# An Update on Rapid Diagnostics for the Antimicrobial Steward


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# Objectives

**At the end of this lecture, the learner will be able to:**

- **List newer microbiology tests that improve antibiotic utilization and the variables affected**
- **Describe point of care testing for infectious diseases**
- **Discuss advanced generation microbiology diagnostic tests and how they might improve antimicrobial use**

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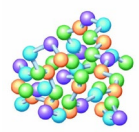
# Technology Revolution - Proteomics

**18th century**

Protein isolation



Crystallization of enzymes (Nobel prize 1946)

**1926**

**1975**

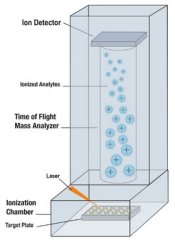
Conceptualization of using mass spectrometry for bacterial identification

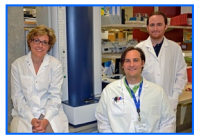
**1985**

Description of MALDI-ToF mass spectrometry (Nobel prize 2002)

MALDI-ToF mass spectrometry for bacterial identification shown to be work in clinical context

**2008**



**2011**

Clinical offering of MALDI-ToF mass spectrometry for bacterial/fungal identification

FDA clearance of MALDI-ToF mass spectrometry for bacterial/fungal identification

**2013**

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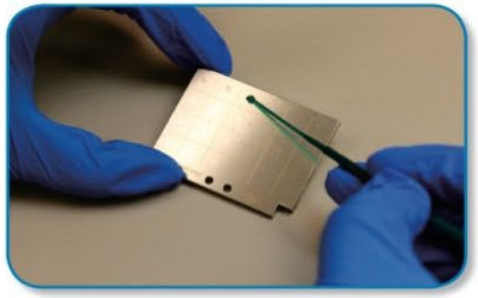
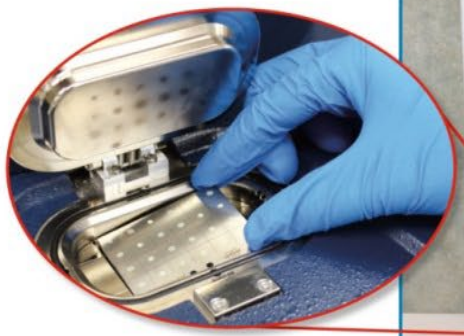
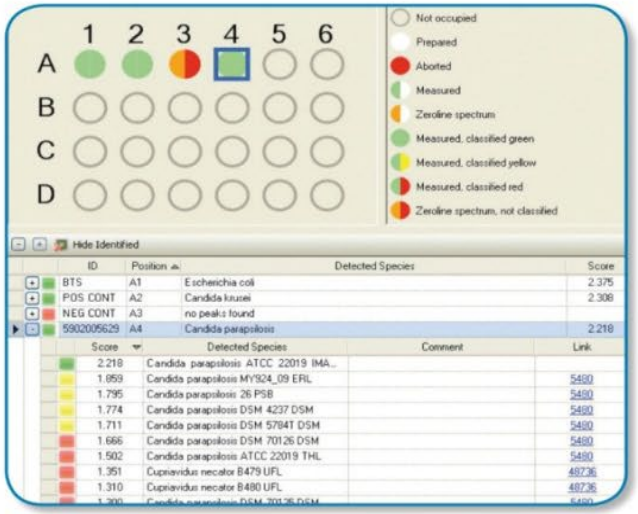
# MALDI ToF Mass Spectrometry



Add Formic Acid and Dry; Add Matrix and Dry



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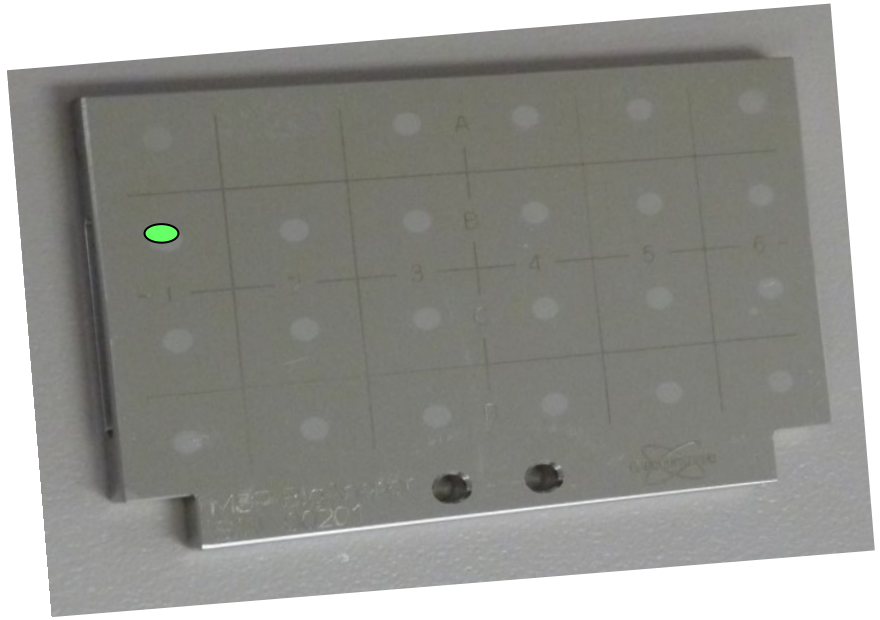
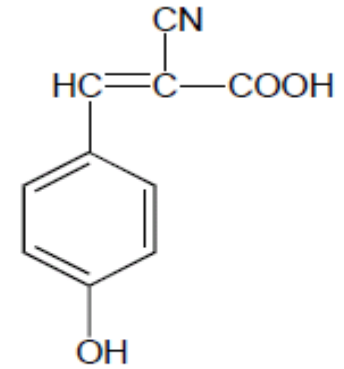
# MALDI ToF Mass Spectrometry

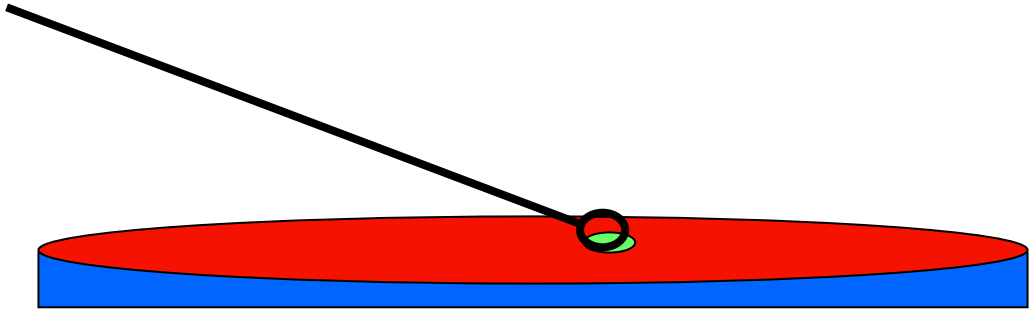


1. **Add colony**
2. **Add matrix (1-2 µl)**




α-cyano-4-hydroxycinnamic acid
(CHCA)

