



Que retenir de 2022 ?

Diagnostic microbiologique

Keyvan Razazi

Service de Médecine intensive-réanimation

Hôpital Henri Mondor, Créteil

Liens d'intérêt

- Aucun
- Trésorier de l'institut Maurice Rapin (journée d'infectiologie)

Plan

1. Détecter le micro-organisme
 2. Identification/comparaison
 3. Obtenir un antibiogramme rapidement
-

Plan

1. Détecter le micro-organisme
 2. Identification/comparaison
 3. Obtenir un antibiogramme rapidement
-

PCR multiplex

- Respiratoire
- Hémoculture
- Gastro-intestinal
- Méningite

Comparaison des mPCR

- 2016-2018, 15 service de réa UK
- Pneumonie nosocomiales (HAP / VAP)
- Aspi B 299, LBA 44, ECBC 272
- Unyvero +FilmArray+ culture

Original research

Multicentre evaluation of two multiplex PCR platforms for the rapid microbiological investigation of nosocomial pneumonia in UK ICUs: the INHALE WP1 study

Virve I Enne,¹ Alp Aydin,¹ Rossella Baldan,^{2,3} Dewi R Owen,¹ Hollian Richardson,³ Federico Ricciardi,⁴ Charlotte Russell,³ Brenda O Nomamiukor-Ikeji,¹ Ann-Marie Swart,⁵ Juliet High,⁵ Antony Colles,⁵ Julie Barber,⁴ Vanya Gant,^{6,7} David M Livermore,³ Justin O'Grady,^{3,8} INHALE WP1 Study Group

Table 6 Scores allocated to PCR tests based on scoring system designed to evaluate overall performance, ease of use and implementability

Criterion	Machine score			
	Curetis Unyvero Pneumonia Panel		BioFire FilmArray Pneumonia Panel	
	Value	Score	Value	Score
Overall concordance (max 45 points)	74.8%	20	71.3%	16
Sensitivity for detection of common pathogens (max 20 points)	3 targets with better performance	6	7 targets with better performance	14
Breadth of panel (max 15 points)	244 unique detections	15	191 unique detections	12
Time to result (max 15 points)	270 min	7	75 min	14
Cost per test (max 15 points)*	+++	10	++	15
Failure rate (max 15 points)	9.1%†	0	1.9%	11
Footprint (max five points)	7.4 sq. ft	1	3.2 sq. ft	5
Customer service (max five points)	–	3	–	4
Consumable logistics (max five points)‡	–	0	–	5
Ease of use (max 10 points)	–	6	–	9
Total (Max 150)	–	68	–	105

mPCR et pneumonie

 Taylor & Francis
Taylor & Francis Group

INFECTIOUS DISEASES,
2020; VOL. 52,
NO. 7, 479–488

ORIGINAL ARTICLE

<https://doi.org/10.1080/23744235.2020.1755053>

OPEN ACCESS 

Evaluation of the Biofire Filmarray Pneumonia panel plus for lower respiratory tract infections

Alicia Edin^{a,b} , Hinnerk Eilers^a and Annika Allard^a

Original Article

Performance of a multiplex PCR pneumonia panel for the identification of respiratory pathogens and the main determinants of resistance from the lower respiratory tract specimens of adult patients in intensive care units



Sze Hwei Lee^{a,b}, Sheng-Yuan Ruan^b, Sung-Ching Pan^b,
Tai-Fen Lee^a, Jung-Yien Chien^{b,*}, Po-Ren Hsueh^{a,b,**}

RESEARCH

Open Access

Multicenter evaluation of a syndromic rapid multiplex PCR test for early adaptation of antimicrobial therapy in adult patients with pneumonia



Céline Monard¹, Jonathan Pehlivan², Gabriel Auger^{3,4}, Sophie Alviset⁵, Alexy Tran Dinh^{6,7}, Paul Duquaire¹, Nabil Gastli⁸, Camille d'Humières^{9,10}, Adel Maamar^{11,12}, André Boibieux¹³, Marion Baldeyrou¹⁴, Julien Loubinoux¹⁵, Olivier Dauwalder^{16,17}, Vincent Cattoir^{3,18,19}, Laurence Armand-Lefèvre^{9,10}, Solen Kernéis^{5,10*} and the ADAPT study group

mPCR et COVID



RESEARCH ARTICLE
November/December 2021 Volume 9 Issue 3 e00695-21
<https://doi.org/10.1128/Spectrum.00695-21>

Diagnosis and Treatment of Bacterial Pneumonia in Critically Ill Patients with COVID-19 Using a Multiplex PCR Assay: A Large Italian Hospital's Five-Month Experience

Brunella Posteraro^{a,b}, Venere Cortazzo^a, Flora Marzia Liotti^{a,c}, Giulia Menchinelli^{a,c}, Chiara Ippoliti^a, Giulia De Angelis^{a,c}, Marilena La Sorda^c, Gennaro Capalbo^d, Joel Vargas^a, Massimo Antonelli^{a,e}, Maurizio Sanguinetti^{a,c}, Gennaro De Pascale^{a,e}, Teresa Spanu^{a,c}

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 Botero¹, Juan Pablo Isaza¹, Luz Elena Cano^{1,3}, Lucelly López¹, Leidy Tamayo¹ and Antoni Torres^{4,5}

European Journal of Clinical Microbiology & Infectious Diseases
<https://doi.org/10.1007/s10096-021-04213-6>

BRIEF REPORT



Impact of rapid multiplex PCR on management of antibiotic therapy in COVID-19-positive patients hospitalized in intensive care unit

Naouale Maataoui^{1,2}, Lotfi Chemali², Juliette Patrier³, Alexy Tran Dinh^{4,5}, Lucie Le Fèvre³, Brice Lortat-Jacob⁴, Mehdi Marzouk³, Camille d'Humières^{1,2}, Emilie Rondinaud^{1,2}, Etienne Ruppé^{1,2}, Philippe Montravers^{4,5}, Jean-François Timsit^{1,3}, Laurence Armand-Lefèvre^{1,2}



Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio



Performance of a multiplex polymerase chain reaction panel for identifying bacterial pathogens causing pneumonia in critically ill patients with COVID-19



François Caméléna^{a,b,1}, Anne-Clotilde Moy^{c,1}, Emmanuel Dudoignon^{c,d}, Thibaut Poncin^{a,b}, Benjamin Deniau^{c,d}, Lucie Guillemet^c, Jérôme Le Goff^a, Mélissa Budoo^a, Mourad Benyamina^c, Maïté Chaussard^c, Maxime Coutrot^c, Matthieu Lafaurie^e, Benoît Plaud^{c,d}, Alexandre Mebazaa^{c,d}, François Depret^{c,d}, Béatrice Berçot^{a,b,*}

Article

Potential of Multiplex Polymerase Chain Reaction Performed on Protected Telescope Catheter Samples for Early Adaptation of Antimicrobial Therapy in ARDS Patients

Keyvan Razazi ^{1,2,*}, Flora Delamaire ^{1,†}, Vincent Fihman ^{3,4}, Mohamed Ahmed Boujelben ^{1,2}, Nicolas Mongardon ^{5,6,7}, Ségolène Gendreau ^{1,2}, Quentin de Roux ^{5,6,7}, Nicolas de Prost ^{1,2,8}, Guillaume Carteaux ^{1,2,8}, Paul-Louis Woerther ^{3,4} and Armand Mekontso Dessap ^{1,2,8}



- 2 réanimations
- Hôpital Henri Mondor
- 125 mPCR parmi 95 patients
 - ❑ 48 CAP/HAP
 - ❑ 77 PAVM

Table 1. Characteristics of patients.

Clinical Characteristics and Comorbidities	Patients <i>n</i> = 95
Age, years, median [IQR]	60 [52–71]
Male gender, <i>n</i> (%)	79 (80%)
SAPS II at ICU admission, median [IQR]	38 [30–50]
Charlson Comorbidity index, median [IQR]	3 [2–5]
Diabetes mellitus, <i>n</i> (%)	40 (40%)
Congestive heart failure (NYHA 3–4), <i>n</i> (%)	6 (6%)
COPD, <i>n</i> (%)	9 (9%)
Immunosuppression condition, <i>n</i> (%)	21 (22%)
Organ failures and outcome	
ARDS	95 (100%)
Extracorporeal membrane oxygenation	28 (29%)
Dialysis	42 (44%)
White blood cell count ($\times 10^9$ /L)	11.4 [8.7–15.9]
C-Reactive Protein, mg/L	143 [91–216]
Procalcitonin, μ g/L	1.0 [0.3–4.8]
Death in ICU	42 (44%)

SAPS, simplified acute physiologic score; ICU, intensive care unit; ARDS, acute respiratory distress syndrome.

BioFire® FilmArray® Pneumonia plus Panel faisable sur les PDP.

Table 2. Analytical performance of BioFire® FilmArray® Pneumonia plus Panel compared with culture, accounting for microbiological thresholds.

Bacterial Target	No. of Specimens				mPCR Performance				Cohen's Kappa Coefficient
	Culture+/FA-PP+	Culture+/FA-PP-	Culture-/FA-PP+	Culture-/FA-PP-	Se (95% CI), %	Sp (95% CI), %	PPV (95% CI), %	NPV (95% CI), %	
<i>Acinetobacter calcoaceticus-baumannii</i> complex	0	0	0	125	NA	100	NA	100	
<i>Enterobacter cloacae</i> complex	2	0	1	122	100	99	67	100	
<i>Escherichiacoli</i>	2	0	2	121	100	98	50	100	
<i>Haemophilus influenzae</i>	0	0	1	124	NA	99	0	100	
<i>Klebsiella aerogenes</i>	2	1	1	121	67	99	99	98	
<i>Klebsiellaoxytoca</i>	0	0	0	125	NA	100	NA	100	
<i>Klebsiellapneumoniae</i> group	0	0	0	125	NA	100	NA	100	
<i>Moraxellacatarhalis</i>	1	0	0	124	100	100	100	100	
<i>Proteus</i> spp.	2	0	0	123	100	100	100	100	
<i>Pseudomonasaeruginosa</i>	11	0	1	113	100	99	92	99	
<i>Serratia marcescens</i>	1	0	0	124	100	100	100	100	
<i>Streptococcus pneumoniae</i>	1	0	1	123	100	99	50	100	
<i>Staphylococcus aureus</i>	5	1	3	116	83	97	63	99	
<i>Streptococcus pyogenes</i>	0	0	0	125	NA	100	NA	100	
<i>Streptococcusagalactiae</i>	0	0	2	123	NA	98	0	100	
<i>Legionella pneumophila</i>	1	0	1	123	100	99	50	100	
TOTAL	28	2	13	1957	93 [84–100]	99 [99–100]	68 [54–83]	100 [100]	0.8 [0.68–0.89]

Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; FA-PP, FilmArray® Pneumonia plus Panel.

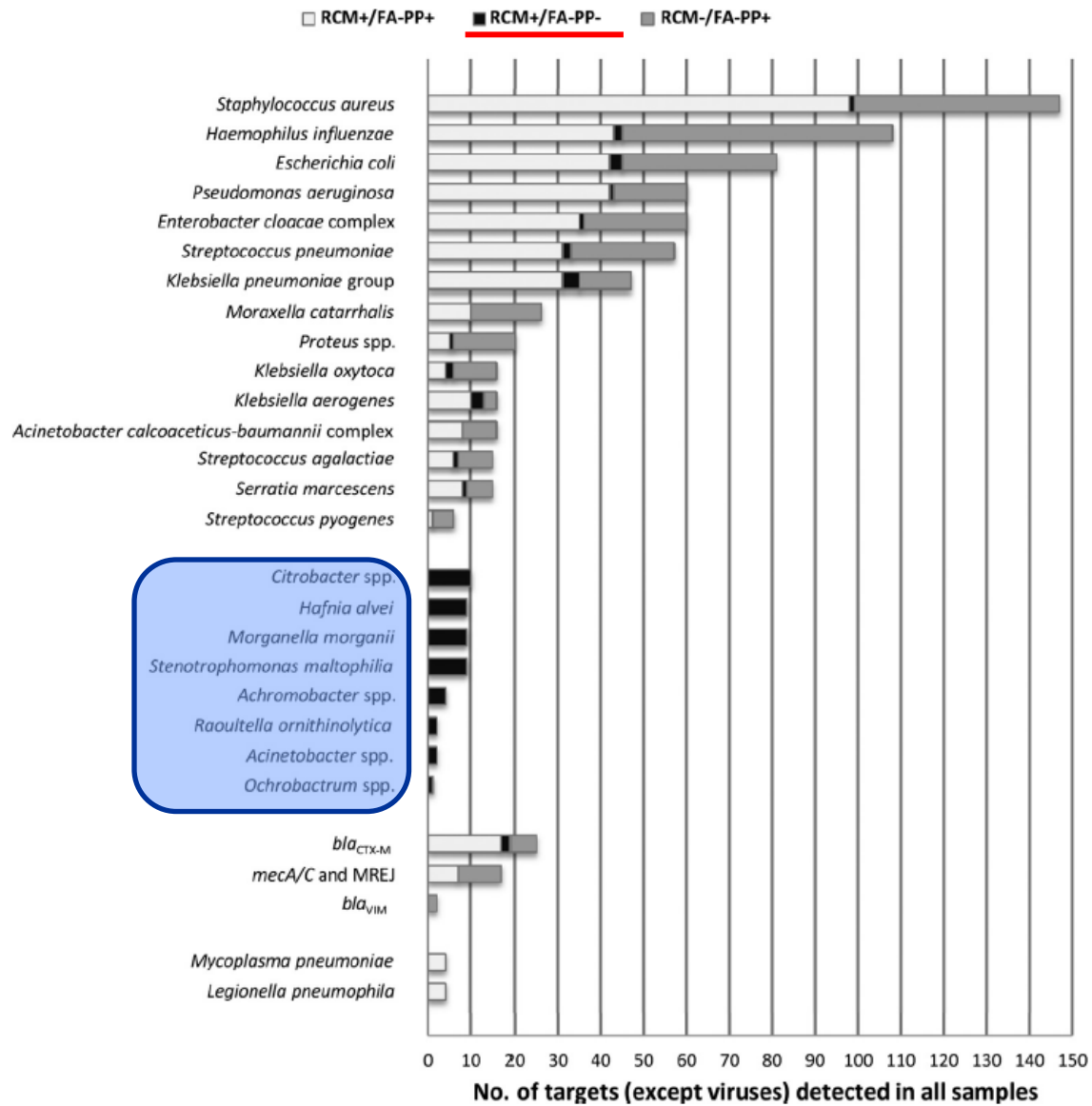
Nb: faux positifs pour SARM: mecA/C with MREJ.

Impact du résultat ?

Table 3. Impact of mPCR results on antibiotic prescription (*n* =125).

		Suspected CAP/HAP Cases (<i>n</i> = 48)		Suspected VAP Cases (<i>n</i> = 77)	
		mPCR – (<i>n</i> = 45)	mPCR + (<i>n</i> = 3)	mPCR – (<i>n</i> = 49)	mPCR + (<i>n</i> = 28)
Antibiotic modification after mPCR		1	3	2	12
• De-escalation		1	3	2	1
	Narrower spectrum antibiotic	0	3	1	1
	Stop antibiotic	1	0	1	0
• Escalation			0		11
	Escalation/Adaptation		0		4
	Escalation usefulness		0		2
	Initiation		0		5
No change after mPCR results		44	0	47	16
• Continuation of antibiotic initiated after suspecting pneumonia		15	0	20	14
• No new antibiotic	Continuation of antibiotic initiated before suspecting pneumonia *	27	0	19	2
	No antibiotic initiation	2	0	8	0

* antibiotic for a previous infectious episode.



