Advancement in Molecular Diagnosis in Infectious Diseases

INTEGRATION OF RAPID DIAGNOSIS INTO LABORATORY OPERATIONS

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Clinical Microbiology Laboratory in 2000s

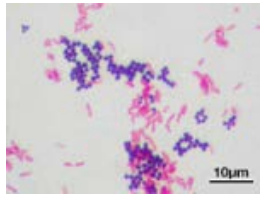
Bacteriology

- Blood culture & CSF
- Urine
- Sputum
- Genital tract
- Stool
- Miscellaneous
  - Wound swab, Tissue, PD Fluid, Pleural fluid, corneal scraping etc etc etc etc…

Mycobacteriology

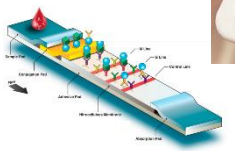
Mycology

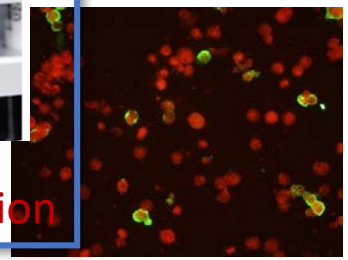
Parasitology



Virology

Molecular diagnostics





Antigen/Antibody detection

Clinical Microbiology Laboratory in 2020s

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- Urine
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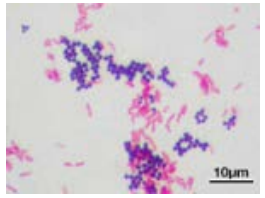

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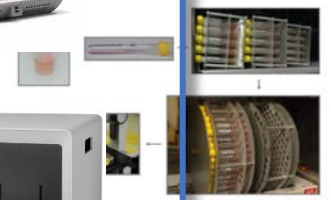

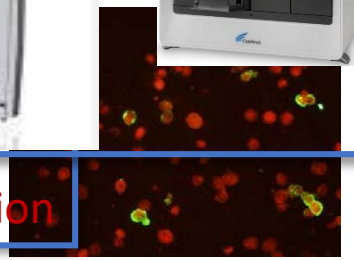

Virology

Antigen/Antibody detection

The need for improved diagnostics for infectious diseases

Better Tests, Better Care: Improved Diagnostics for Infectious Diseases

IDSA PUBLIC POLICY

Table 3. Potential of New Technologies to Address Unmet Clinical Needs

Unmet Need	Example of Pathogen/Syndrome	Potential Technologies
Rapid testing from clinical specimen (≤60 minutes)	HSV-1/2, VZV, enterovirus, parechovirus, influenza, RSV, bacterial resistance (KPC, NDM-1)	Single-step molecular cartridge-based tests
Rapid testing from clinical isolate (≤60 minutes)	Bacterial, fungal, or mycobacterial isolate	MALDI-TOF MS, single-step molecular cartridge-based tests
POC or near-patient testing (≤60 minutes)	Respiratory infections (viral and bacterial), meningitis	Single-step molecular cartridge-based tests, handheld devices for molecular testing, LAMP coupled with Biosensors
Simplicity (CLIA waived)	Influenza, tuberculosis, malaria	Handheld devices for molecular testing, single-step molecular cartridge-based tests
Syndromic testing	Sepsis, pneumonia (HAP, VAP, CAP), meningitis, diarrheal diseases	Highly multiplexed single-step molecular cartridge-based tests, PCR coupled with T2 magnetic resonance
Screening for infection	Biomarkers to distinguish infection from no infection, bacterial from viral infection	Biosensors, biomarkers
Resource-constrained settings	HIV-1, tuberculosis, malaria	Handheld devices for molecular testing, single-step molecular cartridge-based tests
Infection control/hospital epidemiology	Outbreak evaluations of multidrug-resistant organism, rapid strain typing	Next-generation sequencing
Discovery of emerging pathogens	Influenza A H5 and H7, MERS-CoV	PCR coupled with ESI-TOF, next-generation sequencing

Abbreviations: CAP, community-acquired pneumonia; CLIA, Clinical Laboratory Improvement Amendments; ESI-TOF, electrospray ionization time-of-flight; HAP, hospital-associated pneumonia; HIV-1, human immunodeficiency virus type 1; HSV-1/2, herpes simplex viruses 1 and 2; KPC, *Klebsiella pneumoniae* carbapenemase; LAMP, loop-mediated amplification; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MERS-CoV, Middle Eastern respiratory syndrome coronavirus; NDM-1, New Delhi metallo-β-lactamase 1; PCR, polymerase chain reaction; POC, point-of-care; RSV, respiratory syncytial virus; VAP, ventilator-associated pneumonia; VZV, varicella zoster virus.

"…results should be available within 1-2 hours and hence inform critical patient management decisions"

Caliendo AM, Clin Infect Dis. 2014 May;58(9):1346]. *Clin Infect Dis*. 2013;57 Suppl 3(Suppl 3):S139-S170. doi:10.1093/cid/cit578

Key opportunities in Clinical Microbiology

Topic	Key opportunities	Key challenges	Next steps
Widespread use of NGS in clinical microbiology	Rapid strain typing Direct detection from clinical specimens Detection of resistance genes and virulence factors	Defining the specific clinical need Lack of standardized databases, interpretation, and quality control protocols Cost relative to conventional methods	Create guidelines to standardize best laboratory practices
Big data in clinical microbiology	Antimicrobial resistance profiling based on patient risk factors Analysis of protein profiles generated by MALDI-TOF MS AST variance detection Improved image and NGS dataset analyses	Computing power required Microbiologists trained in bioinformatics Artificial intelligence programs needed to perform analyses Variability in microbiology specimens	Performing collaborative outcome studies which include laboratories, information technology, and data scientists
Laboratory diagnostic stewardship	Optimized use of multiplex PCR testing Reduction in unnecessary test utilization Cost control	Integration of LIS with EMR to facilitate data analyses Provider buy-in Ensuring interventions Lack of data demonstrating clinical outcomes of new technology	Generate outcomes data Publish guidelines to establish a laboratory stewardship program Create order templates to assist in test ordering Develop best practices for uniformity in community laboratory stewardship practices Improve laboratory test catalogs
Staffing shortage	Capitalize on technology skills of younger generation microbiologists	Decreasing no. of training programs Pandemic-induced fatigue Extended training required for new hires Limited opportunities for advancement within the laboratory	Promote alternative training mechanisms Advocate for improved pay
Promoting investment in the clinical laboratory	Capitalize on innovations that can be used to improve patient care	Laboratories are often considered a cost-center Siloed thinking in finance departments Indirect benefit of laboratory testing on patient care and cost savings	Perform outcome studies to demonstrate the benefit of investment in laboratory technology Include business analysts in laboratory stewardship programs Engage Chief Financial Officers in ASM-sponsored sessions Participate in pathology management groups and meetings
Rapid susceptibility testing	Improved patient outcomes through faster therapeutic decision making Improved rapid phenotypic testing methods Enhanced predictive value for genotypic susceptibility methods	Cost compared to conventional methods Biological and technical challenges must be overcome Polymicrobial specimens	Microbiology laboratories and industry collaborate to demonstrate improved patient care with rapid AST methods
Point of care testing for infectious diseases	Improved access to testing Near-patient diagnosis facilitating treatment decisions	Easy access promotes overutilization Inaccurate results when performed outside the laboratory Difficult to capture data from POCT Inferior performance to laboratory-based testing	Study the impact of COVID-19 at-home testing
Molecular diagnostics for fungal infections	Improved diagnosis of fungal infections in vulnerable patients	Difficult to validate due to low frequency Cost prohibitive clinical trials False-positive results due to environmental contamination	Develop collaborative groups to create a more efficient test development and evaluation process

Doern CD et al. J Clin Microbiol. 2022 Jul 20;60(7):e0009222. doi: 10.1128/jcm.00092-22.

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Why syndromic panels for HAP/VAP?

- Hospital-acquired pneumonia accounts for >20% nosocomial infections
- Determining the causative infectious agent is pivotal in the prognosis and management of pneumonia

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IDSA
Infectious Diseases Society of America

ATS American Thoracic Society

2019 ATS / IDSA CAP Guidelines:

"Rapid, cost-effective, sensitive, and specific diagnostic tests to identify organisms causing CAP have potential to improve routine care by **supporting the use of targeted therapy…"**

2021 ATS CAP Guidelines:

"Inpatients who are immunocompromised, or immunocompetent with **severe CAP,** should have **multiplex PCR** for non-influenza viral pathogens…"

Am J Respir Crit Care Med. 2019 Oct 1;200(7):e45-e67.

Lancet Respir Med 2022
Published Online May 23, 2022 https://doi.org/10.1016/ S2213-2600(22)00086-8

Fast multiplex bacterial PCR of bronchoalveolar lavage for antibiotic stewardship in hospitalised patients with pneumonia at risk of Gram-negative bacterial infection (Flagship II): a multicentre, randomised controlled trial

Andrei M Darie, Nina Khanna, Kathleen Jahn, Michael Osthoff, Stefano Bassetti, Mirjam Osthoff, Desiree M Schumann, Werner C Albrich, Hans Hirsch, Martin Brutsche, Leticia Grize, Michael Tamm, Daiana Stolz

Summary

Background PCR-based testing has transformed the management of suspected respiratory viral infections. We aimed to determine whether multiplex bacterial PCR of bronchoalveolar lavage fluid aids antibiotic stewardship in patients with pneumonia.

Methods This investigator-initiated, multicentre, randomised controlled trial was conducted at two tertiary care centres in Switzerland (University Hospital of Basel and Kantonsspital St Gallen). Patients aged 18 years or older who were admitted to hospital with suspected pneumonia, had a clinical indication for bronchoscopy with bronchoalveolar lavage, and were at risk of Gram-negative bacterial infection were included. Patients were randomly assigned (1:1) to either the multiplex bacterial PCR group or the conventional microbiology control group using a random allocation sequence. Treating physicians were not masked, but the committee panel was masked to patient randomisation. All patients underwent bronchoscopy with bronchoalveolar lavage and samples were assessed by conventional microbiological culture (and additionally, in the PCR group, by multiplex bacterial PCR for Gram-negative rods using the Unyvero Hospitalized Pneumonia [HPN] Cartridge; Curetis, Holzgerlingen, Germany). Patients received empirical antibiotic therapy as clinically indicated by the treating physician. In the PCR group, a recommendation regarding antibiotic therapy was made approximately 5 h after taking the sample. The primary outcome was the time in hours on inappropriate antibiotic therapy from bronchoscopy to discharge or to 30 days after bronchoscopy. This trial was registered with the International Clinical Trials Registry Platform, ISRCTN95828556.

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- Multicenter, RCT, Switzerland
- Age ≥18
- Working diagnosis of HAP
- At risk of Gram-negative bacterial infection
- Underwent bronchoscopy with BAL
- Randomly assigned 1:1 to PCR group and conventional group
- Treating physicians are not masked

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Table 1. Risk factors of infection with Gram-negative bacteria in patients with CAP(1-4)

Suspicion of or diagnosis of chronic alcoholism
Chronic oral steroid administration (prednisone doses >7·5 mg/d or equivalent for more than 4 weeks) or other immunosuppressive therapy for diseases such as in connective tissue disease, rheumatic disease or solid organ transplantation)
Suspicion of or diagnosis of underlying chronic bronchopulmonary disease such as COPD, bronchiectasis, interstitial lung disease
Suspicion of aspiration
Recent or frequent antibiotic therapy within the last three months
Chemotherapy within the last 3 months
Immunocompromised status due to any condition such as haematological disease, haemodialysis, HIV, solid organ or stem cell transplantation

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PCR group = conventional + Unyvero HPN

Recommendation regarding Abx choice made ~5 hours after taking sample

Primary outcome
Time on inappropriate antibiotic therapy from bronchoscopy to discharge or 30 days after bronchoscopy

PCR group

Hospitalized Pneumonia (HPN) Cartridge

Sample Types

Sputum, bronchoalveolar lavage, tracheal aspirates

Gram-positive bacteria

Staphylococcus aureus
Streptococcus pneumoniae

Enterobacteriaceae

Citrobacter freundii
Escherichia coli
Enterobacter cloacae complex
Klebsiella aerogenes (E. aerogenes)
Proteus spp.
Klebsiella pneumoniae
Klebsiella oxytoca
Klebsiella variicola
Serratia marcescens
Morganella morganii

Non-fermenting bacteria

Moraxella catarrhalis
Pseudomonas aeruginosa
Acinetobacter baumannii complex
Stenotrophomonas maltophilia
Legionella pneumophila

Others/Fungi

Pneumocystis jirovecii
Haemophilus influenzae
Mycoplasma pneumoniae
Chlamydophila pneumoniae

48*

Resistance	Gene
Macrolide/ Lincosamide	*ermB*
Oxacillin	*mecA* *mecC*
Penicillin	*tem* *shv*
3rd generation Cephalosporins	*ctx*-M
Carbapenem	*imp* *kpc* *ndm* *oxa*-23 *oxa*-24/40 *oxa*-48 *oxa*-58 *vim*
Sulfonamide	*sul*1
Fluoroquinolone	*gyrA*83 *gyrA*87

+

Conventional group

Adenovirus
Coronavirus 229E
Coronavirus HKU1
Coronavirus NL63
Coronavirus OC43
Human Bocavirus
Human Metapneumovirus
Human Rhino-Entero-Virus
Influenza A
Influenza B
Parainfluenza 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4
Resp Syncytial Virus A
Resp Syncytial Virus B
Chlamydophila Pneum.
Legionella Pneum.
Mycoplasma Pneum.

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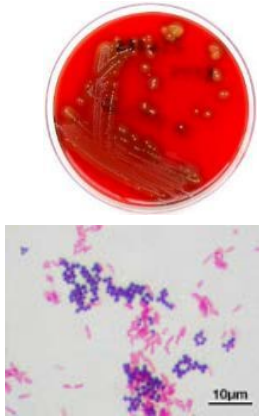


Commercially available multiplex PCR platform Luminex MAGPIX; PathoFinder RespiFinder-22, and Seegene Allplex Respiratory panel

Only results from GNB were disclosed to the attending physician

Table 6. Antibiotic therapy recommendation according to Unyvero Pneumonia HPN results (Antibiotic agents of choice made with consideration of local resistance rate)

Test result	Antibiotic choice	Allergy to 1st choice	Penicillin Allergy Type IV (rash)	Penicillin Allergy Type I (anaphylaxis)
No detection of Gram negative bacteria	Amoxicillin and clavulanic acid or Ceftriaxone	Chose alternative 1st choice If allergies to both seek expert advice (Infectious Disease consult)	Ceftriaxone	Moxifloxacin
Citrobacter freundii	Cefepime or Ertapenem	Chose alternative 1st choice If allergies to both seek expert advice (Infectious Disease consult)	Cefepime or Ertapenem	Ertapenem
Escherichia coli	Ceftriaxone	Piperacillin and Tazobactam	Ceftriaxone	Ertapenem
Enterobacter cloacae complex	Cefepime or Ertapenem	Chose alternative 1st choice If allergies to both seek expert advice (Infectious Disease consult)	Cefepime or Ertapenem	Ertapenem
Enterobacter aerogenes	Cefepime or Ertapenem	Chose alternative 1st choice If allergies to both seek expert advice (Infectious Disease consult)	Cefepime or Ertapenem	Ertapenem
Proteus spp.	Ceftriaxone	Piperacillin and Tazobactam	Ceftriaxone	Ertapenem
Klebsiella pneumoniae	Ceftriaxone	Piperacillin and Tazobactam	Ceftriaxone	Ertapenem
Klebsiella oxytoca	Ceftriaxone	Piperacillin and Tazobactam	Ceftriaxone	Ertapenem
Klebsiella variicola	Ceftriaxone	Piperacillin and Tazobactam	Ceftriaxone	Ertapenem
Serratia marcescens	Cefepime or Ertapenem	Chose alternative 1st choice If allergies to both seek expert advice (Infectious Disease consult)	Cefepime or Ertapenem	Ertapenem
Morganella morganii	Cefepime or Ertapenem	Chose alternative 1st choice If allergies to both seek expert advice (Infectious Disease consult)	Cefepime or Ertapenem	Ertapenem
Moraxella catarrhalis	Amoxicillin and clavulanic acid		Ceftriaxone	Ciprofloxacin
Pseudomonas aeruginosa	Piperacillin and Tazobactam		Cefepime or Ceftazidime	Meropenem
Acinetobacter baumannii complex	Meropenem	Seek expert advice (Infectious Disease consult)	Meropenem	Meropenem
Stenotrophomonas maltophilia	Trimethoprim and Sulfamethoxazol	Seek expert advice (Infectious Disease consult)	Trimethoprim and Sulfamethoxazol	Trimethoprim and Sulfamethoxazol
Haemophilus influenzae	Amoxicillin and clavulanic acid		Ceftriaxone	Ciprofloxacin

- The decision to follow this recommendation was at the physician's discretion

Antibiotic therapy evaluation form

Name of AB	
AB Group	
Reason for stopping or changing the antibiotics therapy	☐ PCR ☐ culture ☐ sensitivity testing ☐ no clinical improvement ☐ death ☐ adverse event ☐ end of i.v. treatment ☐ no information ☐ continuation of therapy longer than 7 days ☐ others→ reason:
Therapy review	Was the AB therapy for atypical microorganism coverage including PCP? (select) ☐ yes ☐ no →☐ appropriate →☐ optimal →☐ not optimal →☐ no information →☐ inappropriate →reason why: ☐ not active to in-vitro susceptibility testing ☐ spectrum too broad ☐ intrinsic resistance ☐ therapy too long ☐ no information ☐ others → reason: ☐ no information

Medications/Modifications of ABT based on BAL analysis ☐ **PCR** ☐ **Control**	Indication to change ABT according to PCR ☐ yes → ☐ therapy changed according to SOP ☐ therapy changed not according to SOP ☐ therapy not changed ☐ no information ☐ no→ ☐ therapy changed not according to SOP ☐ therapy not changed ☐ no information ☐ N.A ☐ no information ☐ others → reason: Indication to change ABT according to culture results ☐ yes → ☐ therapy changed according to culture results ☐ therapy changed not according to culture results ☐ therapy not changed ☐ no information ☐ no → ☐ therapy changed not according to culture results ☐ therapy not changed ☐ no information ☐ N.A ☐ no information ☐ others → reason: Indication to change ABT according to sensitivity test ☐ yes → ☐ therapy changed according to sensitivity test ☐ therapy changed not according to sensitivity test ☐ therapy not changed ☐ no information ☐ no → ☐ therapy changed not according to sensitivity test ☐ therapy not changed ☐ no information ☐ N.A ☐ no information ☐ others → reason:

Results analyzed by the adjudication board a panel of at least 3 physicians
(a respiratory physician, an infectious disease specialist, and an internal medicine specialist)