**Dissolved in acetonitrile (50%) & 2.5% trifluoroacetic acid**



3. **Dry – room air 5 min**

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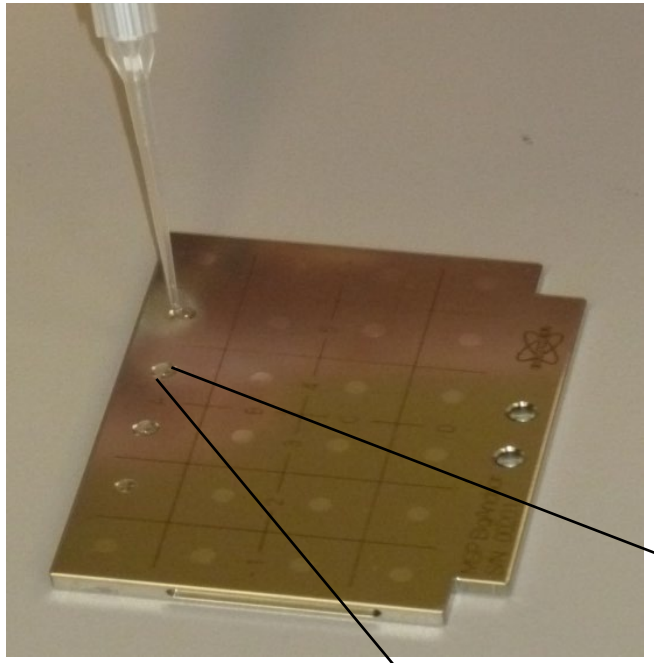
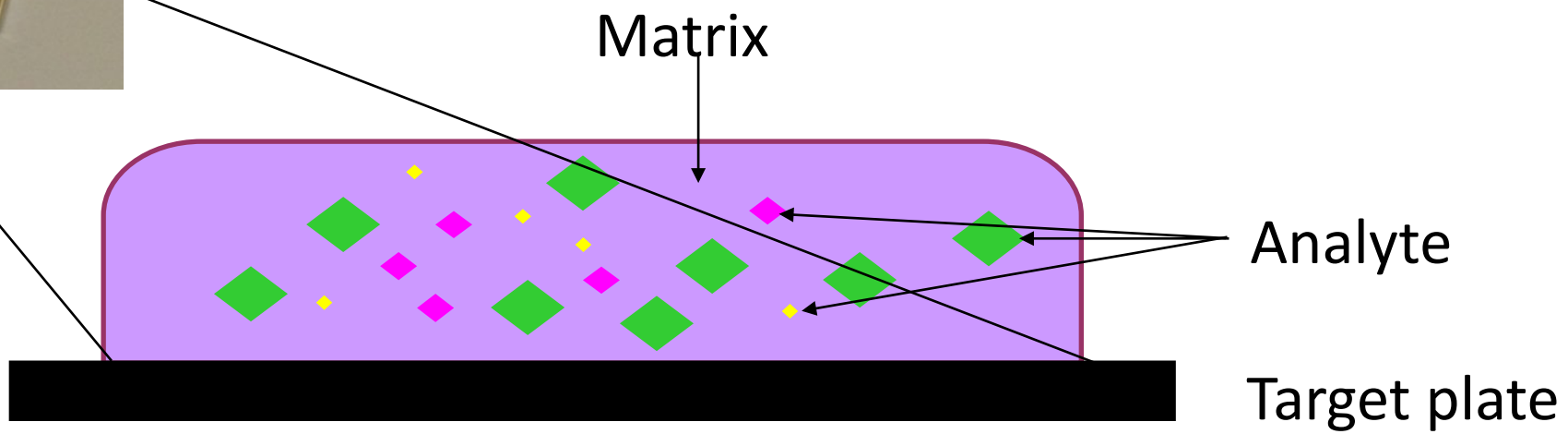
# Matrix Assisted Laser Desorption Ionization





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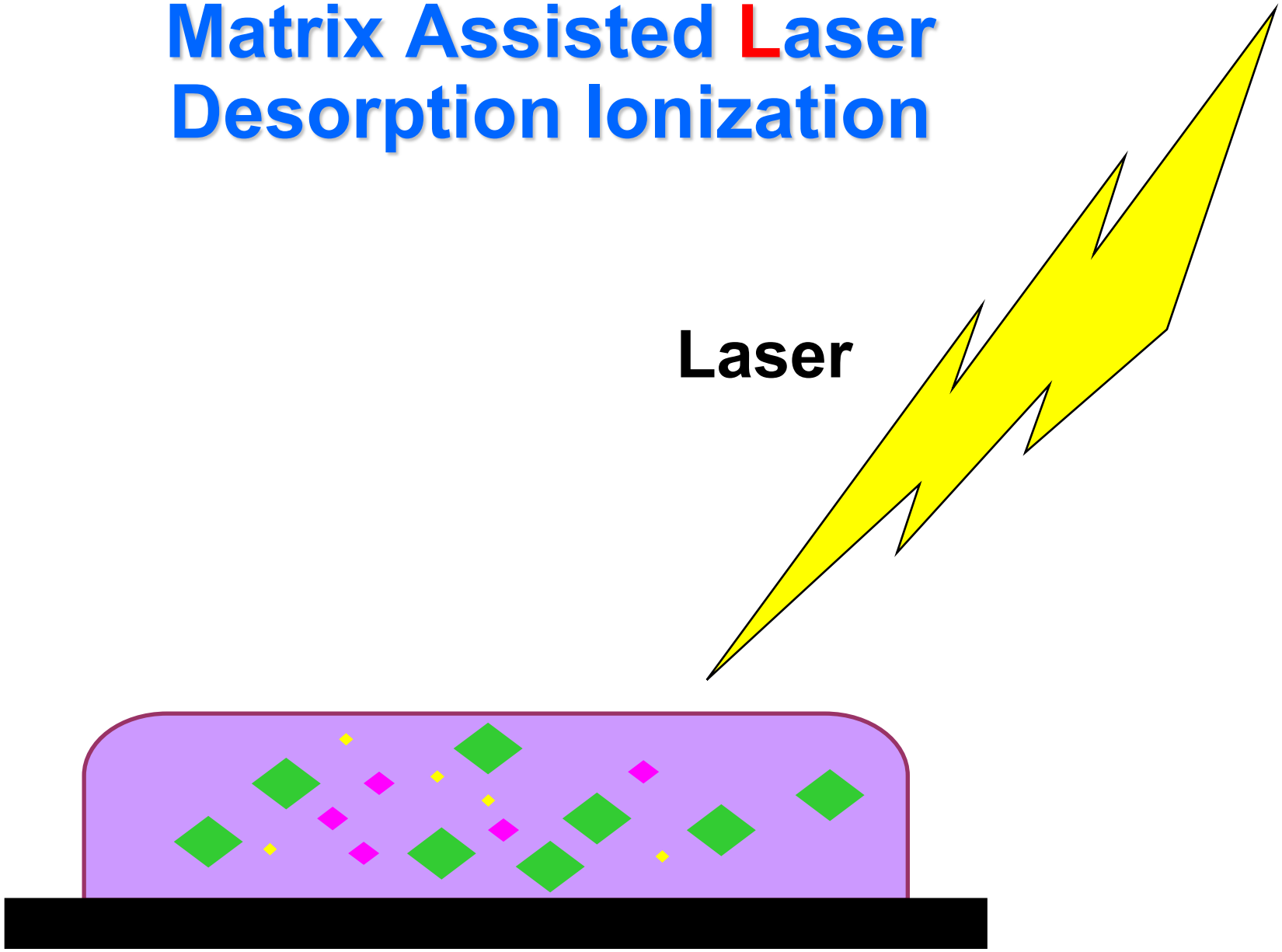
# Matrix Assisted Laser Desorption Ionization