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

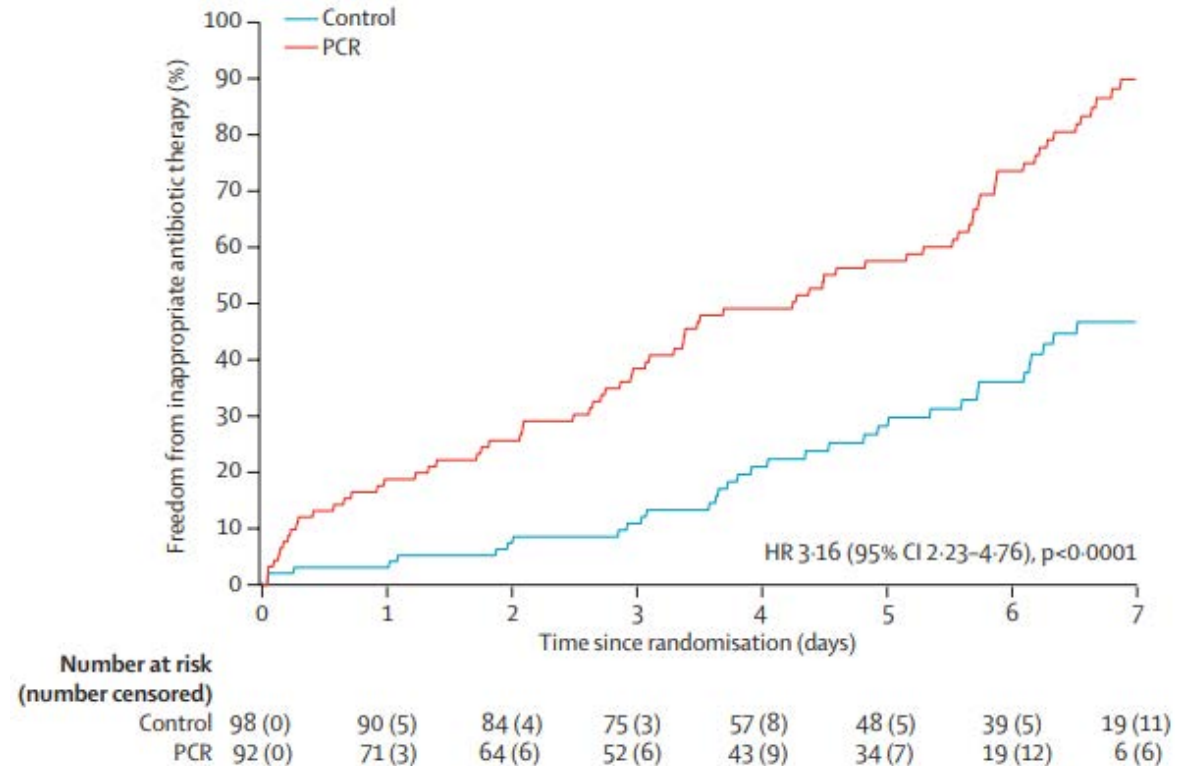
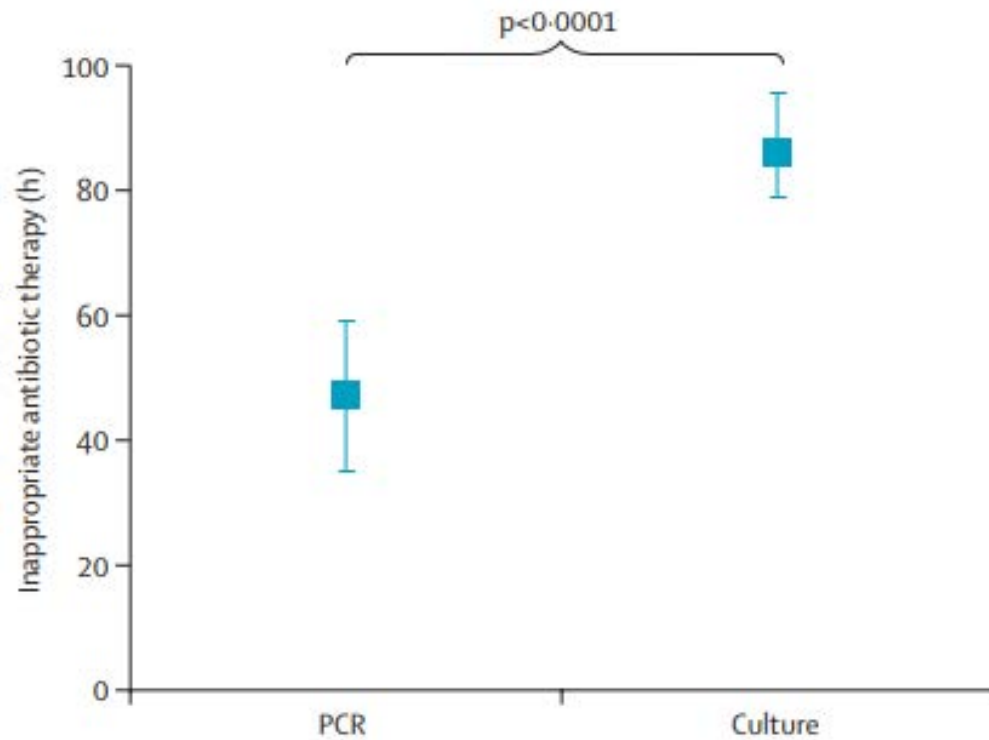
- 2017-2019
 - 2 centres suisses hors réa
 - 208 patients randomisés
 - 75% suspicion de CAP
 - à risque de BGN
 - CURB 1
 - LBA sous fibro B
 - Unyvero + avis ATB
-

BGN retrouvé

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

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

↘ durée ATB inappropriée



Autres approches syndromiques

- Hémoculture

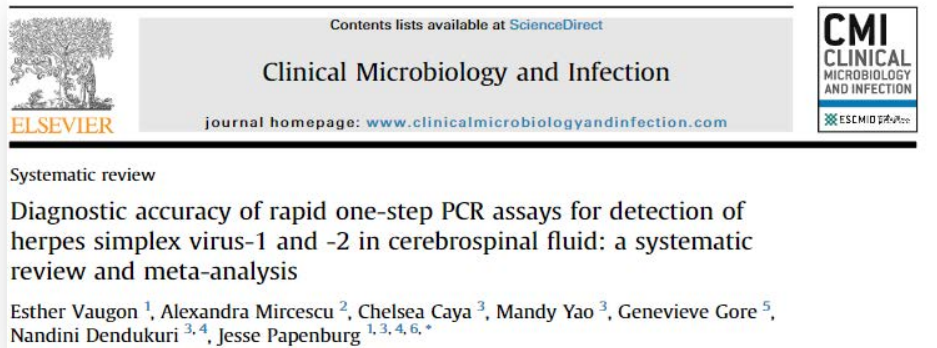
- Gastro-intestinal

Escherichia coli O157:H7

- Méningite

PL après antibiotique

Limites des approches syndromiques



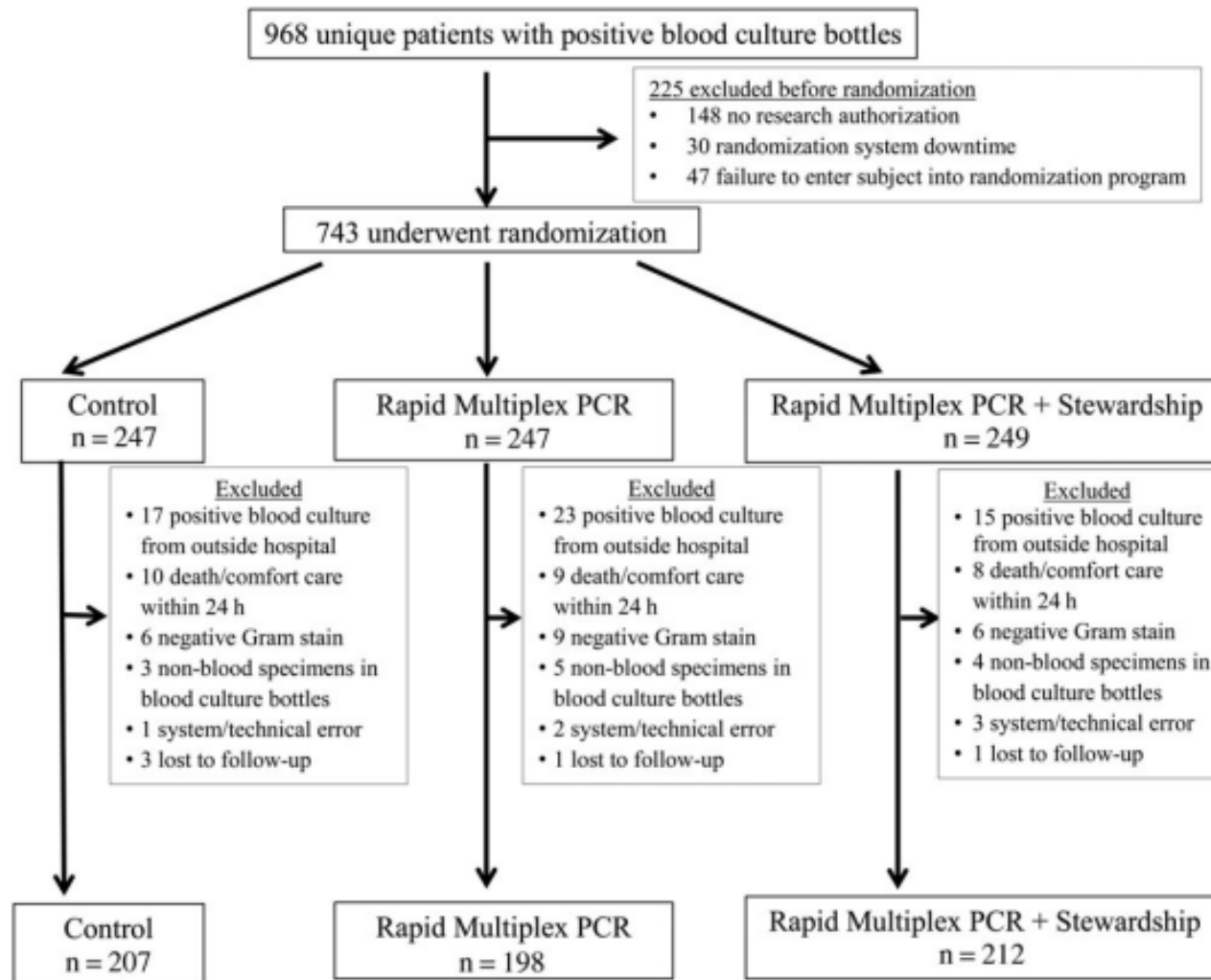
Overall pooled accuracy estimates for rapid one-step PCR assays for HSV-1 and HSV-2 in the CSF, by type of index test

	HSV-1				HSV-2			
	Sensitivity (95% CrI)	<i>n</i>	Specificity (95% CrI)	<i>n</i>	Sensitivity (95% CrI)	<i>n</i>	Specificity (95% CrI)	<i>n</i>
Index test								
FilmArray	84.3 (72.3–93.0)	14	99.8 (99.6–99.9)	27	92.9 (82.0–98.5)	18	99.9 (99.9–100)	27
Simplexa	97.1 (88.1–99.6)	4	98.9 (96.8–99.7)	4	97.9 (89.6–99.9)	4	98.9 (97.1–99.7)	4
Difference	-12.4 (-25.0– -0.7)		0.9 (0.1–3.0)		-4.5 (-15.9–4.4)		1.0 (0.3–2.8)	

CSF, cerebrospinal fluid; CrI, credible interval; HSV, herpes simplex virus; *n*, number of studies informing each estimate.

Randomized Trial of Rapid Multiplex Polymerase Chain Reaction–Based Blood Culture Identification and Susceptibility Testing

Ritu Banerjee,^{1,a} Christine B. Teng,^{2,a} Scott A. Cunningham,³ Sherry M. Ihde,³ James M. Steckelberg,⁴ James P. Moriarty,⁵ Nilay D. Shah,⁵ Jayawant N. Mandrekar,⁶ and Robin Patel^{3,4}



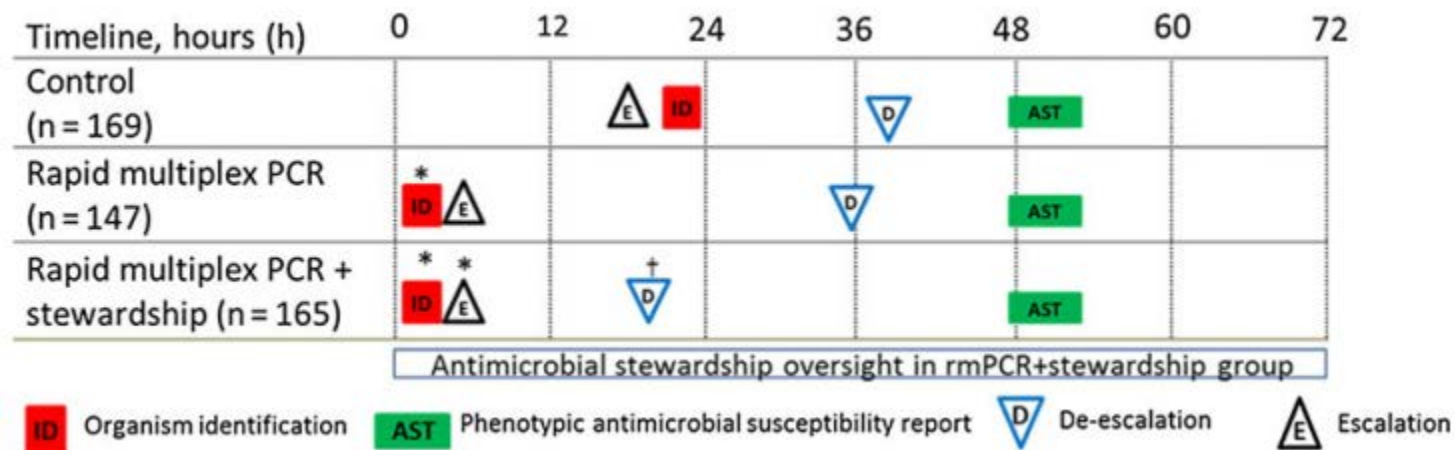


Table 3. Antibiotic Utilization Among All Study Subjects in the First 96 Hours Following Enrollment

Outcome	Control	Rapid Multiplex PCR	Rapid Multiplex PCR + Stewardship	P Value Comparing 3 Groups
Duration of therapy ^a , h				
Vancomycin				
All patients (n = 357)	44 (22–72)	42 (21–93)	42 (19–90)	.92
Organisms not requiring vancomycin ^b (n = 169)	8.2 (0–26)	0 (0–16)	0 (0–3) ^c	.032
Vancomycin-susceptible enterococci (n = 32)	20 (1–59)	70 (48–88) ^c	82 (40–96) ^c	.037
Methicillin-susceptible <i>Staphylococcus aureus</i> (n = 42)	23 (20–53)	11 (0–26)	8 (0–44)	.2
Nafcillin, oxacillin, or cefazolin (n = 50)	42 (24–57)	71 (51–79) ^c	85 (42–92) ^c	.035
Piperacillin-tazobactam (n = 214)	56 (39–82)	44 (27–74) ^c	45 (19–78) ^c	.012
Cefepime (n = 181)	55 (28–96)	71 (43–96)	58 (32–96)	.56
Antibiotic modifications				
Time to first appropriate de-escalation ^d (n = 344)	34 (21–55)	38 (22–66)	21 (7–37) ^{c,e}	<.0001
Time to first appropriate escalation ^f (n = 122)	24 (3–67)	6 (2–36)	5 (2–22) ^c	.04
Time to administration of active antibiotics (n = 123) ^g	11 (2–51)	6 (2–31)	4 (2–20)	.55
Contaminated blood cultures not treated or treated for <24 h, No. (%) ^h	47 (75)	49 (89) ^c	57 (92) ^c	.015

Test rapide seul souvent inefficace

- Bactériémie: *Vardakas Eur J Clin Microbiol Infect* 2015
- Infection cutanée *Terp Clin Infect Dis Off Publ Infect*
2014;58(8):e129-132
- Bactériémie à CG+: *Diagn Microbiol Infect Dis. sept 2016*

Nécessité d'un programme de bon usage des ATB

Prochains résultats

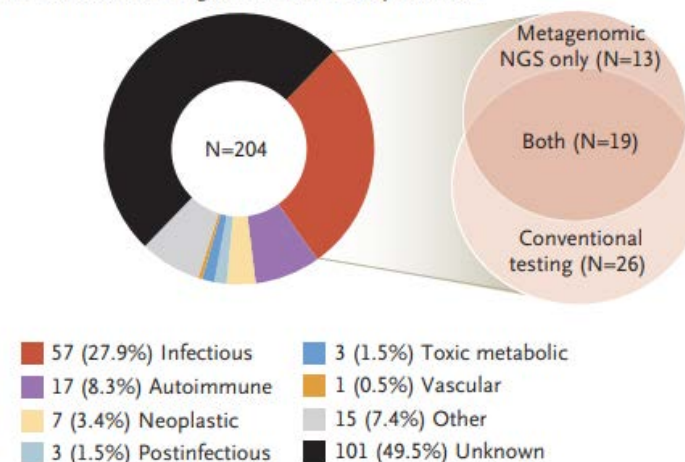
- dans le COVID
 - MULTICAP
 - INHALE II
 - SHARP ...
-