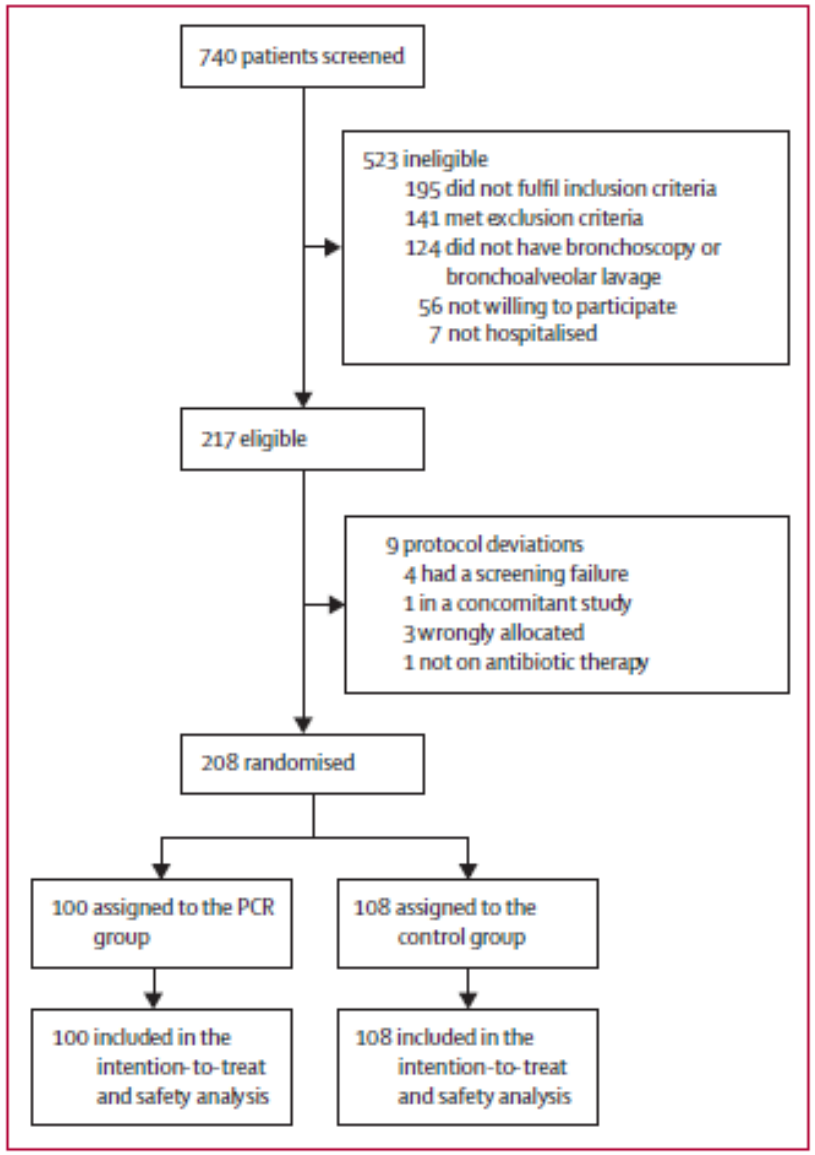


Figure 1: Trial profile

	Control group (n=108)	PCR group (n=100)
Age, years	65·1 (13·9)	66·8 (14·1)
Sex		
Male	64 (59%)	71 (71%)
Female	44 (41%)	29 (29%)
Smoking status		
Current smoker	27 (25%)	18 (18%)
Past smoker	48 (44%)	53 (53%)
Never smoker	32 (30%)	29 (29%)
Pack years*	38·4 (23·2)	36·7 (23·9)
Immunosuppression†	61 (57%)	56 (56%)
Vaccination		
Influenza	42 (39%)	46 (46%)
Pneumococcal	12 (11%)	7 (7%)
Symptoms		
Duration of symptoms before bronchoscopy, days	17·1 (39·7)	11·2 (13·9)
New or increased cough	83 (77%)	71 (71%)
Fever (>38·3°C) or hypothermia (<36·0°C)	50 (46%)	46 (46%)
Dyspnoea	62 (57%)	44 (44%)
Clinical parameters and vital signs		
Respiratory rate, breaths per min	20·8 (5·3)	21·8 (5·0)
Oxygen saturation, % breathing room air	95·2 (3·2)	94·2 (3·9)
Systolic blood pressure, mm Hg	122·2 (22·2)	126·2 (19·6)
Heart rate, beats per min	85·8 (14·1)	82·4 (14·5)
Systemic inflammation	89 (82%)	92 (92%)
White blood cell count, x 10⁹/L	10·4 (6·9)	10·4 (5·1)
C-reactive protein, mg/L	138·9 (105·6)	136·7 (113·8)
Procalcitonin, µg/L‡	0·4 (1·1)	1·0 (3·4)
Prognostic scores		
Charlson Comorbidity Index	3·3 (2·8)	3·5 (2·6)
CURB-65 score	1·0 (0·8)	1·1 (0·9)
Imaging		
Chest x-ray performed	32 (30%)	40 (40%)
Consolidation	28 (26%)	29 (29%)
Interstitial pattern	12 (11%)	15 (15%)
Pulmonary cavitation	1 (<1%)	2 (2%)
Chest CT scan performed	97 (90%)	83 (83%)
Consolidation	84 (78%)	77 (77%)
Interstitial pattern	27 (25%)	25 (25%)
Pulmonary cavitation	1 (<1%)	1 (1%)
Diagnosis at inclusion		
Community-acquired pneumonia	80 (74%)	77 (77%)
Hospital-acquired pneumonia	26 (24%)	22 (22%)
Chronic obstructive pulmonary disease exacerbation	2 (2%)	1 (1%)

Results
patients characteristics

	Control group (n=108)	PCR group (n=100)
(Continued from previous page)		
Patients with both community-acquired pneumonia and risk factors for Gram-negative infection§		
Suspicion or diagnosis of chronic alcoholism	5 (5%)	6 (6%)
Chronic oral steroid or other immunosuppressive drugs	32 (30%)	23 (23%)
Underlying chronic bronchopulmonary disease	41 (38%)	36 (36%)
Aspiration	2 (2%)	3 (3%)
Recent or frequent antibiotic use in the past 3 months	31 (29%)	32 (32%)
Chemotherapy within the past 3 months	18 (17%)	12 (12%)
Immunocompromising condition¶	30 (28%)	21 (21%)

Data are n (%) or mean (SD). *Pack years quantified by multiplying packs of cigarettes smoked per day by the number of years smoking. †Cause of immunosuppression is provided in the appendix (p 6). ‡Procalcitonin was measured in 75 patients. §Full list of risk factors is provided in the appendix (p 1; n=157 patients with community-acquired pneumonia). ¶Immunocompromising condition included haematological disease, HIV, haemodialysis, solid organ transplantation, and stem cell transplantation.

Table 1: Baseline characteristics of patients

Between May 31, 2017, and Sept 25, 2019, 740 patients with pneumonia were screened, and 208 were included and randomly assigned to PCR group (n=100) or conventional microbiology control group (n=108).

- Mean age: 65.9 (SD 14.0)
- 135 (65%) were male

Results: Primary outcome

Daily follow-up until hospital discharge or for a maximum of 30 days:

Overall inappropriate antibiotics:
399 antibiotic regimes for pneumonia were reviewed, 65 (16%) targeted atypical microorganisms including *P. jirovecii*

- Of the 334 remaining regimes, 196 (59%) were deemed to be inappropriate
- Mostly because treatment was unnecessarily broad spectrum 157 (81%),
- And because of extensive prescription duration 23 (12%)
- There were 83 (46%) of 179 inappropriate antibiotic regimes in the PCR group and 113 (73%) of 155 in the control group (p<0.0001)

Duration of inappropriate antibiotics

- the duration of inappropriate antibiotic treatment was significantly shorter by 38·6 hr (95% CI 19·5–57·7) in the PCR group than in the control group (adjusted mean 47·1 h [34·7–59·5] *vs* 85·7 h [78·8–95·6]; p<0·0001),
- which translates as a decrease in the duration of inappropriate antibiotic therapy of 45·0% (37·9–52·1).

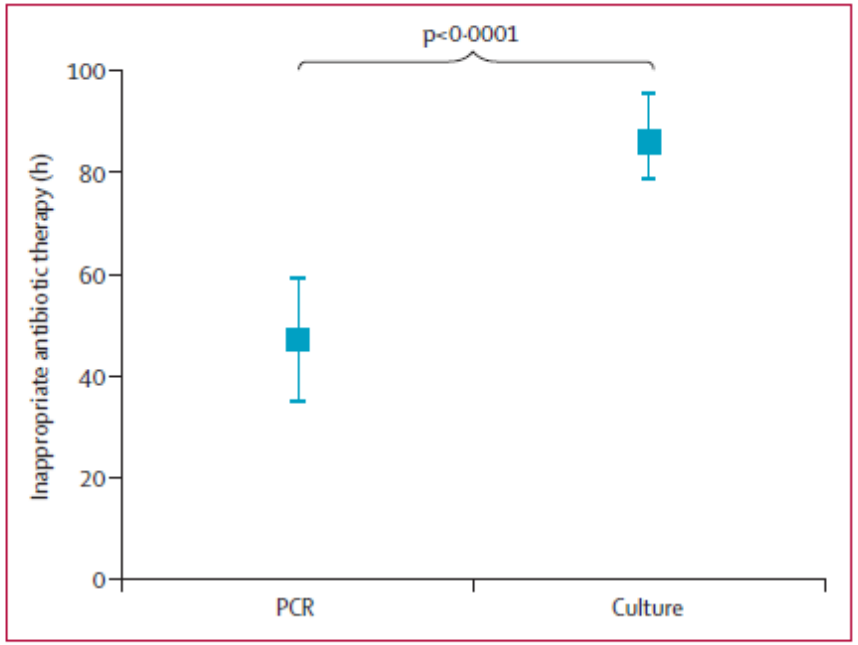


Figure 2: **Duration of inappropriate antibiotic therapy**
Bars indicate 95% CIs.

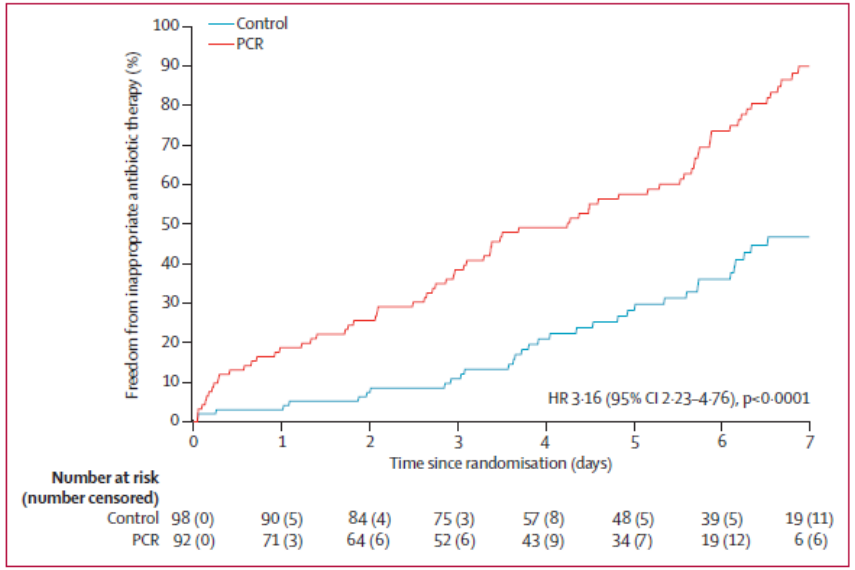


Figure 3: **Freedom from inappropriate antibiotic therapy**

Results: Clinical outcomes

Adverse events due to antimicrobial therapy occurred in nine patients (five [5%] in the PCR group vs four [4%] in the control group)

There were eight (8%) deaths in the PCR group and 11 (10%) in the control group. All in-hospital deaths were attributed to a respiratory cause.

Table 14. Adverse events related to antibiotic therapy

Adverse events related to antibiotic therapy	N (%)
Dermatological Toxicity	5 (2·4%)
Diarrhoea	2 (1·0%)
Neurotoxicity	1 (0·5%)
Muskuloskeletal Toxicity	1 (0·5%)

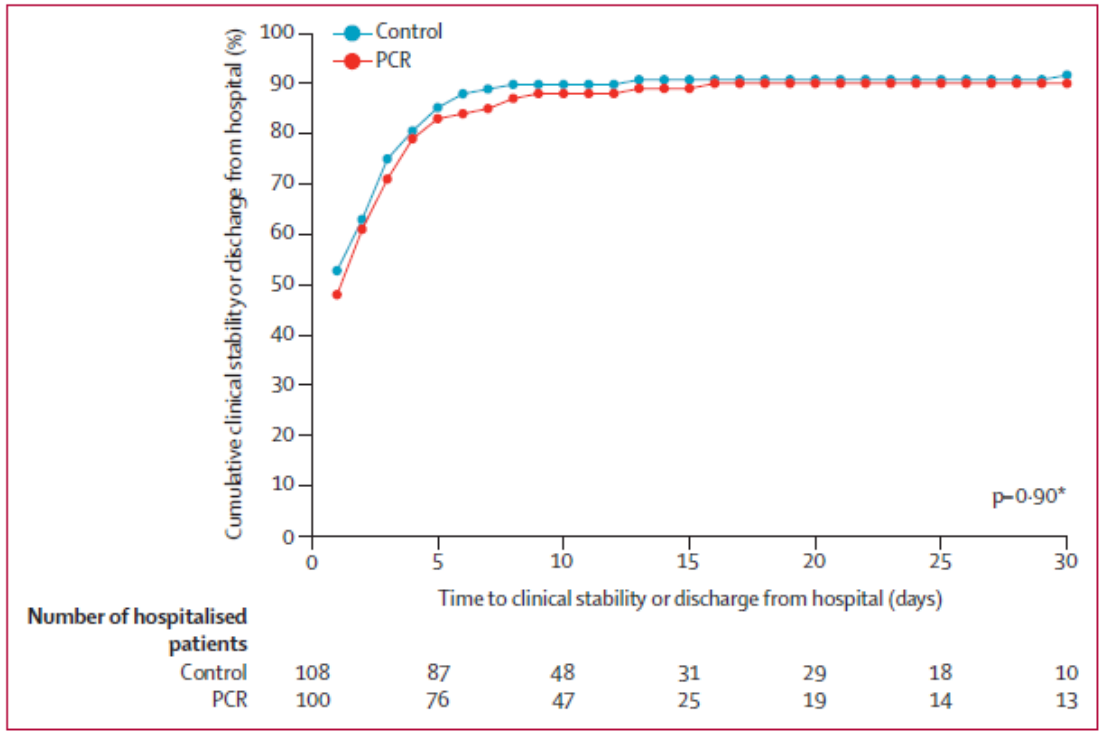


Figure 4: **Time to clinical stability**

*Difference between groups was assessed using the Mann-Whitney *U* test.

Microbiological results

- Growth of any organism was reported in 150 (72%) BAL samples
- Gram NEG rods were detected
  - by PCR in 39 (19%) patient samples
  - by conventional microbiological culture in 30 (14%) cases
- Concurrence with the results of routine culture was observed in 16 cases

	Unyvero PCR		Conventional microbiology	
	Control group (n=108)	PCR group (n=100)	Control group (n=107)*	PCR group (n=100)
Citrobacter freundii	1 (<1%)	0	0	0
Escherichia coli	2 (2%)	3 (3%)	1 (<1%)	2 (2%)
Enterobacter cloacae complex	0	2 (2%)	2 (2%)	4 (4%)
Enterobacter aerogenes	1 (<1%)	0	0	2 (2%)
Proteus spp	1 (<1%)	2 (2%)	2 (2%)	2 (2%)
Klebsiella pneumoniae	1 (<1%)	1 (1%)	2 (2%)	0
Klebsiella oxytoca	0	0	0	0
Klebsiella variicola	0	0	1 (<1%)	0
Serratia marcescens	1 (<1%)	0	1 (<1%)	1 (1%)
Morganella morganii	0	2 (2%)	0	1 (1%)
Moraxella catarrhalis	1 (<1%)	1 (1%)	1 (<1%)	0
Pseudomonas aeruginosa	5 (5%)	4 (4%)	5 (5%)	0
Acinetobacter baumannii complex	0	0	0	1 (1%)
Stenotrophomonas maltophilia	2 (2%)	0	0	0
Haemophilus influenzae	10 (9%)	5 (5%)	4 (4%)	0

*One bronchoscopy and bronchoalveolar lavage sample was not assessed by culture.

Table 2: **Results of multiplex bacterial PCR and conventional microbiology of Gram-negative bacteria**

4 pathogens detected by conventional culture but not by Unyvero HPN panel

Table 17. List of microorganisms detected in the study by different systems

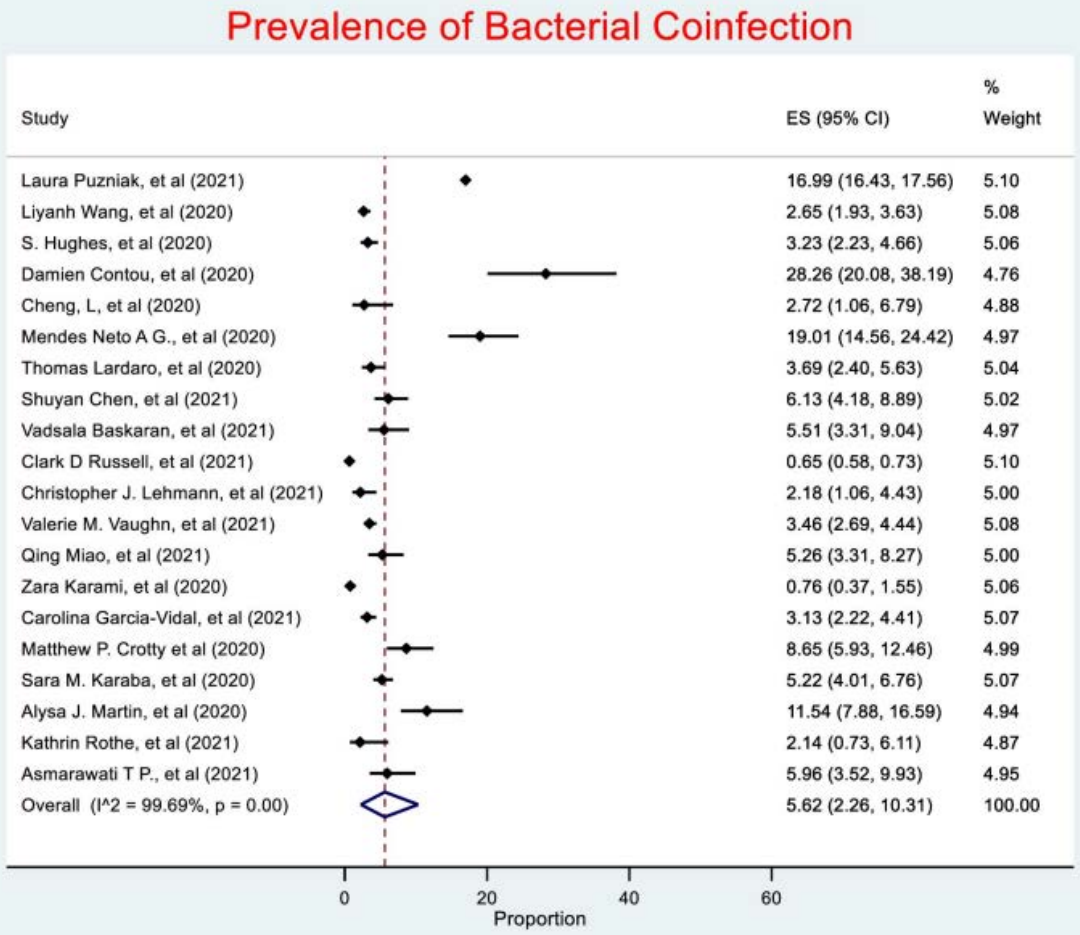
Gram Stain	Detected in Unyvero Pneumonia-Panel*	Detected in Culture	Detected in a second commercially available multiplex PCR assay**
Gram-positive	*Staphylococcus aureus*	*Staphylococcus aureus*	
	Streptococcus pneumoniae	*Streptococcus pneumoniae*	
		Enterococcus faecium	
		Streptococcus agalactiae	
		Streptococcus mitis	
Gram-negative	*Escherichia coli*	*Escherichia coli*	
	Enterobacter cloacae complex	*Enterobacter cloacae complex*	
	Enterobacter aerogenes complex	*Enterobacter aerogenes complex*	
	Proteus spp.	*Proteus spp.*	
	Morganella morganii	*Morganella morganii*	
	Serratia marcescens	*Serratia marcescens*	
	Citrobacter freundii	*Citrobacter freundii*	
	Klebsiella pneumoniae	*Klebsiella pneumoniae*	
	Klebsiella oxytoca	*Klebsiella oxytoca*	
	Klebsiella variicola	*Klebsiella variicola*	
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	
	Acinetobacter baumannii complex	*Acinetobacter baumannii complex*	
	Legionella pneumophila	*Legionella pneumophila*	*Legionella pneumophila*
	Moraxella catarrhalis	*Moraxella catarrhalis*	
	Stenotrophomonas maltophilia	*Stenotrophomonas maltophilia*	
	Haemophilus influenzae	*Haemophilus influenzae*	
	Chlamydophila pneumoniae		*Chlamydophila pneumoniae*
	Mycoplasma pneumoniae		*Mycoplasma pneumoniae*
		Achromobacter xylosoxidans	
			Bordetella Pertussis
Other	*Pneumocystis jirovecii*		

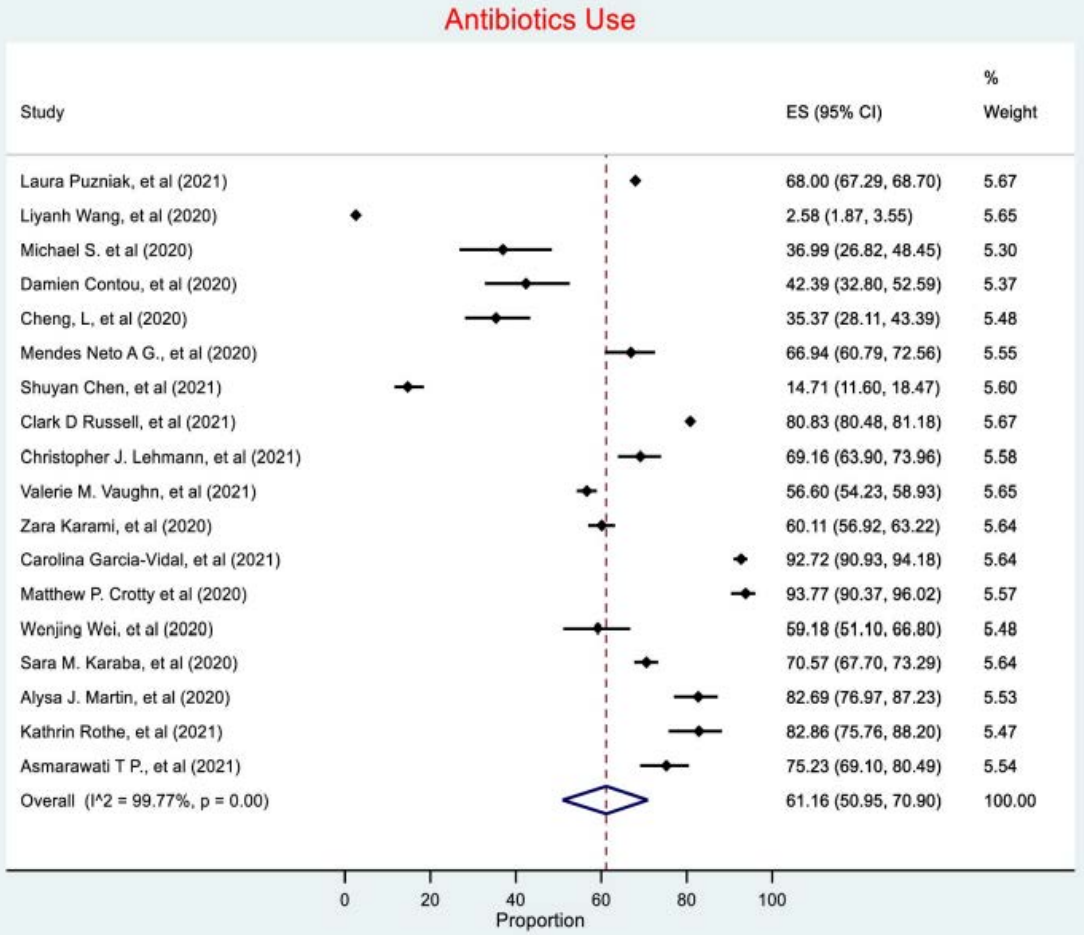
* Considering all pathogens on the multiplex bacterial PCR panel, the Unyvero multiplex bacterial PCR had a sensitivity of 60·0% and a specificity of 85·6% compared to the conventional microbiology.
**University Hospital Basel: MAGPIX (Luminex, MV's-Hertogenbosch, The Netherlands) until 30.11.2018 and RespiFinder-22® (RF-22, PathoFinder, Maastricht, The Netherlands) starting with 01.12.2018. Kantonsspital St. Gallen: Seegene Allplex Respiratory panel (Seegene, Seoul, South Korea).