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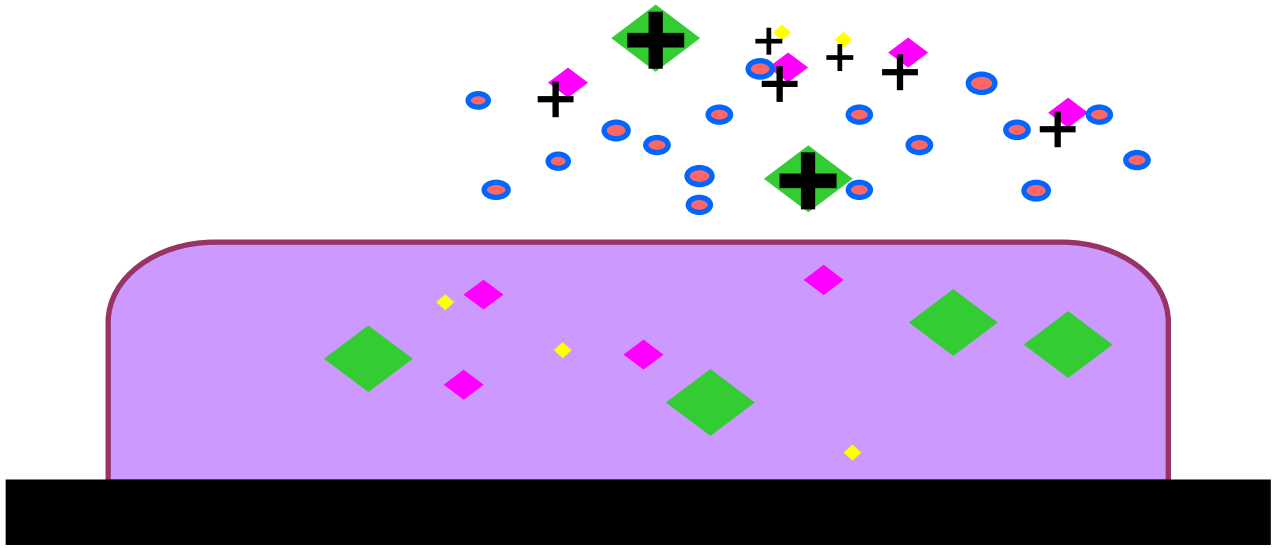
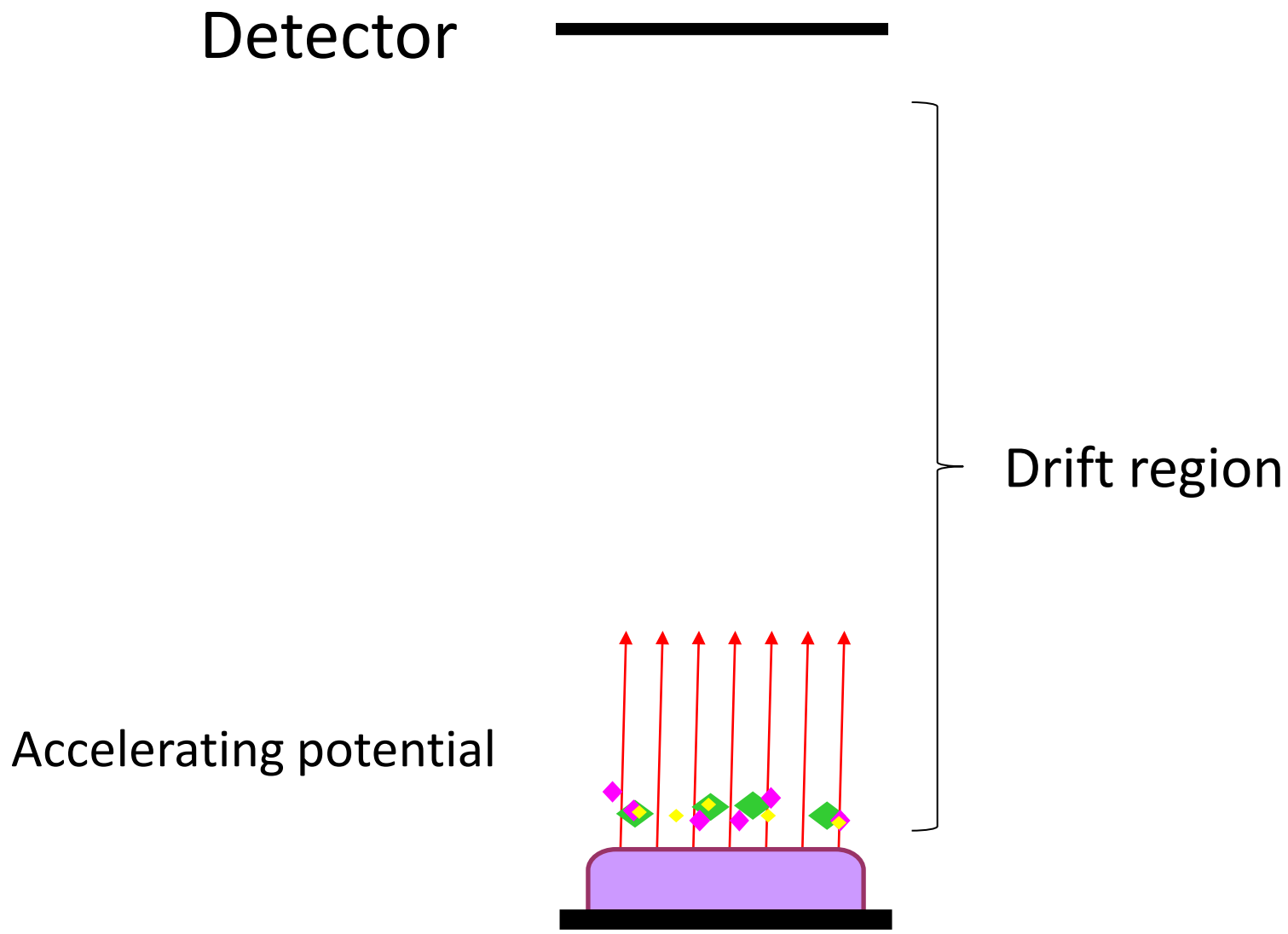
# Matrix Assisted Laser Desorption Ionization



# Time of Flight



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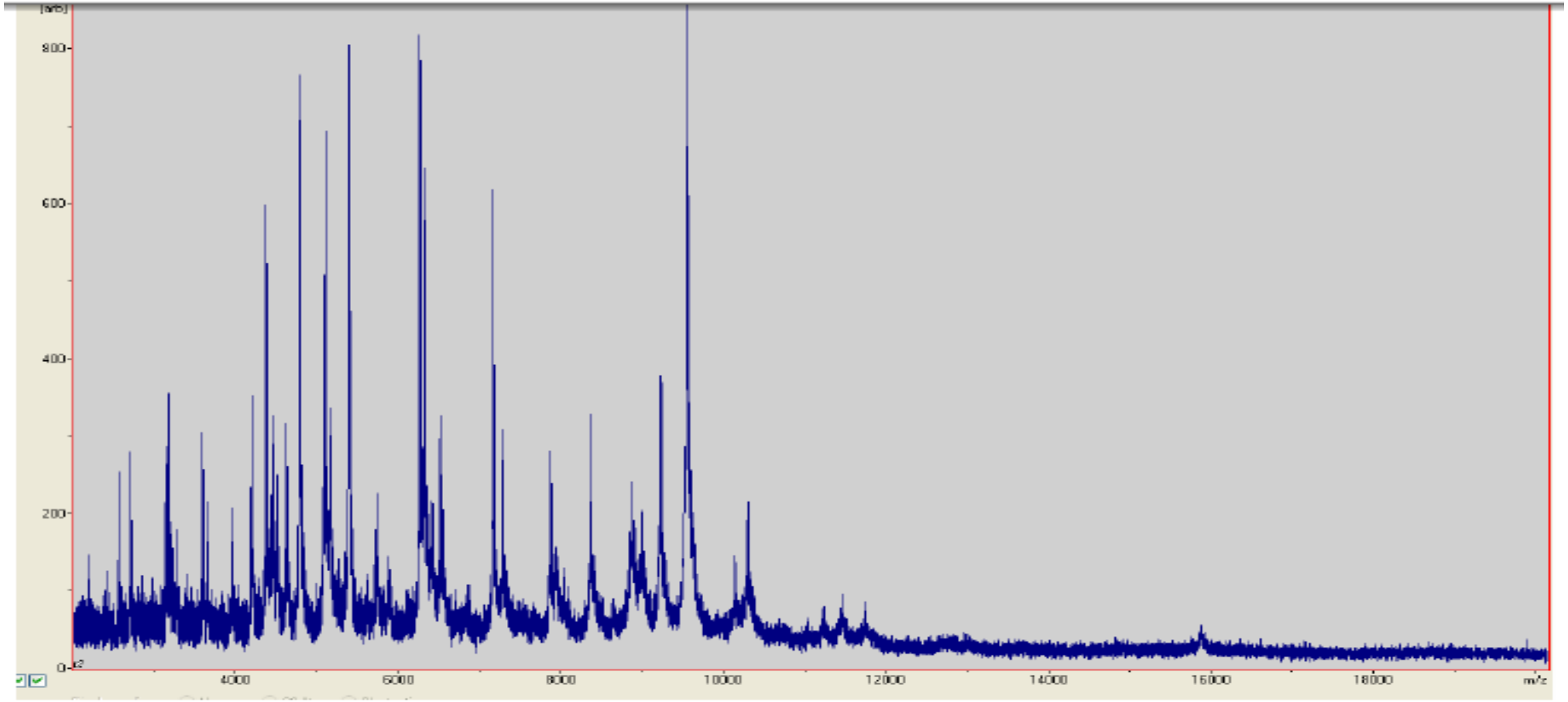
# Mass Spectrum Generated Compared with Library (Database)