ORIGINAL ARTICLE

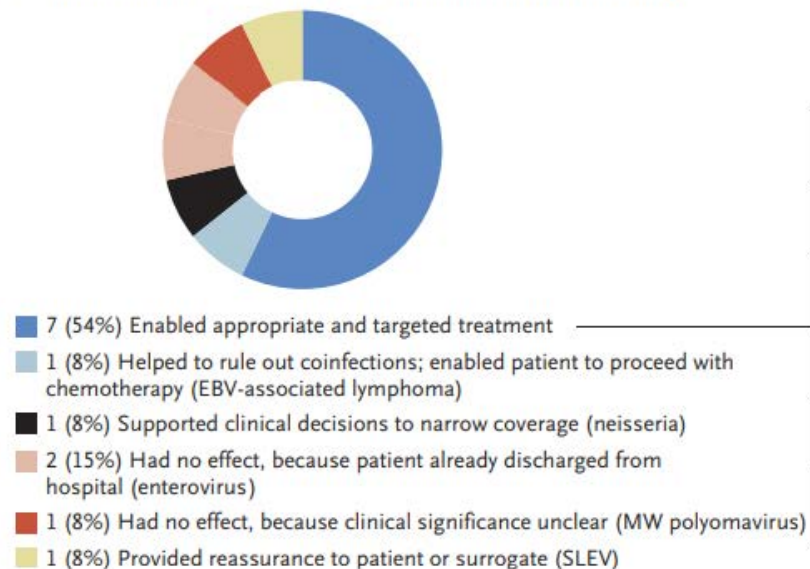
Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis

M.R. Wilson, H.A. Sample, K.C. Zorn, S. Arevalo, G. Yu, J. Neuhaus, S. Federman, D. Stryke, B. Briggs, C. Langelier, A. Berger, V. Douglas, S.A. Josephson, F.C. Chow, B.D. Fulton, J.L. DeRisi, J.M. Gelfand, S.N. Naccache, J. Bender, J. Dien Bard, J. Murkey, M. Carlson, P.M. Vespa, T. Vijayan, P.R. Allyn, S. Campeau, R.M. Humphries, J.D. Klausner, C.D. Ganzon, F. Memar, N.A. Ocampo, L.L. Zimmermann, S.H. Cohen, C.R. Polage, R.L. DeBiasi, B. Haller, R. Dallas, G. Maron, R. Hayden, K. Messacar, S.R. Dominguez, S. Miller, and C.Y. Chiu

A Established Diagnoses in the Study Patients



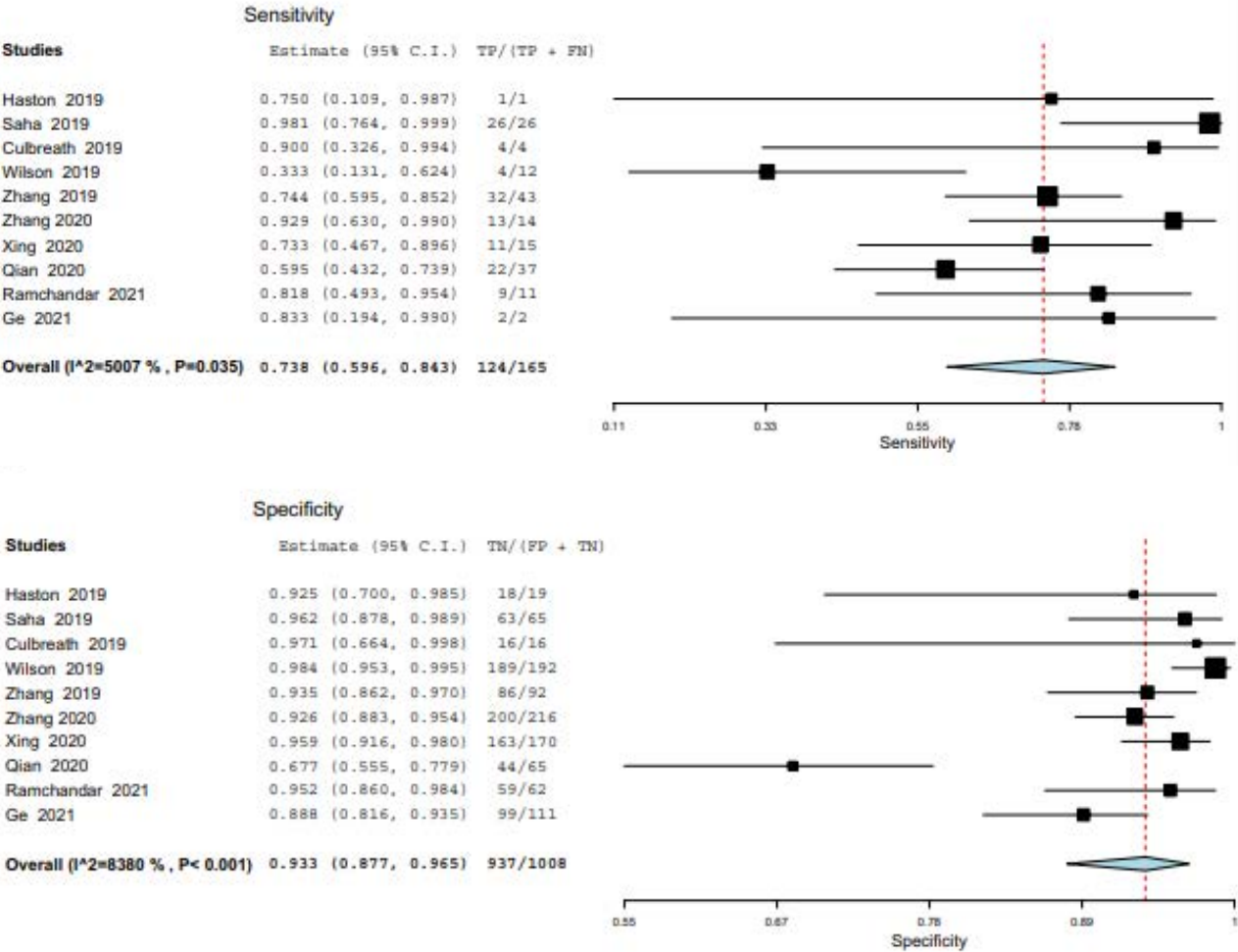
E Clinical Effect (13 cases diagnosed by metagenomic NGS only)



- N. farcinica* — long-term treatment with oral moxifloxacin and minocycline
- Candida tropicalis* — treatment with high-dose fluconazole and liposomal amphotericin B (started empirically for elevated 1,3- β -D-glucan level)
- HEV — successful treatment with IV ribavirin after patient was readmitted with liver failure and consideration of liver transplantation
- E. aerogenes* — narrowing of antibiotic therapy to IV cefepime and oral trimethoprim-sulfamethoxazole
- Enterococcus faecalis* — narrowing of antibiotic therapy to IV vancomycin; discontinuation of meropenem
- S. mitis* — narrowing of antibiotic therapy to IV cefepime; continuation of antibiotics for 4 wk to treat CNS infection
- S. agalactiae* — treatment with an additional 4 wk of therapy with IV ceftriaxone and vancomycin

Diagnostic accuracy of the metagenomic next-generation sequencing (mNGS) for detection of bacterial meningoencephalitis: a systematic review and meta-analysis

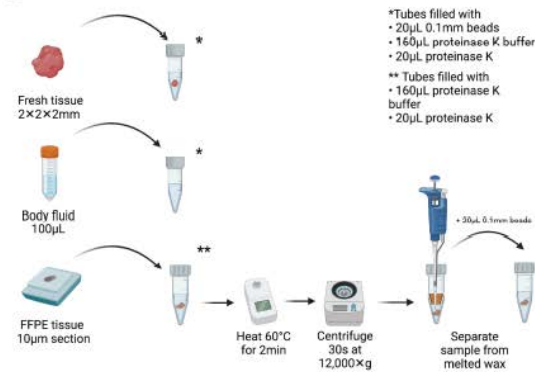
Rimjhim Kanaujia¹ · Manisha Biswal¹ · Archana Angrup¹ · Pallab Ray¹



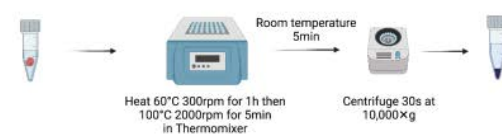
Targeted Metagenomic Sequencing-based Approach Applied to 2146 Tissue and Body Fluid Samples in Routine Clinical Practice

Laure Flurin,^{1,2} Matthew J. Wolf,¹ Melissa M. Mutchler,¹ Matthew L. Daniels,¹ Nancy L. Wengenack,¹ and Robin Patel^{1,3}

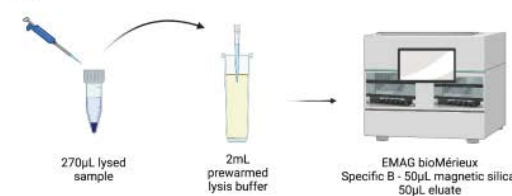
1 Sample processing



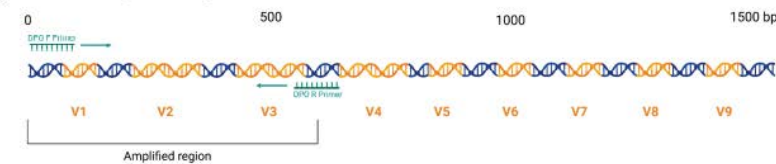
2 Digestion and lysis



3 Extraction



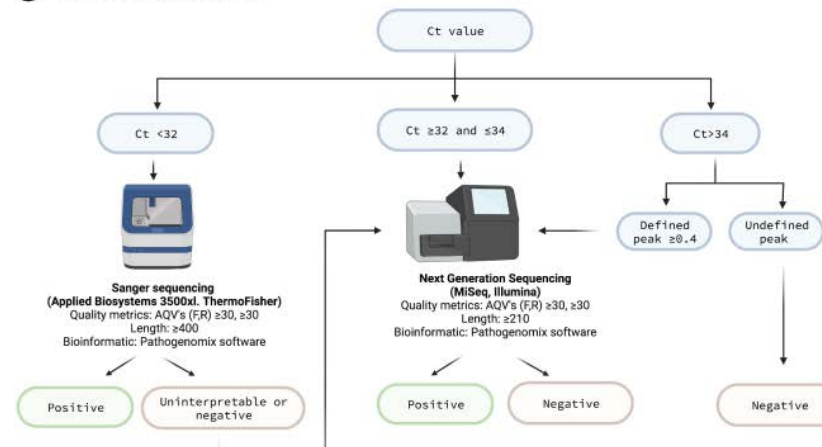
4a 16S rRNA gene and primers



4b 16S rRNA gene PCR

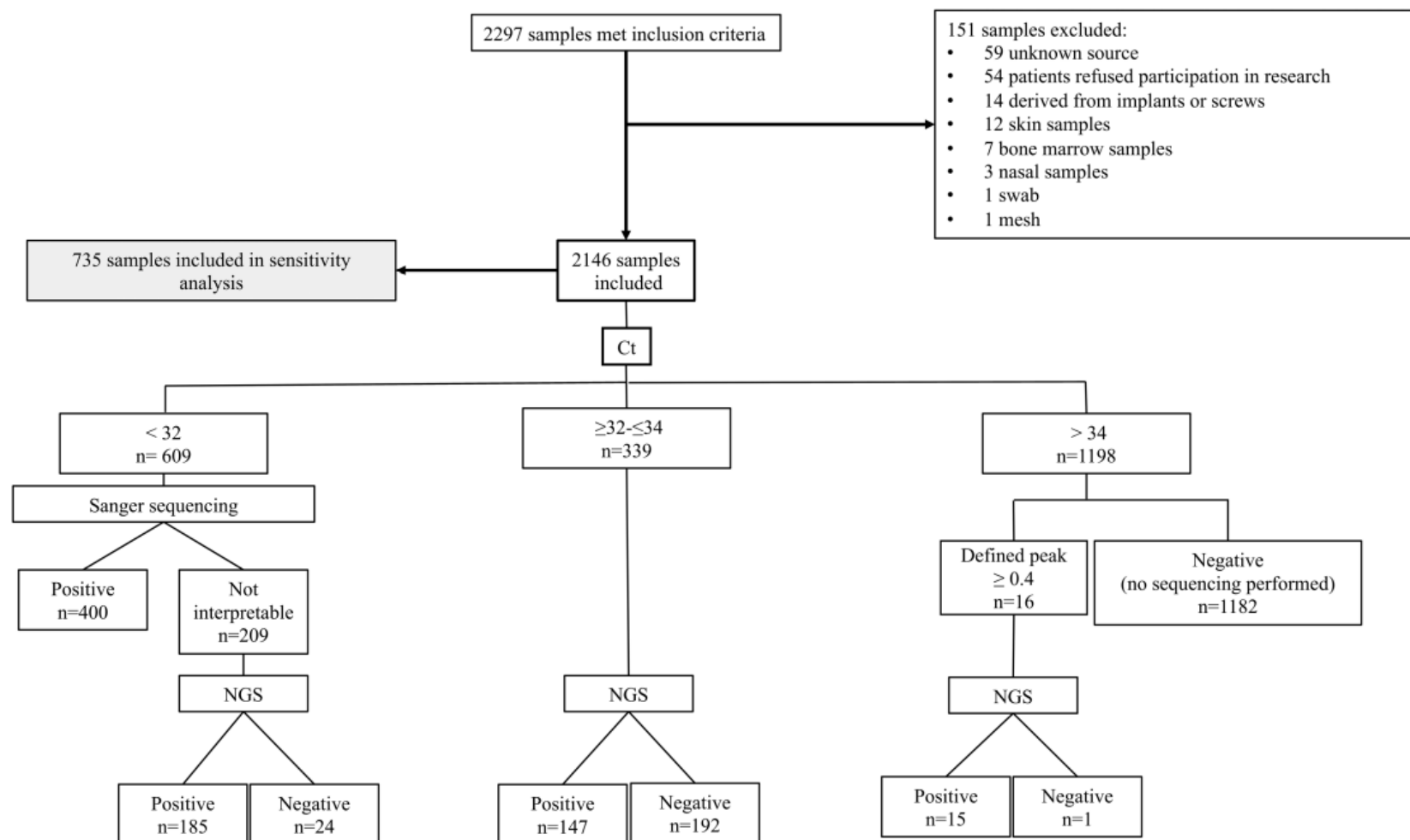


5 Sequencing algorithm

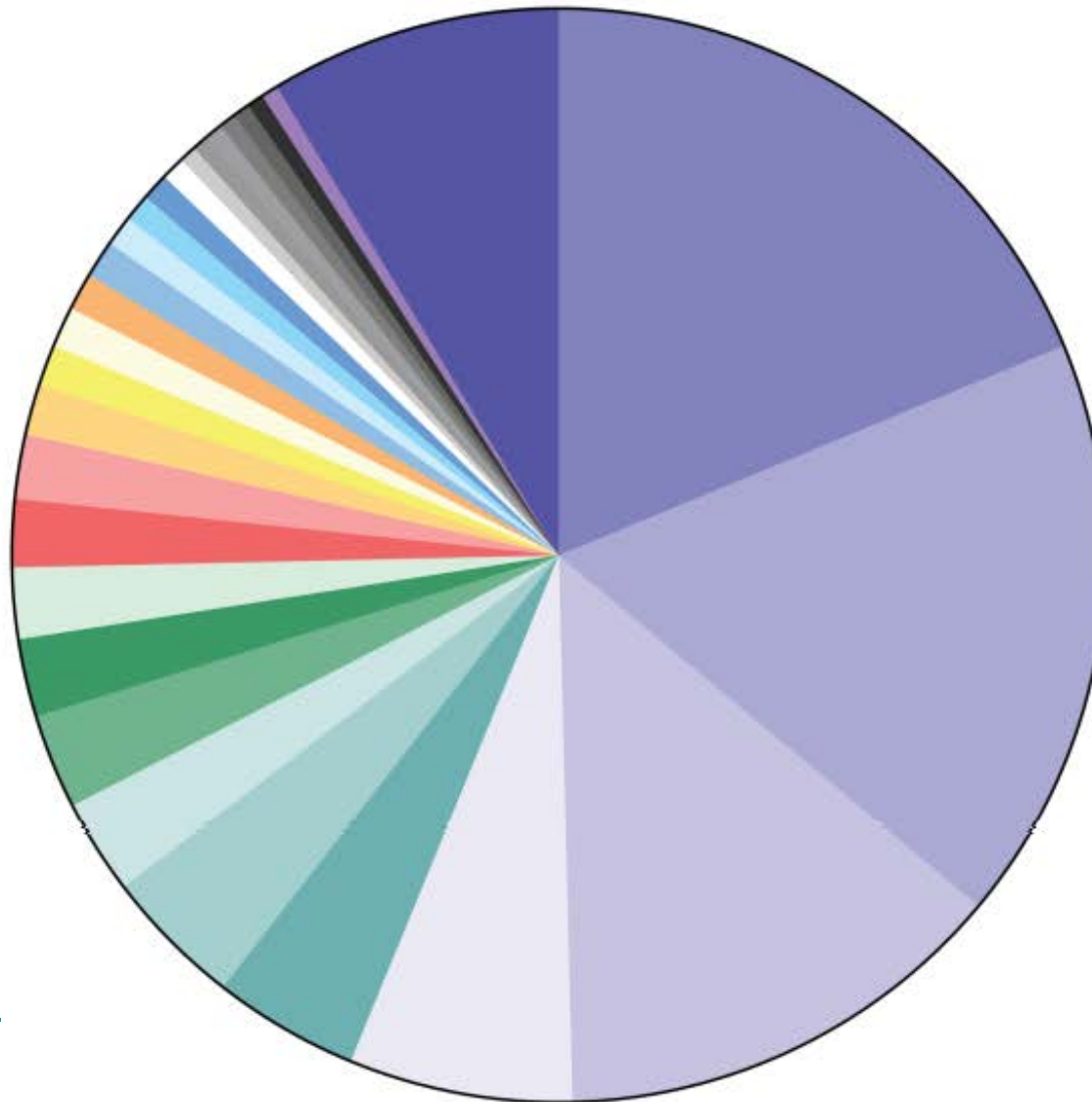


Targeted Metagenomic Sequencing-based Approach Applied to 2146 Tissue and Body Fluid Samples in Routine Clinical Practice