- The multiplex bacterial PCR had a sensitivity of 55.6% and specificity of 86.6% in detecting Gram-negative bacteria.
- The concordance reached 82.5%

Bacterial Co-infection in COVID-19 patients

Prevalence of bacterial coinfection and patterns of antibiotics prescribing in patients with COVID-19: A systematic review and meta-analysis





PLoS ONE 17(8): e0272375. https://doi.org/10.1371/journal.pone.0272375

Molina *et al. Critical Care (2022) 26:130*
https://doi.org/10.1186/s13054-022-04006-z

Critical Care

RESEARCH Open Access

Diagnostic concordance between BioFire® FilmArray® Pneumonia Panel and culture in patients with COVID-19 pneumonia admitted to intensive care units: the experience of the third wave in eight hospitals in Colombia

Francisco José Molina[1,2*], Luz Elena Botero[1], Juan Pablo Isaza[1], Luz Elena Cano[1,3], Lucelly López[1], Leidy Tamayo[1] and Antoni Torres[4,5]

Abstract

Background: The detection of coinfections is important to initiate appropriate antimicrobial therapy. Molecular diagnostic testing identifies pathogens at a greater rate than conventional microbiology. We assessed both bacterial coinfections identified via culture or the BioFire® FilmArray® Pneumonia Panel (FA-PNEU) in patients infected with SARS-CoV-2 in the ICU and the concordance between these techniques.

Methods: This was a prospective study of patients with SARS-CoV-2 who were hospitalized for no more than 48 h and on mechanical ventilation for no longer than 24 h in 8 ICUs in Medellín, Colombia. We studied mini-bronchoalveolar lavage or endotracheal aspirate samples processed via conventional culture and the FA-PNEU. Coinfection was defined as the identification of a respiratory pathogen using the FA-PNEU or cultures. Serum samples of leukocytes, C-reactive protein, and procalcitonin were taken on the first day of intubation. We analyzed the empirical antibiotics and the changes in antibiotic management according to the results of the FA-PNEUM and cultures.

Patients
- 8 ICUs in Columbia
- SARS-CoV-2 positive
- March 1 to July 30, 2021
- Hospitalized for < 48 hours and on mechanical ventilation for < 24 hours
- Mini-BAL or ETA

Endpoint:
- Co-infections
- Empirical antibiotics
- Change in antibiotic management according to FA-PNEUM and culture results

BioFire® PNEUMONIA PANEL*PLUS* - 34 TARGETS

15 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

3 Atypical Bacteria

Qualitative

Legionella pneumophila
Mycoplasma pneumoniae
Chlamydia pneumoniae

9 Viruses

Qualitative (no sub-typing)

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

7 Antibiotic Resistance Genes

Methicilin Resistance

*mec*A/*mec*C and MREJ

ESBL

CTX-M

Carbapenemases

KPC
NDM
OXA48-like
VIM
IMP

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Table 1 Demographic characteristics of patients with COVID-19 pneumonia admitted to intensive care units

Characteristic ($n = 149$)	Frequency (%)
Male	86 (57.7)
Age (Me-P25–P75)	58 (46–66)
Hypertension	69 (46.3)
Diabetes	36 (24.2)
Chronic kidney disease	8 (5.4)
Rheumatologic disease	3 (2.0)
Neoplasm	3 (2.0)
Chronic obstructive pulmonary disease	2 (1.3)
HIV	2 (1.3)
Heart failure	1 (0.7)
Cirrhosis	1 (0.7)

Me, median; P25–P75, 25th and 75th percentiles; HIV, human immunodeficiency virus; %, percentage

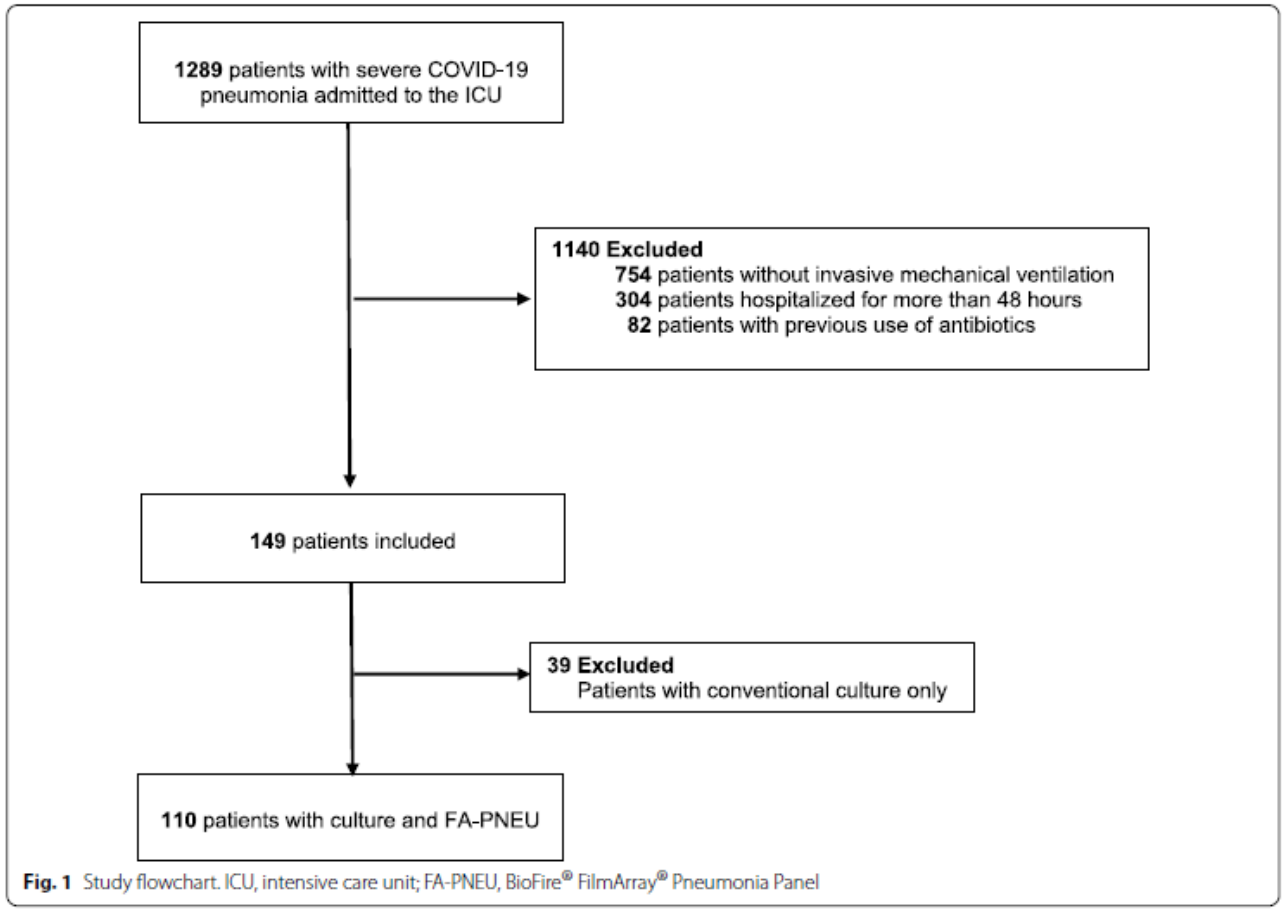

Fig. 1 Study flowchart. ICU, intensive care unit; FA-PNEU, BioFire® FilmArray® Pneumonia Panel

Results (Co-infections):

Table 2 Summary of total, BioFire® FilmArray® Pneumonia Panel, and microbiological culturing detections for all pathogens

Microbial target	FA-PNEU (+) microbiological culture (+)	FA-PNEU (+) microbiological culture (−)	FA-PNEU (−) microbiological culture (+)	Total (+)	FA-PNEU (+)	Microbiological culture (+)	Overall concordance	Concordant positive	Cohen's kappa coefficient (95% CI)
Aspergillus flavus	0	0	1	1	0	1	99.10	0.0	0
Enterobacter cloacae complex	2	2	0	4	4	2	98.20	1.8	65.8 (21.7; 100.9)
Haemophilus influenzae	0	4	0	4	4	0	96.36	0.0	0
Klebsiella pneumoniae	5	0	1	6	5	6	99.10	4.5	90.4 (71.9; 100.9)
Klebsiella oxytoca	1	0	0	1	1	1	100.00	0.9	100 (100; 100)
Pseudomonas aeruginosa	1	0	1	2	1	2	99.10	0.9	66.26 (4.3; 128.2)
Streptococcus agalactiae	0	8	0	8	8	0	92.70	0.0	0
Staphylococcus aureus	8	7	0	15	15	8	93.64	7.3	66.4 (43.7; 89.1)
Streptococcus pneumoniae	2	0	1	3	2	3	99.10	1.8	79.51 (40.4; 108.6)
Global[a]	18	9	1	28	27	19	90.10	16.4	72.7 (57.1; 88.4)

CI, confidence intervals; FA-PNEU (+), positive BioFire® FilmArray® Pneumonia Panel; FA-PNEU (−), negative BioFire® FilmArray® Pneumonia Panel

[a] Patients whose test was positive for at least one microorganism

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Klebsiella pneumoniae	5	0	1					4.5	90.4 (71.9; 100.9)
Klebsiella oxytoca	1	0	0	1	1	1	100.00	0.9	100 (100; 100)
Pseudomonas aeruginosa	1	0	1	2	1	2	99.10	0.9	66.26 (4.3; 128.2)
Streptococcus agalactiae					8	0	92.70	0.0	0
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Streptococcus pneumoniae	2	0	1	3	2	3	99.10	1.8	79.51 (40.4; 108.6)
Global[a]	18	9	1	28	27	19	90.10	16.4	72.7 (57.1; 88.4)

FA-PNEU positive: 25.44%
Culture positive: 17.27%

18 samples positive in both techniques
9 FA-PNEU positive with negative culture
1 culture positive with negative FA-PNEU

CI, confidence intervals; FA-PNEU (+), positive BioFire® FilmArray® Pneumonia Panel; FA-PNEU (−), negative BioFire® FilmArray® Pneumonia Panel

[a] Patients whose test was positive for at least one microorganism

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Klebsiella pneumoniae	5	0	1	6	5	6	99.10	4.5	90.4 (71.9; 100.9)
Klebsiella oxytoca	1	0	0	1	1	1	100.00	0.9	10
Pseudomonas aeruginosa	1	0	1	2	1	2	99.10	0.9	66.
Streptococcus agalactiae	0	8	0	8	8	0	92.70	0.0	0
Staphylococcus aureus	8	7	0	15	15	8	93.64	7.3	66.4 (43.7; 89.1)
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CI, confidence intervals; FA-PNEU (+), positive BioFire® FilmArray® Pneumonia Panel; FA-PNEU (−), negative BioFire® FilmArray® Pneumonia Panel

[a] Patients whose test was positive for at least one microorganism

- 92.7-100% when stratified by organisms
- Overall concordance 90.1%

Results (Co-infections):

Suspect contamination by staff

Table 2 Summary of total, BioFire® FilmArray® Pneumonia Panel, and microbiological culturing detections for all pathogens

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Klebsiella pneumoniae	5	0	1	6	5	6	99.10	4.5	
Klebsiella oxytoca	1	0	0	1	1	1	100.00	0.9	
Pseudomonas aeruginosa	1	0	1	2	1	2	99.10	0.9	
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CI, confidence intervals; FA-PNEU (+), positive BioFire® FilmArray® Pneumonia Panel; FA-PNEU (−), negative BioFire® FilmArray® Pneumonia Panel

[a] Patients whose test was positive for at least one microorganism

Most common FA-PNEU:
- *Staphylococcus aureus*
- *Streptococcus agalactiae*

Most common Culture:
- *Staphylococcus aureus*
- *Klebsiella pneumoniae*

Among the 19 culture samples, 4 were polymicrobial

Among the 27 FA-PNEU samples, 12 were polymicrobial

Results (Co-infections):

Table 2 Summary of total, BioFire® FilmArray® Pneumonia Panel, and microbiological culturing detections for all pathogens

Microbial target	FA-PNEU (+) microbiological culture (+)	FA-PNEU (+) microbiological culture (−)	FA-PNEU (−) microbiological culture (+)	Total (+)	FA-PNEU (+)	Microbiological culture (+)	Overall concordance	Concordant positive	Cohen's kappa coefficient (95% CI)
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Haemophilus influenzae	0	4	0	4	4	0	96.36	0.0	0
Klebsiella pneumoniae	5	0	1	6	5	6			
Klebsiella oxytoca	1	0	0	1	1	1			
Pseudomonas aeruginosa	1	0	1	2	1	2	99.10	0.9	66.26 (4.3; 128.2)
Streptococcus agalactiae	0	8	0	8	8	0	92.70	0.0	0
Staphylococcus aureus	8	7	0	15	15	8	93.64	7.3	66.4 (43.7; 89.1)
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CI, confidence intervals; FA-PNEU (+), positive BioFire® FilmArray® Pneumonia Panel; FA-PNEU (−), negative BioFire® FilmArray® Pneumonia Panel

[a] Patients whose test was positive for at least one microorganism

PPV is low for *E. cloacae complex* & *S. aureus*

Among the 19 culture samples, 4 were polymicrobial

Among the 27 FA-PNEU samples, 12 were polymicrobial

Results: Change of Antibiotics in response to FA-PNEU result

Point 1:
61/110 had antibiotics before LRT sample

- ceftriaxone (45.9%),
- cefepime (31.1%)
- ampicillin/sulbactam (23%)

Table 5 Change of antibiotic management according to the results of BioFire® FilmArray® Pneumonia Panel and cultures

Condition	Test	Result	Change of antibiotic management		*n*
			Yes (%)	No (%)	
With previous antibiotic	FA-PNEUM	Positive	21 (91.3)	2 (8.7)	23
		Negative	37 (97.4)	1 (2.6)	38
		Total	58 (95.1)	3 (4.9)	61
	Culture	Positive	6 (31.6)	13 (68.4)	19
		Negative	1 (100)	0 (0)	1
		Total	7 (35)	13 (65)	20
Without previous antibiotic	FA-PNEUM	Positive	4 (100)	0 (0)	4
		Negative	0 (0)	45 (100)	45
		Total	4 (8.2)	45 (91.8)	49
	Culture	Positive	0 (0)	0 (0)	0
		Negative	0 (0)	81 (100)	81
		Total	0 (0)	81 (100)	81

n, number of observations; FA-PNEU, BioFire® FilmArray® Pneumonia Panel; %, percentage

Point 2:
78/38 patients antibiotics suspended after FA-PNEUM Negative result

Point 3:
4 patients started on Antibiotics with positive FA-PNEUM results

- Oxacillin (33.3%)
- Linezolid 23.8%)

MecA/C/MREJ had specificity of 94.55 and NPV 100%

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RESEARCH Open Access

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Francisco José Molina[1,2*], Luz Elena Botero[1], Juan Pablo Isaza[1], Luz Elena Cano[1,3], Lucelly López[1], Leidy Tamayo[1] and Antoni Torres[4,5]

Salient findings

1. Approximately a quarter of patients with COVID-19 pneumonia admitted to the ICU have bacterial coinfection;
2. A negative FA-PNEU result prevents the inappropriate empirical use of antibiotics;
3. The overall concordance between FA-PNEU and culture was 90.1%,

Example of Algorithm for rational use of multiplex PCR critically ill ventilated COVID-19 patients

A multicenter retrospective analysis of all critically ill patients who were admitted to 6 ICUs from March to May 2020, with COVID-19 and respiratory failure requiring invasive mechanical ventilation in France.

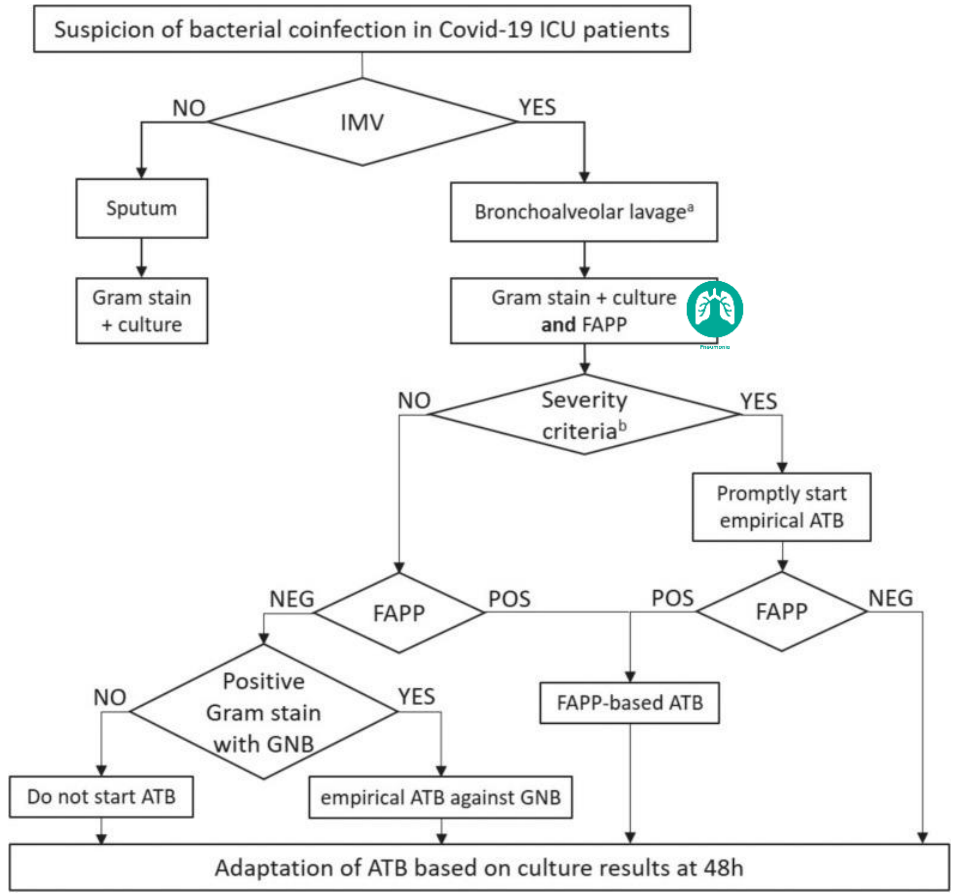
Faster Time Results

- FilmArray Pneumonia Panel (FAPP), conventional culture (CC) and Gram stain were included.
- Results of FAPP and Gram stain were available in **4 hours.**
- A first result of the CC was available after 1 day with a **definitive result within 5 days.**

Impacts of FAPP's Routine Use in ICU

- FAPP-based therapeutic decisions concordance with CC-based therapeutic decisions: 91% for BAL and 69% in ETA (p=0.009)
- Contribution of FAPP-based decision was **antibiotic avoidance: 81.5% (22/27) in CAP and 60.9% (56/92) in VAP**

Clinical algorithm for initiating antibiotics using FAPP in bacterial coinfection of critically ill COVID 19 patients.