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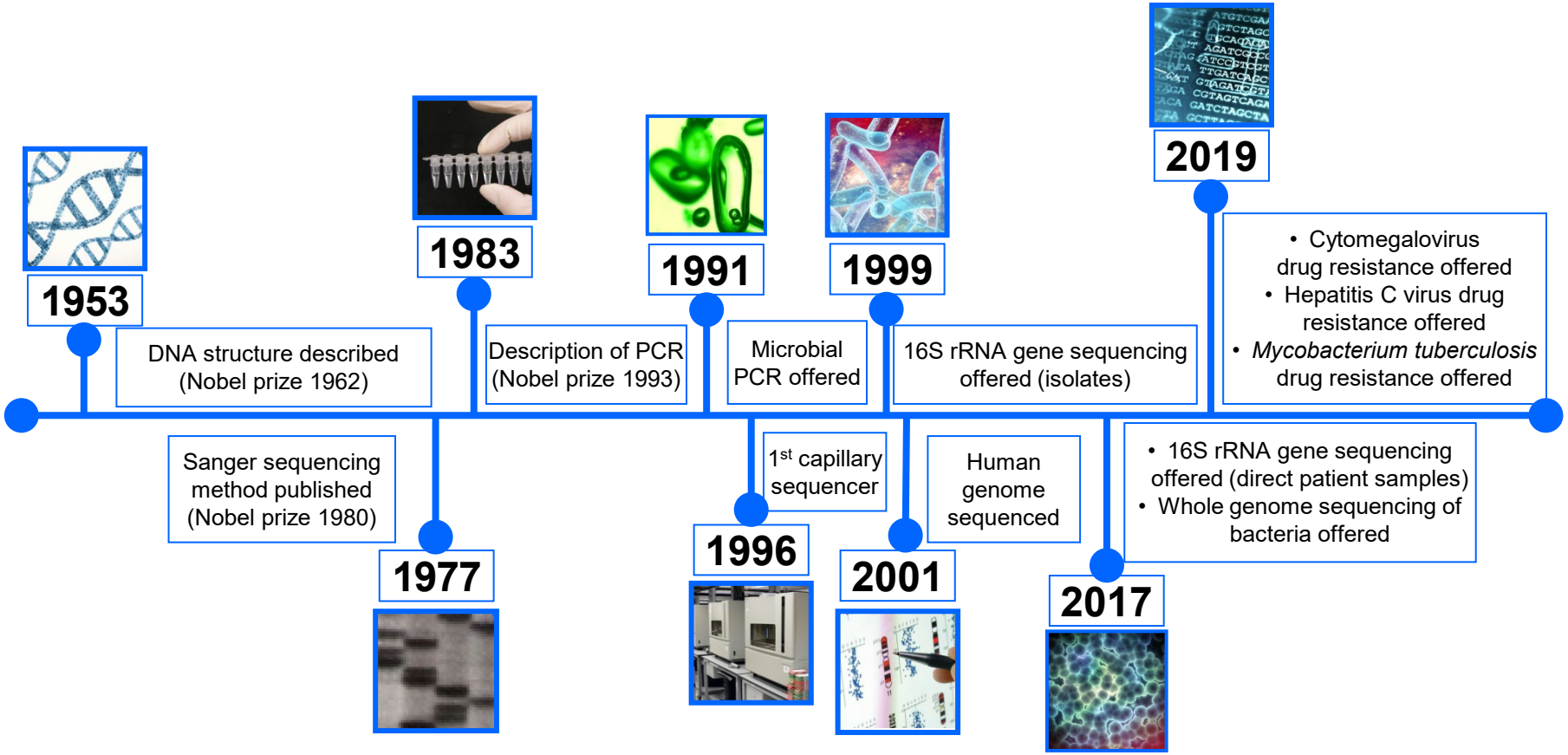


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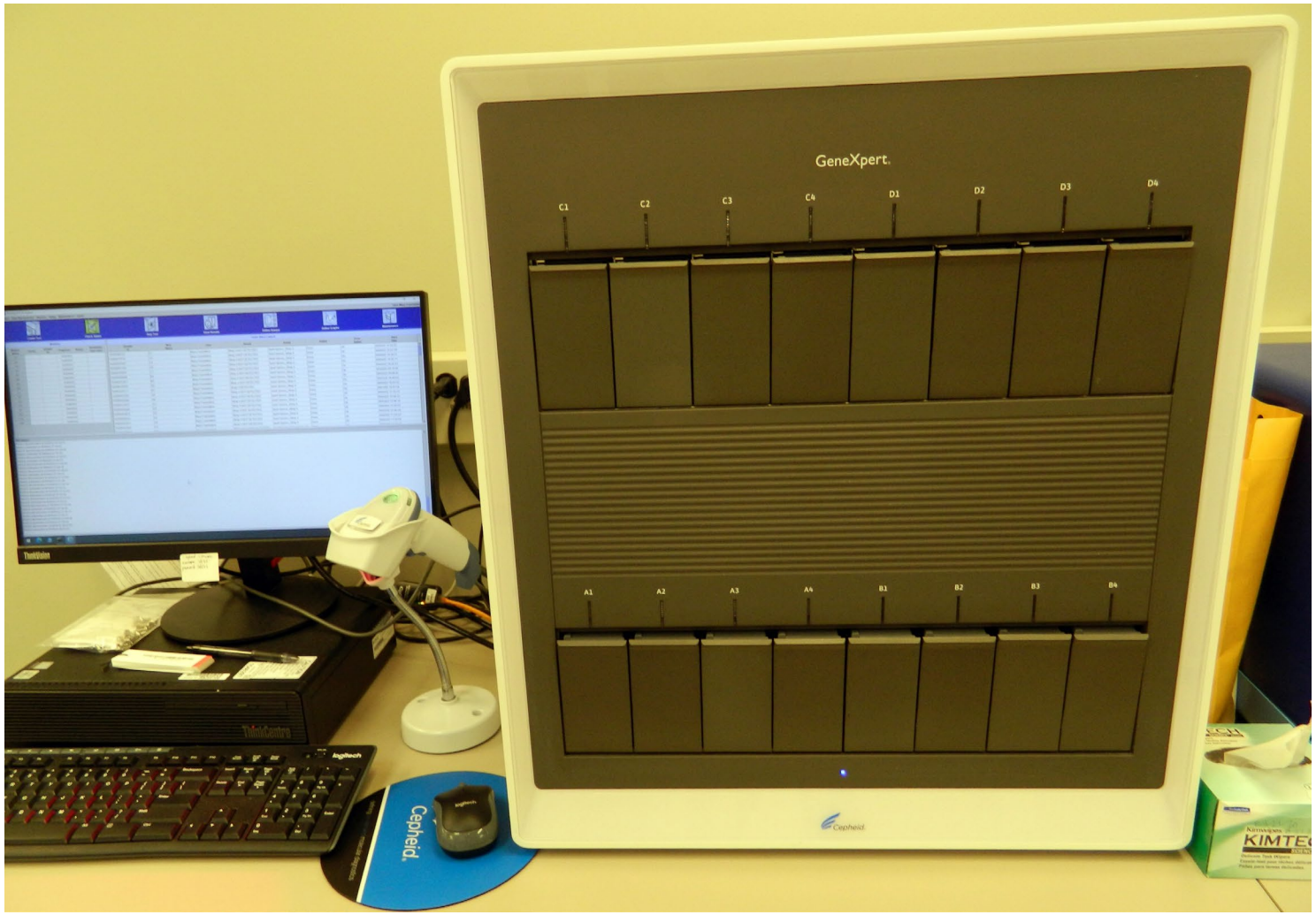
# Technology Revolution: Multiplex and Rapid Automated NAATS

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# Waived testing is an FDA designation for diagnostic assays of which of the following types?

a. Simple to perform and with an insignificant risk of erroneous result
b. Requiring performance by trained medical technologists
c. In need of oversight by a board-certified laboratory director
d. Only suitable for patient-collected specimens

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# Point of Care Diagnostics (examples)


- Xpert® Xpress Flu/RSV. GBS, Flu, Strep A
- cobas Liat Strep A, influenza A/B, influenza A/B and RSV

- ID NOW™ STREP A 2, RSV, Influenza A & B 2
- Visby Medical Sexual Health Click Test (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*)
- BioFire® FilmArray® Respiratory EZ Panel

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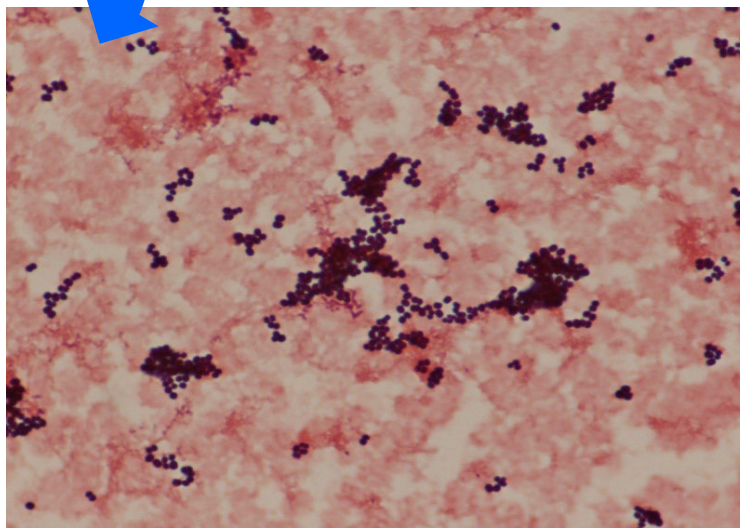
# Multiplex Panels for Direct Detection of Bacteria and Resistance Genes in Positive Blood Culture Bottles

