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What is the Future of Microbiologic Diagnosis?

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Déclaration d'intérêts

- Contracted Research: ContraFect, TenNor Therapeutics Limited, and BioFire
- Consultant: Curetis, PathoQuest, Selux Diagnostics, 1928 Diagnostics, PhAST, Torus Biosystems, Day Zero Diagnostics, Mammoth Biosciences, CARB-X, Qvella, Netflix
- Mayo Clinic and I have a relationship with Adaptive Phage Therapeutics and Pathogenomix
- Patents: *Bordetella pertussis/parapertussis* PCR; device/method for sonication; anti-biofilm substance

Outline

- **The challenge**
- **Technology revolution**
  - Proteomics
  - NAATs & sequencing-based diagnostics
  - Other diagnostic types (imaging, metabolomic)
  - Point of care diagnostics
  - Advanced host response assessment for infectious diseases
- **How to move to the future**
  - Demonstration of clinical utility
- **The deliverables**
  - Improved health
  - Changes to healthcare delivery
  - Better understanding of infectious diseases and their mimics

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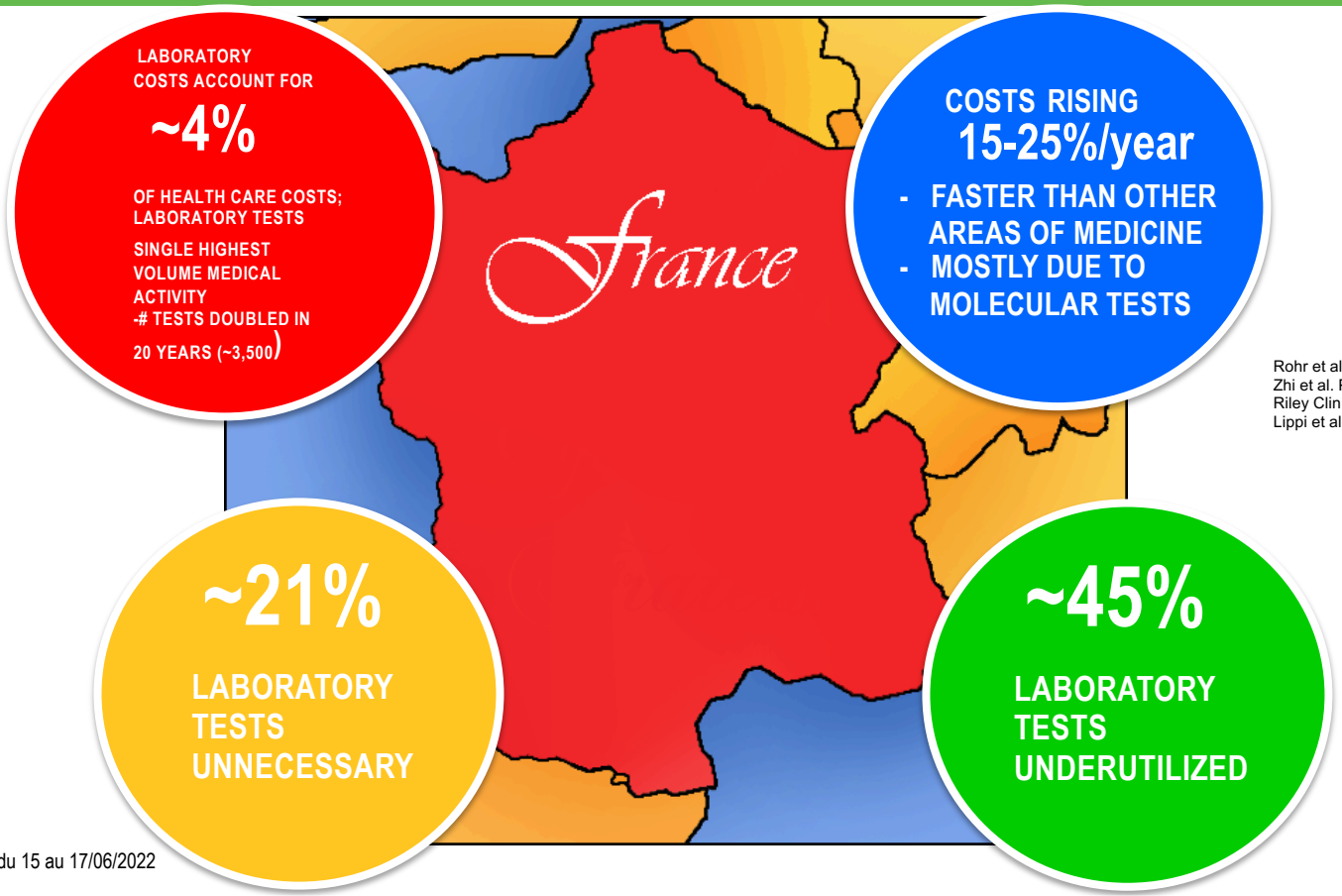
The Challenge

- **The need:**
  - Antimicrobial resistance
  - Emerging infectious diseases
  - Availability of better therapeutics
- **The barriers:**
  - Infectious diseases management predicated on “culture of empiricism”
  - Inappropriate test utilization
  - Lack of understanding of value of advanced diagnostics
  - Cost of advanced diagnostics

70% Clinical Decisions Substantially Based on Results of Diagnostic Tests



Rohr et al. PLoS One 2016;11:e0149856
Zhi et al. PLoS One. 2013;8(11):e78962
Riley Clin Microbiol Newslett 2017;39:69-73
Lippi et al. Ann Transl Med 2017;5(4):82

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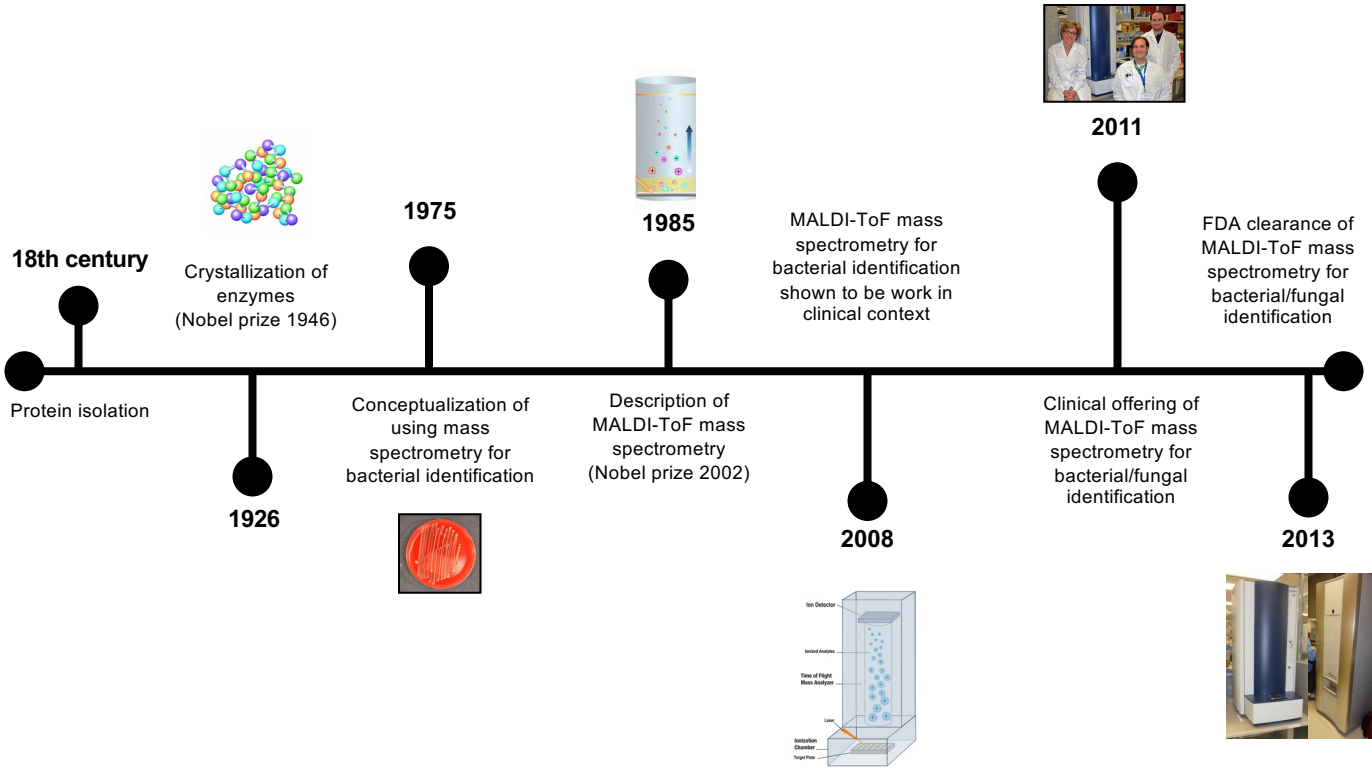
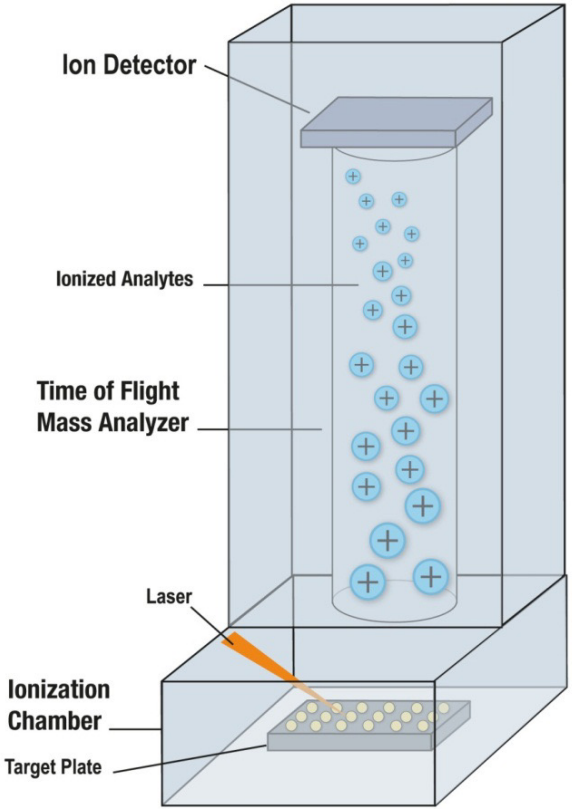
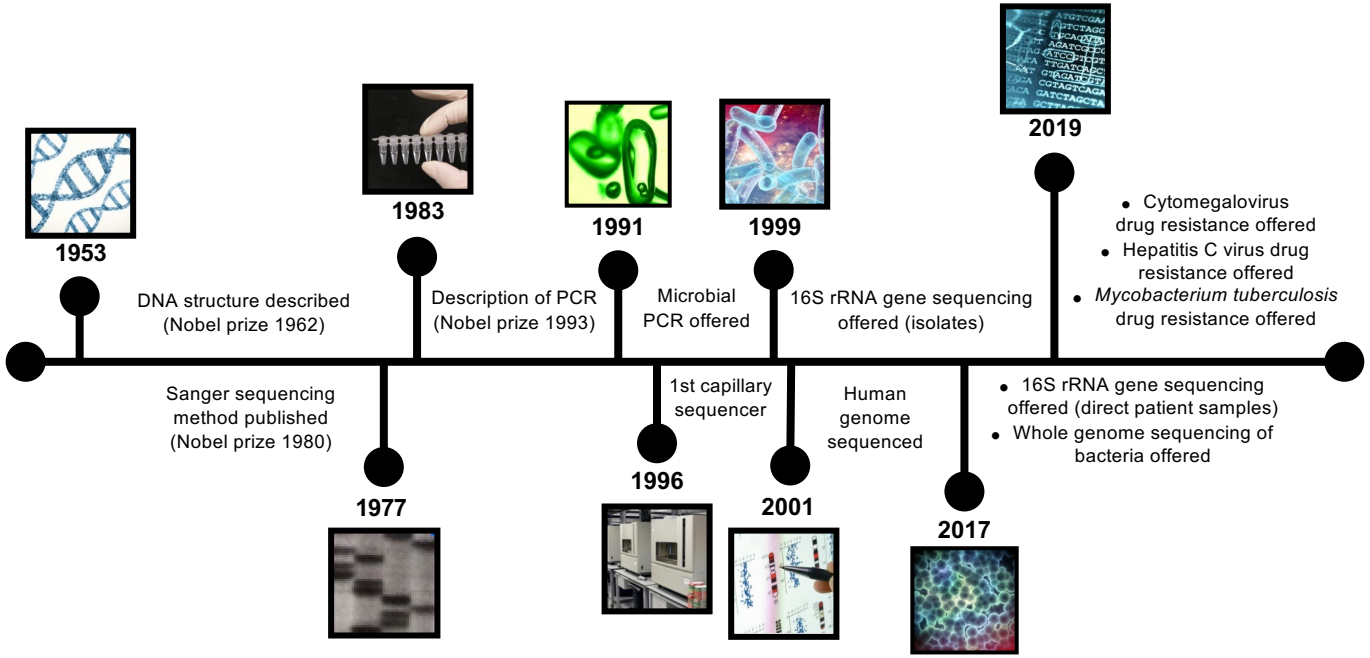
Technology Revolution
Proteomics