IMV, invasive mechanical ventilation; BAL, bronchoalveolar lavage; FAPP, FilmArray Pneumonia Panel; ATB, antibiotics; GNB, Gram-negative bacilli. a Endotracheal aspirate samples could be used but need cautious interpretation regarding the risk of over-diagnosis due to tracheobronchial colonization; b Septic shock (according to SEPSIS-3) or severe ARDS (according to Berlin criteria)

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Original article

Multicentric evaluation of BioFire FilmArray Pneumonia Panel for rapid bacteriological documentation of pneumonia

Nabil Gastli [1], Julien Loubinoux [1], Matthieu Daragon [2], Jean-Philippe Lavigne [3], Pierre Saint-Sardos [4], Hélène Pailhoriès [5], Carole Lemarié [5], Hanaa Benmansour [6], Camille d'Humières [7], Lauranne Broutin [8], Olivier Dauwalder [9], Michael Levy [10], Gabriel Auger [11], Solen Kernéis [12], Vincent Cattoir [11,13,*], the French FA-PP study group[†]

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ABSTRACT

Objectives: To evaluate performances of the rapid multiplex PCR assay BioFire FilmArray Pneumonia Panel (FA-PP) for detection of bacterial pathogens and antibiotic resistance genes in sputum, endotracheal aspirate (ETA) and bronchoalveolar lavage (BAL) specimens.
Methods: This prospective observational study was conducted in 11 French university hospitals (July to December 2018) and assessed performance of FA-PP by comparison with routine conventional methods.
Results: A total of 515 respiratory specimens were studied, including 58 sputa, 217 ETA and 240 BAL. The FA-PP detected at least one pathogen in 384 specimens, yielding an overall positivity rate of 74.6% (384/515). Of them, 353 (68.5%) specimens were positive for typical bacteria while eight atypical bacteria and 42 resistance genes were found. While identifying most bacterial pathogens isolated by culture (374/396, 94.4%), the FA-PP detected 294 additional species in 37.7% (194/515) of specimens. The FA-PP demonstrated positive percentage agreement and negative percentage agreement values of 94.4% (95% CI 91.7%–96.5%) and 96.0% (95% CI 95.5%–96.4%), respectively, when compared with culture. Of FA-PP false-negative results, 67.6% (46/68) corresponded to bacterial species not included in the panel. At the same semi-quantification level (in DNA copies/mL for FA-PP versus in CFU/mL for culture), the concordance rate was 43.4% (142/327) for culture-positive specimens with FA-PP reporting higher semi-quantification of ≥ 1 $\log_{10}$ in 48.6% (159/327) of cases. Interestingly, 90.1% of detected bacteria with $\geq 10^6$ DNA copies/mL grew significantly in culture.
Conclusions: FA-PP is a simple and rapid molecular test that could complement routine conventional methods for improvement of diagnosis accuracy of pneumonia. **Nabil Gastli, Clin Microbiol Infect 2021;27:1308**

Prospective observational study

- 11 French university hospitals
- 515 respiratory specimens (58 sputa, 217 ETA, 240 BAL)

Table 1
Overview of testing of the 515 respiratory specimens

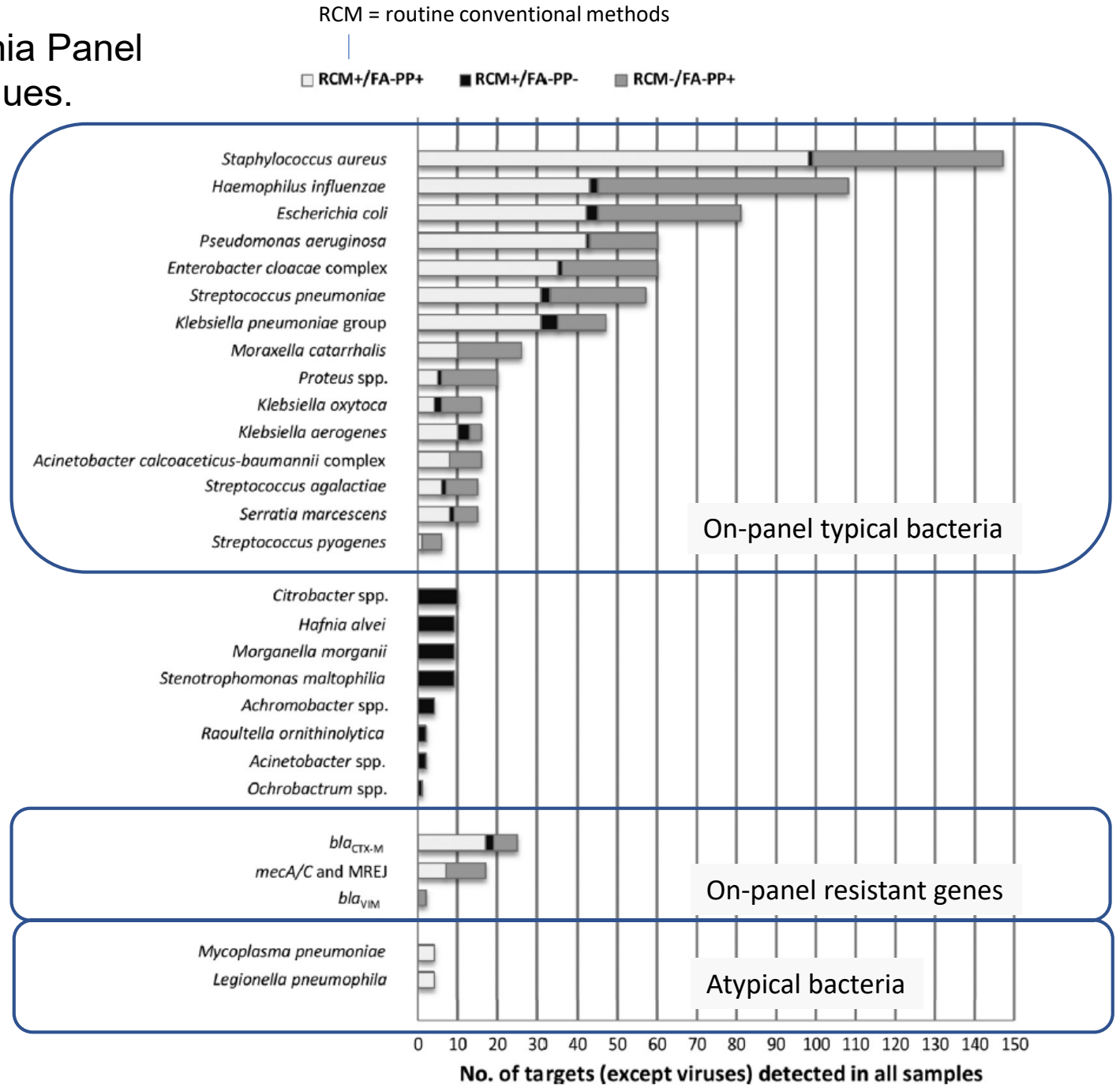
Hospital	Respiratory samples ($n = 515$)				Typical bacterial targets (no. of positive samples)		Atypical bacterial targets (no. of positive samples)		Viral targets (no. of positive samples)
	Sputum	ETA	BAL	Total	RCM ($n = 515$)	FA-PP ($n = 515$)	RCM ($n = 515$)	FA-PP ($n = 515$)	FA-PP ($n = 515$)
Paris, Cochin	27	36	0	63	41	44	2	2	11
Dijon	0	50	10	60	29	38	1	1	15
Nîmes	0	0	59	59	24	27	0	0	2
Clermont-Ferrand	5	13	40	58	22	37	2	2	7
Angers	1	15	41	57	36	40	1	1	9
Paris, Lariboisière	9	10	38	57	28	41	0	0	7
Paris, Bichat	5	4	30	39	29	28	1	1	8
Poitiers	5	32	1	38	37	37	0	0	3
Rennes	1	15	15	31	23	23	0	0	4
Hospices Civils de Lyon	4	20	5	29	9	20	1	1	8
Paris, Robert-Debré	1	22	1	24	16	18	0	0	5
Total, *n* (%)	58 (11.3)	217 (42.1)	240 (46.6)	515	294 (57.1)	353 (68.5)	8 (1.6)	8 (1.6)	79 (15.3)

Abbreviations: BAL, bronchoalveolar lavage; ETA, endotracheal aspirate; FA-PP, FilmArray Pneumonia panel; RCM, routine conventional methods.

Performance of the FilmArray Pneumonia Panel compared with standard of care techniques.

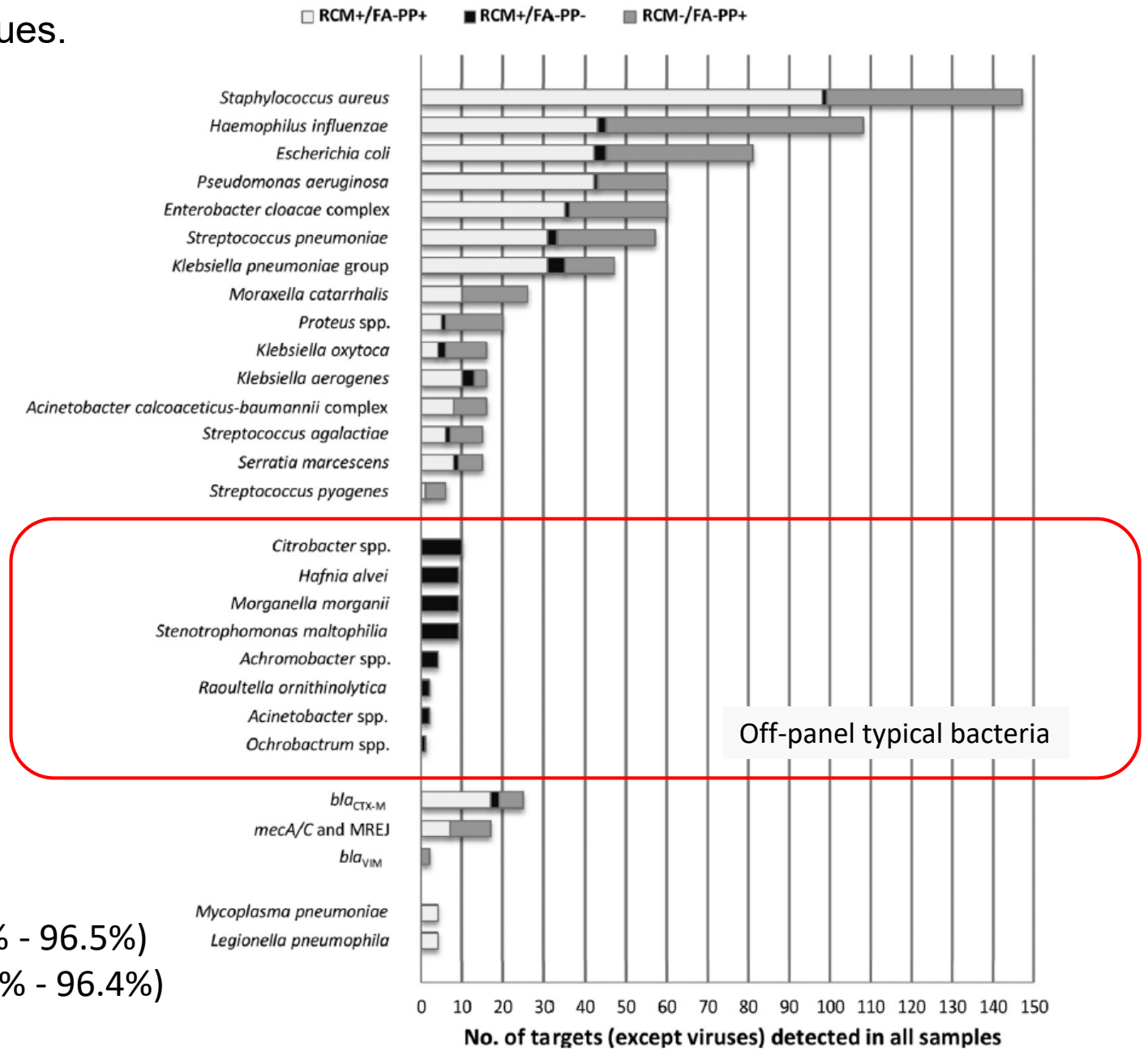
FA-PP ≥1 pathogen in 384 specimens, positive rate of 74.6%

- 353 typical bacteria
- 8 atypical bacteria
- 42 resistance genes



Performance of the FilmArray Pneumonia Panel compared with standard of care techniques.

RCM = routine conventional methods



FA-PP Identify most bacterial pathogens isolated by culture 374/396
But cannot detect off panel organisms

Positive percentage agreement 94.4% (91.7% - 96.5%)
Negative percentage agreement 96.0% (95.5% - 96.4%)

Performance of the FilmArray Pneumonia Panel compared with standard of care techniques.

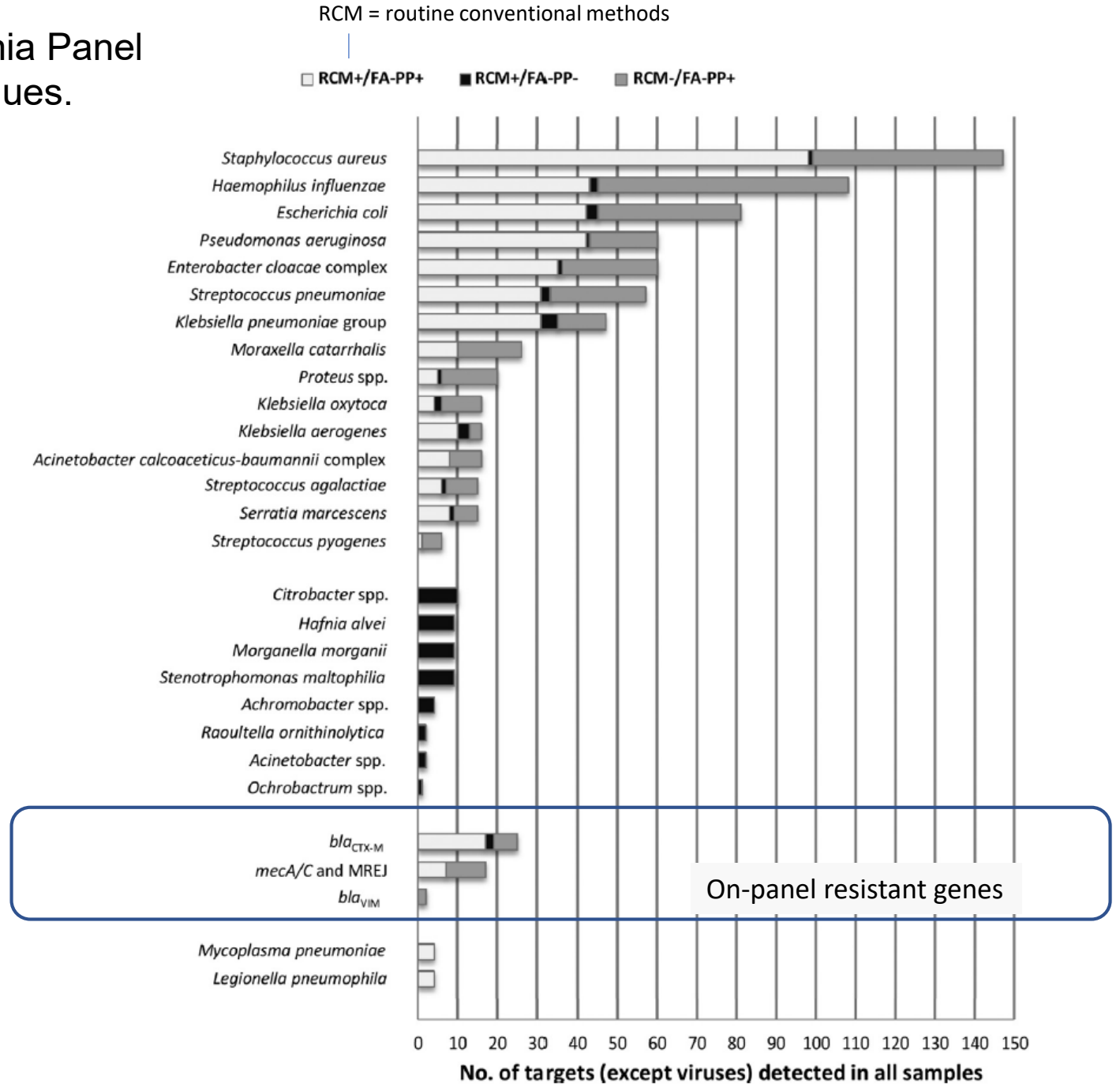
Of the 42 resistance genes detected by the FA-PP, 24 markers were confirmed by routine antimicrobial susceptibility testing methods:

- ESBL-producing Enterobacteriaceae (n = 17)
- and methicillin-resistant Staphylococcus aureus (n = 7).

In addition, FA-PP detected bla_{CTX-M} (n = 6) and bla_{VIM} (n = 2) targets in eight samples with no Gram-negative rods in culture.

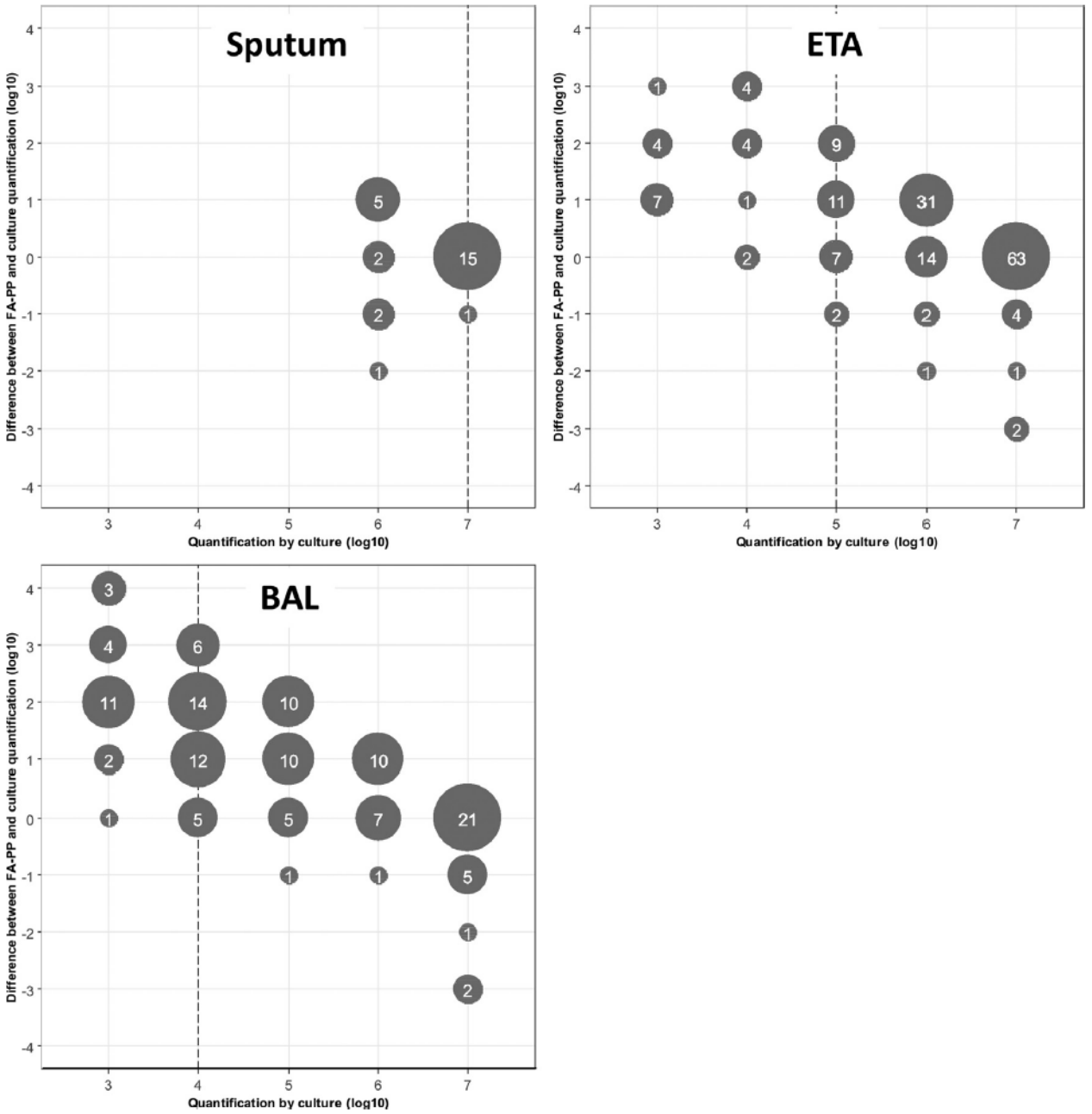
1 strain of ESBL-producing *Citrobacter freundii* and another of *Morganella morganii*, neither of which is included in the FA-PP, were only reported by culture.