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# FDA-Approved Multiplex Panels for Detection of Gram-Positive Bacteria in Positive Blood Cultures

| | FilmArray BCID2 | VERIGENE® | GenMark$_{Dx}$ | |
|---|---|---|---|---|
| | | Gram-Positive Blood Culture Test | ePlex BCID-GP Panel | ePlex BCID-GN Panel |
| ***Staphylococcus* species** | ✓ | ✓ | ✓ | |
| ***Staphylococcus aureus*** | ✓ | ✓ | ✓ | |
| ***Staphylococcus epidermidis*** | ✓ | ✓ | ✓ | |
| ***Staphylococcus lugdunensis*** | ✓ | ✓ | ✓ | |
| ***Streptococcus* species** | ✓ | ✓ | ✓ | |
| ***Streptococcus agalactiae*** | ✓ | ✓ | ✓ | |
| ***Streptococcus pyogenes*** | ✓ | ✓ | ✓ | |
| ***Streptococcus pneumoniae*** | ✓ | ✓ | ✓ | |
| ***Streptococcus anginosus* group** | | ✓ | ✓ | |
| ***Enterococcus* species** | | | ✓ | |
| ***Enterococcus faecalis*** | ✓ | ✓ | ✓ | |
| ***Enterococcus faecium*** | ✓ | ✓ | ✓ | |
| ***Listeria* species** | | ✓ | ✓ | |
| ***Listeria monocytogenes*** | ✓ | | ✓ | |
| ***Bacillus cereus* group** | | | ✓ | |
| ***Bacillus subtilis* group** | | | ✓ | |
| ***Corynebacterium* species** | | | ✓ | |
| ***Cutibacterium acnes*** | | | ✓ | |
| ***Lactobacillus* species** | | | ✓ | |
| ***Micrococcus* species** | | ✓ | ✓ | |
| **Pan Gram-Positive** | | | | ✓ |

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# FDA-Approved Multiplex Panels for Detection of Gram-Negative Bacteria in Positive Blood Cultures, continued

| | FilmArray BCID2 | VERIGENE® | GenMark$_{Dx}$ | |
|---|---|---|---|---|
| | | Gram-Negative Blood Culture Test | ePlex BCID-GP Panel | ePlex BCID-GN Panel |
| ***Klebsiella oxytoca*** | ✓ | ✓ | | ✓ |
| ***Klebsiella pneumoniae*** | | ✓ | | |
| ***Klebsiella pneumoniae* group** | ✓ | | | ✓ |
| ***Klebsiella aerogenes*** | ✓ | * | | * |
| ***Salmonella* species** | ✓ | | | ✓ |
| ***Morganella morganii*** | | | | ✓ |
| ***Stenotrophomonas maltophilia*** | ✓ | | | ✓ |
| ***Serratia* species** | | | | ✓ |
| ***Serratia marcescens*** | ✓ | ✓ | | ✓ |
| ***Proteus* species** | ✓ | ✓ | | ✓ |
| ***Proteus mirabilis*** | | | | ✓ |
| ***Acinetobacter* species** | | ✓ | | |
| ***Acinetobacter baumannii*** | | | | ✓ |
| ***Acinetobacter calcoaceticus-baumannii* complex** | ✓ | | | |
| ***Hemophilus influenzae*** | ✓ | | | ✓ |
| ***Cronobacter sakazakii*** | | | | ✓ |
| ***Neisseria meningitidis*** | ✓ | | | ✓ |
| ***Pseudomonas aeruginosa*** | ✓ | ✓ | | ✓ |
| **Enterobacterales** | ✓ | | | |
| ***Escherichia coli*** | ✓ | ✓ | | ✓ |
| ***Enterobacter* species** | | ✓ | | ✓ |
| ***Enterobacter cloacae* complex** | ✓ | | | ✓ |
| ***Citrobacter* species** | | ✓ | | ✓ |
| ***Bacteroides fragilis*** | ✓ | | | ✓ |
| ***Fusobacterium necrophorum*** | | | | ✓ |
| ***Fusobacterium nucleatum*** | | | | ✓ |
| **Pan Gram-Negative** | | | ✓ | |

*Detected as *Enterobacter* species

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# FDA-Approved Multiplex Panels for Detection of Select Resistance Genes in Positive Blood Cultures, continued

| | FilmArray BCID2 | VERIGENE® | | GenMark$_{Dx}$ | |
|---|---|---|---|---|---|
| | | Gram-Positive Blood Culture Test | Gram-Negative Blood Culture Test | ePlex BCID-GP Panel | ePlex BCID-GN Panel |
| ***mecA*** | | ✓ | | ✓ | |
| ***mecC*** | | | | ✓ | |
| ***mecA/C*** | ✓ | | | | |
| ***mecA/C and MREJ*** | ✓ | | | | |
| ***vanA*** | | ✓ | | ✓ | |
| ***vanB*** | | ✓ | | ✓ | |
| ***vanA/B*** | ✓ | | | | |
| ***bla$_{KPC}$*** | ✓ | | ✓ | | ✓ |
| ***bla$_{NDM}$*** | ✓ | | ✓ | | ✓ |
| ***bla$_{OXA}$*** | ✓ | | ✓ | | ✓ |
| ***bla$_{VIM}$*** | ✓ | | ✓ | | ✓ |
| ***bla$_{IMP}$*** | ✓ | | ✓ | | ✓ |
| ***bla$_{CTX-M}$*** | ✓ | | ✓ | | ✓ |
| ***mcr-1*** | ✓ | | | | |

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# FDA-Approved Multiplex Panels for Detection of Fungi in Positive Blood Cultures, continued

| | FilmArray BCID2 | GenMark$_{Dx}$ | | |
|---|---|---|---|---|
| | | ePlex BCID-GP Panel | ePlex BCID-FP Panel | ePlex BCID-GN Panel |
| ***Candida albicans*** | ✓ | | ✓ | |
| ***Candida auris*** | ✓ | | ✓ | |
| ***Candida dubliniensis*** | | | ✓ | |
| ***Candida famata*** | | | ✓ | |
| ***Nakaseomyces glabrata*** | ✓ | | ✓ | |
| ***Candida guilliermondii*** | | | ✓ | |
| ***Candida kefyr*** | | | ✓ | |
| ***Pichia kudriavzevii*** | ✓ | | ✓ | |
| ***Candida lusitaniae*** | | | ✓ | |
| ***Candida parapsilosis*** | ✓ | | ✓ | |
| ***Candida tropicalis*** | ✓ | | ✓ | |
| ***Cryptococcus gattii*** | | | ✓ | |
| ***Cryptococcus neoformans*** | | | ✓ | |
| ***C. neoformans/gattii*** | ✓ | | | |
| ***Fusarium* species** | | | ✓ | |
| ***Rhodotorula* species** | | | ✓ | |
| **Pan *Candida*** | | ✓ | | ✓ |