Laure Flurin,^{1,2} Matthew J. Wolf,¹ Melissa M. Mutchler,¹ Matthew L. Daniels,¹ Nancy L. Wengenack,¹ and Robin Patel^{1,3,6}



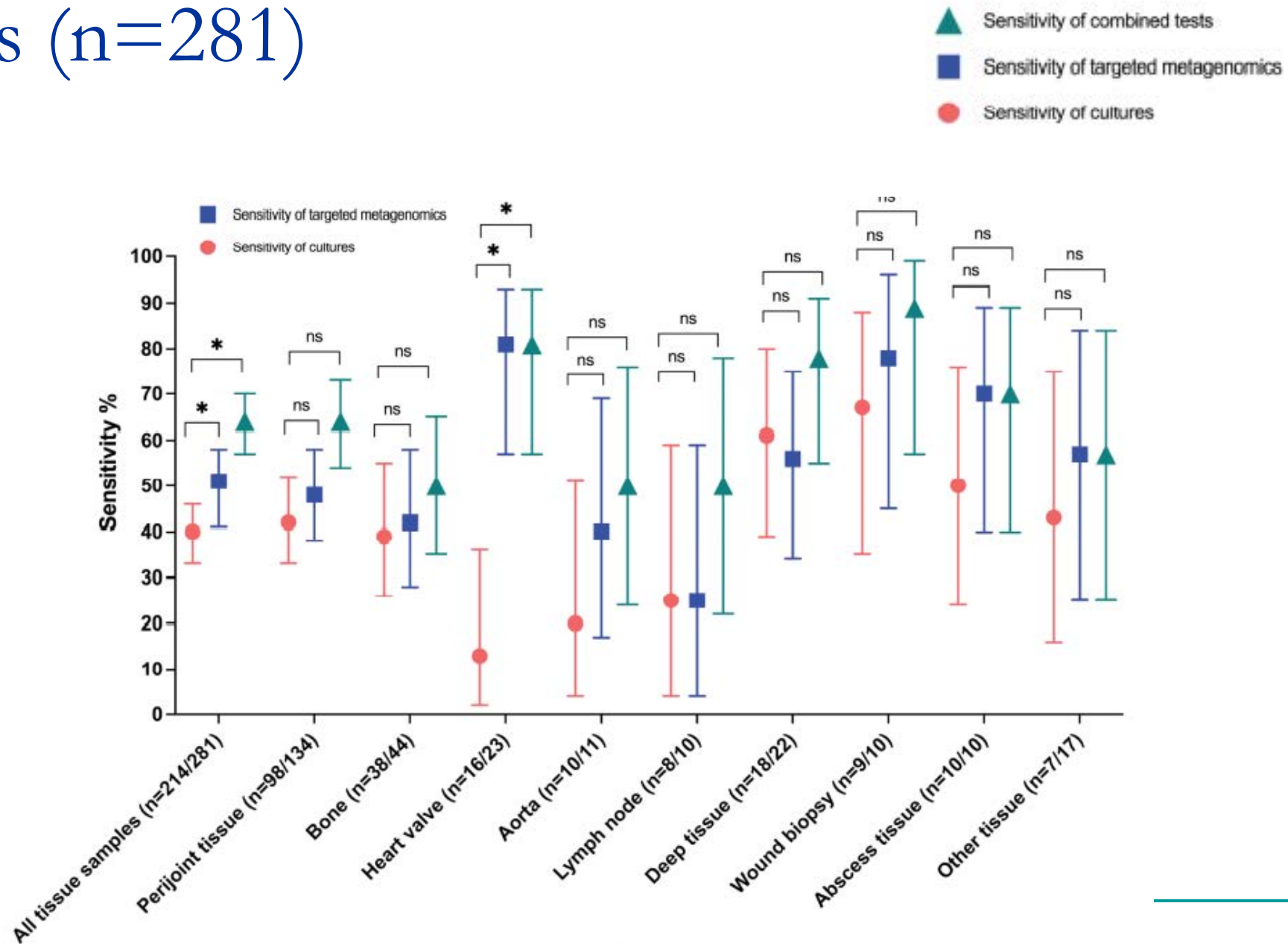
Identification by targeted metagenomics



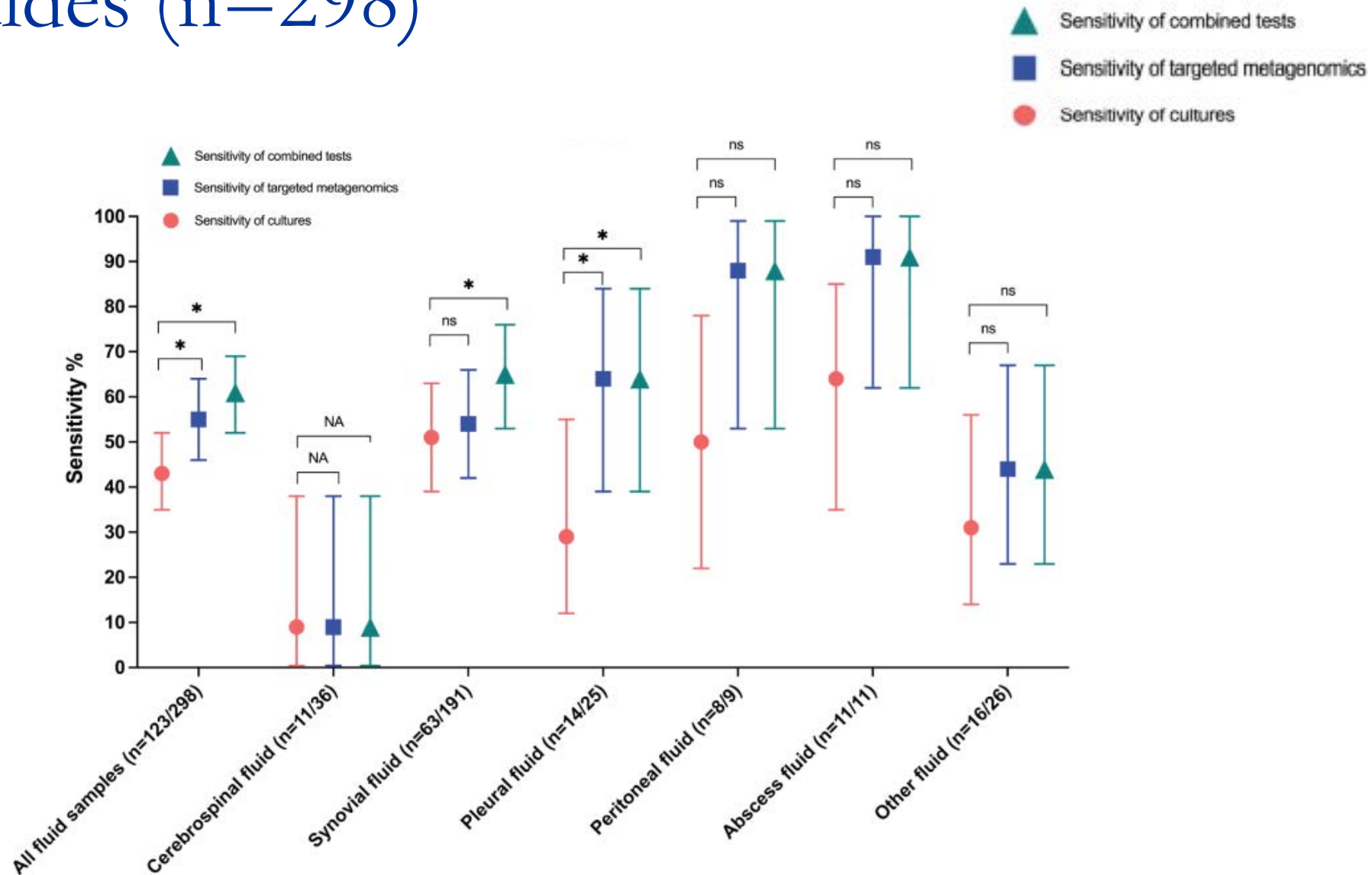
Positive samples (n=747)

- 18.7% Polymicrobial
- 17.3% *Streptococcus* sp.
- 13.5% *Staphylococcus aureus* complex
- 6.6% *Staphylococcus epidermidis*
- 4.3% *Mycobacterium* sp.
- 4.1% *Enterococcus* sp.
- 2.8% *Corynebacterium* sp.
- 2.8% *Pseudomonas aeruginosa*
- 2.3% Enterobacterales
- 2.1% *Cutibacterium acnes*
- 2.0% *Fusobacterium* sp.
- 1.9% *Serratia* sp.
- 1.5% *Bartonella* sp.
- 1.2% *Escherichia/Shigella* sp.
- 1.2% *Staphylococcus lugdunensis*
- 1.1% Other coagulase negative staphylococci
- 1.1% *Proteus mirabilis*
- 0.9% *Nocardia* sp.
- 0.8% *Granulicatella* sp.
- 0.8% *Haemophilus* sp.
- 0.7% *Ureaplasma* sp.
- 0.5% *Klebsiella* sp.
- 0.5% *Klebsiella/Raoultella* sp.
- 0.5% *Morococcus/Neisseria* sp.
- 0.5% *Mycoplasma* sp.
- 0.5% *Parvimonas* sp.
- 0.5% *Pasteurella multocida*
- 0.5% *Tropheryma whipplei*
- 8.6% Others

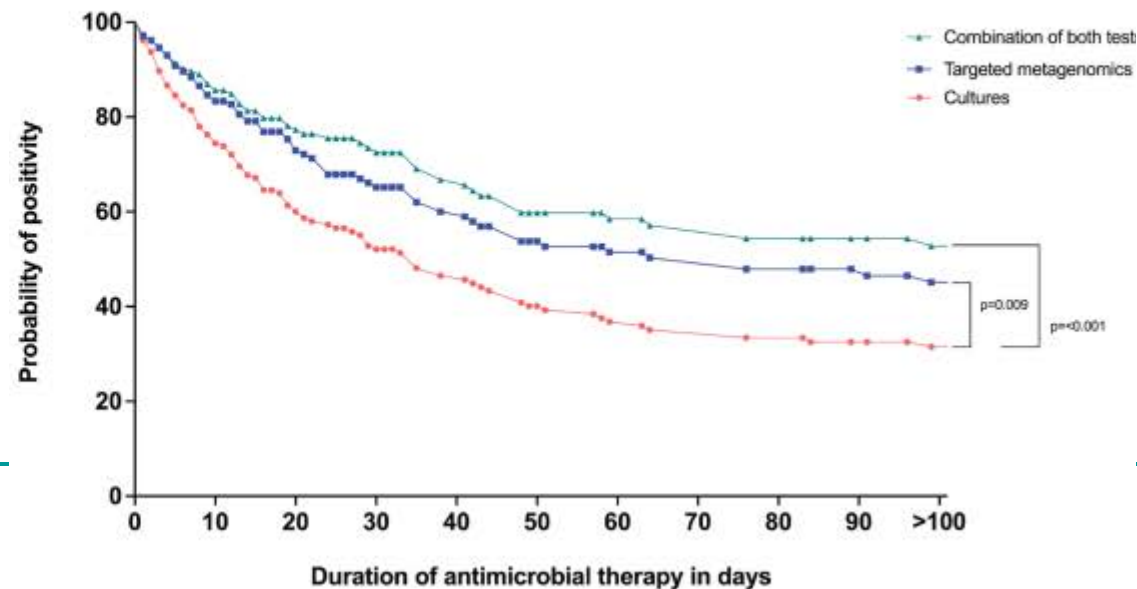
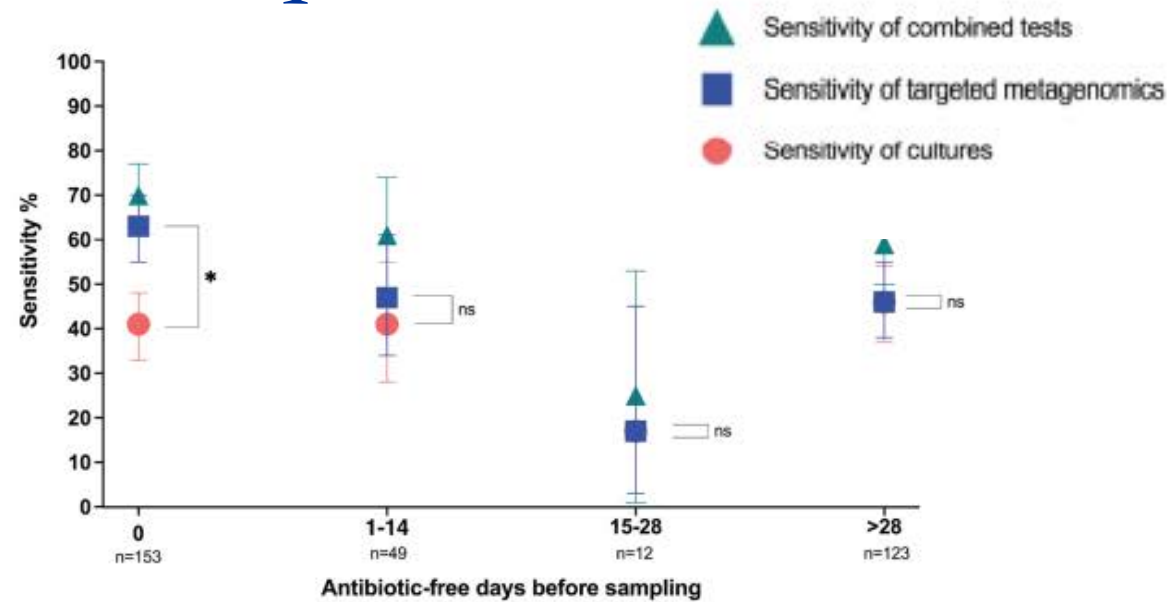
Tissue (n=281)



Liquides (n=298)