The highest rate (10/17, 58.8%) of discrepancies was related to methicillin resistance where detection of mecA/C and MREJ was either discordant with routine antimicrobial susceptibility testing (n = 3) or reported in Staphylococcus aureus-culture-free samples (n = 7).



Results in Semi-quantitation

- In DNA copies/mL for FA-PP versus in CFU/mL for culture, the concordance rate was 43.4% (142/327) for culture-positive specimens
- FA-PP reporting a higher semi-quantification of 1 $\log_{10}$ in 48.6% (159/327) of cases
- The overestimation of bacterial load by FA-PP may be attributed to the detection of dead or non-cultivable bacteria
- 90.1% of detected bacteria with 10^6 DNA copies/mL grew significantly in culture.



Syndromic panels for HAP/VAP - Summary

1. Faster time to targeted therapy
2. Increased pathogen detection, including **viruses** and bacteria
3. Early de-escalation of antibiotics
4. Improved antimicrobial stewardship
5. Rapid, effective **antimicrobial resistance screening**

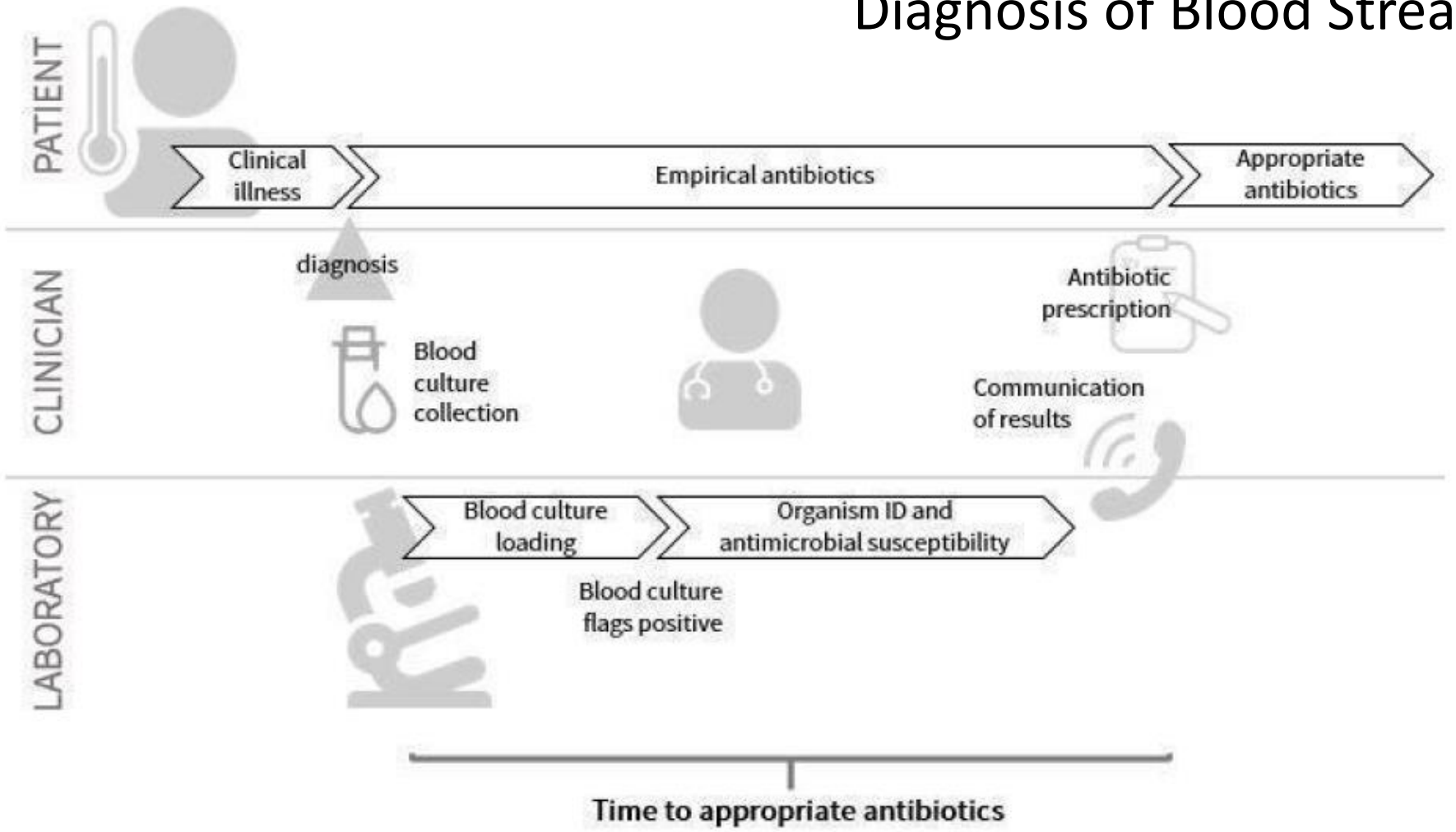
Early diagnosis for Blood Stream Infections

- **The survival rate of patients with sepsis drops by 7.4% per hour of delayed treatment**

- The lack of accurate and rapid techniques for the timely elucidation of causative pathogens necessitates the use of broad-spectrum antibiotic agents.

- The administration of broad-spectrum antibiotics can lead to complications, including toxicity, increased antibiotic resistance, and *Clostridioides difficile* toxin-related diseases. Thus, it is essential to determine the nature of the infecting organism(s) and corresponding antibiotic susceptibilities as soon as possible to allow the selection of the appropriate and targeted therapy.

Diagnosis of Blood Stream Infection



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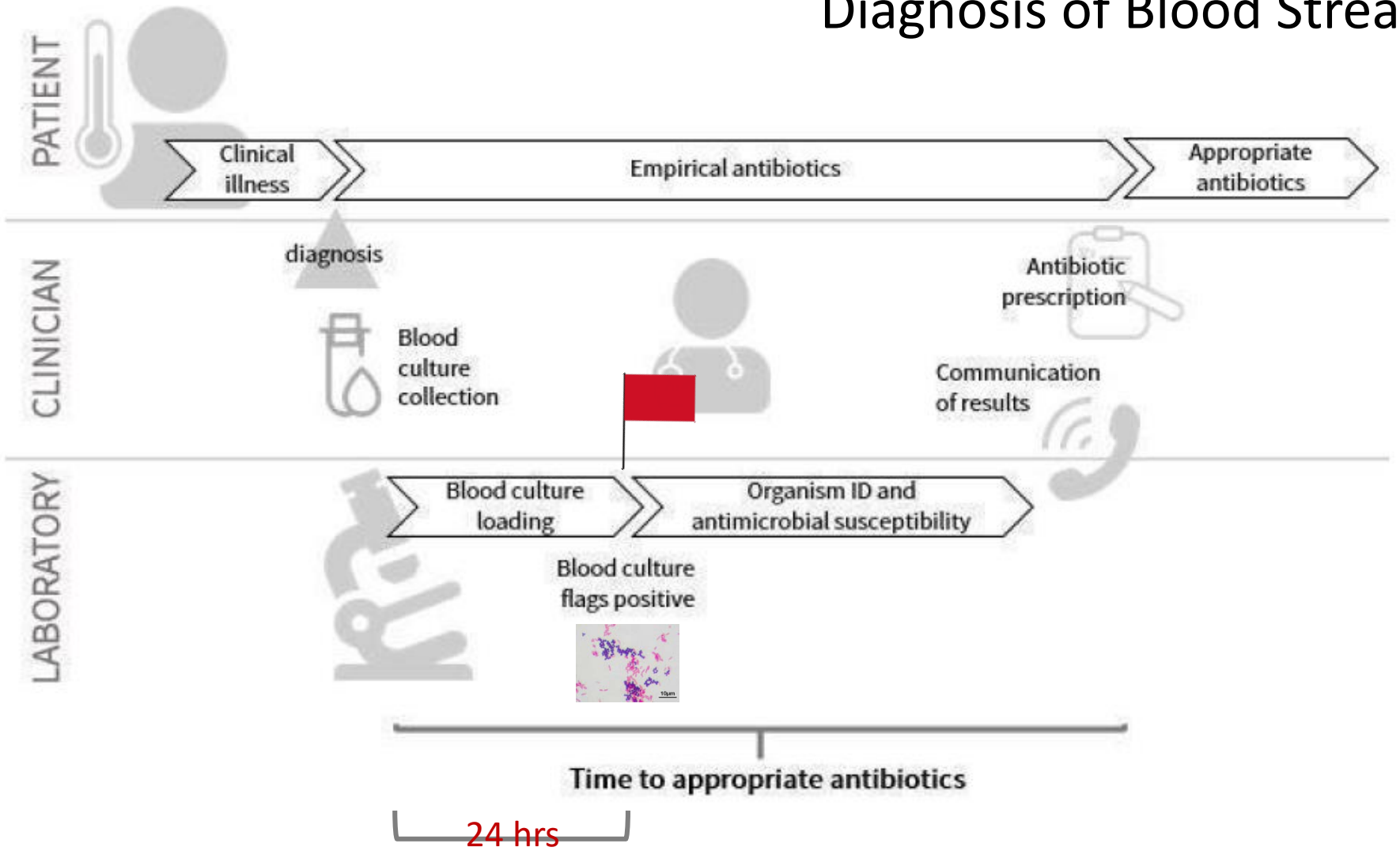

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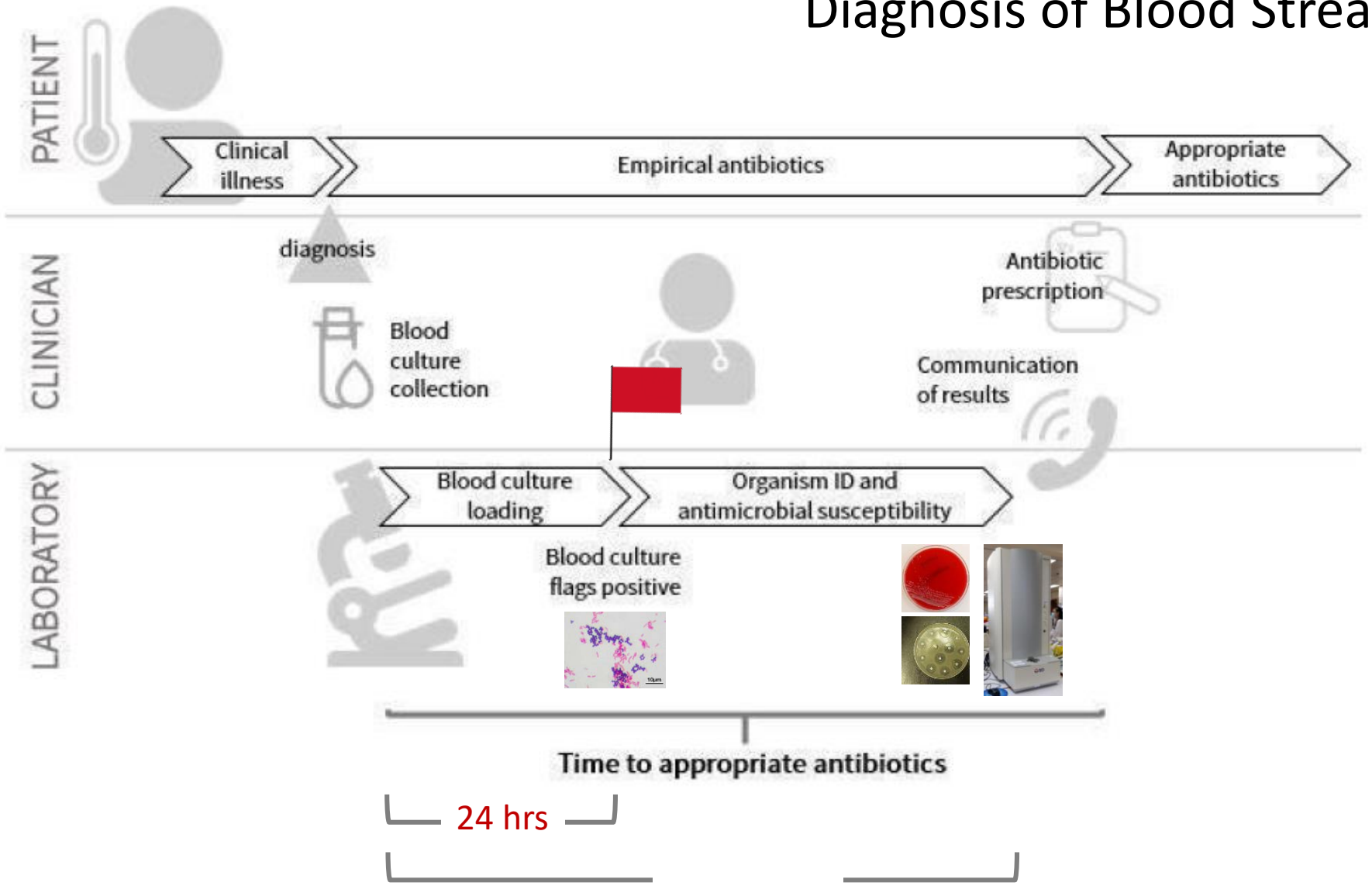

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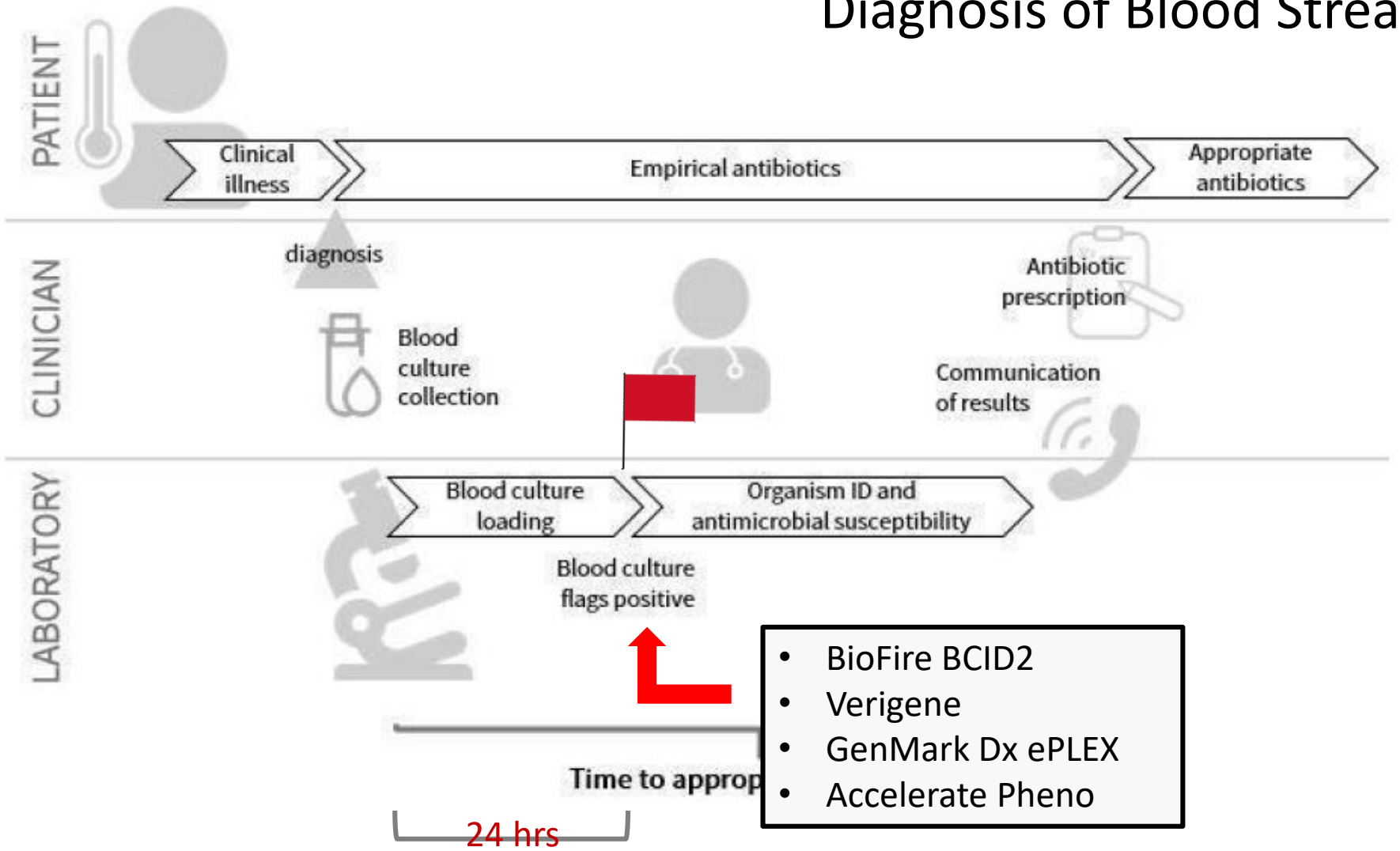
List of commercial and developing technologies for BSI diagnosis

No.	Company	System	Approach	Status *	Sample Prep ‡	Detection/ ID	AST	TAT ^
1	Abacus Diagnostica	Genomera CDX	Rapid/ Real-Time PCR	Dev.	BC (+)	✓		50 min
2	Affinity Biosensors	LifeScale AST	Microorganism mass measurement	CE-IVD	BC (+)		✓	4 h
3	Amplex Diagnostics, GmbH, Germany	Eazyplex MRSA	LAMP ultra-rapid MRSA detection	CE-IVD	BC (+)	✓		30 min
4	Arc Bio	Galileo pathogen solution	Shotgun Sequencing	Dev.	WB	✓		48 h
5	BD	GeneOhm MRSA	Real-Time PCR	FDA, CE-IVD	BC (+)	✓		2 h
6	Becton Dickinson	BD Max StaphSR	Real-Time PCR	FDA	BC (+)	✓		~1.5 h
7	BioFire/bioMerieux Diagnostics	FilmArray DIRECT (new)	Nested PCR	FDA, CE-IVD	WB	✓		1 h
8	BioRad	Droplet dPCR	dPCR; absolute quantification using Poisson's statistics without requiring a standard curve	CE-IVD	BC (+)	✓		No report
9	BioSense Solutions (Denmark)	oCelloScope	3D optical scanning microscopy imaging	Dev.	BC (+)		✓	1 to 4 h
10	Bruker Daltonics	MALDI Biotyper + DxM MicroScan WalkAway System	Mass spectrometry	FDA, CE-IVD	BC (+)	✓		12 to 24 h
11	DNAe (electronic)	LiDia Bloodstream Infection Test	WGS/NGS/miniaturised sequencing	Dev.	WB	✓		3 to 4 h
12	FASTinov	Flow cytometry	Cell sorting fluorescence-based AST	Dev.	BC (+)		✓	<2 h
14	Roche	Smarticles	Bacteriophage-based	Dev.	BC (+)	✓		No report
15	GenMarkDx USA	ePlex BCID	Multiplex PCR	CE-IVD	BC (+)	✓		1.5 h
16	Gradientech AB	Rapid IVD; QuickMIC and CellDirector	Microfluidics Phenotypic multiplex chip	Dev.	BC (+)		✓	2 h
17	Great Basin Corporation (Bringham Young Univ.)	OptoFluidic Platform	Single molecule fluorescence hybridization	Dev.	WB	✓		1 h
18	Hologic	AccuProbe	In situ hybridization	CE-IVD	BC (+)	✓		1 h
19	iCubate	iC GPC	Multiplex amplification assay	FDA, CE-IVD	BC (+)	✓		4 to 5 h
20	IRIDICA	BAC BSI Assay	PCR/ESI-MS	withdrawn	WB	✓		8 h
21	Karius, Inc.	Karius Test	NextGen Seq cfDNA; Genomic; Bioinformatics	Dev.	WB	✓		48 h
22	Luminex	Verigene Gram+ BC	Microarray	FDA.	BC (+)	✓		2.5 h
23	Luminex	Verigene Gram− BC	Microarray	FDA	BC (+)	✓		2.5 h
24	Master Diagnostica, Spain	Sepsis Flow Chip	Microarray	CE-IVD	BC (+)	✓		3 to 4 h
25	Molzym, Germany	SeptiTest; UMD SelectNA	Real Time PCR	CE-IVD	WB	✓		8 to 12 h
26	Momentum Biosciences (Cardiff, UK)	TBD Cognitor Minus	Enzymatic template generation and amplification	awaiting clearance	BC (+)	✓		No report
27	OpGen USA	PNA FISH	In situ hybridization	CE-IVD	BC (+)	✓		2.5 h
28	OpGen USA	Quick FISH	In situ hybridization	CE-IVD	BC (+)	✓		30 min
29	QLinea (Uppsala, Sweden)	AsTAR	High-speed time-lapse microscopy imaging of bacteria in broth	Dev.	BC (+)		✓	6 h
30	Resistell (Switzerland)	Rapid AST antibiogram	AFM, Cantilever, Nanomotion detection-based AST	unknown	BC (+)		✓	No report
31	Roche Molecular System, Switzerland	LightCycler SeptiFast	Real-Time PCR	CE-IVD	WB	✓		6 h
32	SeeGene, Korea	Magicplex Sepsis RT test	Real-Time PCR	CE-IVD	WB	✓		3 to 6 h
33	Specific Diagnostics Inc	Reveal phenotypic AST	Detection of volatile organic compounds	Dev.	BC (+)		✓	~5 h (with MIC)
34	T2Biosystem	T2 Candida Panel T2MR	Nuclear Magnetic Resonance	FDA, CE-IVD	WB	✓		3 to 5 h
35	QuantaMatrix	QMAC-dRAST	Optical Microscopy	Dev.	BC (+)		✓	4 to 6 h

* Platforms on this list are either U.S. Food and Drug Administration (FDA) and/or European CE Marking for In Vitro Diagnostic (CE-IVD) certified or under research development (Dev.); ‡ BC (+): blood culture-positive; WB: whole blood; ^ TAT: turnaround time.