Technology Revolution
MALDI ToF Mass Spectrometry



Technology Revolution
NAAT and Sequencing-Based Diagnostics



Technology Revolution: Microbial Real-Time PCR Assays Developed @ Mayo Clinic, 2000–2020

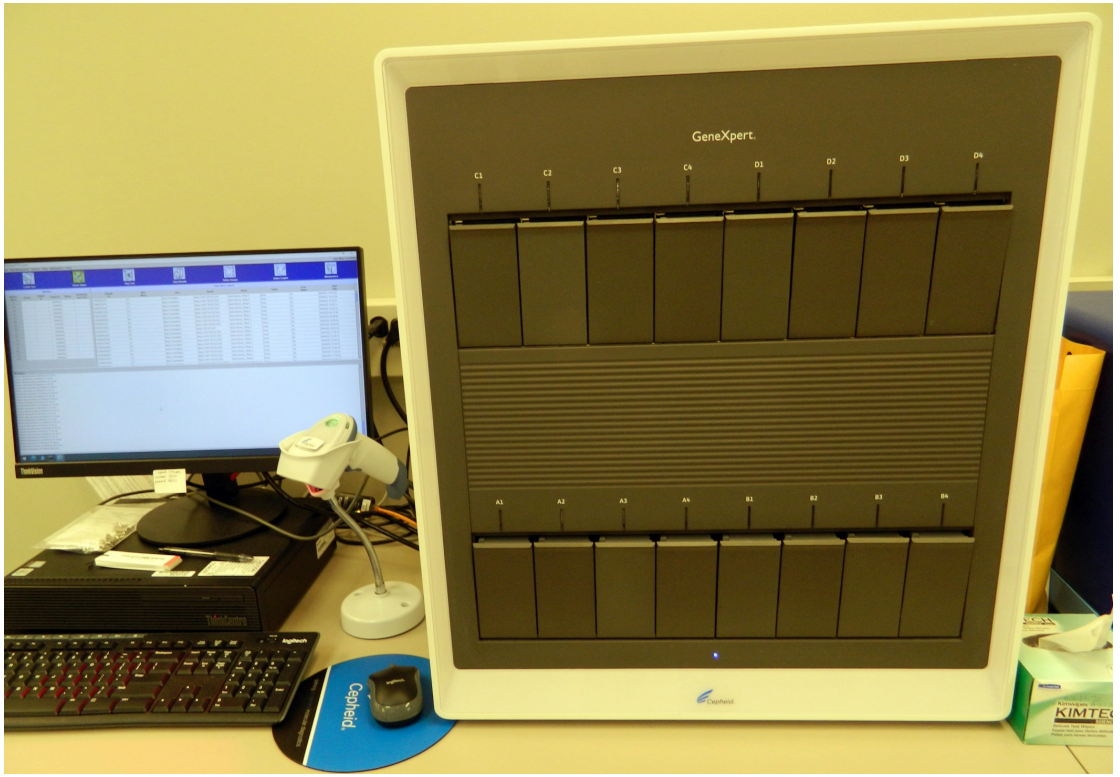
2000	Herpes simplex virus*
2001	*Bacillus anthracis*
	Cytomegalovirus*
	*Toxoplasma gondii**
2002	*Bordetella pertussis/parapertussis**
	Streptococcus pyogenes
	Enterovirus
	Varicella zoster virus*
2003	*Tropheryma whipplei**
	*Babesia microti**
	BK virus*
	Ehrlichia/Anaplasma species*
	Epstein–Barr virus (qualitative)*
	JC virus*
	*Borrelia burgdorferi**
2004	*Bartonella* species*
	*vanA/vanB**
	Parvovirus B19*
2005	Influenza A/B
	Pneumocystis jirovecii
	BK virus (quantitative)
	Epstein–Barr virus (quantitative)
	Human herpesvirus-6
	Plasmodium species
	West Nile virus
2007	*Clostridioides difficile*
	Coccidioides immitis/posadasii
	Staphylococcus aureus
	Mycobacterium chelonae/abcessus
2008	Adenovirus

2009	*Legionella* species
	bla^{KPC}
	Mycobacterium tuberculosis complex
2010	*Salmonella* species
	Shigella species
	Campylobacter species
	Yersinia species
	Shiga toxin
2011	*Mycoplasma genitalium*
	Mycoplasma hominis
	Ureaplasma urealyticum/parvum
	Histoplasma/Blastomyces species
2012	*Mycobacterium tuberculosis* complex species
2013	*Coxiella burnetii*
	bla^{NDM}
	Mycoplasma pneumoniae
2014	*Babesia* species
	Borrelia mayonii
2015	Microsporidia
2016	*Borrelia miyamotoi*
2017	*bla*$^{OXA48\text{-}like}$
	bla^{VIM}
	Kingella kingae
	Acanthamoeba species
	Free-living amoeba
	Norovirus
2020	*mecA*
	Helicobacter pylori
	Candida auris
	SARS coronavirus-2

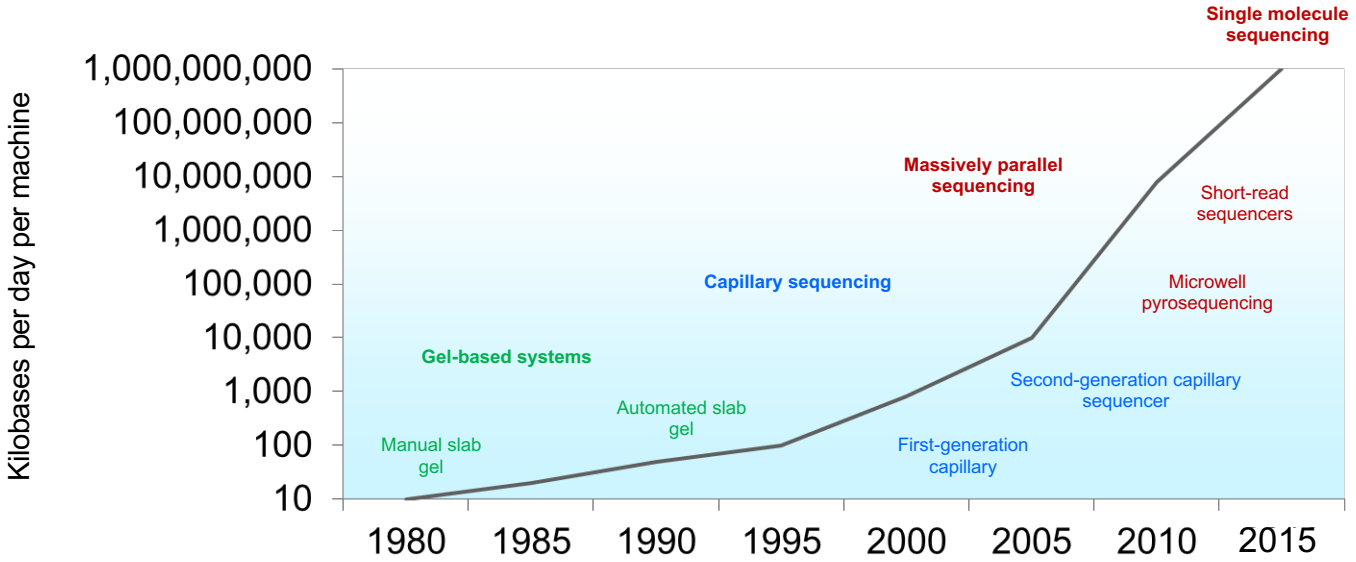
Microorganisms marked by an asterisk (*) were detected with conventional PCR assays with Southern blot detection developed and deployed in the 1990s prior to conversion to real-time PCR assays