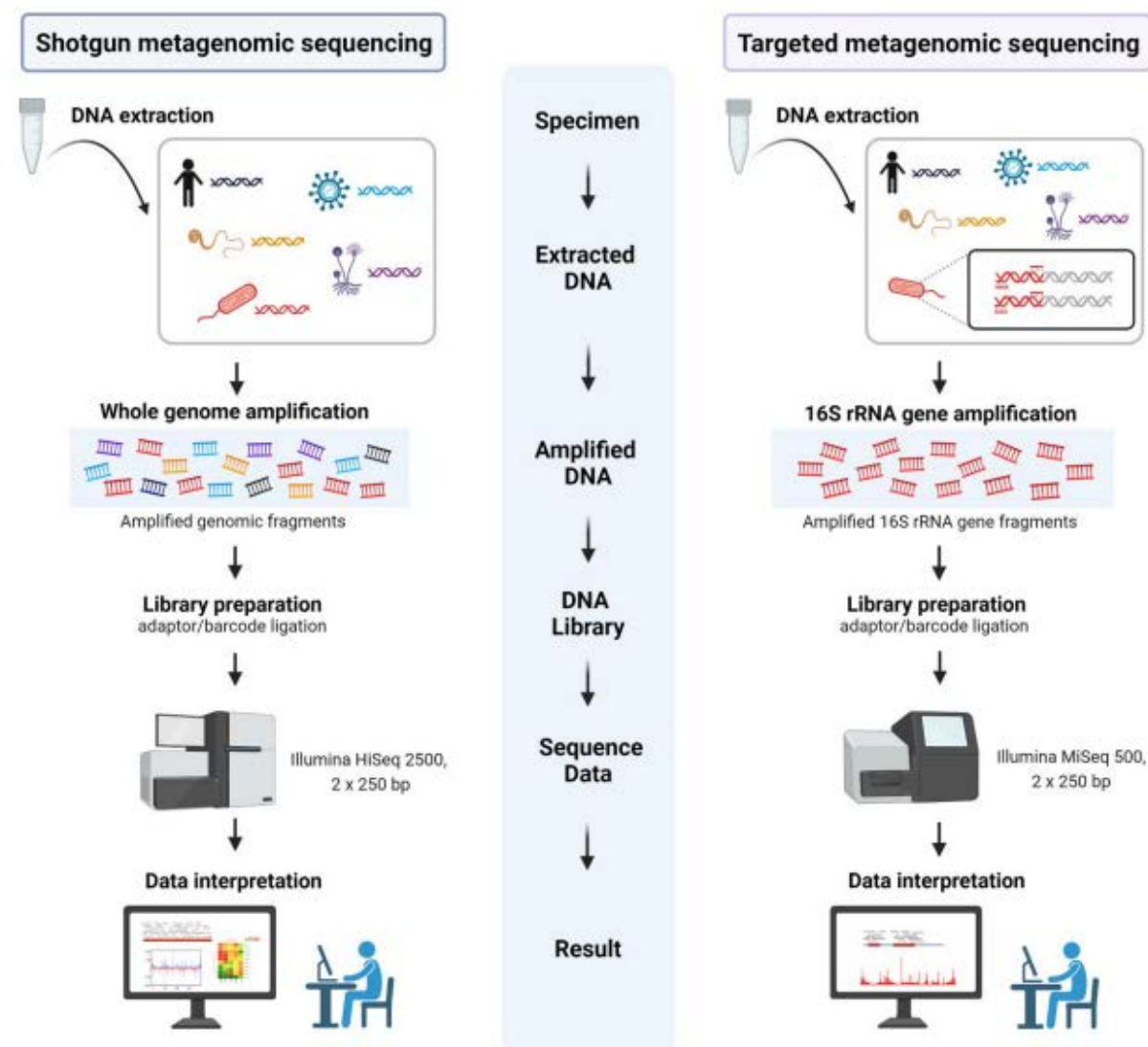


Sensibilité meilleure que culture si ATB reçu



Targeted Versus Shotgun Metagenomic Sequencing-based Detection of Microorganisms in Sonicate Fluid for Periprosthetic Joint Infection Diagnosis

Hyo-Lim Hong,^{1,2} Laure Flurin,^{1,3} Matthew J. Thoendel,⁴ Matthew J. Wolf,¹ Matthew P. Abdel,⁵ Kerry E. Greenwood-Quaintance,¹ and Robin Patel^{1,4,6}

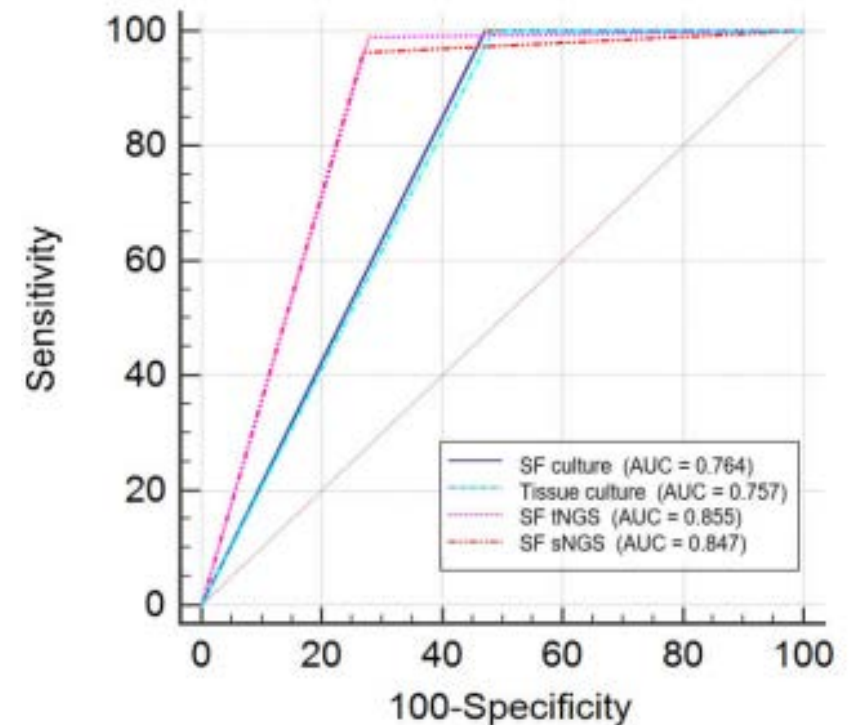


tNGS vs sNGS

■ Pas de différence

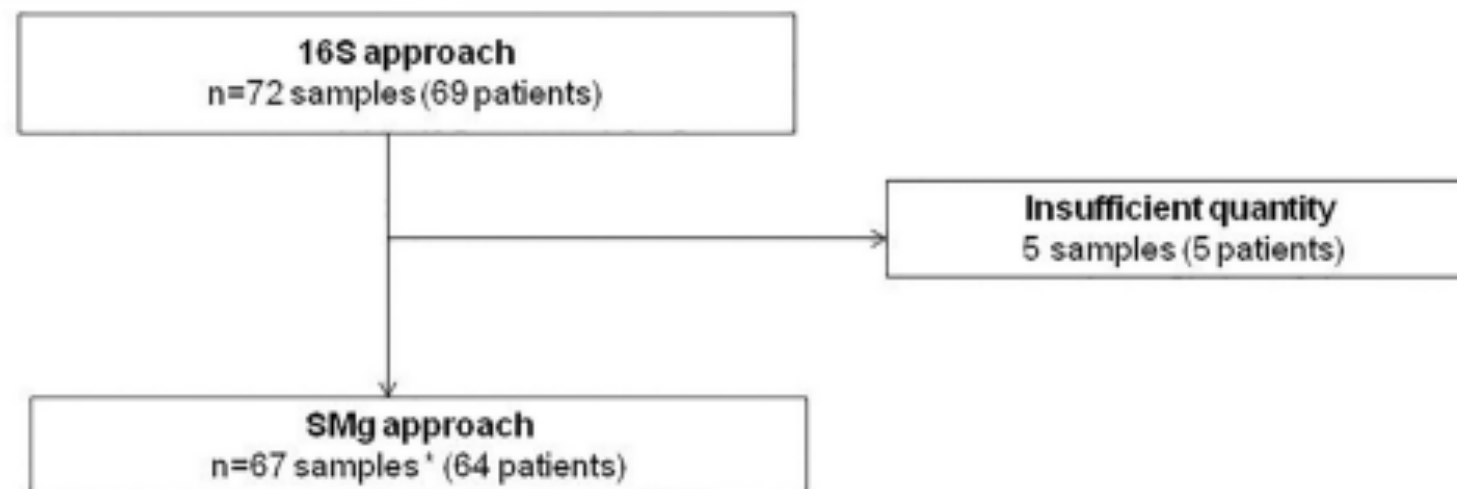
Table 4. Performance of Sonicate Fluid 16S Ribosomal RNA Gene-based Targeted Metagenomic Sequencing (tNGS) Versus Sonicate Fluid Shotgun Metagenomic Sequencing (sNGS)

Case Classification	Number of Samples	Identical Findings	Organisms Identified by tNGS but not sNGS	Organisms Identified by sNGS but not tNGS
NIAF	187	178 (95.2%)	2 (1.1%)	7 (3.7%)
Culture-positive PJI	110	92 (83.6%)	6 (5.5%)	13 (11.8%) ^a
Culture-negative PJI	98	76 (77.6%)	12 (12.2%) ^b	11 (11.2%) ^c



Prospective Comparison Between Shotgun Metagenomics and Sanger Sequencing of the 16S rRNA Gene for the Etiological Diagnosis of Infections

Claudie Lamoureux^{1,2,3}, Laure Surgers^{4,5}, Vincent Fihman^{1,6}, Guillaume Gricourt⁷, Vanessa Demontant⁷, Elisabeth Trawinski⁷, Melissa N'Debi⁷, Camille Gomart¹, Guilhem Royer¹, Nathalie Launay¹, Jeanne-Marie Le Glaunec¹, Charlotte Wemmert⁸, Giulia La Martire⁸, Geoffrey Rossi⁸, Raphaël Lepeule⁸, Jean-Michel Pawlotsky^{1,5}, Christophe Rodriguez^{1,5,7†} and Paul-Louis Woerther^{1,6,8*†}



* Description of the number of samples according to the suspicion of infection:

Bone and joint	Cardiovascular	Pulmonary	Intra-abdominal	Genito-urinary	Skin and soft tissue	Central nervous system
n=33 (49.3%)	n=21 (31.3%)	n=4 (6.0%)	n=3 (4.5%)	n=3 (4.5%)	n=2 (3.0%)	n=1 (1.5%)

tNGS vs sNGS

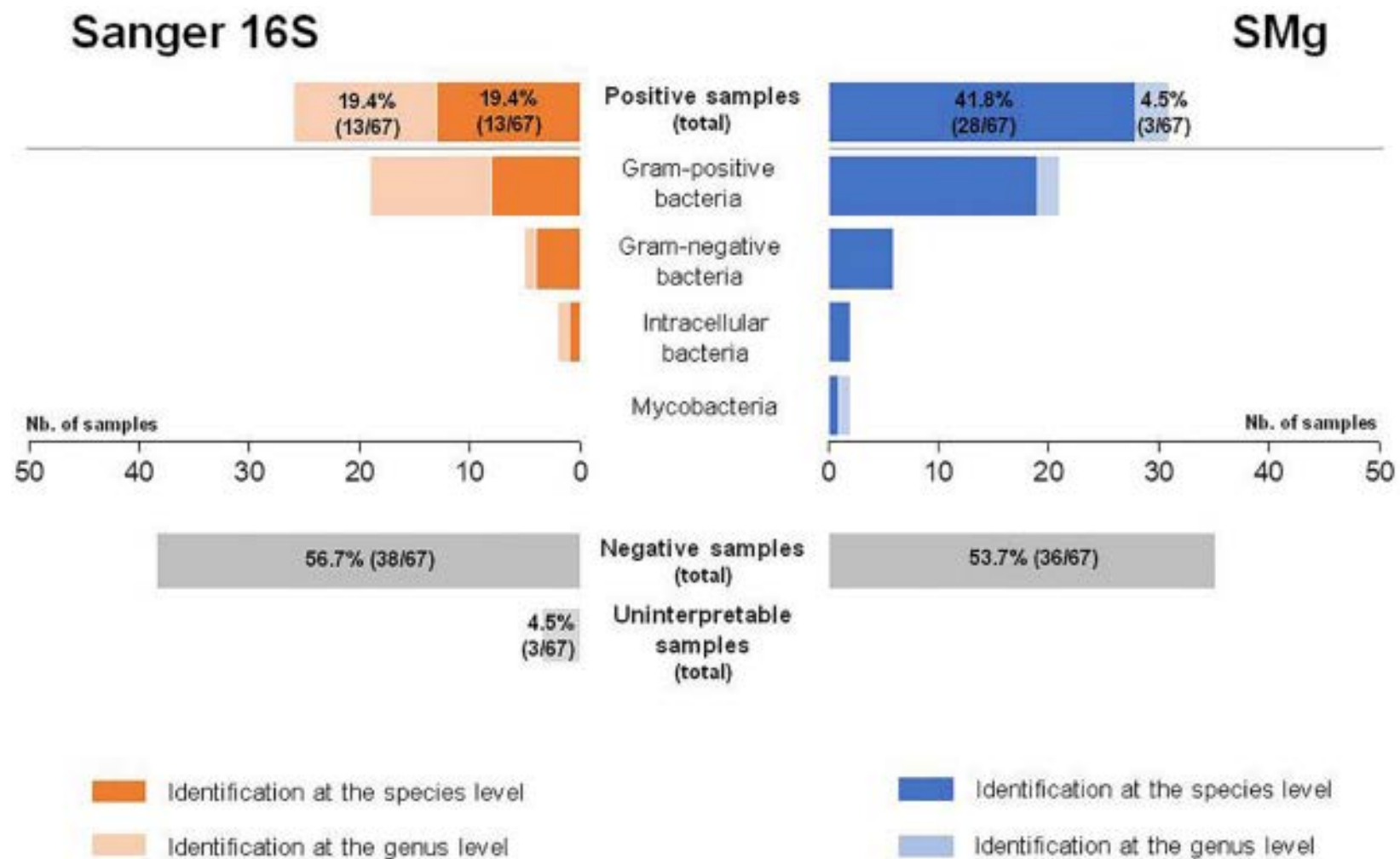


TABLE 1 | Clinical and biological data of the 34 positive samples detected positive with Sanger sequencing of the 16S rRNA gene (Sanger 16S) and/or shotgun metagenomics (SMg).