Tjandra K.C. et al. Antibiotics 2022, 11,511 https://doi.org/10.3390/antibiotics11040511

Diagnosis of Blood Stream Infection



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Clinical Infectious Diseases

MAJOR ARTICLE

Real-World Impact of the Accelerate PhenoTest BC Kit on Patients With Bloodstream Infections in the Improving Outcomes and Antimicrobial Stewardship Study: A Quasiexperimental Multicenter Study

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Background. Bloodstream infections (BSIs) are a leading cause of morbidity and mortality. The Improving Outcomes and Antimicrobial Stewardship study seeks to evaluate the impact of the Accelerate PhenoTest BC Kit (AXDX) on antimicrobial use and clinical outcomes in BSIs.

Methods. This multicenter, quasiexperimental study compared clinical and antimicrobial stewardship metrics, prior to and after implementation of AXDX, to evaluate the impact this technology has on patients with BSIs. Laboratory and clinical data from hospitalized patients with BSIs (excluding contaminants) were compared between 2 arms, 1 that underwent testing on AXDX (post-AXDX) and 1 that underwent alternative organism identification and susceptibility testing (pre-AXDX). The primary outcomes were time to optimal therapy (TTOT) and 30-day mortality.

Methods:

- 5 center, retrospective observational, quasiexperimental study
- Hospitalized patients with BSI (i.e. positive peripheral blood culture results and not a contaminant)
- With or without Accelerate PhenoTest BC Kit (AXDX)

Outcome:

- Time to optimal therapy (investigator-defined, site-specific practice determined by practicing clinical pharmacists or infectious diseases physician)
- 30-day mortality

Inclusion

- Hospitalized patients with PBCs deemed clinically significant by the participating sites (ie, not a contaminant) were eligible
- for inclusion in the IOAS study.

Exclusion

- Patients who were not admitted to the hospital at the time of PBC,
- those with a history of PBC in the prior 14 days with the same organism,
- patients who experienced early mortality (expired within 48 hours of PBC),
- and patients treated with palliative care and not expected to survive were excluded.

PBC = positive blood culture

Table 1. Demographics and Baseline Patient Characteristics

Demographics and Characteristics	Pre-AXDX (n = 435)	Post-AXDX (n = 419)	*P* Value
Demographics			
Male sex	226 (51.2)	224 (53.5)	.66
Age, mean ± SD, years	58.2 ± 20.1	59.1 ± 21.1	.22
Age <18 years	16 (3.7)	24 (5.7)	
Coexisting conditions			
Charlson comorbidity score, mean ± SD	5.1 ± 3.4	5.3 ± 3.6	.46
Malignancy	179 (41.1)	168 (40.0)	.75
Leukemia, lymphoma, local tumor	144 (33.1)	115 (27.5)	
Metastatic tumor	35 (8.1)	53 (12.7)	**.03**
Diabetes mellitus	142 (32.6)	136 (32.5)	.89
Chronic kidney disease	107 (24.6)	92 (22.0)	.36
Chronic liver disease	62 (14.3)	68 (16.4)	.33
Clinical characteristics at blood culture positivity			
Source of bacteremia[a]			.19
Bone/joint	14 (3.2)	18 (4.3)	
Cardiovascular	13 (3.0)	11 (2.6)	
Central venous catheter	64 (14.7)	45 (10.7)	
Intraabdominal	70 (16.1)	87 (20.8)	
Respiratory	23 (5.3)	12 (2.9)	
Skin/soft tissue	16 (3.7)	7 (1.7)	
Urinary	94 (21.6)	96 (22.9)	
Other	16 (3.7)	7 (1.7)	
Unidentified	121 (27.8)	119 (28.4)	
Immunosuppressant use[b]	135 (31.0)	128 (30.6)	.88
Concurrent infection requiring antimicrobial therapy[c]	75 (17.2)	76 (18.1)	.73
Acquisition type			
Community acquired[d]	314 (72.2)	303 (72.3)	.97
Intensive care unit residence	126 (29.0)	107 (25.5)	.26
Pitt bacteremia score[e]	2.0 ± 2.3	2.2 ± 2.0	.28
Quick sequential organ failure assessment score[e]	0.78 ± 0.72	0.72 ± 0.71	.24
Serum creatinine, mg/dL[e] ± SD	1.6 ± 1.5	1.6 ± 1.6	.97
Requiring mechanical ventilation	61 (14.0)	62 (14.8)	.74
Hypotension (systolic blood pressure <90 mm Hg)	103 (23.7)	113 (27.0)	.26
Required intravenous vasopressors	73 (16.8)	59 (14.1)	.28

Data are presented as n (%) of patients, unless specified otherwise. Significant differences are highlighted in bold.

Abbreviations: AXDX, Accelerate PhenoTest BC Kit; SD, standard deviation.

[a]Source of bacteremia: (i) for a bloodstream infection to be determined secondary to another site of infection, at least 1 organism from the blood specimen must match an organism identified from the site-specific infection; (ii) if there is not another site of infection with organism growth, a clinician may determine the likely source of the bacteremia based on their clinical judgment; and (iii) unidentified: unknown or no clear source of bacteria.

[b]Immunosuppression included any of the following: active systemic chemotherapy, tacrolimus, mycophenolate mofetil, azathioprine, cyclosporine (or equivalent therapy) for more than 7 days *or* a systemic steroid for more than 10 days in the previous month; or absolute neutrophil count <1500.

[c]A patient was classified as having a concurrent infection when a culture from the concomitant infection site grew at least 1 organism that was not isolated from blood or had a suspected infection that required additional antimicrobial therapy.

[d]Occurred prior to hospitalization or within ≤2 days of hospital admission.

[e]Evaluated for patients aged ≥18 years.

Results:

85% had organisms that were “on-panel” targets for AXDX

Total 854 patients with BSIs were included (435 pre-AXDX, 419 post-AXDX)

Table 2. Blood Culture Organisms

Organism	Pre-AXDX (n = 435)	Post-AXDX (n = 419)
Total organisms isolated	487	430
Gram-positive, by isolate	155 (31.8)	143 (33.3)
CoNS	45 (9.2)	39 (9.1)
Staphylococcus aureus	36 (7.4)	45 (10.5)
Enterococcus spp. *(E. faecium, E. faecalis)*	27 (5.5)	18 (4.2)
Streptococcus spp.	32 (6.6)	35 (8.1)
Other, gram-positive	15 (3.1)	6 (1.4)
Gram-negative, by isolate	328 (67.4)	276 (64.2)
Acinetobacter baumannii	2 (0.4)	1 (0.2)
Citrobacter spp.	5 (1.0)	4 (0.9)
Escherichia coli	140 (28.8)	123 (28.6)
Enterobacter spp.	21 (4.3)	22 (5.1)
Klebsiella spp.	53 (10.9)	53 (12.3)
Proteus spp.	10 (2.1)	9 (2.1)
Pseudomonas aeruginosa	33 (6.8)	27 (6.3)
Serratia marcescens	13 (2.7)	6 (1.4)
Other, gram-negative	51 (10.5)	31 (7.2)
Yeast, by isolate *(C. albicans, C. glabrata)*	4 (0.8)	11 (2.6)
AXDX off-panel organism isolated	86 (17.7)	62 (14.4)
Polymicrobial blood culture	58 (13.3)	47 (11.2)
Proportion of blood cultures with all organisms on AXDX identification/ antimicrobial susceptibility testing panel	360/435 (82.8)	365/419 (87.1)
MDR in blood culture isolates[a]	54(12.4)	69(16.5)
Methicillin-resistant *S. aureus*	9/36(25.0)	20/45(44.4)
Vancomycin-resistant enterococci	7/27 (25.9)	2/18 (11.1)
Extended-spectrum cephalosporin-resistant Enterobacterales	36/242 (14.9)	35/217 (16.1)
MDR *Acinetobacter* spp.	1/2	0/1
MDR *P. aeruginosa*	1/33 (0.5)	11/27 (40.7)

Data are presented as n (%) of patients, unless specified otherwise.

Abbreviations: AXDX, Accelerate PhenoTest BC Kit; CoNS, coagulase-negative staphylococci; MDR, multidrug resistant.

[a]The isolation of a MDR organism includes vancomycin-resistant enterococci, methicillin-resistant *S. aureus*, extended-spectrum cephalosporin-resistant Enterobacterales, and *P. aeruginosa* and *Acinetobacter* species nonsusceptible to at least 1 agent in ≥3 antimicrobial categories as described by Magiorakos et al [18]. (i) Extended-spectrum cephalosporin-resistant Enterobacterales defined as intermediate or resistant to a third-generation cephalosporin. (ii) Carbapenem-resistant Enterobacterales defined as intermediate or resistant to imipenem, doripenem, ertapenem (R only), or meropenem. If the sensitivity test indicated the specimen was resistant to any of those medications, the specimen was categorized as "carbapenem nonsusceptible."

Other organisms in the pre-AXDX arm: Gram-positive: *Abiotrophia defectiva, Actinomyces odontolyticus, Anaerococcus prevotii, Bacillus* spp., *Clostridium* spp. (3), *Corynebacterium* spp. (3), *Finegoldia magna, Nocardia farcinica, Paenibacillus* spp., *Peptoniphilus harei, Peptostreptococcus* spp. Gram-negative: *Acinetobacter* spp. [non-baumannii] (4), *Aeromonas* spp. (2), *Alcaligenes xylosoxidans*, anaerobic gram-negative rod [unable to further identify], *Bacteroides* spp. (7), *Elizabethkingae meningiosepticum* group, *Flavobacterium meningosepticum* (2), *Fusobacterium* spp. (4), *Haemophilus* spp. (4), *Moraxella* spp. (2), *Morganella morganii* (3), *Pantoea* spp. (2), *Prevotella* spp. (2), *Pseudomonas* spp. [non-aeruginosa] (2), *Salmonella* spp. (4), *Sphingomonas paucimobilis* (1), *Stenotrophomonas maltophilia* (6), *Veillonella* spp. (2), *Vibrio* spp.

Other organisms in the post-AXDX arm: Gram-positive: *Bacillus* spp. (3), *Corynebacterium* spp., *Finegoldia magna, Lactobacillus* spp. Gram-negative: *Achromobacter xyloxidans, Bacteroides* spp. (12), *Chryseobacterium indologenes, Fusobacterium* spp. (2), *Haemophilus* spp. (2), *Morganella morganii, Pantoea* spp. (2), *Pasteurella multocida, Prevotella* spp. (2), *Pseudomonas* spp. [non-aeruginosa], *Salmonella* spp. (3), *Sphingomonas paucimobilis, Stenotrophomonas maltophilia* (2).

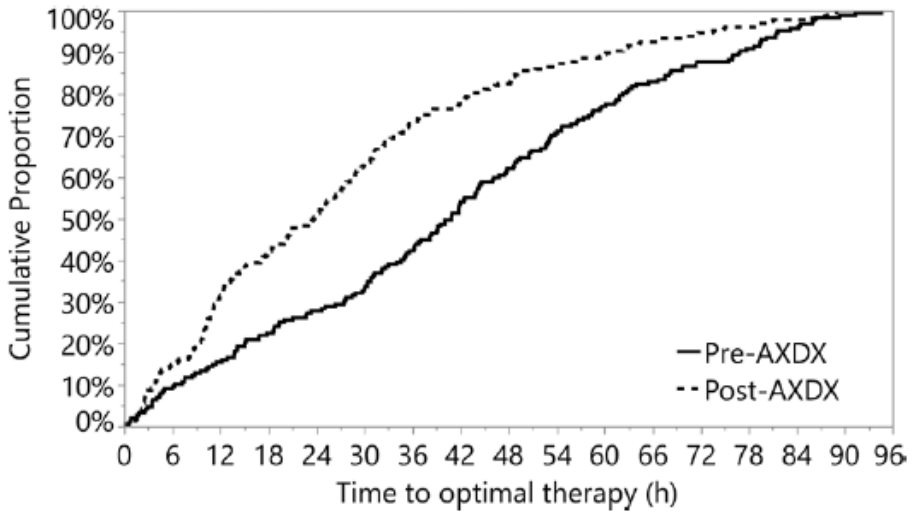
- The median time to PBC from the time of blood culture collection was similar between arms (pre-AXDX 15.3 vs post-AXDX 15.0 hours).
- **Time from PBC to organism identification:** 22.3 hours shorter in the post-AXDX arm than in the pre-AXDX arm (median 2.5 vs 24.8 hours; *P* < .0001)
- **Time to AST result:** 31.6 hours shorter in the post-AXDX arm than in the pre-AXDX arm (median 7.9 vs 39.5 hours; *P* < .0001).
- **Time to optimal therapy**: 17.2 hours shorter in the post-AXDX arm (23.7 hours) compared with the pre-AXDX arm (40.9 hours; P<.0001)
- Clinical endpoints: no significant difference

Table 4. Antimicrobial Modifications and Clinical Outcomes

	All[a]			Gram-Negative[b]		
Endpoint	Pre-AXDX	Post-AXDX	*P* Value	Pre-AXDX	Post-AXDX	*P* Value
Antimicrobial modification[c]						
Time to first antimicrobial modification[d]	24.2 (7.3–46.2)	13.9 (5.0–31.1)	**<.0001**	22.8 (7.0–45.3)	13.6 (5.8–30.9)	**.01**
Time to first gram-positive antimicrobial modification[e]	30.1 (11.2–52.8)	18.3 (6.7–41.8)	**.0013**	28.1 (10.5–51.7)	18.6 (9.4–42.1)	.11
Time to first gram-negative antimicrobial modification[f]	34.6 (9.2–53.4)	18.6 (8.2–36.8)	**<.0001**	30.2 (7.6–52.8)	16.7 (8.6–35.2)	**.003**
Time to first antimicrobial escalation[g]	9.5 (3.4–28.9)	9.0 (3.7–18.4)	.22	9.5 (3.7–31.6)	9.6 (3.9–18.4)	.44
Time to first antimicrobial deescalation[h]	36.0 (17.1–54.5)	27.2 (13.5–43.6)	**.0004**	34.5 (16.6–52.8)	25.4 (12.0–42.5)	**.003**
Time to effective therapy[i]	13.3 (3.1–35.9)	6.7 (3.1–16.2)	**.02**	13.7 (3.3–38.1)	10.0 (3.6–18.6)	.10
Clinical outcome						
30-day mortality	38 (8.7)	25 (6.0)	.12	25 (8.3)	19 (6.7)	.47
Post-blood culture length of stay, median (interquartile range), days	7.0 (4.0–12.4)	6.5 (3.7–12.0)	.43	6.4 (3.7–11.7)	5.4 (3.4–9.7)	**.03**
Acute kidney injury (aged ≥18 years)	92 (23.2)	78 (21.1)	.49	64 (22.7)	57 (21.6)	.76
14-day renal replacement therapy	15 (3.5)	9 (2.2)	.25	10 (3.3)	5 (1.8)	.24
30-day *Clostridioides difficile* infection (day 3–30)	3 (0.7)	4 (1.0)	.67	0	1 (0.4)	.48
Acquisition of new multidrug-resistant organisms within 30 days	22 (5.1)	15 (3.6)	.29	17 (5.7)	9 (3.2)	.15
Readmission within 30 days	76 (19.4)	91 (23.8)	.14	52 (18.6)	51 (19.4)	.82
Readmission within 30 days from bacteremia	15 (3.8)	16 (4.2)	.68	7 (2.5)	11 (4.2)	.54

All data are reported as n (%), unless specified otherwise. Significant differences are highlighted in bold.

The isolation of a multidrug-resistant organism includes vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, extended-spectrum cephalosporin-resistant Enterobacterales, and *Pseudomonas aeruginosa* and *Acinetobacter* species nonsusceptible to at least 1 agent in ≥3 antimicrobial categories as described by Magiorakos et al [18]. (i) Extended-spectrum cephalosporin-resistant Enterobacterales defined as intermediate or resistant to a third-generation cephalosporin. (ii) Carbapenem-resistant Enterobacterales defined as intermediate or resistant to imipenem, doripenem, ertapenem (R only), or meropenem. If the susceptibility test indicated the specimen was resistant to any of those medications, the specimen was categorized as "carbapenem nonsusceptible."



Kaplan-Meier analysis of the time from blood culture positivity to optimal antimicrobial therapy. Log-rank *P* <.0001

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Usefulness of BioFire FilmArray BCID2 for Blood Culture Processing in Clinical Practice

Benjamin Berinson,[a] Anna Both,[a] Laura Berneking,[a] Martin Christner,[a] Marc Lütgehetmann,[a] Martin Aepfelbacher,[a] Holger Rohde[a]

[a]Institute for Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

ABSTRACT Rapid pathogen characterization from positive blood cultures (BC) can improve management of patients with bloodstream infections (BSI). The FilmArray blood culture identification (BCID) assay is a molecular test approved for direct identification of BSI causing pathogens from positive BC. A recently updated version of the panel (BCID2) comprises improved species identification characteristics and allows for the detection of one expanded-spectrum β-lactamase (ESBL)- and several carbapenemase-encoding genes. Here, the clinical performance of the BCID2 assay for species identification in 180 positive BCs was evaluated. BCID2 results were concordant with the standard of care (SOC) in 159/180 (88.3%) BCs; 68/74 (91.9%) and 71/74 (96.0%) of all samples growing monobacterial, Gram-positive or Gram-negative pathogens, respectively, were identified, in agreement with SOC results. Nonconcordance was related to the detection of additional pathogens by the BCID2 assay (n = 4), discrepant species identification (n = 4), or failure of BCID2 to detect on-panel pathogens (n = 1). A number (12/31; 38.7%) of discordant results became evident in polymicrobial BC specimens. BCID2 identified the presence of bla_{CTX-M}-carrying species in 12 BC specimens but failed to predict third-generation cephalosporin resistance in four isolates exhibiting independent cephalosporin resistance mechanisms. Carbapenem resistance related to the presence of bla_{VIM-2} or bla_{Oxa-48}-like was correctly predicted in two isolates. In conclusion, the BCID2 assay is a reliable tool for rapid BC processing and species identification. Despite inclusion of common ESBL- or carbapenemase-encoding markers, the multifactorial nature of β-lactam resistance in Gram-negative organisms warrants combination of BCID2 with (rapid) phenotypic susceptibility assays.