Patel. Clin Chem. 2022;68:10-15

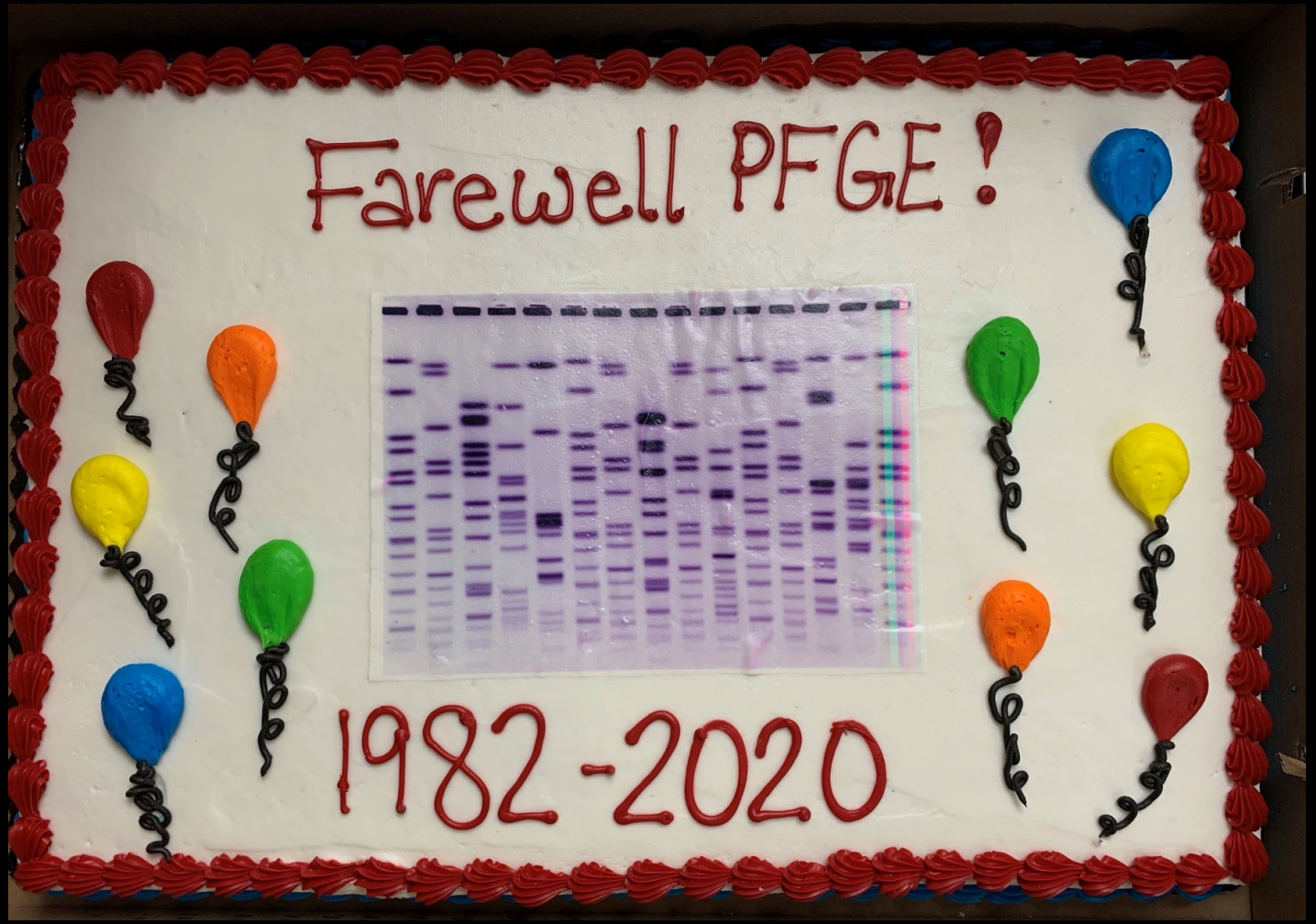
Technology Revolution:
Multiplex and Rapid Automated NAATS



Technology Revolution: Improvements in DNA Sequencing Rates

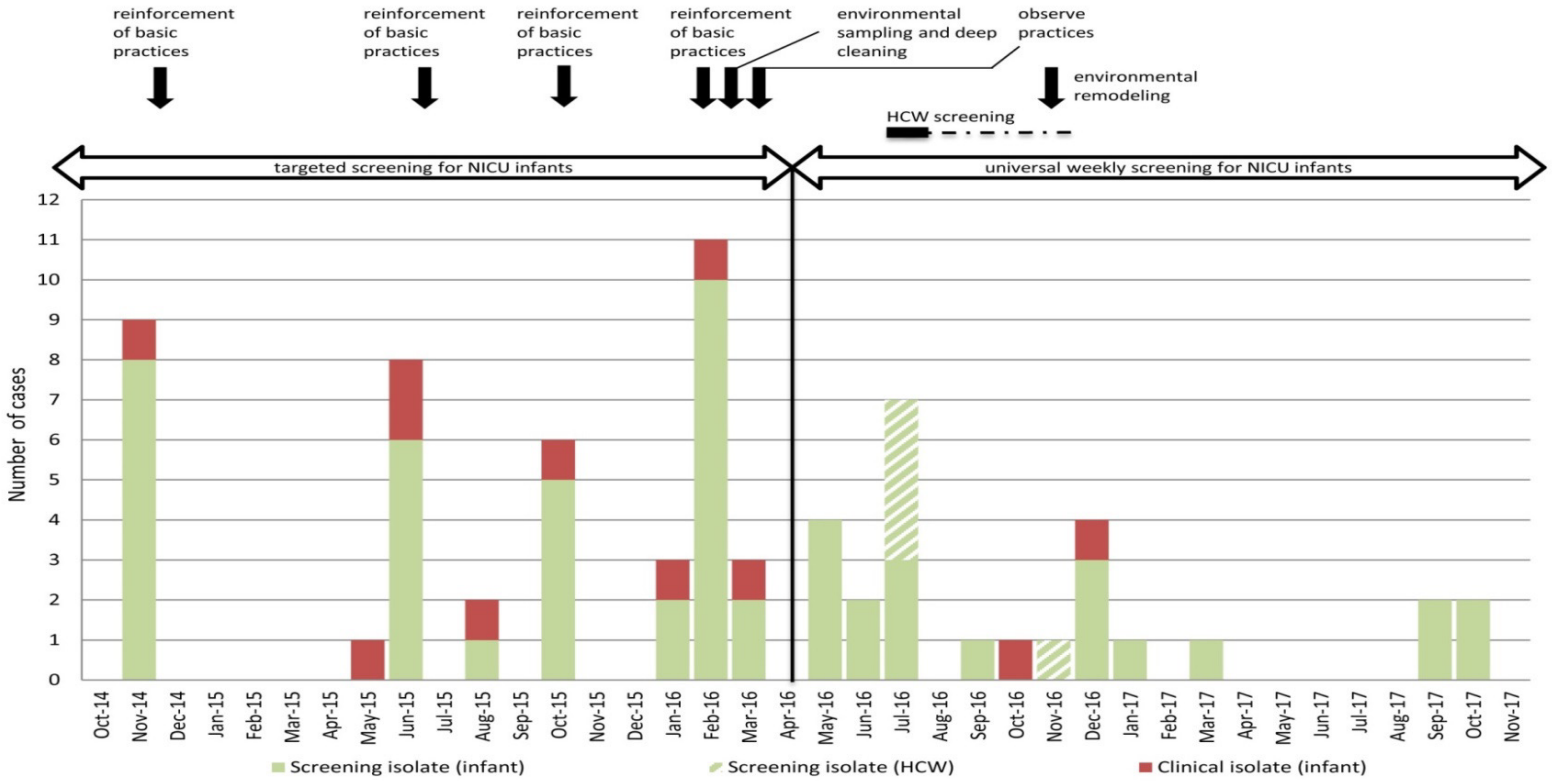


Based on Stratton et al. Nature 2009;458:719


Farewell PFGE!
1982-2020

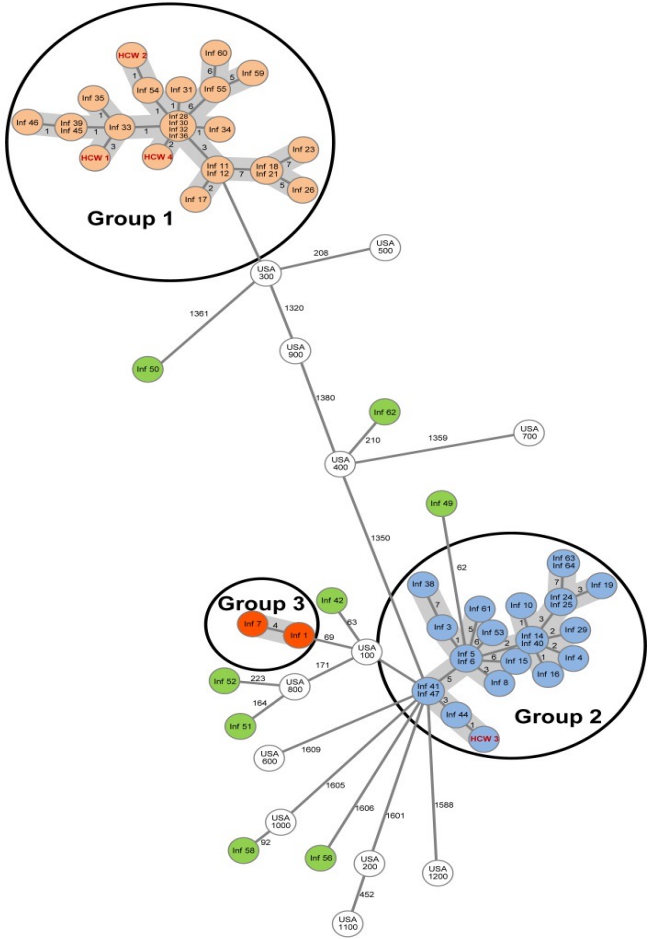
Technology Revolution: Whole Genome Sequencing