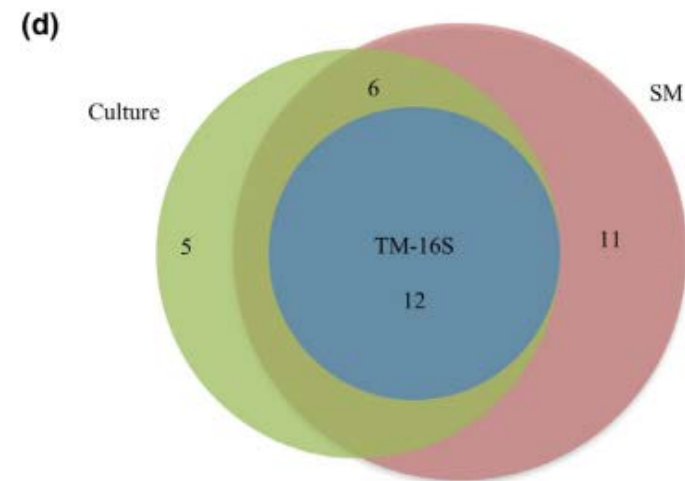
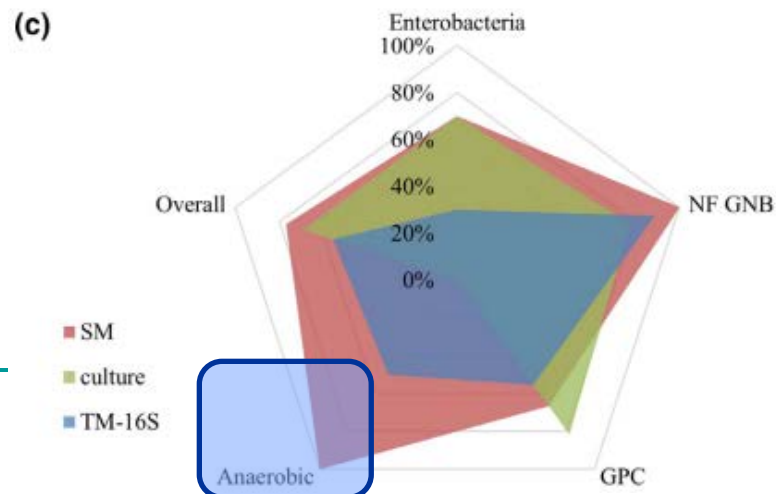
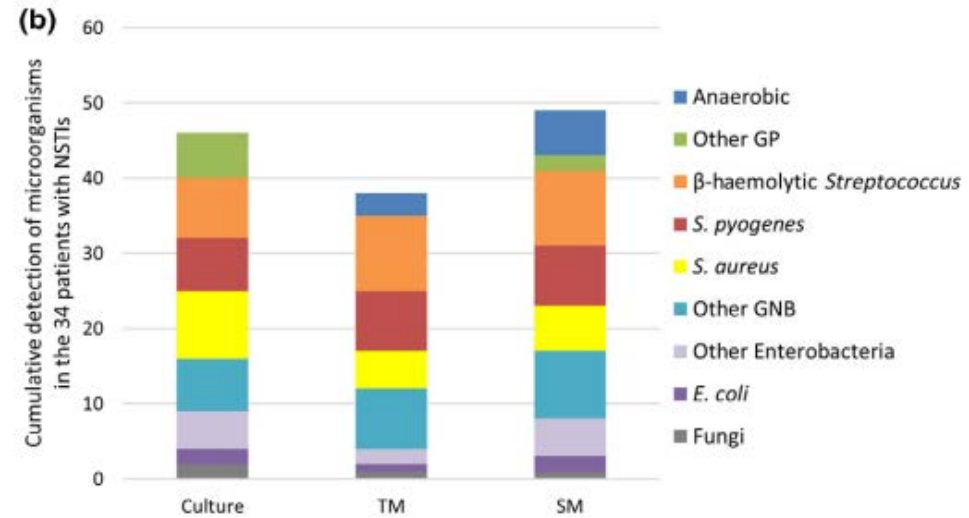
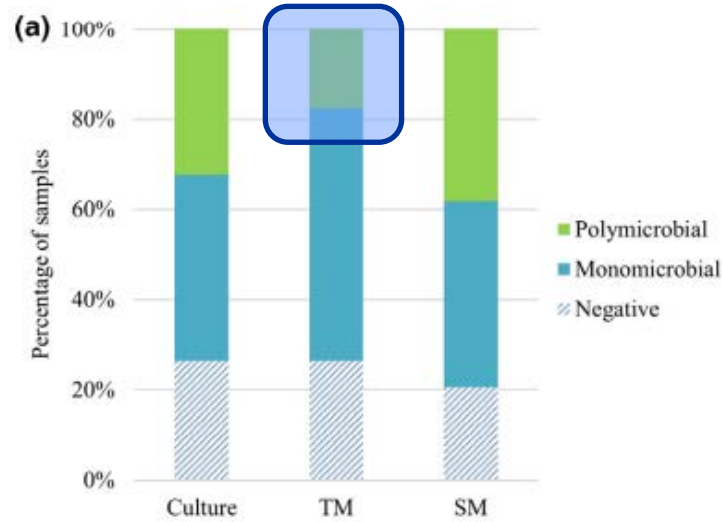
Suspected type of infection (%;n/N)*	Sample type	Antibiotic therapy**	Identification by SMg	Identification by Sanger 16S	Clinical diagnosis
Bone and joint (48.5%; 16/33)	Joint fluid	N	<i>Klebsiella pneumoniae</i>	Negative	Septic arthritis
	Joint fluid (Knee)	Y	<i>Mycoplasma hominis</i>	<i>Mycoplasma hominis</i>	Septic arthritis
	Joint fluid (Knee)	N	<i>Neisseria meningitidis</i>	<i>Neisseria meningitidis</i>	Septic arthritis
	Joint fluid	N	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	Septic arthritis
	Joint fluid	Y	<i>Streptococcus dysgalactiae</i>	<i>Streptococcus dysgalactiae</i>	Septic arthritis
	Joint fluid (Knee)	Y	Negative	<i>Streptococcus</i> sp. ^C <i>Moraxella</i> sp. ^C	Microcrystalline arthritis
	Biopsy (Tissue: sternum)	N	<i>Corynebacterium</i> sp. ^C	Negative	Non-documented infection
	Biopsy (Tissue: hip)	N	<i>Cutibacterium acnes</i>	Negative	Prosthetic joint infection
	Biopsy (Tissue: hip)	Y	<i>Escherichia coli</i>	<i>Escherichia coli</i>	Prosthetic joint infection
	Biopsy (Tissue: elbow)	Y	<i>Mycobacterium tuberculosis</i>	Negative	Osteoarthritis
	Biopsy (Tissue: foot)	Y	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	Osteoarthritis
	Biopsy (Tissue: spine)	N	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	Prosthetic joint infection
	Biopsy (Tissue)	Y	Negative	<i>Streptococcus</i> sp.	Septic arthritis
	Abscess	N	<i>Cutibacterium acnes</i>	<i>Cutibacterium acnes</i>	Prosthetic joint infection
	Abscess (Knee)	Y	Negative	<i>Pseudomonas fluorescens</i> group ^C	Non-documented septic arthritis
	Abscess (Psoas)	Y	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	Vertebral osteomyelitis
Cardiovascular (61.9%; 13/21)	Biopsy (Tissue: aortic aneurysm)	Y	<i>Bacteroides fragilis</i>	<i>Bacteroides fragilis</i>	Endocarditis
	Biopsy (Tissue: vegetation)	N	<i>Bartonella quintana</i>	<i>Bartonella</i> sp.	Endocarditis
	Biopsy (Tissue: vegetation)	Y	<i>Enterococcus faecalis</i>	<i>Enterococcus</i> sp.	Endocarditis
	Biopsy (Tissue: vegetation)	Y	<i>Staphylococcus aureus</i>	<i>Staphylococcus</i> sp.	Endocarditis
	Biopsy (Tissue: aortic valve)	Y	<i>Staphylococcus epidermidis</i>	Negative	Endocarditis
	Biopsy (Tissue: valve prosthesis)	N	<i>Streptococcus anginosus</i>	<i>Streptococcus milleri</i> group	Endocarditis
	Biopsy (Tissue: vegetation)	Y	<i>Streptococcus dysgalactiae</i>	β-haemolytic <i>Streptococcus</i>	Endocarditis
	Biopsy (Tissue: mitral valve)	Y	<i>Streptococcus gordonii</i>	<i>Streptococcus gordonii</i>	Endocarditis
	Biopsy (Tissue: vegetation)	Y	<i>Streptococcus mitis</i> group	<i>Streptococcus</i> sp.	Endocarditis
	Biopsy (Tissue: carotid)	Y	<i>Streptococcus pneumoniae</i>	α-haemolytic <i>Streptococcus</i>	Aortitis
	Biopsy (Tissue)	Y	<i>Streptococcus pyogenes</i>	<i>Streptococcus pyogenes</i>	Endocarditis
	Biopsy (Tissue: aortic valve)	Y	<i>Streptococcus sanguinis</i>	<i>Streptococcus mitis</i> group	Endocarditis
	Abscess (Mediastinum)	Y	<i>Cutibacterium acnes</i> (associated with cutaneous flora)	Negative	Mediastinitis
Intra-abdominal (100%; 3/3)	Abscess (Liver)	Y	<i>Fusobacterium nucleatum</i>	<i>Fusobacterium nucleatum</i>	Liver abscess
	Abscess (Liver)	Y	<i>Klebsiella pneumoniae</i>	Negative	Liver abscess
	Abscess (Intra-abdominal)	Y	<i>Staphylococcus lugdunensis</i>	<i>Staphylococcus</i> sp.	Abdominal abscess
Genito-urinary (33.3%; 1/3)	Abscess (Kidney)	Y	<i>Enterococcus faecalis</i>	<i>Enterococcus</i> sp.	Kidney graft abscess
Skin and soft tissue (50.0%; 1/2)	Granuloma	N	<i>Mycobacterium tuberculosis</i> complex	Negative	Post-BCG infection

tNGS vs sNGS

Pathogen identification by shotgun metagenomics of patients with necrotizing soft-tissue infections*

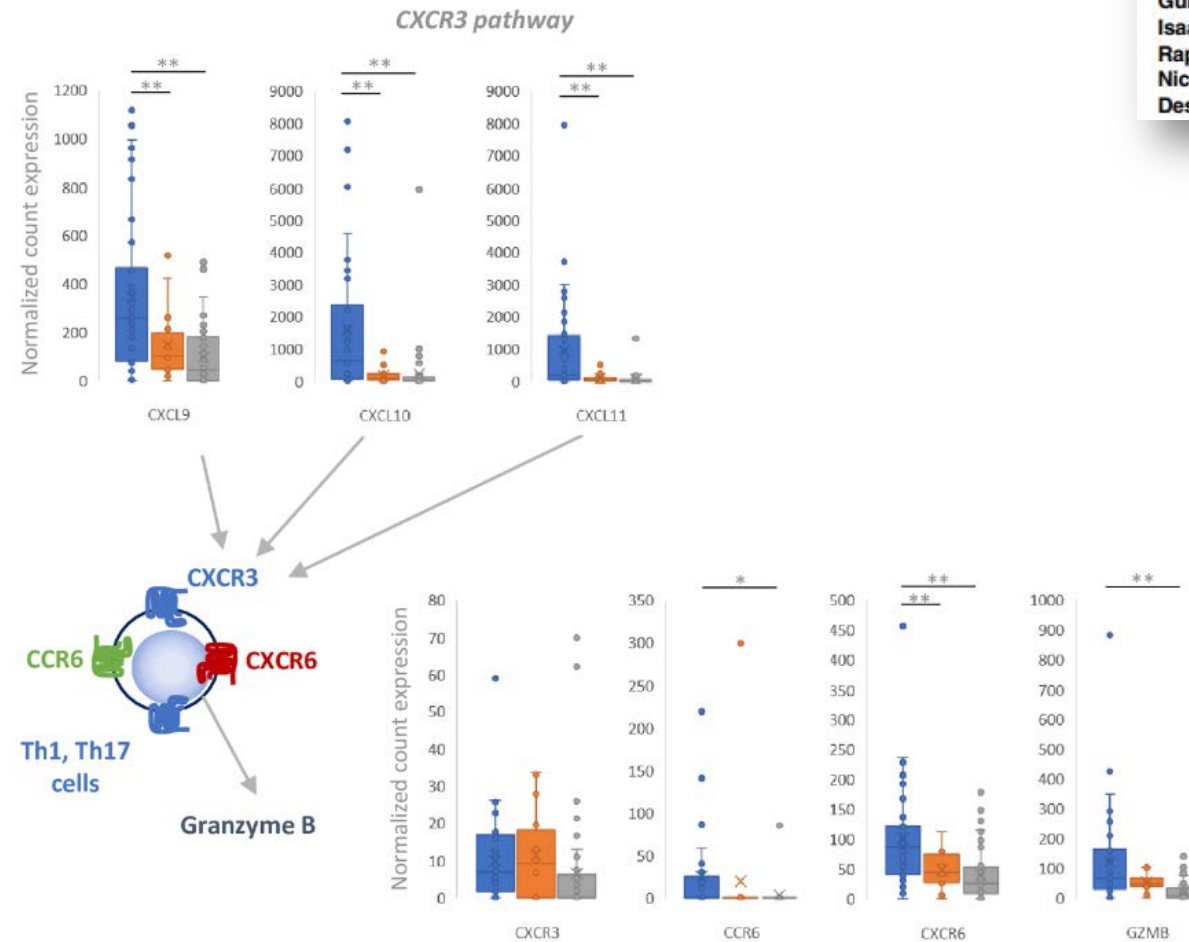
C. Rodriguez^{1,2,3}, A. Jary,¹ C. Hua,⁴ P.-L. Woerther,¹ R. Bosc,⁵ M. Desroches,¹ E. Sitterlé,⁶ G. Gricourt,³ N. De Prost^{1,2}, J.-M. Pawlotsky,^{1,2} O. Chosidow^{1,2}, E. Sbidian^{4,7} and J.-W. Decusser¹ on behalf of the Multidisciplinary Necrotizing Fasciitis Study Group

- 34 patients prélèvements per op



Viral genomic, metagenomic and human transcriptomic characterization and prediction of the clinical forms of COVID-19

Christophe Rodriguez^{1,2}*, Nicolas de Prost^{3,4}, Slim Fourati^{1,2}, Claudie Lamoureux¹, Guillaume Gricourt^{1,2}, Melissa N'deji^{1,2}, Florence Canoui-Poitrine^{5,6}, Isaac Désveaux¹, Oriane Picard¹, Vanessa Demontant¹, Elisabeth Trawinski¹, Raphaël Lepeule¹, Laure Surgers¹, William Vindrios⁷, Jean-Daniel Lelièvre⁷, Nicolas Mongardon⁸, Olivier Langeron⁸, José L. Cohen^{9,10}, Armand Mekontso-Dessap^{3,4}, Paul-Louis Woerther^{1,11}, Jean-Michel Pawlotsky^{1,2}



C

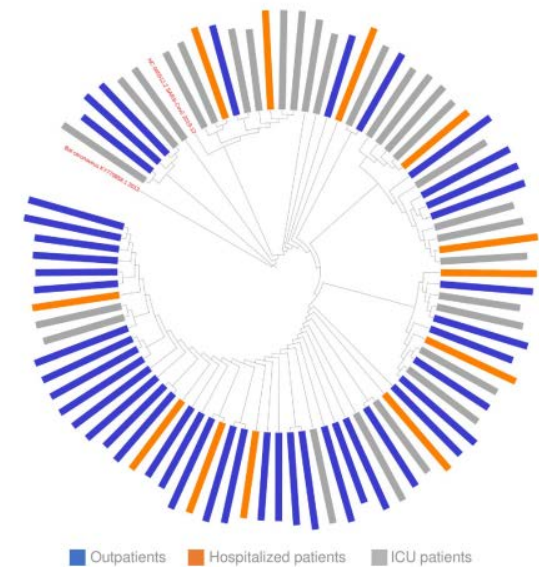
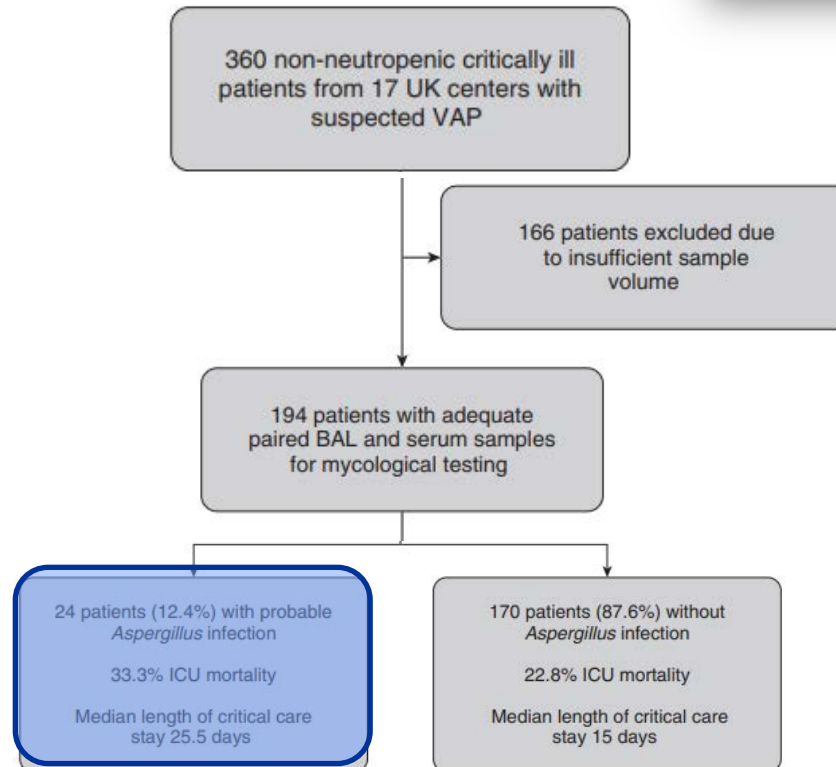


Fig 2. Relationship between SARS-CoV-2 characteristics and the severity of COVID-19 disease. (A) Relationship between

Pulmonary Aspergillosis in Patients with Suspected Ventilator-associated Pneumonia in UK ICUs

Laura Loughlin¹, Thomas P. Hellyer², P. Lewis White³, Danny F. McAuley¹, Andrew Conway Morris⁴, Raquel B. Posso³, Malcolm D. Richardson⁵, David W. Denning⁶, A. John Simpson^{2*}, and Ronan McMullan^{1*}



On ne trouve que ce qu'on cherche!!!!