KEYWORDS sepsis, blood culture, diagnostics, multiplex PCR, FilmArray, species identification, resistance, molecular methods, rapid tests, technical evaluation

A significant improvement of the BCID2 panel compared to BCID1 is the built-in ability to differentiate between *E. faecalis* and *E. faecium*. In combination with the ability to detect vanA-vanB

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.
  - *Salmonella* 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*
- *mec*A/C and MREJ
- bla_{NDM}
- $bla_{OXA-48-like}$
- bla_{VIM}
- *van*A/B

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KEYWORDS sepsis, blood culture, diagnostics, multiplex PCR, FilmArray, species identification, resistance, molecular methods, rapid tests, technical evaluation

Method

- Prospective single-center study
- Age ≥18, ICU/ED
- No positive blood culture in previous 7 days
- TTP < 20 hr (because CoNS usually >20 hours)

Results

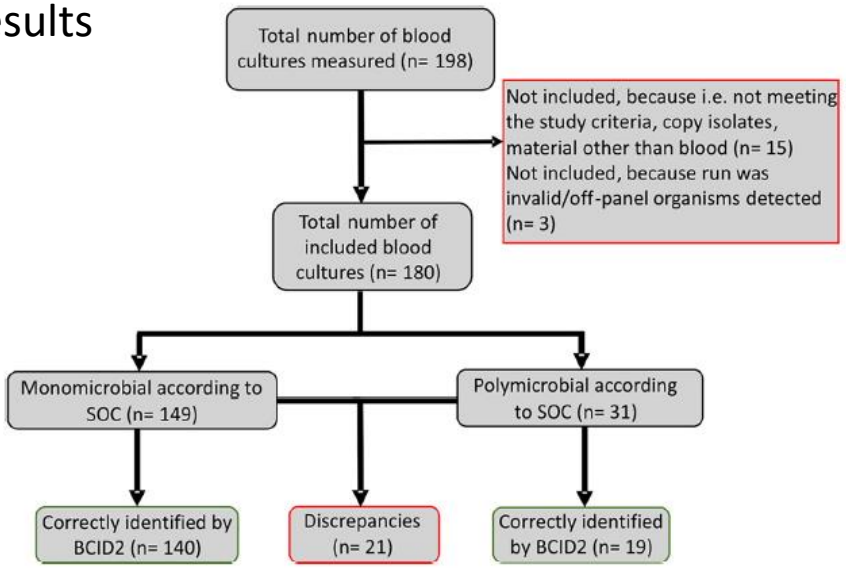

FIG 1 Flowchart showing the inclusion and results/interpretation of isolates. The discrepancies are presented in more detail in Table 1.

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Results

Concordant with SOC 159/180 (88.3%)

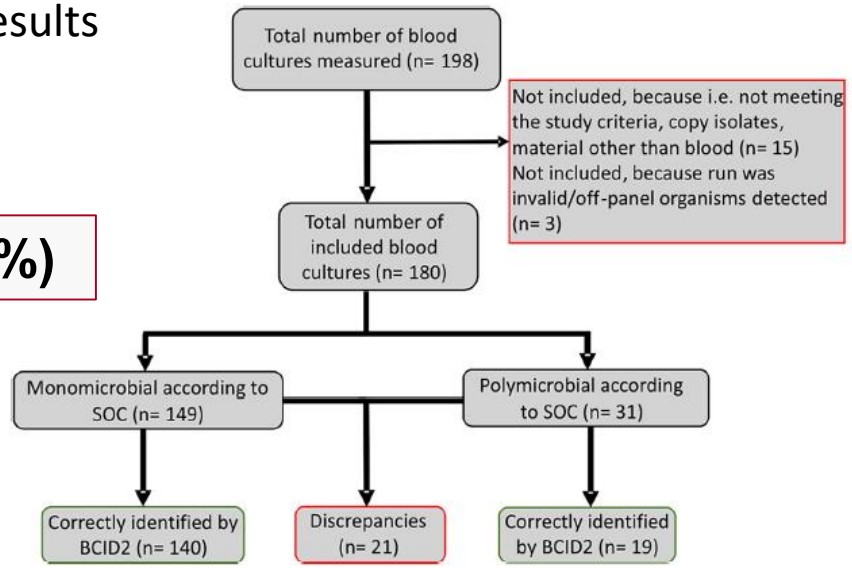

FIG 1 Flowchart showing the inclusion and results/interpretation of isolates. The discrepancies are presented in more detail in Table 1.

Discrepant result analysis

Out of the 68 Gram-positive monomicrobial results, 6 were discordant

Out of the 78 Gram-negative monomicrobial results, 3 were discordant

Out of the 31 polymicrobial blood cultures, BCID2 produce discordant results in 12 cases.

TABLE 1 Overview on discordant species identification by SOC analytics and the BCID2 assay system

Study no.	SOC identification	BCID2 identification
Monomicrobial Gram positive		
6	*E. faecalis*	*E. faecalis*, *Staphylococcus* spp.
47	*S. haemolyticus*	*S. epidermidis*
54	*E. faecalis*	*E. faecalis*, *S. epidermidis*
62	*S. haemolyticus*	*S. epidermidis*
97	*S. haemolyticus*	*S. epidermidis*
118	*S. haemolyticus*	*S. epidermidis*
Monomicrobial Gram negative		
17	*K. pneumoniae*	None
28	*E. coli*	*E. coli*, *S. epidermidis*
70	*E. coli*	*E. coli*, *S. epidermidis*
Polymicrobial culture		
5	*K. pneumoniae*, *S. capitis*	*K. pneumoniae* group
14	*P. aeruginosa*, *S. maltophilia*	*P. aeruginosa*
20	*E. faecium*, *S. haemolyticus*	*E. faecium*, *S. epidermidis*
51	*E. faecium*, *S. epidermidis*	*E. faecium*
58	*E. coli*, *A. veronii*	*E. coli*, *K. pneumoniae* group
73	*E. coli*, *S. epidermidis*	*E. coli*, *Staphylococcus* spp.
75	*S. haemolyticus*, *C. krusei*	*S. epidermidis*, *C. krusei*
82	*E. coli*, *S. anginosus* group	*E. coli*, *B. fragilis*, *Streptococcus* spp.
123	*C. perfringens*, *S. epidermidis*	None
127	*E. faecalis*, *E. faecium*, *Candida albicans*	*E. faecalis*, *E. faecium*
129	*K. oxytoca*, *E. faecium*	*K. oxytoca*
178	*P. agglomerans*, *S. haemolyticus*	*Enterobacterales*, *S. epidermidis*

Other limitations of note

An urgent field safety notice was released by *bioMérieux* due to the detection of Proteus species DNA in Proteus species-negative blood culture bottles in February 2020

Results on resistant genes

Clinical samples:

SOC identified:

- 16 3GC-R isolates
  - *E. coli* (n=12)
  - *K. pneumoniae* group (n=3)
  - *K. oxytoca* (n=1)
- 2 Carbapenem-R isolates
  - *K. pneumoniae* group (n=1)
  - *P. aeruginosa* (n=1)

BCID2 failed to detect

- *E.coli* bla_{TEM} (n=1)
- *K. pneumoniae* bla_{SHV} (n=1)
- *K. pneumoniae* bla_{SHV}, bla_{TEM} (n=1)

Spiked samples:

- correct 10 out of 10

TABLE 3 Usefulness of BCID2 to detect *mcr1*, bla_{CTX-M}, or carbapenemase-encoding genes from isolates grown in blood culture bottles

Isolate (resistance determinant)	BCID2 result
S. marcescens (bla_{OXA-48}-like, bla_{VIM-2}, bla_{CTX-M})[a]	*S. marcescens* (bla_{OXA-48}-like, bla_{VIM}, bla_{CTX-M})
E. cloacae complex (bla_{OXA-48}-like, bla_{NDM})[a]	*E. cloacae* complex (bla_{OXA-48}-like, bla_{NDM})
E. cloacae complex ($bla_{CTX-M-3}$, bla_{OXA-48}, bla_{TEM-1})[b]	*E. cloacae* complex (bla_{CTX-M}, bla_{OXA-48}-like)
E. cloacae complex ($bla_{CTX-M-9}$, bla_{OXA-48})[b]	*E. cloacae* complex (bla_{CTX-M}, bla_{OXA-48}-like)
E. coli ($bla_{CTX-M-15}$, $bla_{OXA-244}$)[b]	*E. coli* (bla_{CTX-M}, bla_{OXA-48}-like)
E. coli ($bla_{CTX-M-24}$, bla_{OXA-48})[b]	*E. coli* (bla_{CTX-M}, $blaa_{OXA-48}$-like)
E. coli (bla_{NDM-5}, bla_{TEM-1})[b]	*E. coli* (bla_{NDM})
E. coli ($bla_{CTX-M-3}$, $bla_{OXA-181}$, bla_{TEM-35})[b]	*E. coli* (bla_{CTX-M}, bla_{OXA-48}-like)
E. coli ($bla_{CTX-M-1}$, *mcr1.1*)[b]	*E. coli* (bla_{CTX-M}, *mcr1*)
K. oxytoca ($bla_{CTX-M-15}$, bla_{VIM-4})[b]	*K. oxytoca* (bla_{CTX-M}, $blaa_{VIM}$)

[a] β-Lactamase-encoding genes were detected by PCR (15).
[b] β-Lactamase-encoding genes were identified from whole-genome assemblies using abricate (https://github.com/tseemann/abricate) and the NCBI bacterial antimicrobial resistance reference gene database (https://www.ncbi.nlm.nih.gov/bioproject/313047).

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

A Multicenter Clinical Study To Demonstrate the Diagnostic Accuracy of the GenMark Dx ePlex Blood Culture Identification Gram-Negative Panel

Donna M. Wolk,[a] Stephen Young,[b] Natalie N. Whitfield,[c] Jennifer L. Reid,[c] Adam Thornberg,[c] Karen C. Carroll,[d] Blake W. Buchan,[e] Thomas E. Davis,[f] Hossein Salimnia[g]

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ABSTRACT Bacteremia can progress to septic shock and death without appropriate medical intervention. Increasing evidence supports the role of molecular diagnostic panels in reducing the clinical impact of these infections through rapid identification of the infecting organism and associated antimicrobial resistance genes. We report the results of a multicenter clinical study assessing the performance of the GenMark Dx ePlex investigational-use-only blood culture identification Gram-negative panel (BCID-GN), a rapid diagnostic assay for detection of bloodstream pathogens in positive blood culture (PBC) bottles. Prospective, retrospective, and contrived samples were tested. Results from the BCID-GN were compared to standard-of-care bacterial identification methods. Antimicrobial resistance genes (ARGs) were identified using PCR and sequence analysis. The final BCID-GN analysis included 2,444 PBC samples, of which 926 were clinical samples with negative Gram stain results. Of these, 109 samples had false-negative and/or -positive results, resulting in an overall sample accuracy of 88.2% (817/926). After discordant resolution, overall sample accuracy increased to 92.9% (860/926). Pre- and postdiscordant resolution sample accuracy excludes 37 Gram-negative organisms representing 20 uncommon genera, 10 Gram-positive organisms, and 1 *Candida* species present in 5% of samples that are not targeted by the BCID-GN. The overall weighted positive percent agreement (PPA), which averages the individual PPAs from the 27 targets (Gram-negative and ARG), was 94.9%. The limit of detection ranged from 10^4 to 10^7 CFU/ml, except for one strain of *Fusobacterium necrophorum* at 10^8 CFU/mL

GenMark Dx

ePlex® BCID-GP Panel	ePlex® BCID-GN Panel	ePlex® BCID-FP Panel
Gram-Positive Organisms	**Gram-Negative Organisms**	**Fungal Organisms**
Bacillus cereus group	*Acinetobacter baumannii*	*Candida albicans*
Bacillus subtilis group	*Bacteroides fragilis*	*Candida auris*
Corynebacterium	*Citrobacter*	*Candida dubliniensis*
Cutibacterium acnes	*Cronobacter sakazakii*	*Candida famata*
(Propionibacterium acnes)	*Enterobacter (non-cloacae complex)*	*Candida glabrata*
Enterococcus	*Enterobacter cloacae* complex	*Candida guilliermondii*
Enterococcus faecalis	*Escherichia coli*	*Candida kefyr*
Enterococcus faecium	*Fusobacterium nucleatum*	*Candida krusei*
Lactobacillus	*Fusobacterium necrophorum*	*Candida lusitaniae*
Listeria	*Haemophilus influenzae*	*Candida parapsilosis*
Listeria monocytogenes	*Klebsiella oxytoca*	*Candida tropicalis*
Micrococcus	*Klebsiella pneumoniae*	*Cryptococcus gattii*
Staphylococcus	*Morganella morganii*	*Cryptococcus neoformans*
Staphylococcus aureus	*Neisseria meningitidis*	*Fusarium*
Staphylococcus epidermidis	*Proteus*	*Rhodotorula*
Staphylococcus lugdunensis	*Proteus mirabilis*	
Streptococcus	*Pseudomonas aeruginosa*	
Streptococcus agalactiae (GBS)	*Salmonella*	
Streptococcus anginosus group	*Serratia*	
Streptococcus pneumoniae	*Serratia marcescens*	
Streptococcus pyogenes (GAS)	*Stenotrophomonas maltophilia*	
Resistance Genes	**Resistance Genes**	
mecA	CTX-M	
mecC	IMP	
vanA	KPC	
vanB	NDM	
	OXA	
	VIM	
Pan Targets	**Pan Targets**	
Pan Gram-Negative	Pan Gram-Positive	
Pan *Candida*	Pan *Candida*	

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Investigational-use-only

GenMark Dx

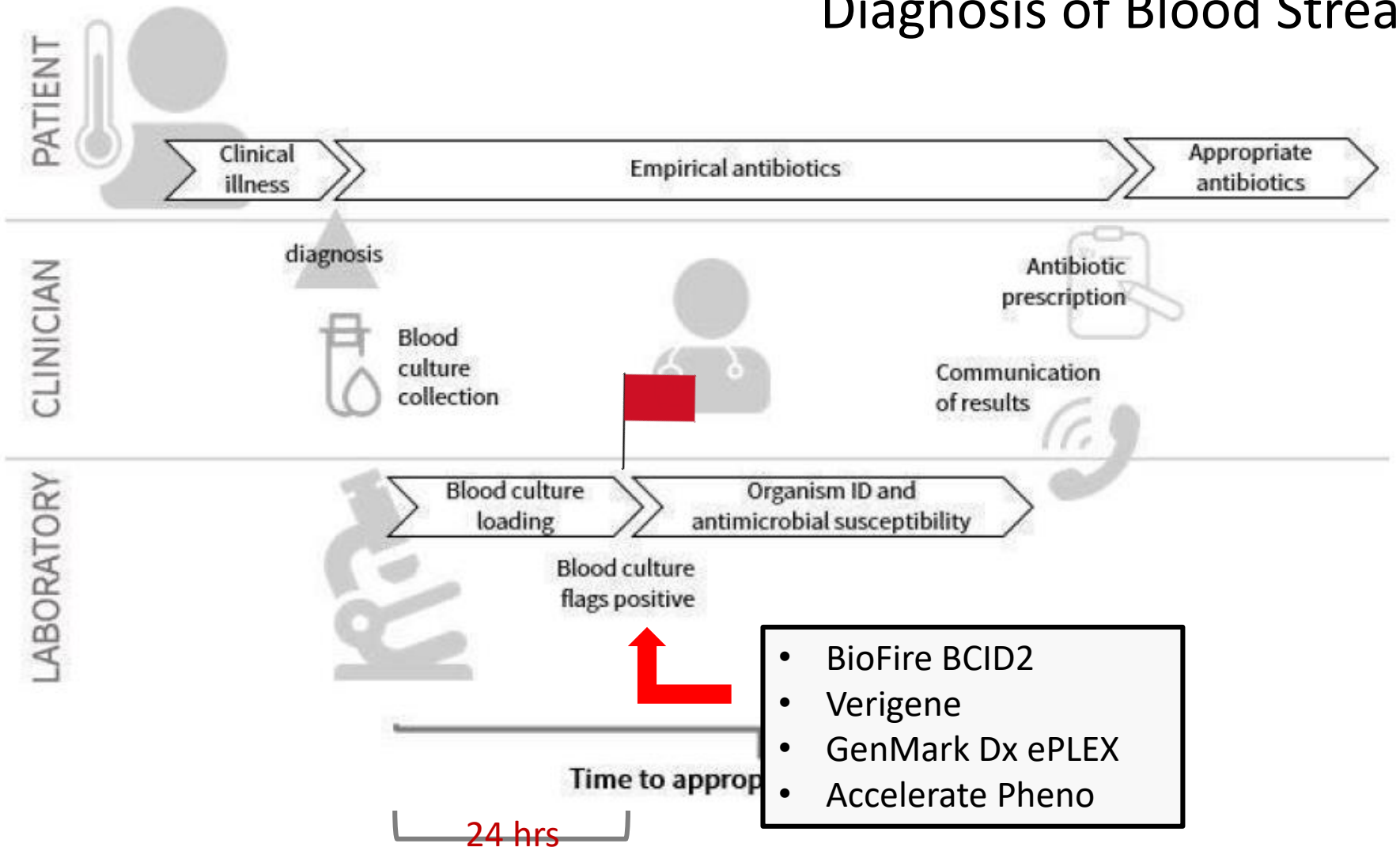
Study
- All ages (with 10% <18 years old)
- 10 geographically diverse regions within the USA
- 13 different blood culture bottle systems from 3 different manufacturers

- A combination of 354 prospectively enrolled, 1,326 retrospectively selected (from frozen, banked PBC samples), and 780 contrived (isolates spiked into whole blood and blood culture bottles) samples were tested with the BCID-GN.

- Compare the Gram-Negative panel only

Results
- Overall sample accuracy 88.2%

Diagnosis of Blood Stream Infection



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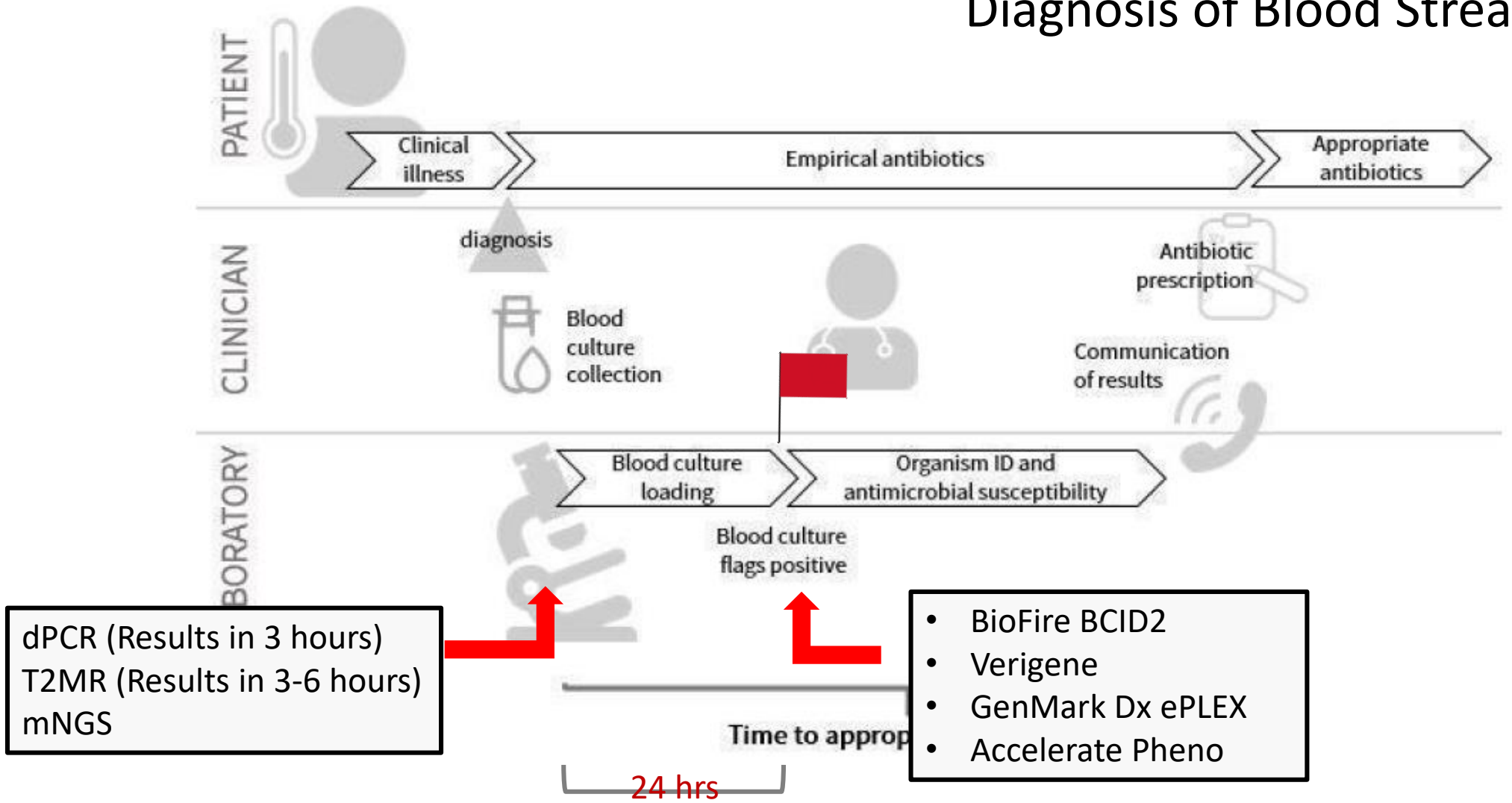
Diagnosis of Blood Stream Infection



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BMC Infectious Diseases

RESEARCH Open Access

Role of the T2Dx magnetic resonance assay in patients with suspected bloodstream infection: a single-centre real-world experience

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Abstract

Background: T2Dx was approved by the US Food and Drug Administration for the rapid detection of a modified panel of ESKAPE bacterial species or *Candida* spp. causing bloodstream infection (BSI).