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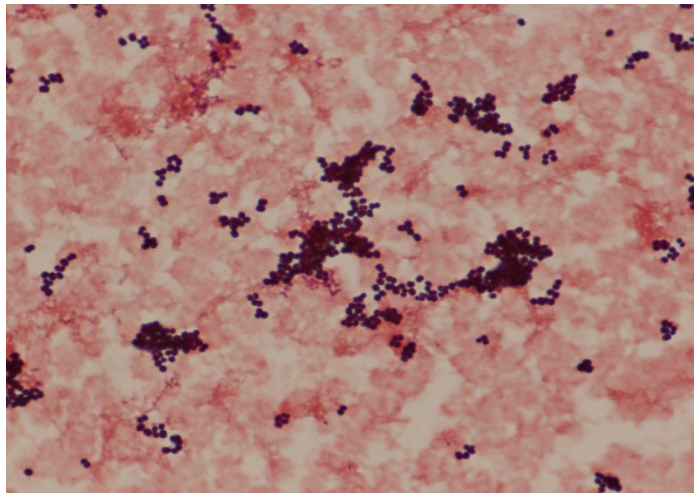
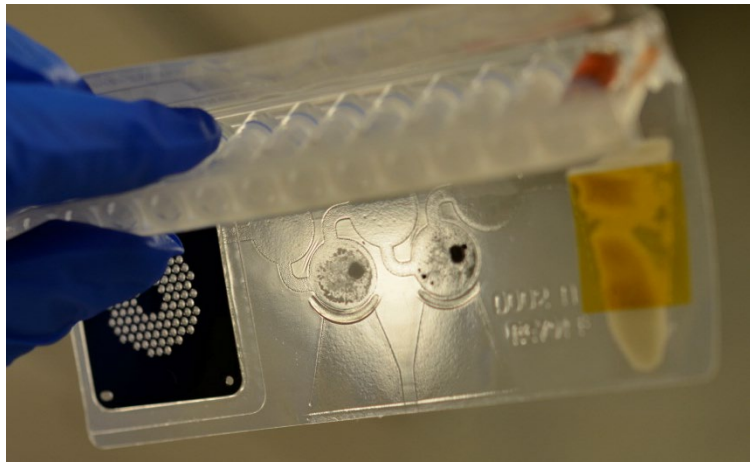
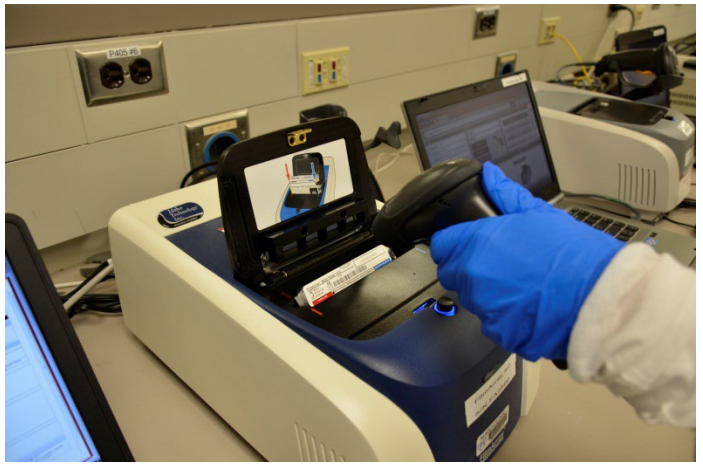
# Multiplex PCR Panel Example: FilmArray® BCID (BioFire)





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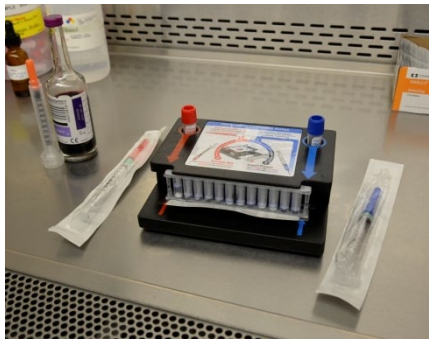
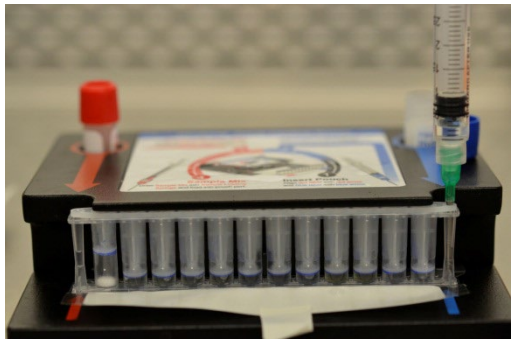
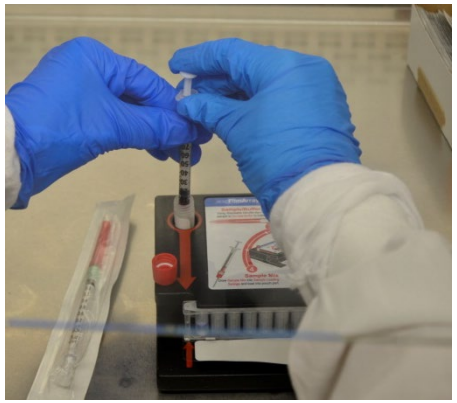
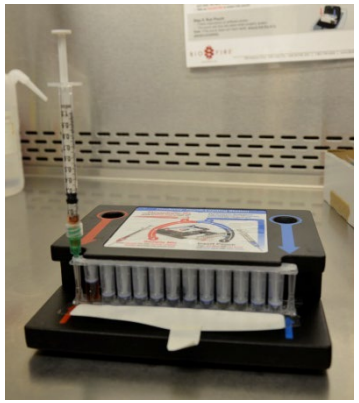
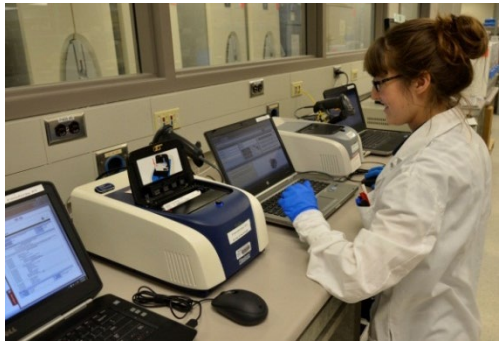
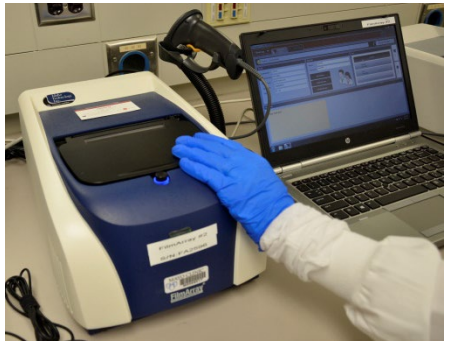
# FilmArray® BCID







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# FilmArray® BCID

| Gram Positive Bacteria | Gram Negative Bacteria | Fungi | Resistance Genes |
|---|---|---|---|
| *Staphylococcus* | *Klebsiella oxytoca* | *Candida albicans* | *bla*$_{KPC}$ |
| *Staphylococcus aureus* | *Klebsiella pneumoniae* | *Nakaseomyces glabrata* | *mecA* |
| *Streptococcus* | *Serratia* | *Pichia kudriavzevii* | *vanA/vanB* |
| *Streptococcus agalactiae* | *Proteus* | *Candida parapsilosis* | |
| *Streptococcus pyogenes* | *Acinetobacter baumannii* | *Candida tropicalis* | |
| *Streptococcus pneumoniae* | *Haemophilus influenzae* | | |
| *Enterococcus* | *Neisseria meningitidis* | | |
| *Listeria monocytogenes* | *Pseudomonas aeruginosa* | | |
| | *Enterobacteriaceae* | | |
| | *Escherichia coli* | | |
| | *Enterobacter cloacae* complex | | |

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# Randomized Controlled Clinical Trial 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<br>• Standard subculture and susceptibility (1-3 d) | • Gram stain called to service<br>• Standard subculture and susceptibility (1-3 d)<br>• Rapid test plus lab call with comments (1 h) | • Gram stain called to service<br>• Standard subculture and susceptibility (1-3 d)<br>• Rapid test plus lab call with comments (1 h)<br>• 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

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# Clinical Outcomes

| Outcome | Control (n= 207) | Rapid Test (n=198) | Rapid Test + Stewardship (n=212) | P –value |
|---|---|---|---|---|
| Length of stay (days) | 8 (5,15) | 8 (5,15) | 8 (5,16) | 0.60 |
| 30-day mortality | 22 (10.6%) | 20 (10.1%) | 18 (8.5%) | 0.74 |
| 30-day readmission w/same organism | 6 (2.9%) | 6 (3%) | 8 (3.8%) | 0.88 |
| Toxicity/adverse drug reaction | 3 (1.4%) | 3 (1.5%) | 2 (0.9%) | 0.82 |
| Blood culture clearance in 3 days | 147 (71%) | 131 (66.2%) | 146 (68.9%) | 0.79 |
| *C. difficile*/Drug-resistant organism[1] within 30d | 15 (7.2%) | 16 (8.1%) | 21 (9.9%) | 0.62 |