Neonatal Intensive Care Unit MRSA Cases by Initial Source & Interventions Taken



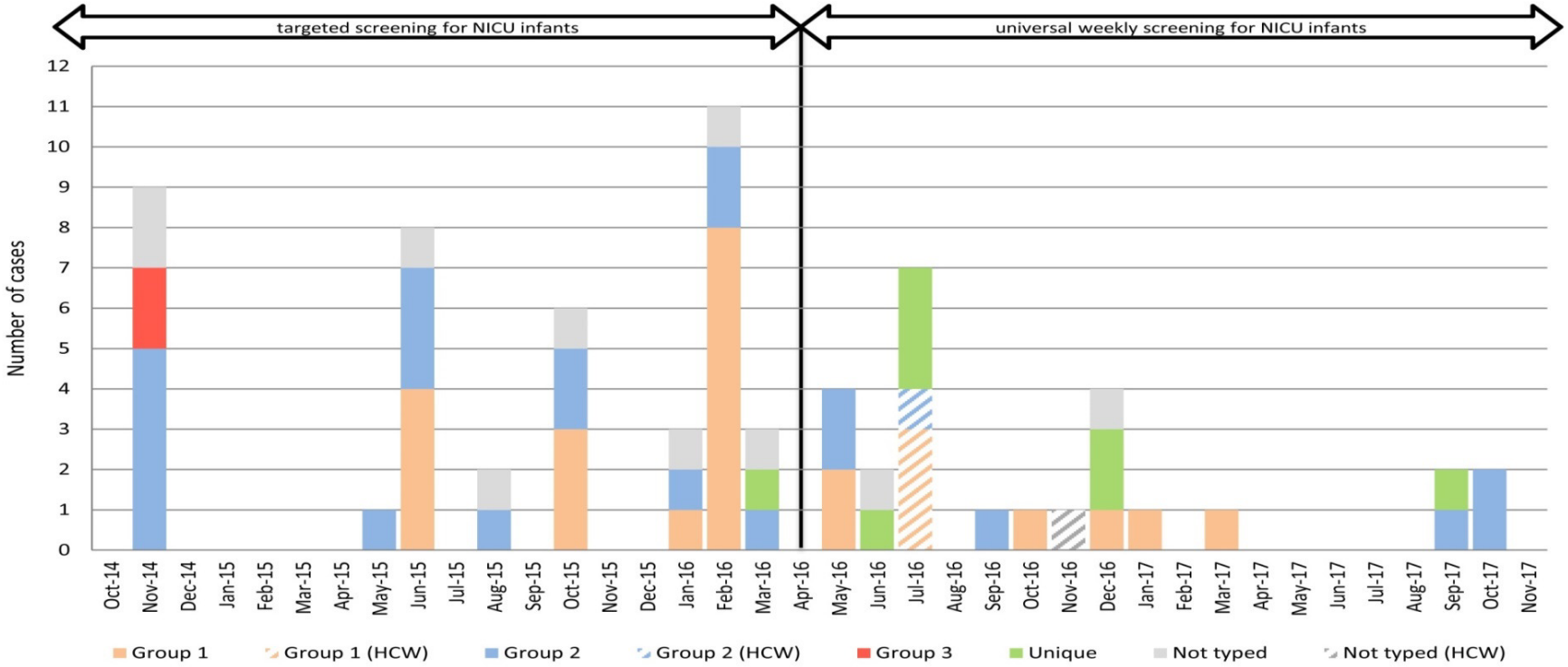
Madigan et al. Infect Cont Hosp Epi 2018;39:1412-18

Neonatal Intensive Care Unit Isolates (Clinical & Surveillance) Neonates & Healthcare Workers



Madigan et al. Infect Cont Hosp Epi 2018;39:1412-18

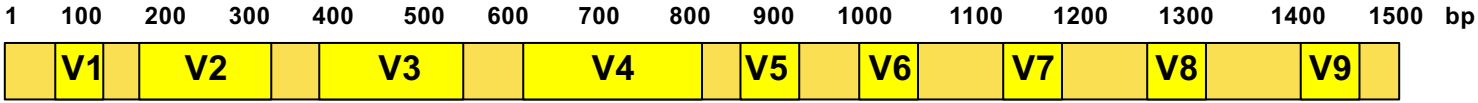
NICU Outbreaks (!) Timeline
MRSA Cases Shown by WGS Group



Species Validated for WGS (cgMLST)

- *Acinetobacter baumannii*
- *Campylobacter jejuni/coli*
- *Clostridioides difficile*
- *Cutibacterium acnes*
- *Enterobacter cloacae*
- *Enterococcus faecalis*
- *Enterococcus faecium*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Legionella pneumophila*
- *Pseudomonas aeruginosa*
- *Serratia marcescens*
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Staphylococcus lugdunensis*
- *Streptococcus agalactiae*
- *Streptococcus pyogenes*

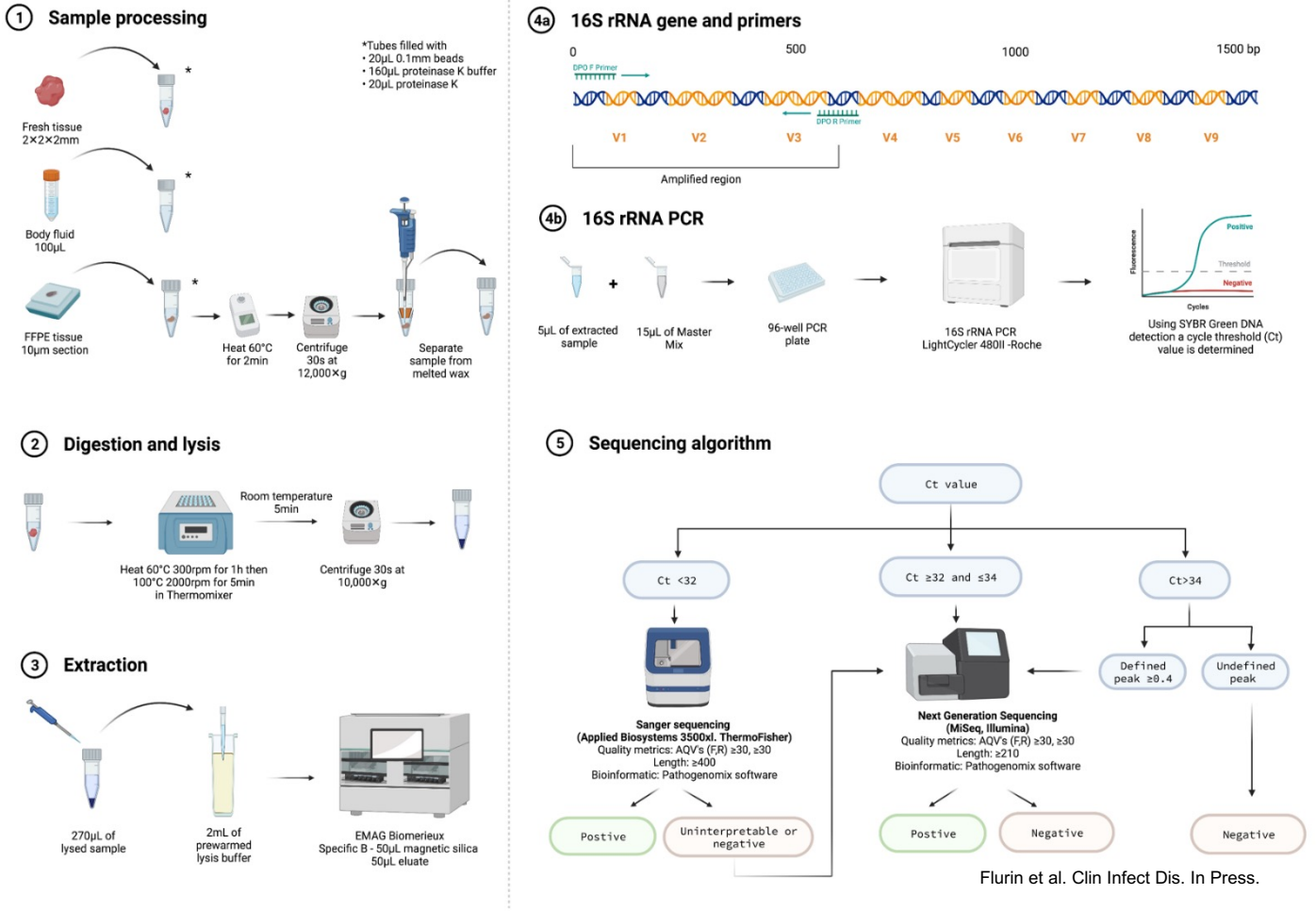
Technology Revolution:
Targeted Metagenomic Sequencing (16S Ribosomal RNA Gene)