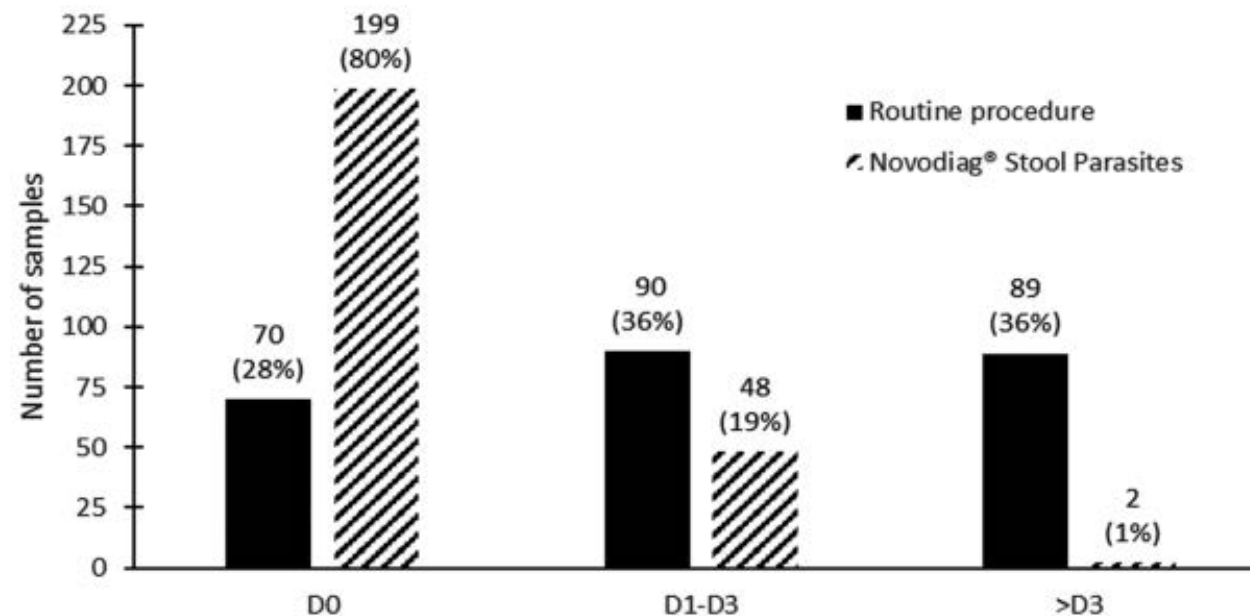
Parasites gastro-intestinaux

The Novodiag[®] Stool parasites assay, an innovative high-plex technique for fast detection of protozoa, helminths and microsporidia in stool samples: a retrospective and prospective study

Sophie Hartuis¹, Rose-Anne Lavergne¹, Céline Nourrisson², Jaco Verweij³, Guillaume Desoubieux⁴, Florian Lussac-Sorton⁵, Jean-Philippe Lemoine⁶, Estelle Cateau⁷, Fakhri Jeddi¹, Philippe Poirier², Patrice Le Pape¹, and Florent Morio^{1,*}



- 26 cibles dans selles (protozoaires, helminthes et microsporidies)



Plan

1. Trouver le micro-organisme
 2. Identification/comparaison
 3. Obtenir un antibiogramme rapidement
-

Techniques rapide d'identification / comparaison

■ PCR

- **Identification:** Spectromètre de masse + une source d'ionisation laser + un analyseur à temps de vol + banques de spectre = MALDI-TOF (Matrix Assisted Laser Desorption Ionisation/Time Of Flight) = **5 minutes**

■ Comparaison de bactéries / levures:

spectrométrie infrarouge = spectre d'absorption + transformée de Fourier = **15 minutes**

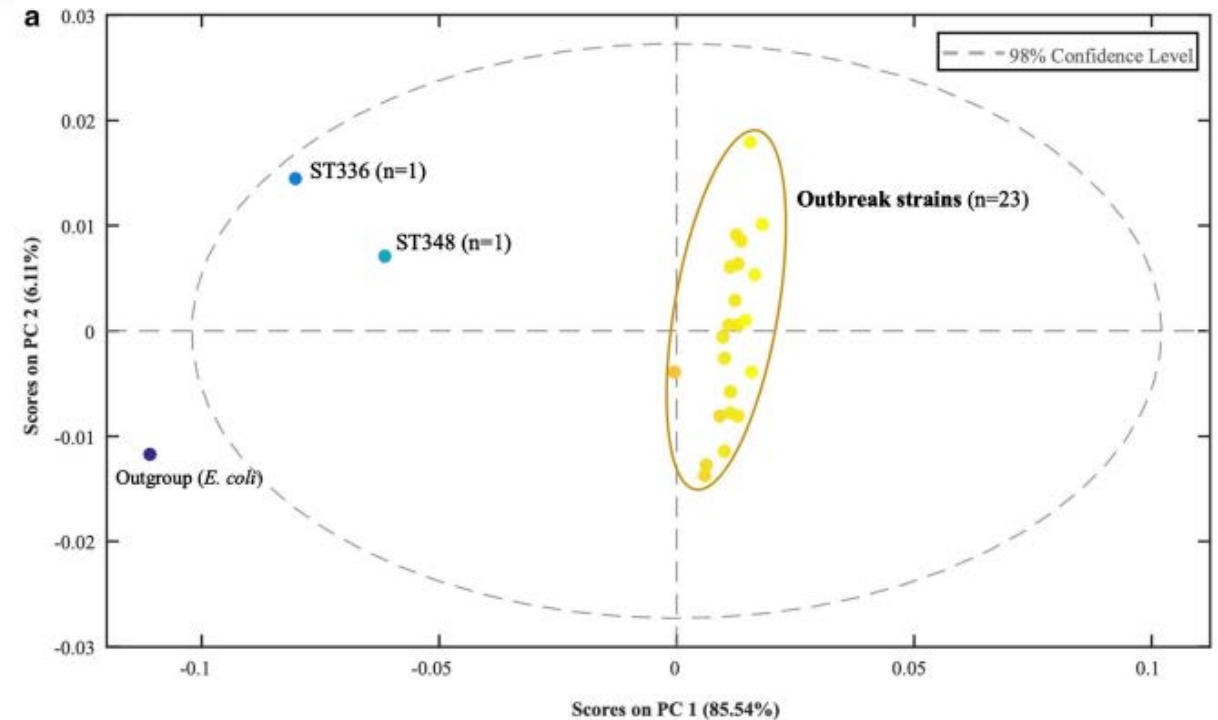


Fourier transform infrared (FT-IR) spectroscopy typing: a real-time analysis of an outbreak by carbapenem-resistant *Klebsiella pneumoniae*

Liliana Silva^{1,2} · Carla Rodrigues^{1,3} · Agostinho Lira⁴ · Mariana Leão⁴ · Margarida Mota⁴ · Paulo Lopes⁴ · Ângela Novais¹ · Luísa Peixe¹



- Utilisation prospective possible
- Intérêt dans les enquêtes épidémiologiques



Plan

1. Détecter le micro-organisme
 2. Identification/comparaison
 3. Obtenir un antibiogramme rapidement
-

Antibiogramme directement à partir d'HC+

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2003, p. 4751–4754
0095-1137/03/\$08.00+0 DOI: 10.1128/JCM.41.10.4751–4754.2003
Copyright © 2003, American Society for Microbiology. All Rights Reserved.

Vol. 41, No. 10

Direct Susceptibility Testing of Positive Blood Cultures by Using Sensititre Broth Microdilution Plates

Kimberle C. Chapin* and Michael C. Musgnug

Department of Laboratory Medicine, Lahey Clinic Medical Center, Burlington, Massachusetts 01805

Received 7 May 2003/Returned for modification 19 June 2003/Accepted 11 July 2003

Beuving et al. *BMC Microbiology* 2011, **11**:156
<http://www.biomedcentral.com/1471-2180/11/156>



RESEARCH ARTICLE

Open Access

Evaluation of direct inoculation of the BD PHOENIX system from positive BACTEC blood cultures for both Gram-positive cocci and Gram-negative rods

Judith Beuving¹, Christina FM van der Donk¹, Catharina FM Linssen¹, Petra FG Wolffs^{1*} and Annelies Verbon^{1,2}



Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Antimicrobial Susceptibility Testing

Evgeny A. Idelevich,^{a,b} Karsten Becker^a

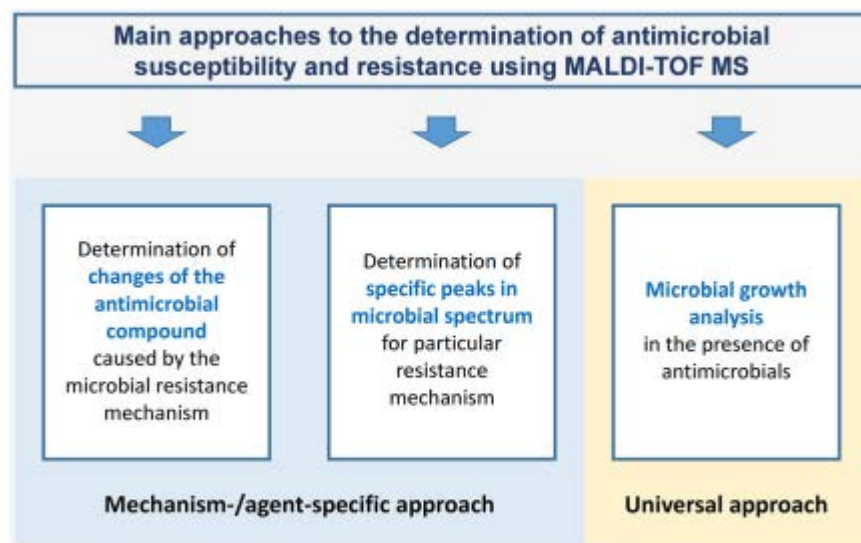
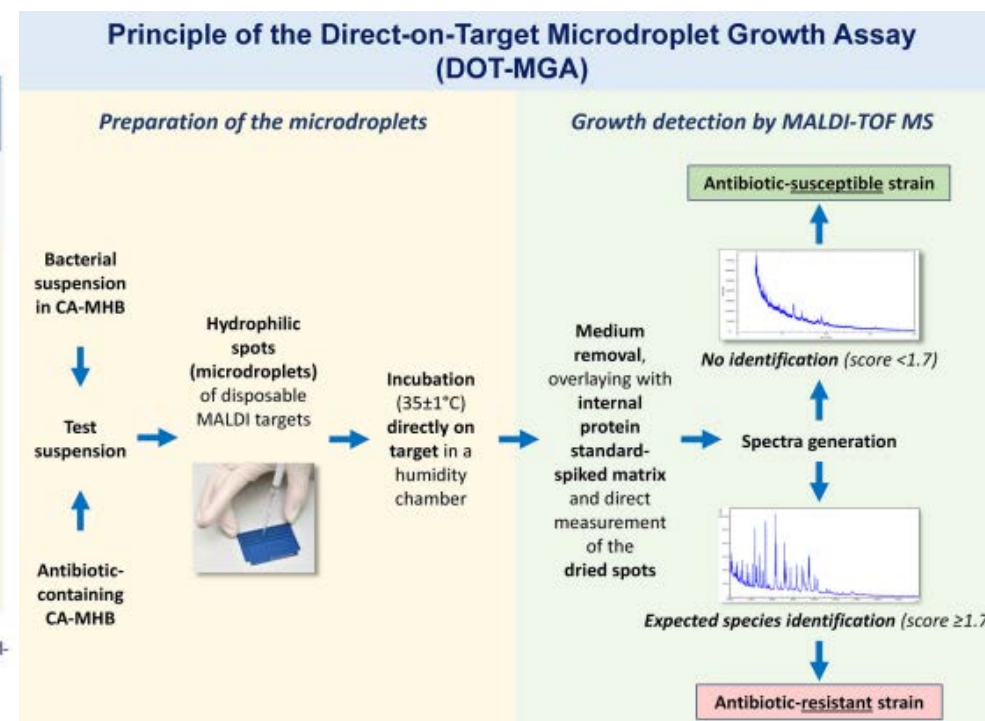


FIG 1 Main approaches to the determination of antimicrobial susceptibility and resistance using MALDI-TOF MS.



Original article

Rapid detection of antibiotic resistance by MALDI-TOF mass spectrometry using a novel direct-on-target microdroplet growth assay[☆]

E.A. Idelevich¹, K. Sparbier², M. Kostrzewa², K. Becker^{1,*}

Table 1

Accuracy of detection of carbapenem-non-susceptibility in *Klebsiella pneumoniae*, n = 24

Incubation time	Microdroplet total volume														
	2 µL			4 µL			6 µL			8 µL			10 µL		
	Valid ^a	Sensitivity ^b	Specificity ^b	Valid	Sensitivity	Specificity	Valid	Sensitivity	Specificity	Valid	Sensitivity	Specificity	Valid	Sensitivity	Specificity
3 hours	45.8%	50%	100%	70.8%	50%	100%	87.5%	90%	100%	87.5%	90%	90.9%	79.2%	87.5%	90.9%
4 hours	100%	58.3%	100%	100%	66.7%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
18 hours	83.3%	91.7%	100%	95.8%	100%	100%	100%	100%	100%	95.8%	100%	100%	87.5%	100%	100%

The values in bold indicate the microdroplet volume and the incubation times which resulted in best assay performance.

^a Valid test—the growth control was detected.

^b Calculated for valid tests.

Table 2

Accuracy of detection of carbapenem-non-susceptibility in *Pseudomonas aeruginosa*, n = 24

Incubation time	Microdroplet total volume														
	2 µL			4 µL			6 µL			8 µL			10 µL		
	Valid ^a	Sensitivity ^b	Specificity ^b	Valid	Sensitivity	Specificity	Valid	Sensitivity	Specificity	Valid	Sensitivity	Specificity	Valid	Sensitivity	Specificity
4 hours	0%	—	—	20.8%	50.0%	100%	54.2%	40.0%	100%	62.5%	83.3%	100%	66.7%	85.7%	100%
5 hours	37.5%	75.0%	100%	66.7%	100%	100%	83.3%	100%	100%	91.7%	100%	90.9%	91.7%	100%	90.9%
18 hours	95.8%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	91.7%	91.7%

The values in bold indicate the microdroplet volume and the incubation times which resulted in best assay performance.

^a Valid test—the growth control was detected.

^b Calculated for valid tests.