[1]VRE, MRSA, ESBLs, Gram negative bacilli resistant to ≥3 drug classes

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

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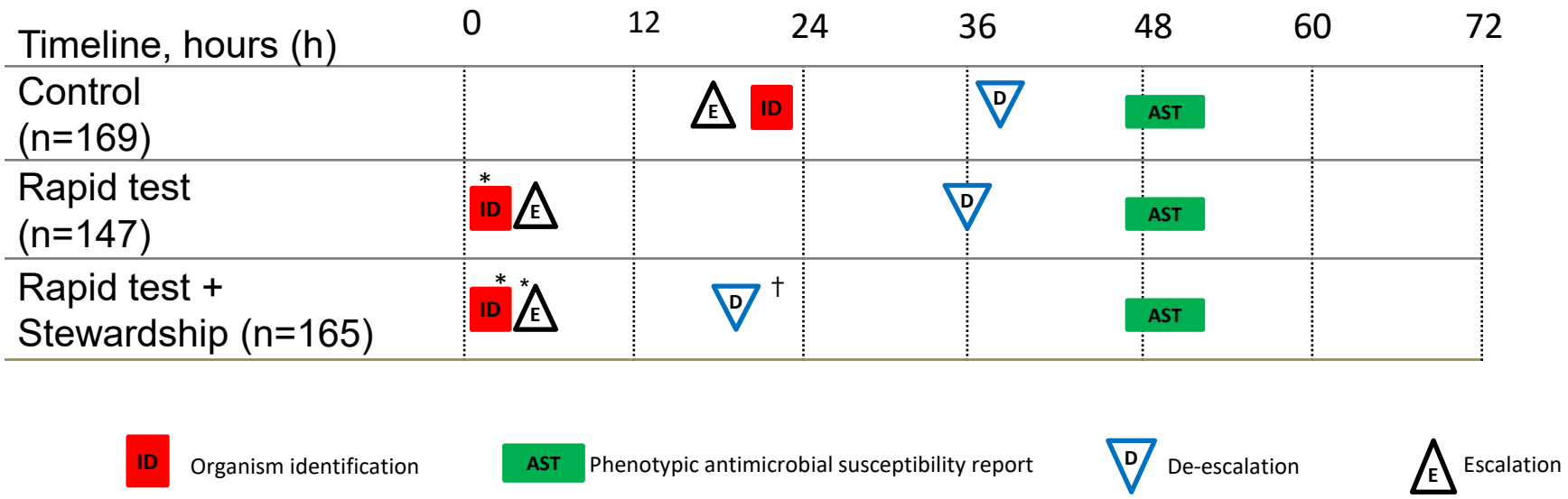
# Comparison of Time To Identification, Susceptibility Results, and Antibiotic Modifications




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

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

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# Findings

- Rapid test → more judicious antibiotic use, without worsening clinical outcomes
  - ↑ Narrow-spectrum antibiotics for Gram positives
  - ↓ Piperacillin-tazobactam use
  - ↓ Treatment of contaminants
  - ↑'d & faster de-escalation/escalation
    - Rapid test + stewardship

    → fastest de-escalation

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

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| FilmArray BCID2 Result (What the Laboratory "Sees") | What the Laboratory Reports | |
|---|---|---|
| | | Isolate Comments |
| *Staphylococcus* +<br>*S. aureus* +<br>*mecA/C* and MREJ (MRSA) + | *Staphylococcus aureus* complex | *mecA* detected<br>Methicillin (oxacillin)-resistant *Staphylococcus aureus* complex (MRSA).<br>MRSA is predictably resistant to beta-lactam antibiotics (except ceftaroline).<br>Vancomycin or other anti-MRSA treatment recommended for initial therapy pending susceptibility results.<br>Semi-Urgent Result<br>Consult Infectious Diseases |
| *Staphylococcus* +<br>*S. aureus* +<br>*mecA/C* and MREJ (MRSA) - | *Staphylococcus aureus* complex | *mecA* not detected<br>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.<br>Consult Infectious Diseases |
| *Staphylococcus* +<br>*S. epidermidis* +<br>*mecA/C* + | *Staphylococcus epidermidis* | *mecA* detected<br>Methicillin (oxacillin)-resistant *Staphylococcus epidermidis.*<br>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. |

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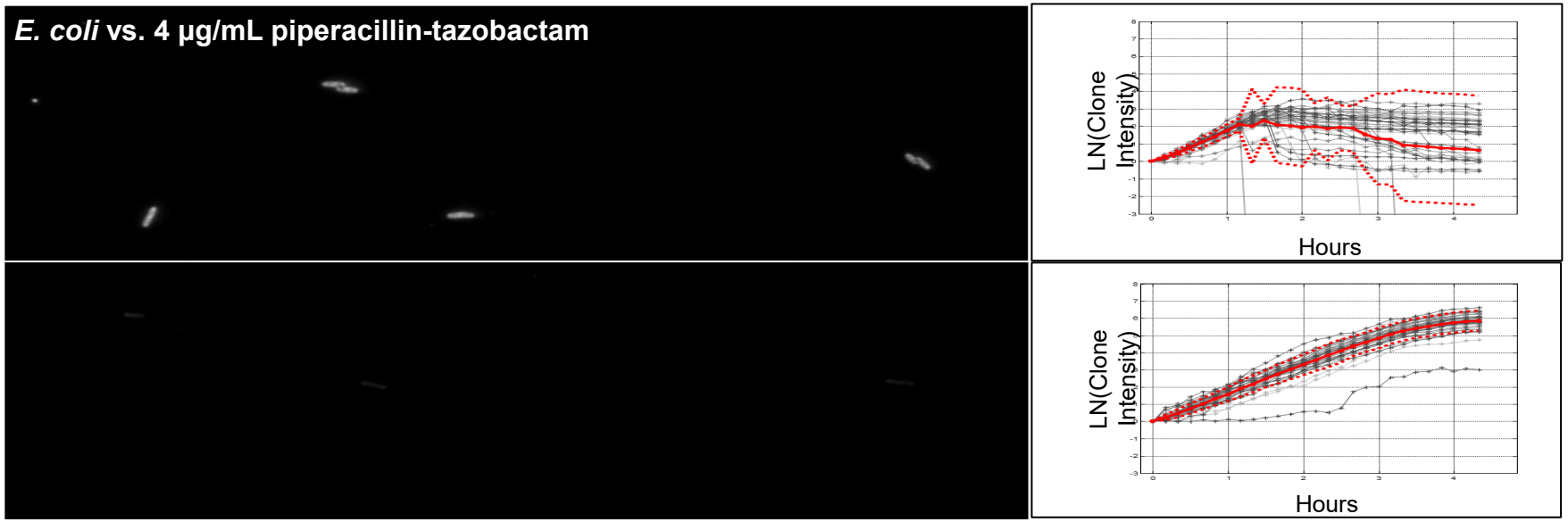
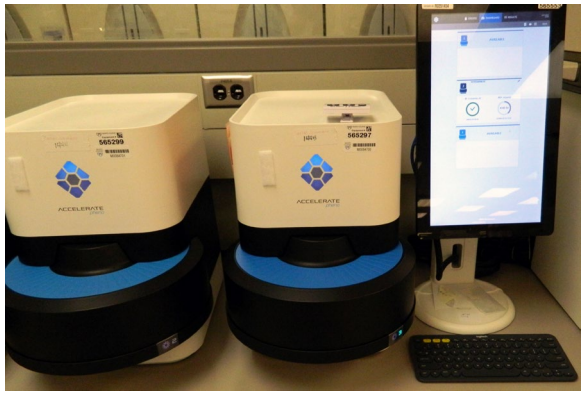
# Technology Revolution: Single Cell Imaging Application: Antibacterial Susceptibility Testing