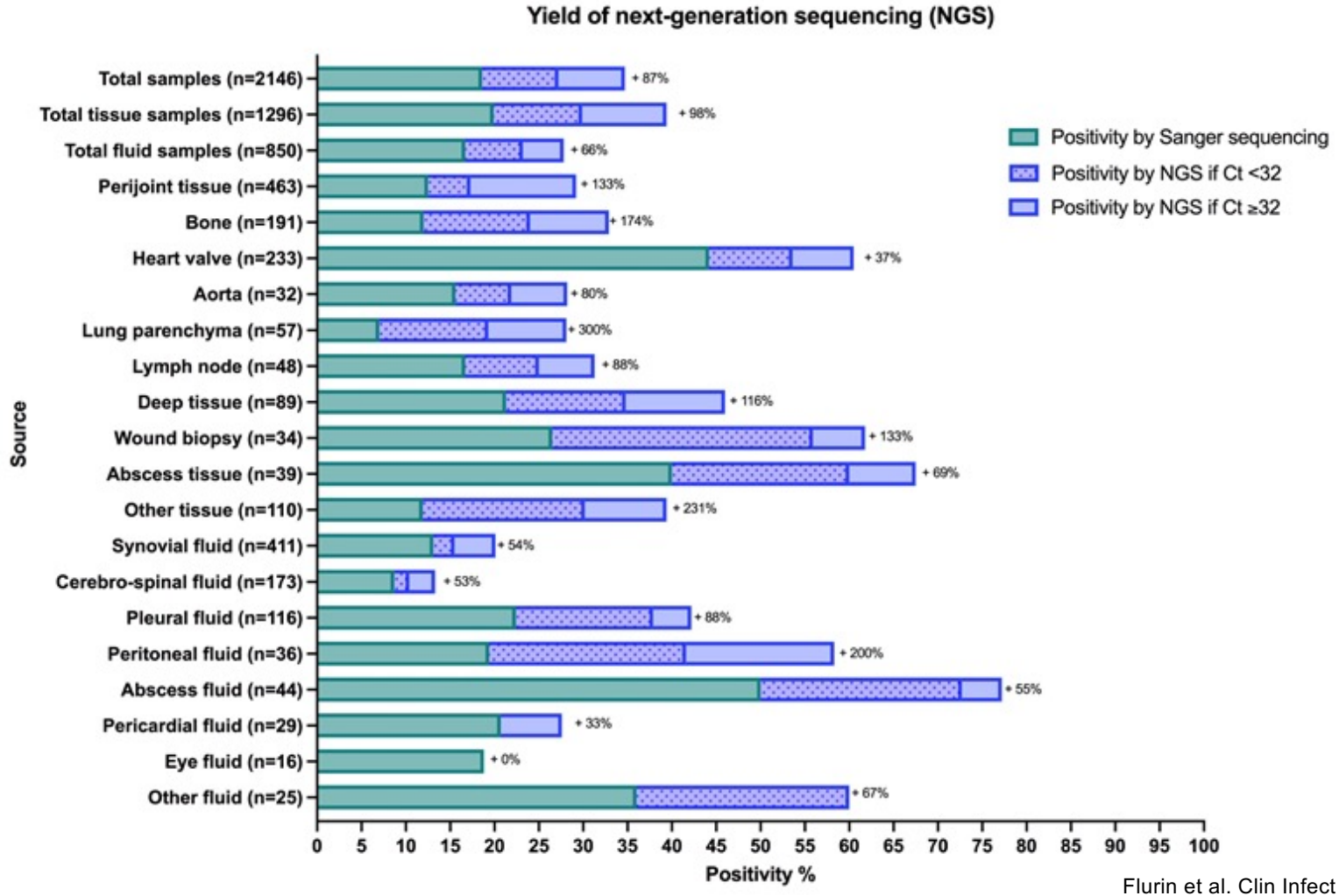
16S rRNA Gene PCR/Sanger Sequencing in Clinical Practice

- **Retrospective analysis specimens clinically analyzed by 16S rRNA PCR/Sanger sequencing (04-2017→ 03-2019)**
- **566 specimens, 460 patients**
- **17% (97/566)16S rRNA gene PCR/Sanger sequencing positive**
  - 90% (88/97) positive results → clinical infections
- **22% positive specimens (21/97), 16S rRNA gene detected but bacterium not identified by Sanger sequencing (mixtures)**

Fida et al. Clin Infect Dis. 2021;73:961–968



Flurin et al. Clin Infect Dis. In Press.



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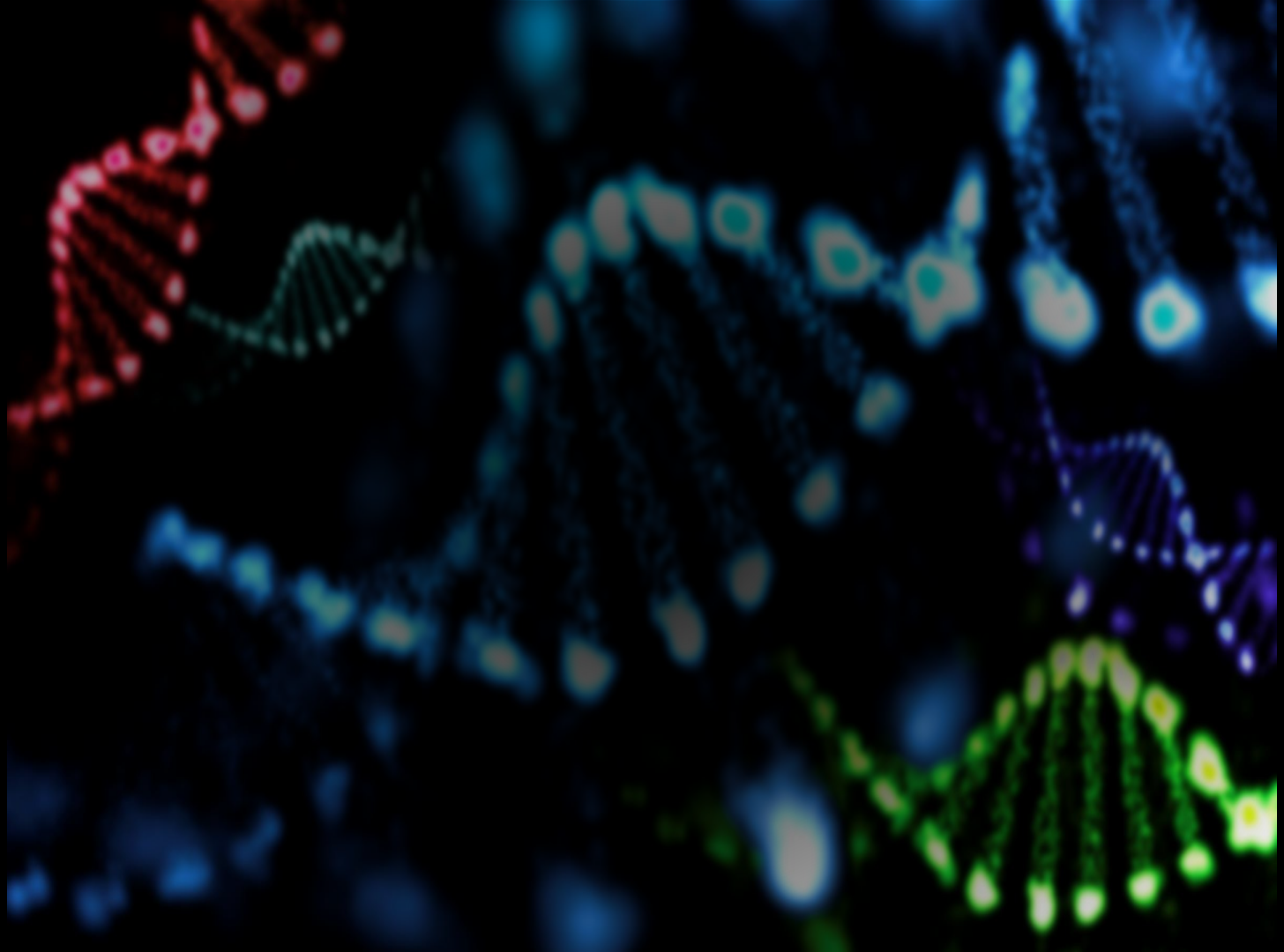
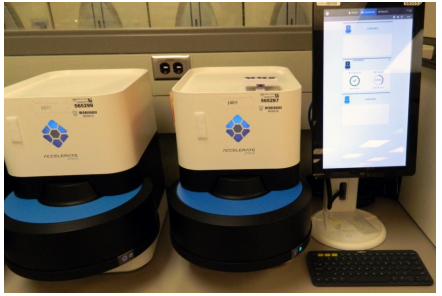
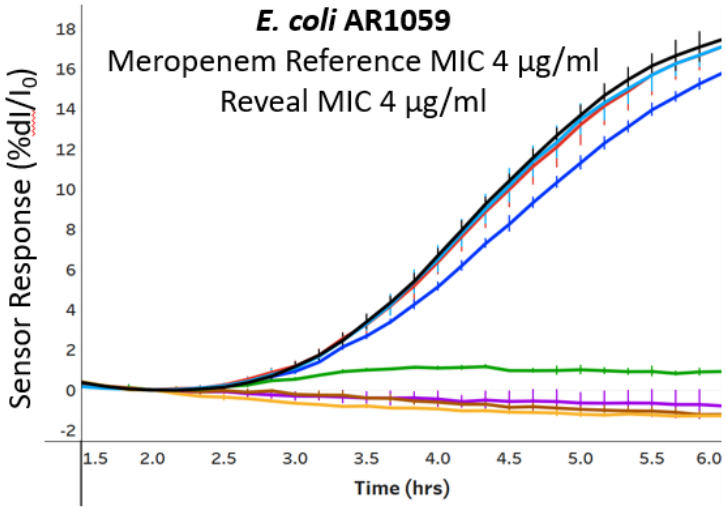


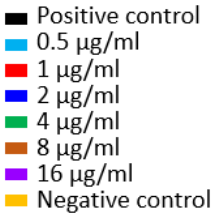
Technology Revolution: Single Cell Imaging
Application: Antibacterial Susceptibility Testing

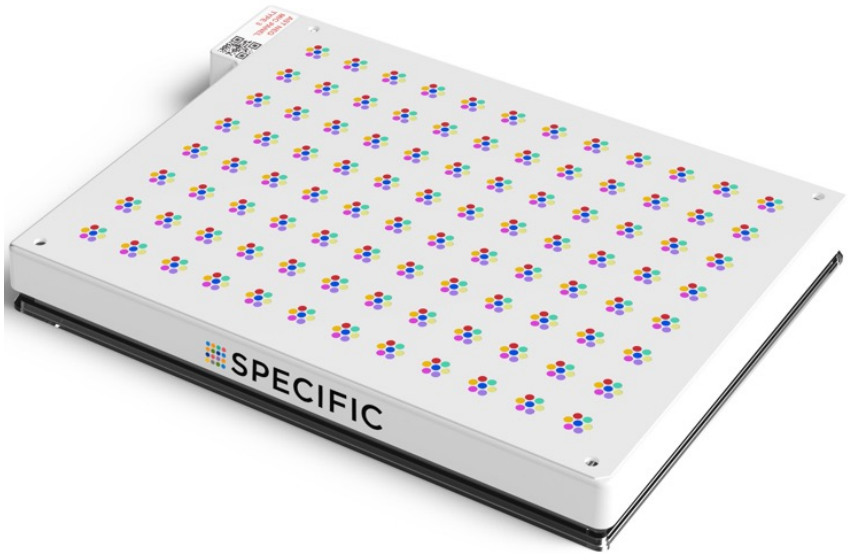
- **Time-lapse darkfield imaging, analysis of bacterial growth**
  - Individual bacterial response to single antibiotic concentration over time