EUCAST rapid antimicrobial susceptibility testing (RAST) in blood cultures: validation in 55 European laboratories

Anna Åkerlund^{1,2,3*}, Emma Jonasson^{4,5}, Erika Matuschek⁵, Lena Serrander^{2,3}, Martin Sundqvist⁶ and Gunnar Kahlmeter^{4,5} on behalf of the RAST Study Group†

- 55 laboratoires en Europe
- 580 *E. Coli*, 338 *S.aureus*, 69 *P.aeruginosa*, 36 *S.pneumoniae*

Table 4. Theoretical and actual numbers of tests, the proportions of tests that could be read and interpreted as S or R after 4, 6 and 8 h and the categorical errors with RAST versus standard DD by EUCAST breakpoint tables version 8.0 at each reading time for all species in NE + SE [RAST breakpoint table version 0 (v. 0) and version 1 (v. 1.0)]

	Incubation time (h)					
	4		6		8	
Breakpoint table	v. 0	v. 1.0	v. 0/v. 1.0		v. 0/v. 1.0	
Theoretical number of tests ^a	7024	7361	7361		7361	
Number of completed tests ^b	6398	6718	7210		6655	
Readable zones ^c (% of completed tests)	5624 (88)	5811 (87)	6921 (96)		6561 (99)	
Results calculated on readable zones (%)						
breakpoint table	v. 0	v. 1.0	v. 0	v. 1.0	v. 0	v. 1.0
not interpreted as S or R (ATU)	20	16	16	7.5	14	5.7
interpreted as S	71	75	76	84	78	86
interpreted as R	8.8	8.8	7.6	8.5	7.7	8.6
Errors calculated on the total number of zones interpreted as S or R (%)						
breakpoint table	v. 0	v. 1.0	v. 0	v. 1.0	v. 0	v. 1.0
mEs	0.7	0.6	0.5	0.6	0.6	0.8
MEs	2.2	2.1	0.9	1.1	0.7	0.9
VMEs	0.2	0.2	0.4	0.4	0.6	0.5
total errors	3.1	3.0	1.8	2.1	1.8	2.2

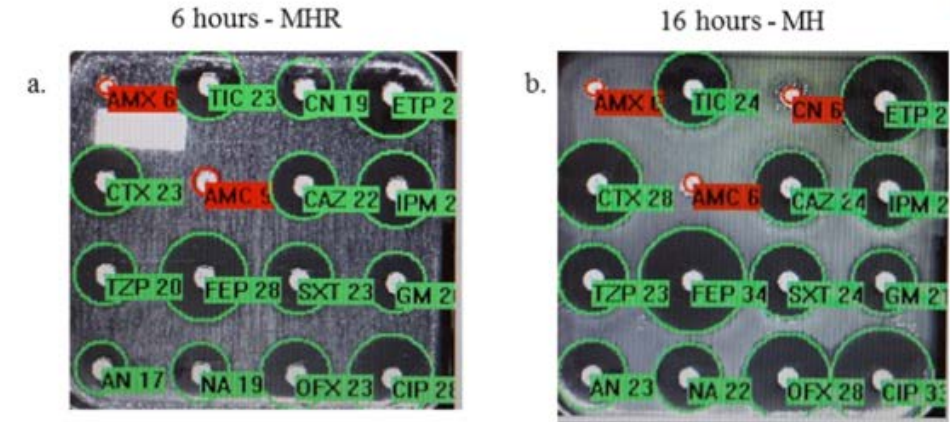
Prospective evaluation of rapid antimicrobial susceptibility testing by disk diffusion on Mueller-Hinton rapid-SIR directly on blood cultures

Claire Périllaud ^a, Benoît Pilmis ^b, Julien Diep ^b, Gauthier Péan de Ponfilly ^a, Barbara Vidal ^b, Carine Couzigou ^b, Assaf Mizrahi ^a, Julie Lourtet-Hascoët ^a, Alban Le Monnier ^a, Jean-Claude Nguyen Van ^{a,*}

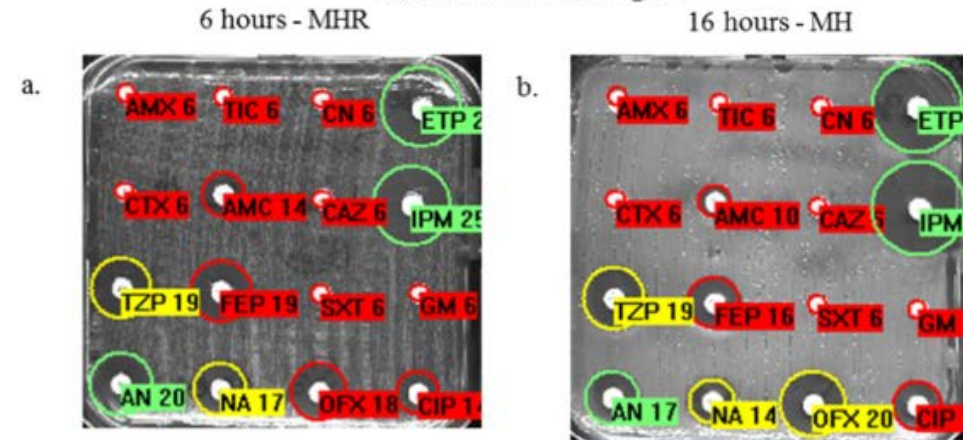


■ 1847 tests Staph/ Enterobacterales

- ❑ Concordances 1804 (97.7%)
- ❑ minor errors 25 (1.4%)
- ❑ ME 8 (0.4%)
- ❑ VME 10 (0.5%)



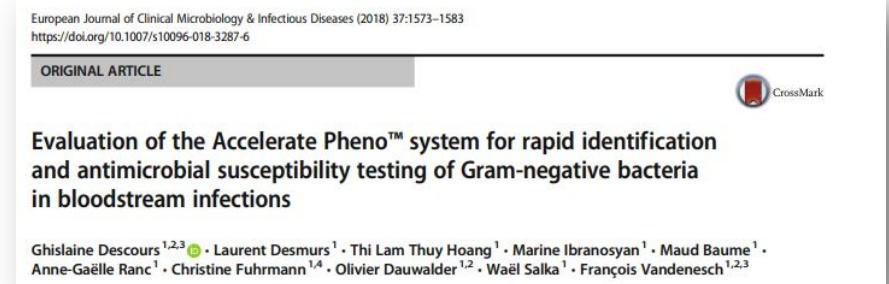
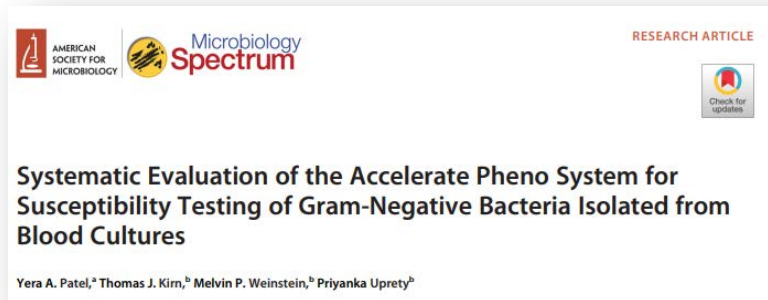
A) *Enterobacter aerogenes*



B) *Klebsiella pneumoniae*

Difficile à appliquer, nécessité de technicien spécialisé

Automatisation



■ Trop d'erreurs !!!!!

Table 3 Overall essential agreement of antimicrobial susceptibility testing results obtained with the Accelerate Pheno™ system and the routine culture-based method (VITEK®2; *n* = 90). The number of results in agreement is indicated between brackets

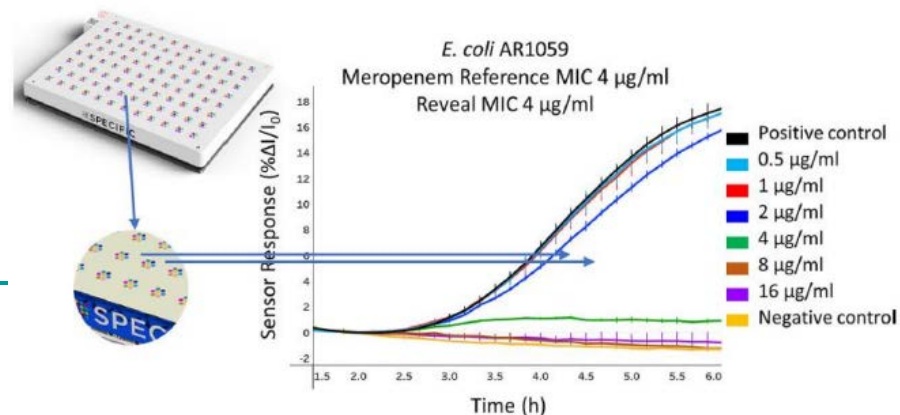
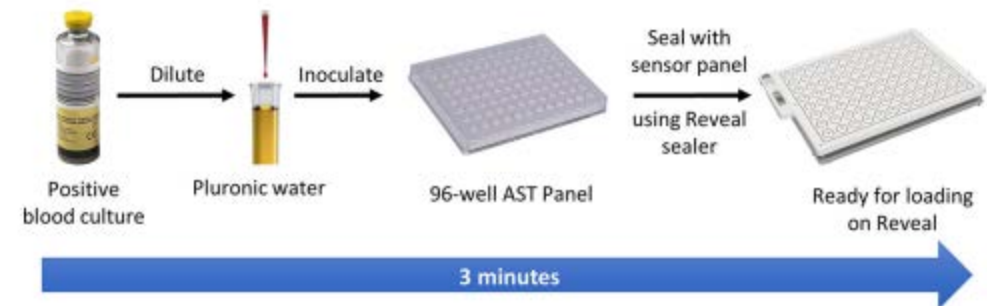
	All species combined	<i>Escherichia coli</i> (<i>n</i> = 43)	<i>Klebsiella</i> spp. (<i>n</i> = 17)	<i>Enterobacter</i> spp. (<i>n</i> = 9)	<i>Proteus</i> spp. (<i>n</i> = 6)	<i>Serratia marcescens</i> (<i>n</i> = 5)	<i>Citrobacter</i> spp. (<i>n</i> = 1)	<i>Pseudomonas aeruginosa</i> (<i>n</i> = 9)
All antibiotics combined	92.3% (1006/1090)	95.8% (522/545)	90.0% (197/219)	91.6% (98/107)	100% (64/64)	98.1% (53/54)	91.7% (11/12)	68.5% (61/89)
SAM	83.3% (50/60)	93.0% (40/43)	58.8% (10/17)	Not evaluated ^a	Not evaluated ^a	Not evaluated ^a	Not evaluated ^a	Not evaluated ^a
CRO	97.5% (79/81)	97.7% (42/43)	94.1% (16/17)	100% (9/9)	100% (6/6)	100% (5/5)	100% (1/1)	Not evaluated ^a
FEP	87.8% (79/90)	97.7% (42/43)	76.5% (13/17)	100% (9/9)	100% (6/6)	100% (5/5)	100% (1/1)	33.3% (3/9)
CAZ	88.8% (79/89)	97.6% (41/42)	94.1% (16/17)	100% (9/9)	100% (6/6)	100% (5/5)	100% (1/1)	11.1% (1/9)
TZP	89.8% (79/89)	90.5% (38/42)	94.1% (16/17)	66.7% (6/9)	100% (6/6)	100% (5/5)	100% (1/1)	77.8% (7/9)
ATM	89.9% (80/89)	97.6% (41/42)	82.3% (14/17)	77.8% (7/9)	100% (6/6)	100% (5/5)	0% (0/1)	77.8% (7/9)
ETP	97.5% (79/80)	100% (42/42)	94.1% (16/17)	88.9% (8/9)	100% (6/6)	100% (5/5)	100% (1/1)	Not evaluated ^a
MEM	91.1% (82/90)	100% (43/43)	94.1% (16/17)	88.9% (8/9)	100% (6/6)	100% (5/5)	100% (1/1)	33.3% (3/9)
CIP	95.5% (85/89)	97.6% (41/42)	94.1% (16/17)	100% (9/9)	100% (6/6)	80% (4/5)	100% (1/1)	88.9% (8/9)
AMK	94% (79/84)	95.2% (40/42)	93.8% (15/16)	100% (8/8)	100% (4/4)	100% (4/4)	100% (1/1)	77.8% (7/9)
GEN	97.8% (87/89)	97.6% (41/42)	100% (17/17)	100% (9/9)	100% (6/6)	100% (5/5)	100% (1/1)	88.9% (8/9)
TOB	94.4% (84/89)	90.5% (38/42)	94.1% (16/17)	100% (9/9)	100% (6/6)	100% (5/5)	100% (1/1)	100% (9/9)
CST	91.5% (65/71)	89.2% (33/37)	100% (16/16)	77.8% (7/9)	Not evaluated ^a	Not evaluated ^a	100% (1/1)	100% (8/8)

Performance of the Reveal Rapid Antibiotic Susceptibility Testing System on Gram-Negative Blood Cultures at a Large Urban Hospital

June 2022 Volume 60 Issue 6

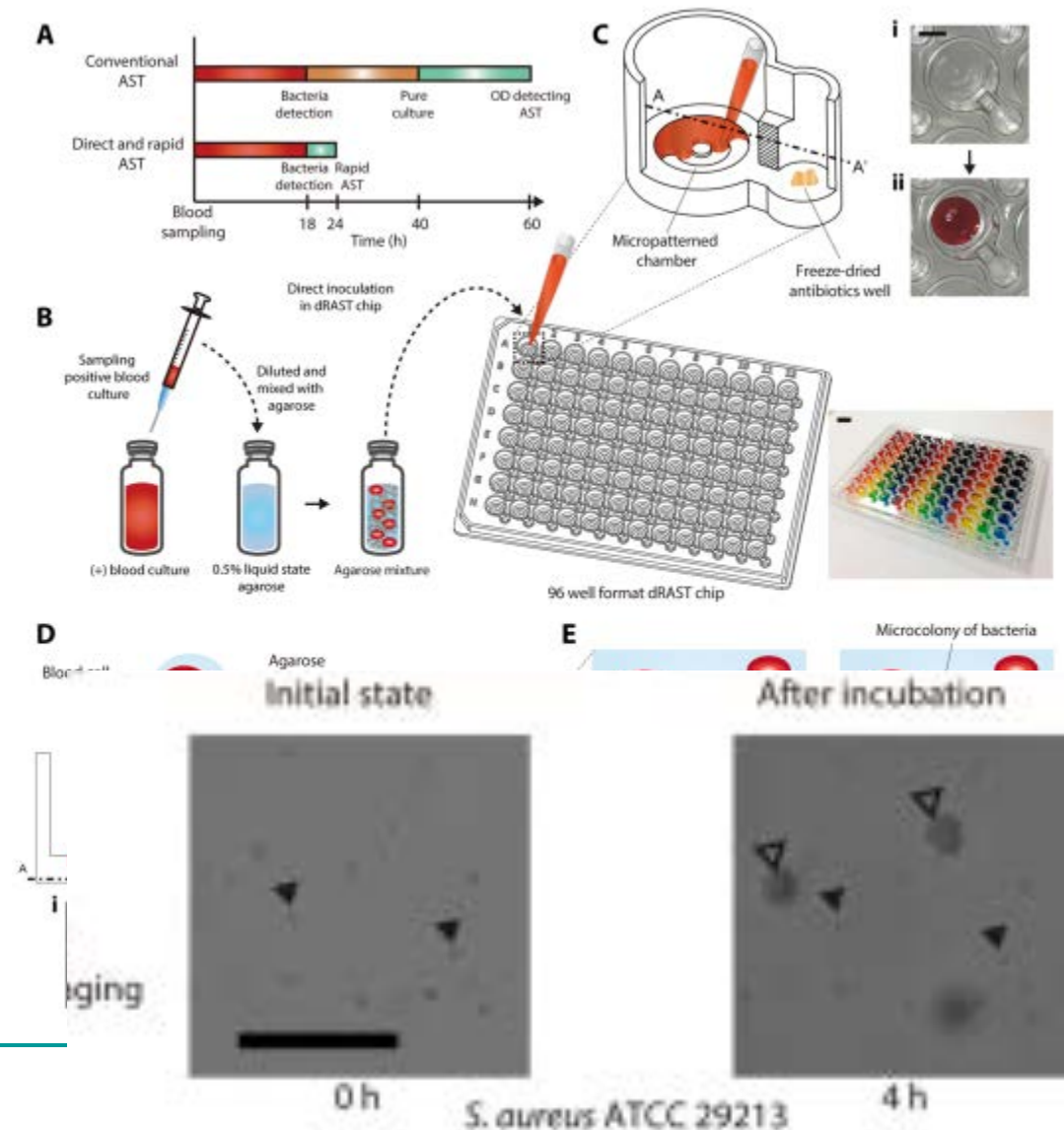
- Pour les BGN
- Déterminer la sensibilité bactérienne aux antibiotiques (CMI) par microdilution, à partir des flacons d'hémoculture, grâce à la détection de composés organiques volatils.
- Résultats en 5h en moyenne /nécessité d'identification

A Practical Method of Rapid AST for Blood Cultures



Rapid antimicrobial susceptibility testing (dRAST)

- Lecture plus rapide des antibiogrammes
standard: 4-6-8-vs 16/20 heures
- Détection des colonies



QMAC-dRAST

- 124 HC + à Entérobacterales
- Test de 16 antibiotiques, CMI
- Rendu 6,5 vs 18h / Nécessité d'identification

Method used on PBCB	Total tests	Agreement (%)		Errors (%)		
		Essential	Categorical	VME	ME	mE
QMAC-dRAST	2082	95.7	93.5	3.0	4.0	2.8
MicroScan	2064	98.8	98.5	0.6	0.9	0.7
Disk diffusion	1868	/	95.8	1.7	2.0	1.9

In press

Efficacité sur les *pseudomonas* ?

Conclusion

- Beaucoup de nouveaux outils
- Nécessité de prélèvements de qualité et d'une culture standard
- Discussion avec le microbiologiste/ formation/ guide d'utilisation
- Qu'attendez vous du résultat ?
- Données sur l'impact clinique prochainement
- Changement des organisations des laboratoires?