Patients and methods: We performed a retrospective, observational study from January 1, 2018 to December 31, 2019 of all hospitalised patients with suspected BSI who underwent assessment using T2Dx in addition to standard blood culture (BC). T2-positive patients (cases) were compared to a matched group of patients with BSI documented only by BC (1:2 ratio) to investigate the possible impact of T2Dx on the appropriateness of empirical antimicrobial therapy and 21-day mortality.

Results: In total, 78 T2Dx-analysed samples (49 patients) were analysed. The T2Dx assay result was positive for18 patients and negative for 31 patients. The concordance rates of the T2Bacteria Panel and T2Candida Panel results with those of standard BC were 74.4% and 91.4%, respectively. In the matched analysis, inappropriate empiric antimicrobial therapy administration was significantly less frequent in cases than in comparators (5.5% vs. 38.8%). The 21-day mortality rate was twofold lower in cases than in comparators (22.2% vs. 44.4%), although the difference was not significant. No other analysed variables were significantly different between the two groups.

Conclusions: This study illustrated that T2Dx might be associated with an increase in the appropriateness of empiric antimicrobial therapy in patients with BSI. Further studies are needed to evaluate whether the T2Dx assay can improve patient outcomes.

Keywords: Blood stream infections, T2Dx, ESKAPE, Antimicrobial therapy

Directly from whole blood sample
ESKAPE bacterial species

- *Enterococcus faecium*
- *Staphylococcus aureus*
- *Klebsiella pneumoniae*
- *Acinetobacter baumannii*
- *Pseudomonas aeruginosa*
- *Enterobacter species*

Single center

- 2 year period
- Retrospective observational case-control study
- Consecutive patients hospitalized and suspected BSI

- Outcome
- Appropriateness of empirical antimicrobial
- 21 day mortality

Results

Inappropriate empirical antimicrobial therapy

- T2Bacteria/T2candida: 5.5%
- Standard: 38.8%

78 samples from 49 patients

Concordance result

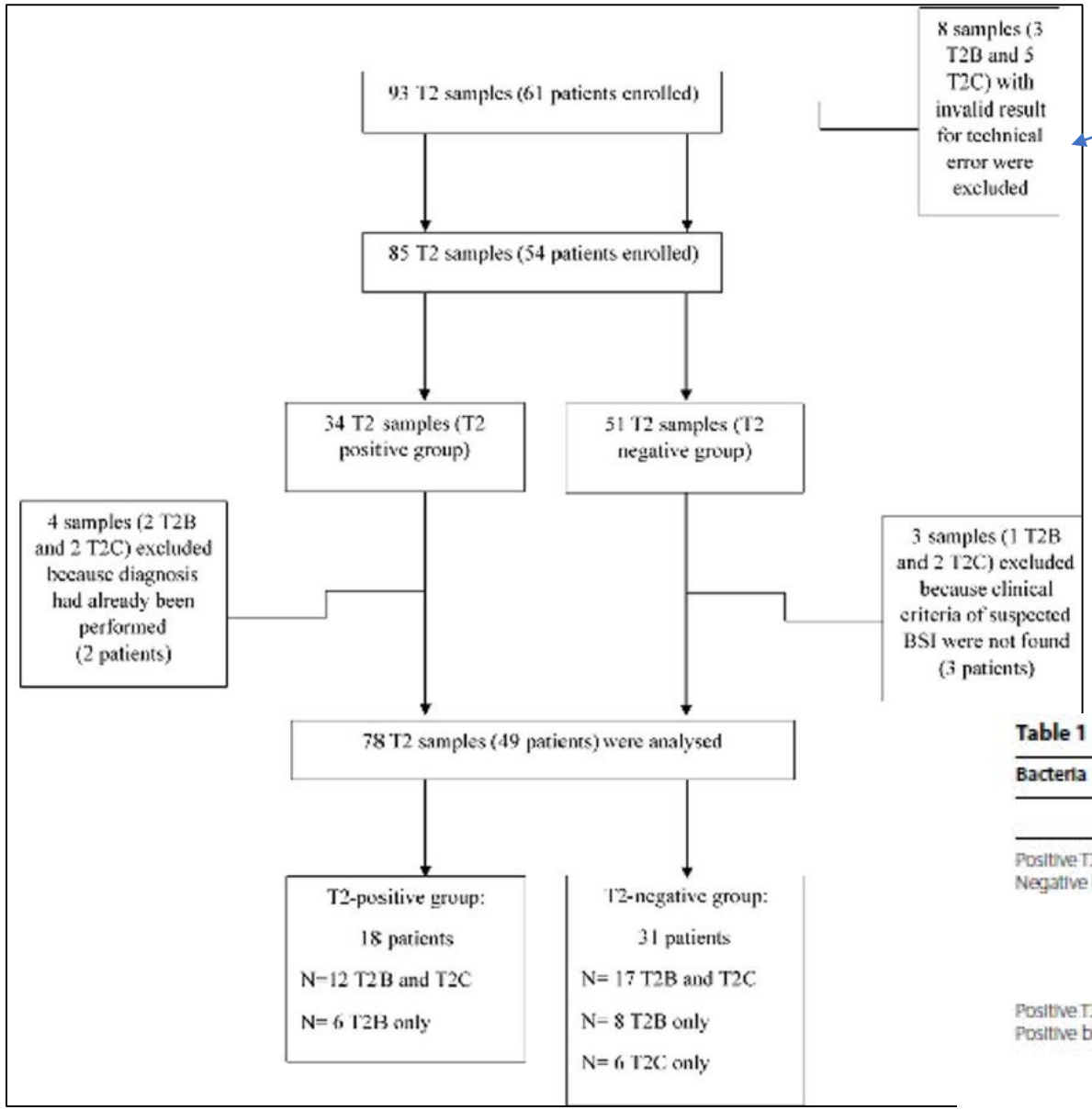
- T2Bacteria Panel: 74.4%
- T2Candida panel: 91.4%

21-day mortality rate was not statistically different between the T2-positive and comparator groups, a twofold difference was recorded between the groups

Variables	T2-positive group (n = 18)	Standard blood culture (n = 36)	p value
Demographic information			
Age (years), mean (SD)	59.7 (21.1)	60.2 (16.0)	0.923
Male gender	13 (72.2)	24 (66.6)	0.678
Patient's co-morbidities			
Chronic kidney disease	3 (16.6)	7 (19.4)	0.804
Hypertension	9 (50.0)	12 (33.3)	0.236
Diabetes	3 (16.6)	6 (16.6)	1.000
Ischaemic heart disease	4 (22.2)	8 (22.2)	1.000
Neurological disease	4 (22.2)	4 (11.1)	0.278
COPD	1 (5.5)	7 (19.4)	0.175
Obesity	1 (5.5)	6 (16.6)	0.251
Cancer (all)	6 (33.3)	12 (33.3)	1.000
Solid cancer	4 (22.2)	8 (22.2)	1.000
Haematological cancer	2 (11.1)	5 (13.8)	0.774
Hospitalisation area			
Intensive care unit	11 (61.1)	20 (55.5)	0.697
Medical unit	5 (27.7)	11 (30.5)	0.833
Surgical unit	2 (11.1)	5 (13.8)	0.774
Microorganism			
Escherichia coli	4 (22.2)	10 (27.7)	0.660
Klebsiella pneumoniae	4 (22.2)	9 (25.0)	0.821
Pseudomonas aeruginosa	2 (11.1)	4 (11.1)	1.000
Acinetobacter baumannii	6 (33.3)	11 (30.5)	0.835
Enterococcus faecium	2 (11.1)	4 (11.1)	1.000
Candida spp.	5 (27.7)	5 (13.8)	0.215
Mixed species	5 (27.7)	6 (16.6)	0.339
Primary site of BSI			
Respiratory	8 (44.4)	16 (44.4)	1.000
Urinary	3 (16.6)	4 (11.1)	0.566
Abdominal	4 (22.2)	8 (22.2)	1.000
Heart	0	1 (2.7)	0.475
Skin and soft tissue	2 (11.1)	3 (8.3)	0.739
Unknown	1 (5.5)	5 (13.8)	0.358
Hospitalisation data			
Length of hospitalisation (days), mean (SD)	32.7 (14.9)	25.1 (17.5)	0.115
Time from admission to infection (days), mean (SD)	16.1 (15.7)	12.3 (15.5)	0.411
Time from infection to discharge/death (days), mean (SD)	16.8 (11.1)	12.8 (11.1)	0.219
Miscellaneous			
PCT at baseline, mean (SD)	18.6 (42.8)	20.6 (37.2)	0.858
Mechanical ventilation	10 (55.5)	17 (47.2)	0.563
Inotrope drugs	9 (50)	17 (47.2)	0.847
ECMO	4 (22.2)	4 (11.1)	0.278
CVVH	2 (11.1)	7 (19.4)	0.438
Inappropriate empiric antibiotic therapy	1 (5.5)	14 (38.8)	**0.009**
21-day mortality	4 (22.2)	16 (44.4)	0.110

COPD chronic obstructive pulmonary disease, *PCT* procalcitonin, *ECMO* extracorporeal membrane oxygenation, *CVVH* continuous veno-venous hemofiltration

All values are presented as n (%) unless otherwise specified



Technical errors noted

Missed detecting on-panel organisms

Table 1 Results of T2Dx and standard blood culture for bacteria and fungi

Bacteria			
		T2Dx	**Blood culture**
Positive T2Dx Negative blood culture	n = 6	*E. faecium* (1) *K. pneumoniae/A. baumannii* (1) *A. baumannii* (1) *P. aeruginosa* (2) *E. coli* (1)	
Positive T2Dx Positive blood culture	n = 8	*A. baumannii* (2) *E. coli* (1) *K. pneumoniae/A. baumannii* (1) *K. pneumoniae/E. coli* (2) *E. faecium* (1) *A. baumannii* (1)	*A. baumannii* (2) *E. coli* (1) *K. pneumoniae* (1) *E. coli* (2) *Acinetobacter* spp. (1) *E. cloacae* complex (1) *S. epidermidis* (5) *S. epidermidis/A. baumannii* (1) *K. pneumoniae* (1)
Negative T2Dx Positive blood culture	n = 7		

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Duplex dPCR System for Rapid Identification of Gram-Negative Pathogens in the Blood of Patients with Bloodstream Infection: A Culture-Independent Approach

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Early and accurate detection of pathogens is important to improve clinical outcomes of bloodstream infections (BSI), especially in the case of drug-resistant pathogens. In this study, we aimed to develop a culture-independent digital PCR (dPCR) system for multiplex detection of major sepsis-causing gram-negative pathogens and antimicrobial resistance genes using plasma DNA from BSI patients. Our duplex dPCR system successfully detected nine targets (five bacteria-specific targets and four antimicrobial resistance genes) through five reactions within 3 hours. The minimum detection limit was 50 ag of bacterial DNA, suggesting that 1 CFU/ml of bacteria in the blood can be detected. To validate the clinical applicability, cell-free DNA samples from febrile patients were tested with our system and confirmed high consistency with conventional blood culture. This system can support early identification of some drug-resistant gram-negative pathogens, which can help improving treatment outcomes of BSI.

Keywords: Digital PCR, bloodstream infection, Gram-negative, blood-stream infection, antimicrobial resistance

Advantage of dPCR over conventional PCR
→ much higher sensitivity

Table 1. Primer/probe sequences of targeted genes for dPCR.

	Target gene	Probe	Primer/probe sequences
A. baumanii	*ompA*	VIC	F: ACGTAGTTCTTGGTGGTCACTTGA
			R: AGGTCTTCAGTTAACTCTTGTGGTTGT
			P: CTCCTGTAGTAGAAGTTG
E. coli	*uidA*	VIC	F: GGGCGAACAGTTCCTGATCA
			R: TCATGACGACCAAAGCCAGTAA
			P: CCACAAACCGTTCTAC
	lacY	FAM	F: CTGGTCTGTTTCTGCTTCTTTAAGC
			R: TGCCCGCCAGTACAGACA
			P: ACTGGCGATGATTTT
K. pneumoniae	*phoE*	VIC	F: TGCAGTACCAGGGTAAAAACGA
			R: CGCCGTCGCCGTTCT
			P: CCGTGAAGCGAAGAA
P. aeruginosa	*ecf*	FAM	F: CCGCGCGCATTTCTTTT
			R: CCAATGGTCGCGCAACA
			P: CAGATCGCCCGCAAC
Resistance genes	$bla_{\text{TEM-type}}$	VIC	F: TGCTGCCATAACCATGAGTGA
			R: GGTCCTCCGATCGTTGTCA
			P: TGCTGCCAACTTACT
	$bla_{\text{CTX-M}}$ group 1	FAM	F: GACGCTGGGTAAAGCATTGG
			R: GGTATTGCCTTTCATCCATGTCA
			P: ACAGCCAACGGGC
	$bla_{\text{NDM-1}}$	VIC	F: GACCGCCCAGATCCTCAA
			R: CGCGACCGGCAGGTT
			P: TGGATCAAGCAGGAGAT
	$bla_{\text{IMP-type}}$	FAM	F: CGATCTATCCCCACGTATGCA
			R: GGCTTGAACCTTACCGTCTTTTT
			P: CTGAATTAACAAATGAACTGC

The Gibbs free energy (ΔG) of each prob[illegible] ?q=mfold/); the probes with ΔG ≥ 0 were [illegible]

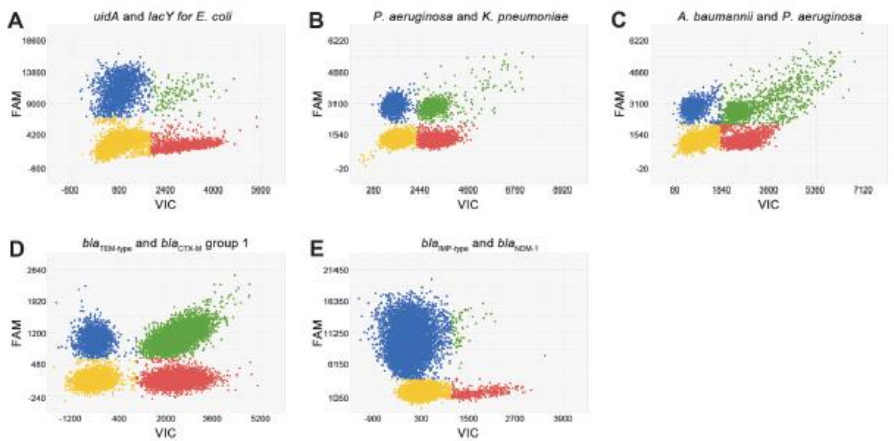

Fig. 3. Duplex identification of target genes by digital PCR. (**A**) The *uidA* (x-axis, red) and *lacY* (y-axis, blue) genes specifically identified in a duplex reaction condition from the *E. coli* reference strain (ATCC 25922). (**B**) The *ecfX* (y-axis, blue) and *phoE* (x-axis, red) genes identified from the DNA mixture of *P. aeruginosa* and *K. pneumoniae*. (**C**) The *ompA* (x-axis, red) and *ecfX* (y-axis, blue) genes identified from the DNA mixture of *A. baumannii* and *P. aeruginosa*. (**D**) The $bla_{\text{TEM-type}}$ (x-axis, red) and $bla_{\text{CTX-M}}$ group 1 (y-axis, blue) genes identified from the *E. coli* isolate cm241. (**E**) The $bla_{\text{IMP-type}}$ (y-axis, blue) and $bla_{\text{NDM-1}}$ (x-axis, red) genes identified from the DNA mixture of *P. aeruginosa* cmPA-1 and *E. coli* cmEC-1 isolates.

Study Protocol Clinical Trial

Medicine

OPEN

Next-generation sequencing diagnostics of bacteremia in pediatric sepsis

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Abstract

Introduction: Sepsis and septic shock are the most severe forms of infection affecting predominantly elderly people, preterm and term neonates, and young infants. Even in high-income countries sepsis causes about 8% of admissions to pediatric intensive care units (PICUs). Early diagnosis, rapid anti-infective treatment, and prompt hemodynamic stabilization are crucial for patient survival. In this context, it is essential to identify the causative pathogen as soon as possible to optimize antimicrobial treatment. To date, culture-based diagnostic procedures (e.g., blood cultures) represent the standard of care. However, they have 2 major problems: on the one hand, in the case of very small sample volumes (and thus usually in children), they are not sufficiently sensitive. On the other hand, with a time-to-result of 2 to 5 days, blood cultures need a relatively long time for the anti-infective therapy to be calculated. To overcome these problems, culture-independent molecular diagnostic procedures such as unbiased sequence analysis of circulating cell-free DNA (cfDNA) from plasma samples of septic patients by next-generation sequencing (NGS) have been tested successfully in adult septic patients. However, these results still need to be transferred to the pediatric setting.

Methods: The Next GeneSIPS-Trial is a prospective, observational, non-interventional, multicenter study used to assess the diagnostic performance of an NGS-based approach for the identification of causative pathogens in (preterm and term) neonates (d1–d28, n = 50), infants (d29 to <1 yr, n = 50), and toddlers (1 yr to <5 yr, n = 50) with suspected or proven severe sepsis or septic shock (according to the pediatric sepsis definition) by the use of the quantitative sepsis indicating quantifier (SIQ) score in comparison to standard of care (culture-based) microbiological diagnostics. Potential changes in anti-infective treatment regimens based on these NGS results will be estimated retrospectively by a panel of 3 independent clinical specialists.

Discussion: Neonates, infants, and young children are significantly affected by sepsis. Fast and more sensitive diagnostic approaches are urgently needed. This prospective, observational, non-interventional, multicenter study seeks to evaluate an NGS-based approach in critically ill children suffering from sepsis.

Trial registration: DRKS-ID: DRKS00015705 (registered October 24, 2018). https://www.drks.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00015705

The Next-GeneSIPS-trial is funded by the Dietmar Hopp Foundation, St. Leon-Rot (Germany). Publication rights are owned by the investigators.

Ethics approval: The clinical trial protocol and the corresponding documents will be approved by the IRB of all participating centers. A first positive ethical vote has been given by the Ethics Committee of the Medical Faculty of Heidelberg, Trial Codes No. S-605/2018.

Consent of publication: Not applicable.

Availability of data: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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frontiers in Cellular and Infection Microbiology

METHODS
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Optimising Treatment Outcomes for Children and Adults Through Rapid Genome Sequencing of Sepsis Pathogens. A Study Protocol for a Prospective, Multi-Centre Trial (DIRECT)

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Background: Sepsis contributes significantly to morbidity and mortality globally. In Australia, 20,000 develop sepsis every year, resulting in 5,000 deaths, and more than AUD$846 million in expenditure. Prompt, appropriate antibiotic therapy is effective in improving outcomes in sepsis. Conventional culture-based methods to identify appropriate therapy have limited yield and take days to complete. Recently, nanopore technology has enabled rapid sequencing with real-time analysis of pathogen DNA. We set out to demonstrate the feasibility and diagnostic accuracy of pathogen sequencing direct from clinical samples, and estimate the impact of this approach on time to effective therapy when integrated with personalised software-guided antimicrobial dosing in children and adults on ICU with sepsis.

Methods: The DIRECT study is a pilot prospective, non-randomized multicentre trial of an integrated diagnostic and therapeutic algorithm combining rapid direct pathogen sequencing and software-guided, personalised antibiotic dosing in children and adults with sepsis on ICU.

Participants and interventions: DIRECT will collect microbiological and pharmacokinetic samples from approximately 200 children and adults with sepsis admitted to one of four

Rapid diagnosis in Blood stream infections - Summary

- Current technologies can provide accurate and rapid organism ID & AST results (genotype or phenotype)
- Lead to reduce time to appropriate therapy
- Newer technologies such as dPCR and NGS could further improve sepsis care

THANK YOU!

DISCUSSIONS WELCOMED