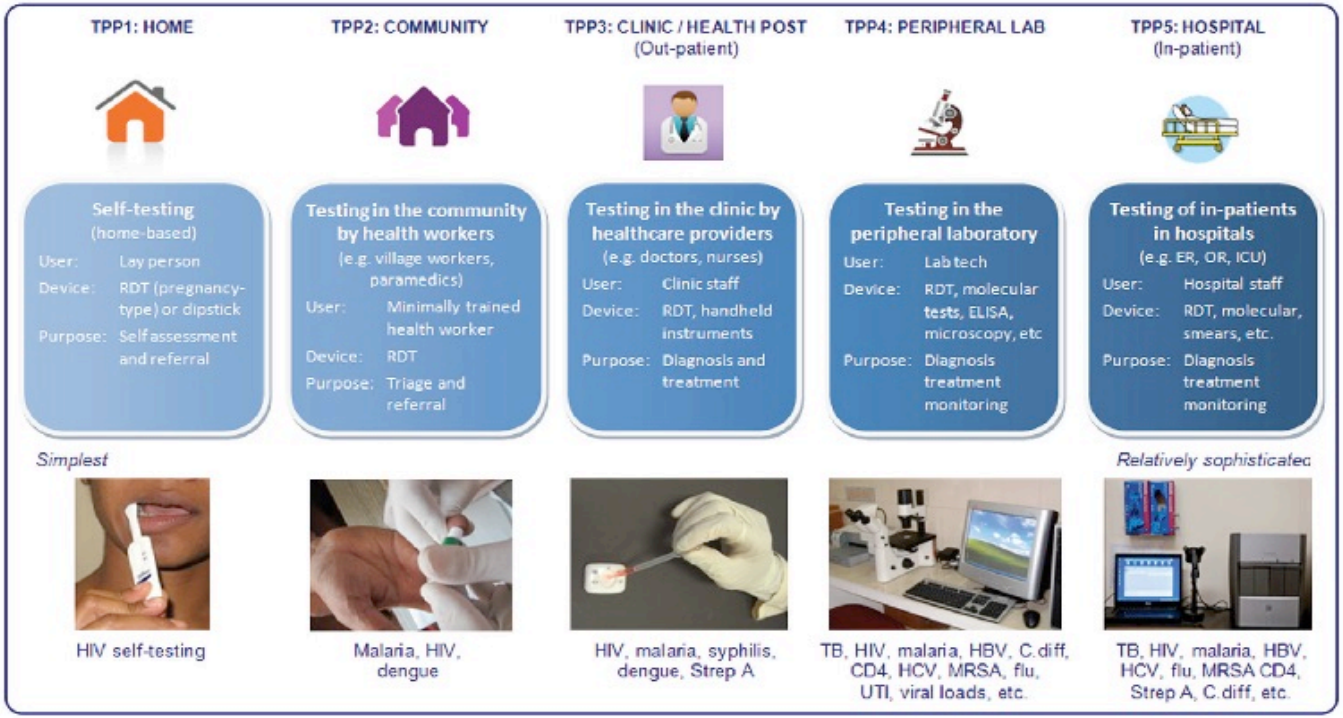
Technology Revolution: Metabolomics
Application: Antibacterial Susceptibility Testing







Technology Revolution
Point of Care Diagnostics



www.asm.org/index.php/colloquium-reports/item/6421-changing-diagnostic-paradigms-for-microbiology

Technology Revolution: Advanced Host Response Assessment for Infectious Diseases

- **Advanced human transcriptomic and/or proteomic analyses may be able to**
  - Point to underlying viral, bacterial, fungal, parasitic infections and non-infectious processes
  - Possibly, point to specific microbial etiologies
  - Blood, body fluids, tissues?

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How to Move to the Future?

- **MALDI-TOF mass spectrometry example**
- **Demonstration of clinical utility**
  - COVID-19 example
  - Advanced diagnostics - sometimes not so clear…

Randomized Controlled Clinical Trial
BioFire BCID, Mayo Clinic 2013-2014

Patients with positive blood cultures
Stratified randomization (age, ICU, transplant service)

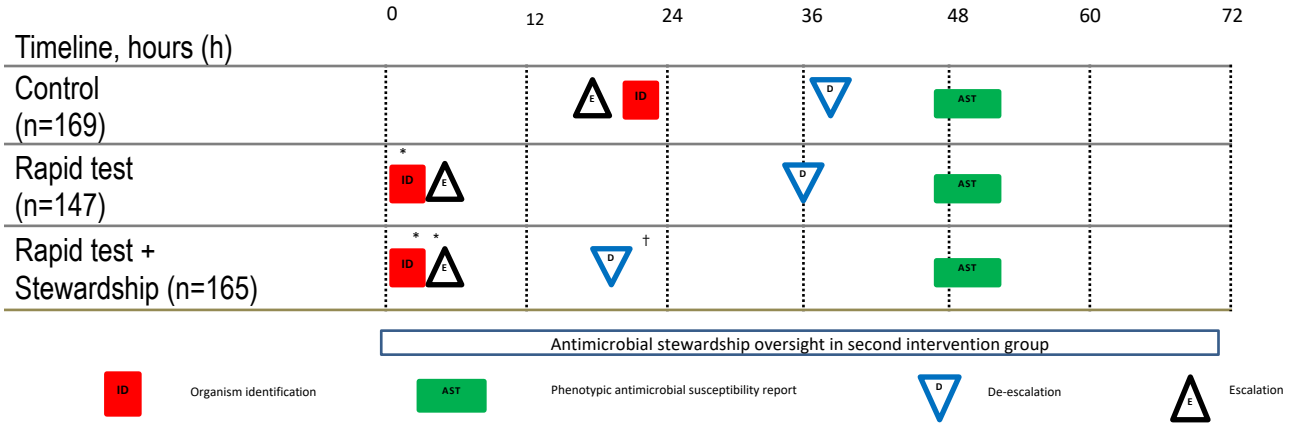
CONTROL	RAPID TEST ALONE	RAPID TEST/STEWARDSHIP
• Gram stain called to service • Standard subculture and susceptibility (1-3 d)	• Gram stain called to service • Standard subculture and susceptibility (1-3 d) • Rapid test plus lab call with comments (1 h)	• Gram stain called to service • Standard subculture and susceptibility (1-3 d) • Rapid test plus lab call with comments (1 h) • ID MD/pharmacist call with specific treatment recommendations

Banerjee et al. Clin Infect Dis 2015:61:1071

Supported by the National Institute of Allergy And Infectious Diseases of the National Institutes of Health under Award Number UM1AI104681 (Antibacterial Resistance Leadership Group)

ARLG
Antibacterial Resistance Leadership Group

Comparison of Time To Identification, Susceptibility Results, and Antibiotic Modifications



*Significant vs. control; †Significant vs. control and rapid multiplex PCR alone

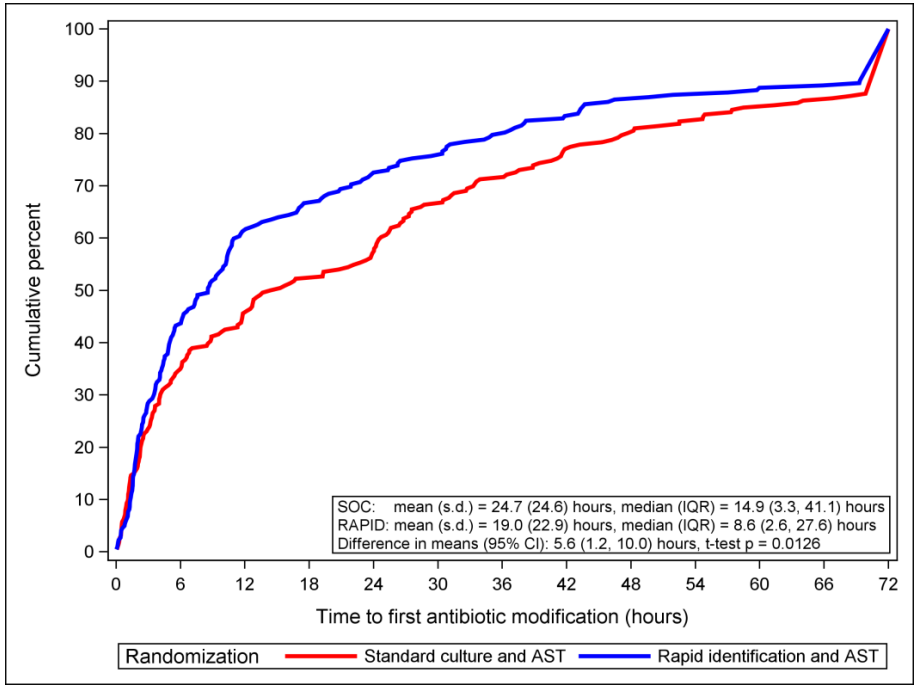
Banerjee et al. Clin Infect Dis 2015:61:1071

