannii*, $\geq 10^7$ copies/ml	
			2. *Serratia marcescens*, 10^4 copies/ml	
Nb 9 16/1	**BAL**	*Candida* spp.*	Influenza A	**Yes**
	Βρογχικές	*Candida* spp.*	Influenza A	
Nb 12 21/1	**BAL**	*A. baumannii*, 10^2 cfu/ml	*A. baumannii*, 10^5 copies/ml	**Yes**
			CTX-M	
	Βρογχικές	1. *A baumannii*, 10^5 cfu/ml	*A. baumannii*, 10^6 copies/ml	
		2. *K. pneumoniae*, 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	*Candida* spp.*	1. *S. aureus* MRSA, 10^4 copies/ml 2. Influenza A	
Nb 4. 3/1/2019	**BAL**	Αρνητική	None detected	No
	Βρογχικές	*Candida* spp.*	1. *Serratia marcescens*, 10^4 copies/ml **2.** Human Rhinovirus/enterovirus	
Nb 14 30/1	**BAL**	Αρνητική	Influenza A	Yes
	Βρογχικές	*Candida* spp.*	Influenza A	
Nb 15 24/1	**BAL**	Αρνητική	None detected	No
	Βρογχικές	*A. baumannii*, 10^2 cfu/ml (2 αποικίες μόνο)	Influenza A	
Nb 1.	**BAL**	Αρνητική	None detected	No
	Βρογχικές	*A. baumannii*, 10^5 cfu/ml	*A. baumannii*, 10^4 copies/ml	

THANK YOU FOR YOUR ATTENTION!