Thoughtful, Clear and Informative Test Reporting

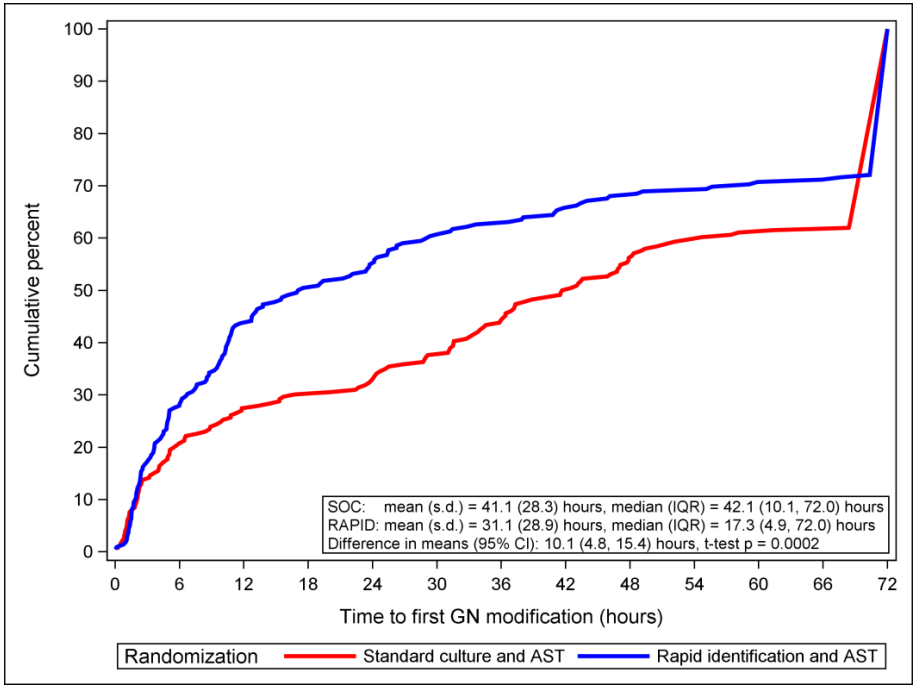
FilmArray BCID2 Result	Report	Isolate Comments
Staphylococcus + *S. aureus* + *mecA/C* and MREJ (MRSA) +	*Staphylococcus aureus* complex	*mecA* detected Methicillin (oxacillin)-resistant *Staphylococcus aureus* complex (MRSA). MRSA is predictably resistant to beta-lactam antibiotics (except ceftaroline). Vancomycin or other anti-MRSA treatment recommended for initial therapy pending susceptibility results. Semi-Urgent Result Consult Infectious Diseases
Staphylococcus + *S. aureus* + *mecA/C* and MREJ (MRSA) -	*Staphylococcus aureus* complex	*mecA* not detected Methicillin (oxacillin)-susceptible *Staphylococcus aureus* complex. An anti-staphylococcal beta-lactam, such as cefazolin, nafcillin or oxacillin, is recommended for empiric therapy, unless clinically contraindicated. Consult Infectious Diseases
Staphylococcus + *S. epidermidis* + *mecA/C* +	*Staphylococcus epidermidis*	*mecA* detected Methicillin (oxacillin)-resistant *Staphylococcus epidermidis.* Possible blood culture contaminant (unless isolated from more than one blood culture draw or clinical case suggests pathogenicity). No antibiotic treatment is indicated for blood culture contaminants.

Randomized Trial Evaluating Clinical Impact of RAPid IDentification and Susceptibility Testing for Gram-negative Bacteremia: RAPIDS-GN

Time to any antibiotic change



Time to GN antibiotic change

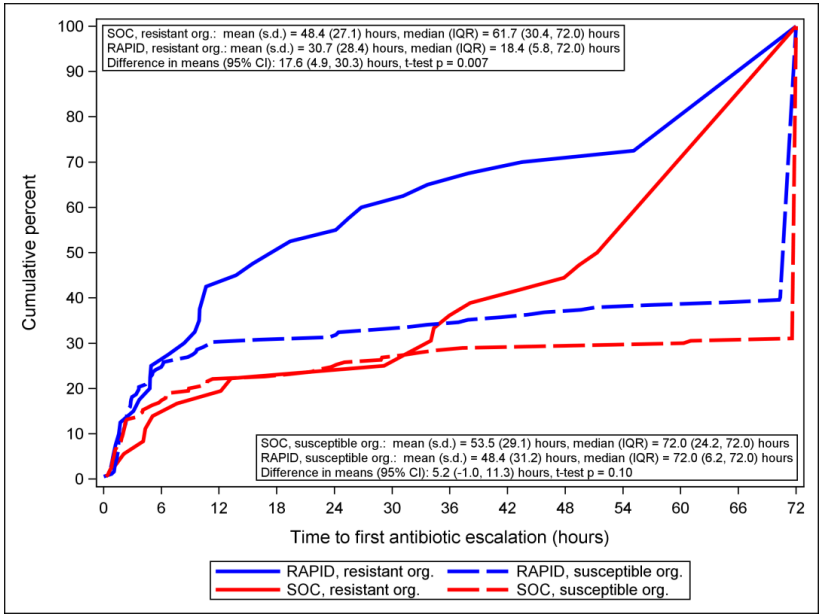


RAPIDS-GN
Antibacterial Resistance Leadership Group

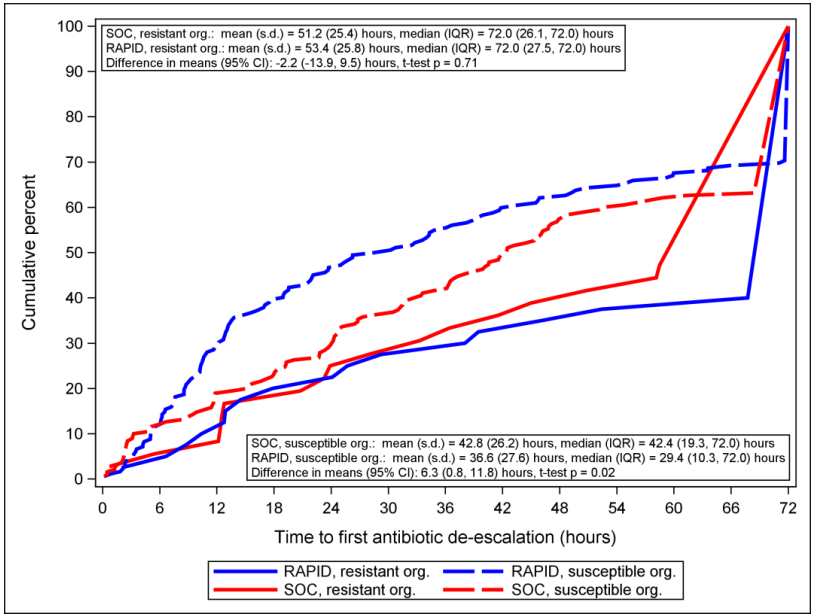
Banerjee et al. Clin Infect Dis 2021;73:e39-e46

Randomized Trial Evaluating Clinical Impact of RAPid IDentification and Susceptibility Testing for Gram-negative Bacteremia: RAPIDS-GN

Time to antibiotic escalation



Time to antibiotic de-escalation

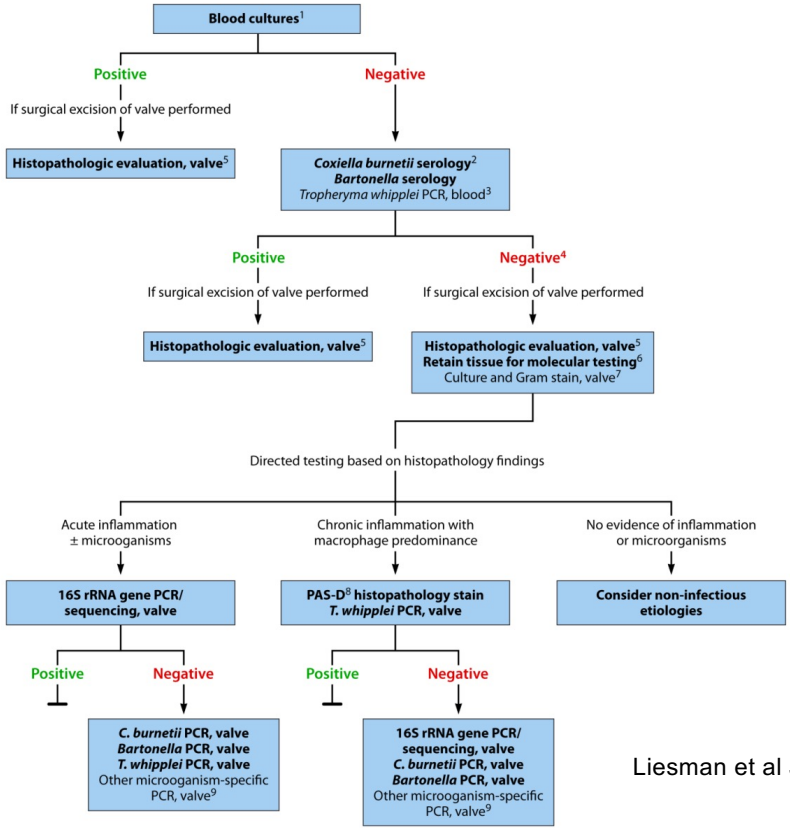


RAPIDS-GN Antibacterial Resistance Leadership Group

Banerjee et al. Clin Infect Dis 2021;73:e39-e46

Importance of Guidelines, Diagnostic Algorithms, Diagnostic Stewardship

Microbiologic and Pathological Diagnostic Algorithm For Endocarditis



Liesman et al J Clin Microbiol. 201;55(9):2599-608

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The Deliverables: Improved Patient Health

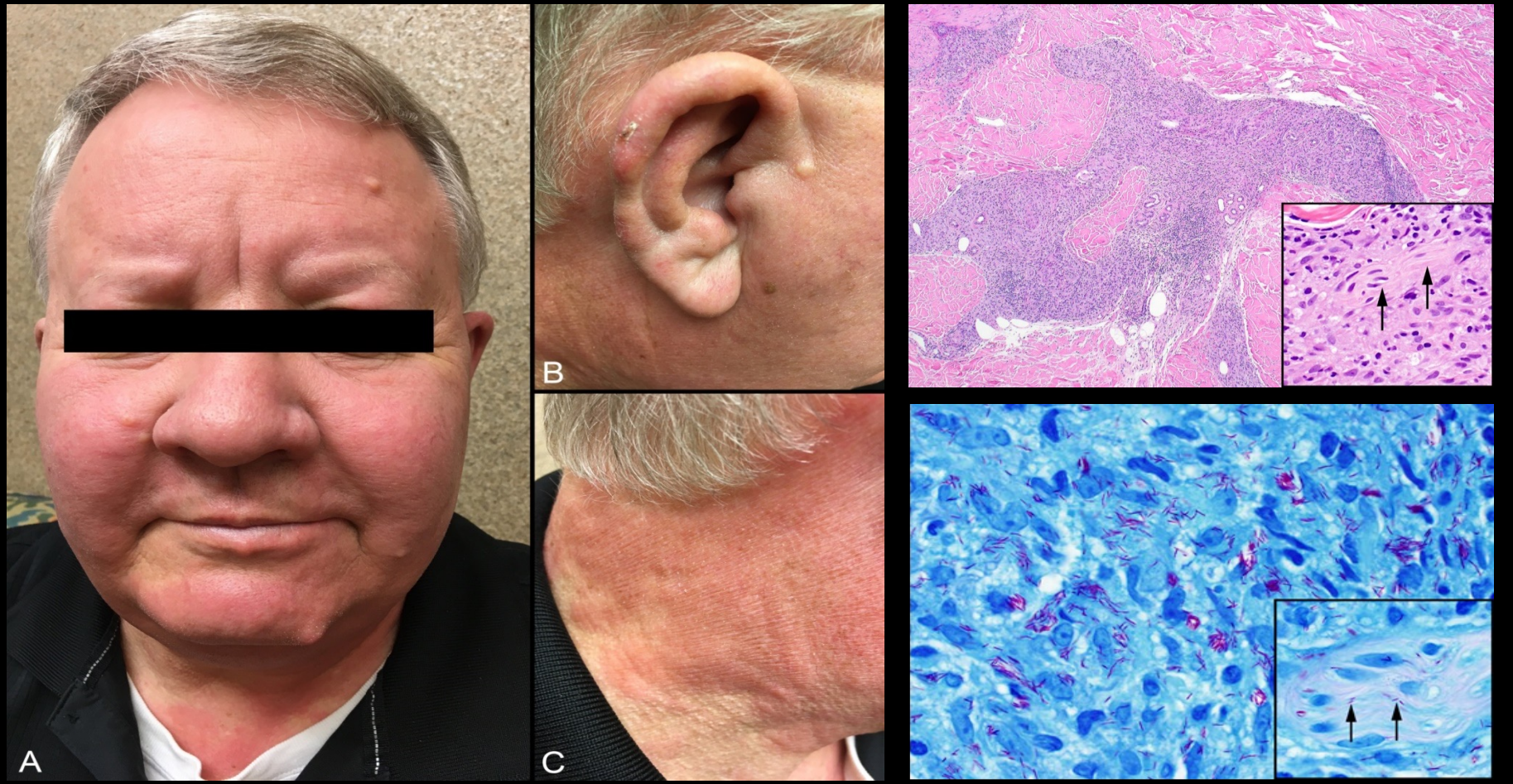
- **Improved patient health and hopefully…**
  - Avoid unneeded testing and treatment (patient benefit)
  - Decrease transmission of infectious diseases (societal benefit)
  - Curb emergence of antimicrobial resistance (patient and societal benefit)

The Deliverables: Changes in Healthcare Delivery

- Home-based testing
- Patient self-testing
- Testing @ non-traditional sites
- Test prioritization approaches in traditional healthcare sites (e.g., emergency departments, urgent care settings)
- etc.

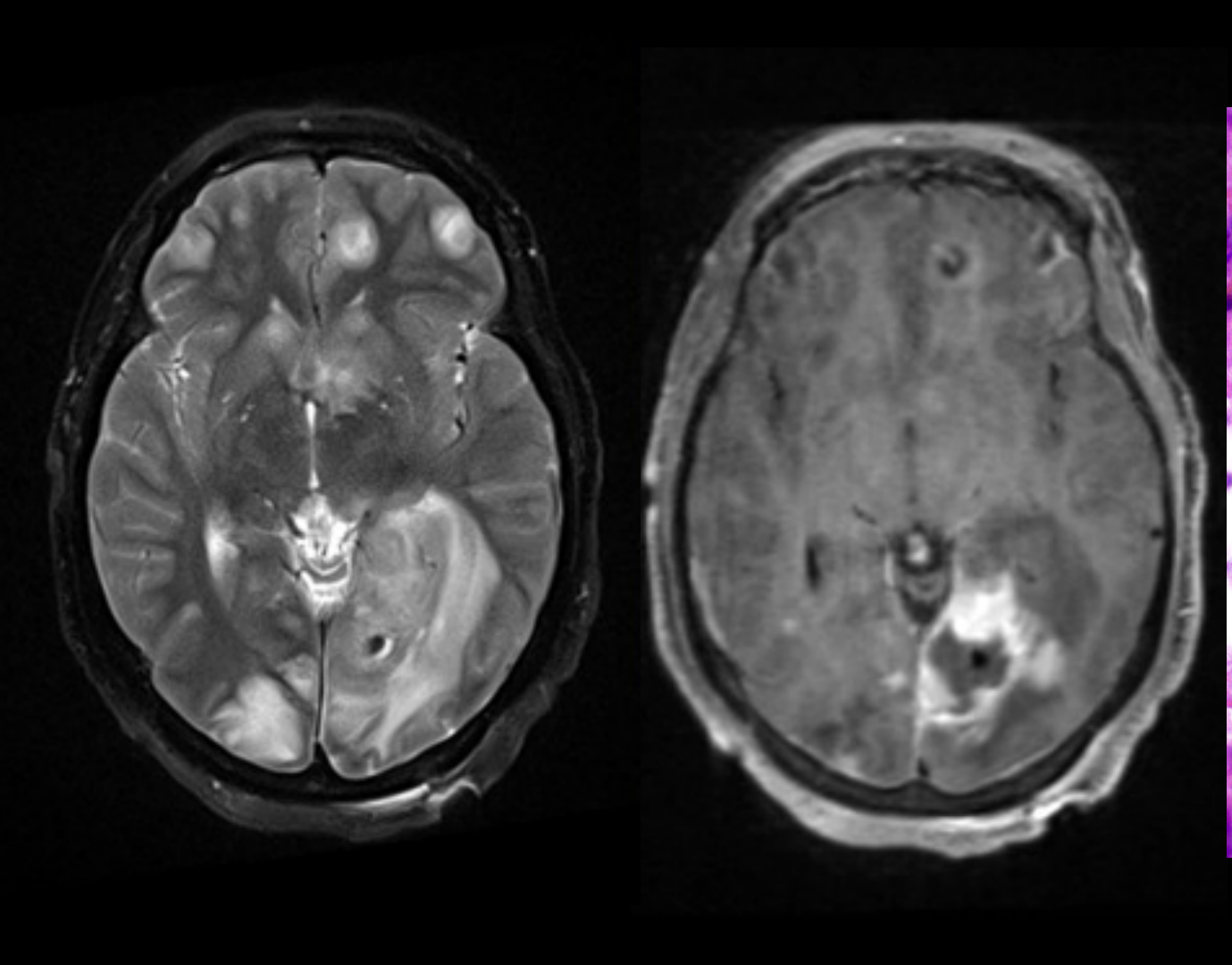
The Deliverables: Better Understanding of Infectious Diseases and Their Mimics

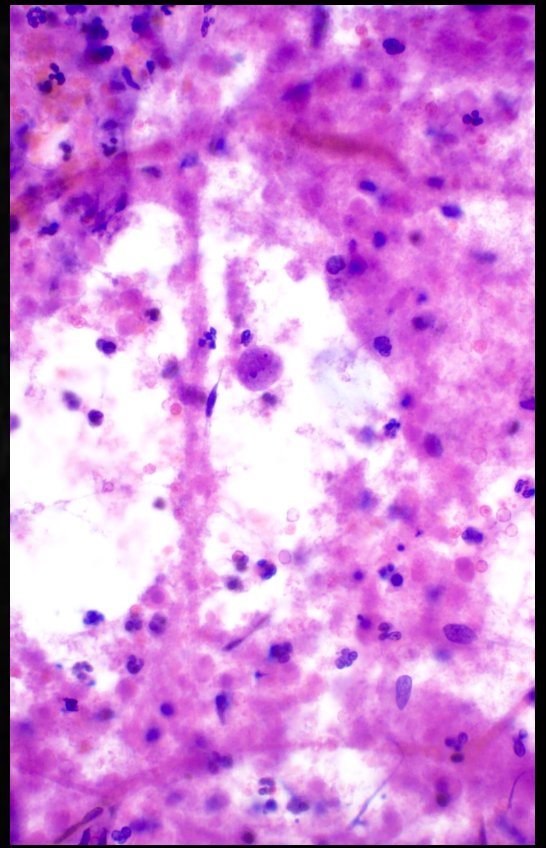
- **Discovery through testing**
  - *Borrelia mayonii*, *E. muris* subspecies *eauclairensis*, *Yersinia rochesterensis*
    - Many more novel species remain to be described
  - Understanding antimicrobial resistance
    - Characterization of vancomycin resistance in *Paenibacillus popilliae*
    - Recognition of the frequency of resistance in *Helicobacter pylori*
  - New diseases – hyperammonemia associated with *Ureaplasma* species



Virk et al. Emerg Infect Dis 2017:23:1864

Source:	BRAIN, RIGHT- Tissue. RIght frontal lobe			
Fresh vs FFPE:	Fresh			
Cp value:	31.86			
FORWARD				
Abundance	Read length		Result	% ID
22606		222	Amoebophilus	96.8
405		225	Cutibacterium acnes **	100
274		224	Methylotenera	95.5
222		222	Amoebophilus	96.4
211		223	Chryseobacterium lactis / Chryseobacterium aquaticum / Chryseobacterium aurantiacum / Chryseobacterium vietnamense	99.1
148		224	Amoebophilus	96.4
140		222	Enterococcus faecium / Enterococcus hirae / Enterococcus pseudoavium	100
138		222	Amoebophilus	96.8
128		222	Amoebophilus	96
117		222	Amoebophilus	96.4
106		222	Amoebophilus	96.4
REVERSE				
19971		228	Amoebophilus	98.2
754		229	Amoebophilus	98.3
353		226	Cutibacterium acnes / Cutibacterium namnetense *	100
179		227	Methylophilus	98.7
170		228	Amoebophilus	97.8
144		228	Amoebophilus	97.8
139		227	Enterococcus durans / Enterococcus faecium / Enterococcus hirae / Enterococcus ratti / Enterococcus thailandicus / Enterococcus villorum / Enterococcus xinjiangensis / Enterococcus lactis / Enterococcus pseudoavium	100
131		228	Chryseobacterium artocarpi / Chryseobacterium antibioticum / Chryseobacterium carnipullorum / Chryseobacterium shigense / Chryseobacterium ureilyticum / Chryseobacterium vrystaatense	99.6
118		228	Amoebophilus	97.4
118		228	Amoebophilus	97.4
104		228	Amoebophilus	97.8





Conclusions

- **Many current microbiology diagnostics antiquated, poorly used**
- **Technology revolution**
  - Bringing major advances
- **How to move to the future**
  - Demonstration of value
- **The deliverables**
  - Improved health (patients, society, antimicrobial resistance)
  - Changes to healthcare delivery
  - Better understanding of infectious diseases and their mimics (discovery through testing)

R01 AR056647
R01 AI91594
R21 AI125870
UM1 AI104681