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



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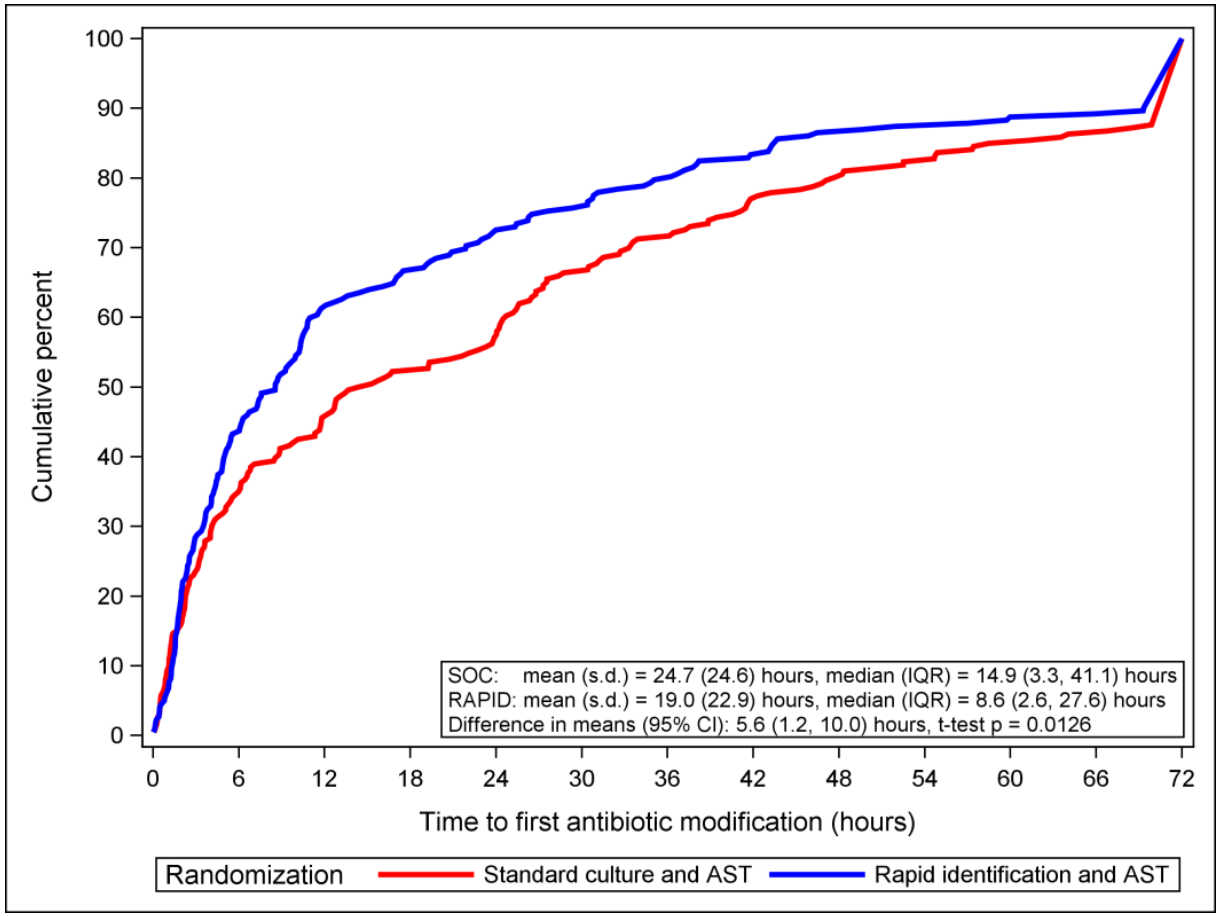
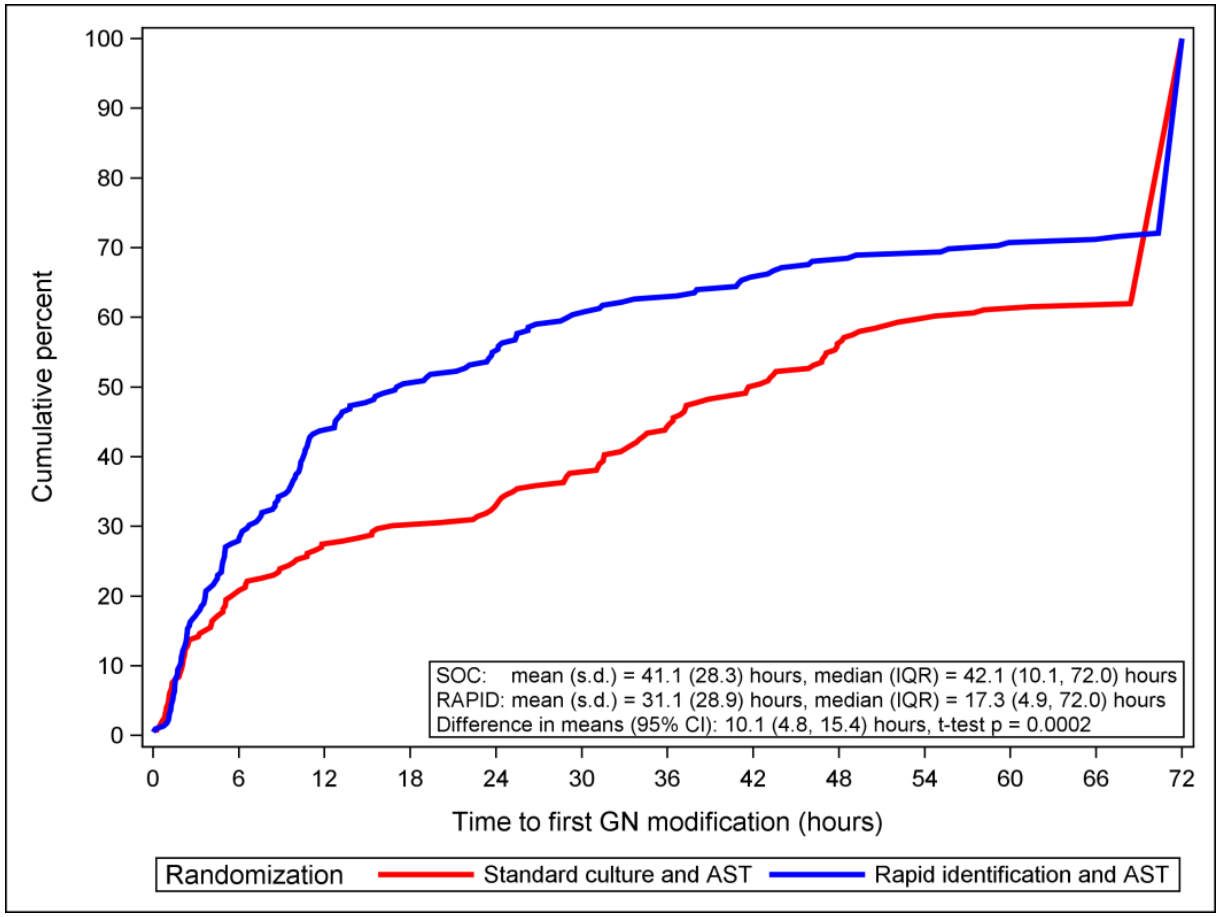
# Randomized Trial Evaluating Clinical Impact of RAPid IDentification and Susceptibility Testing for Gram-negative Bacteremia: RAPIDS-GN

## 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

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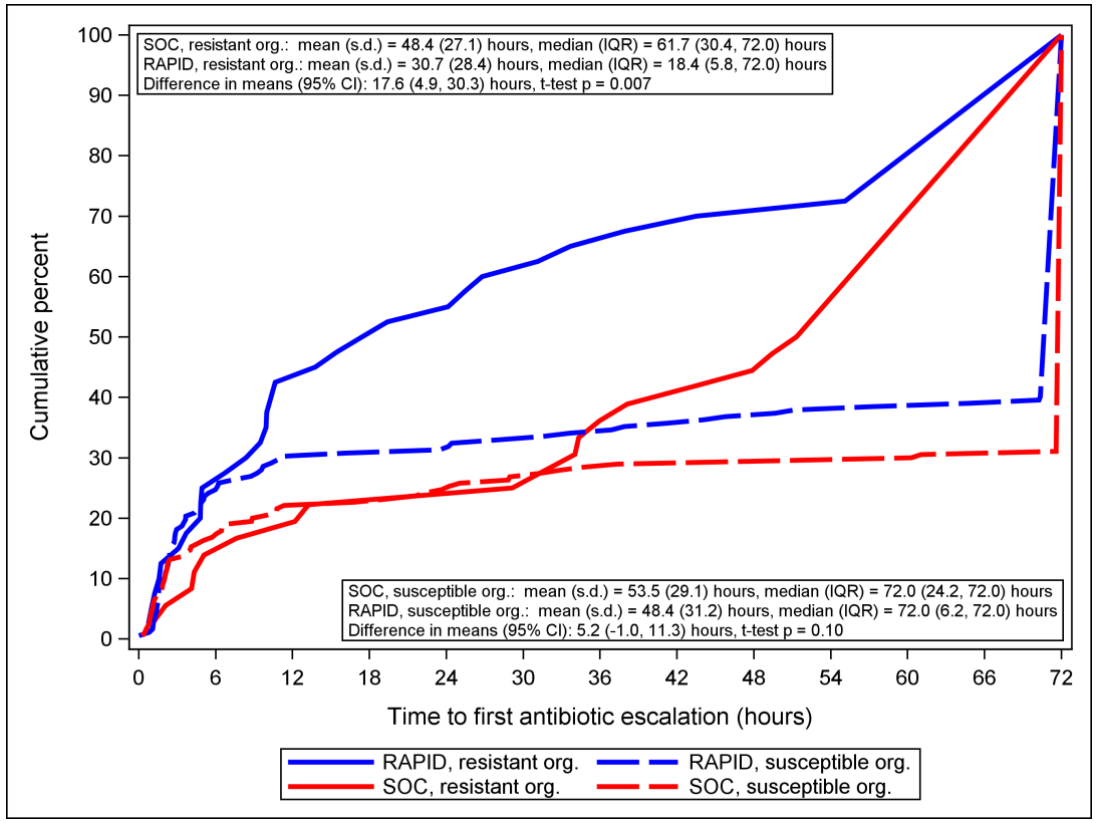
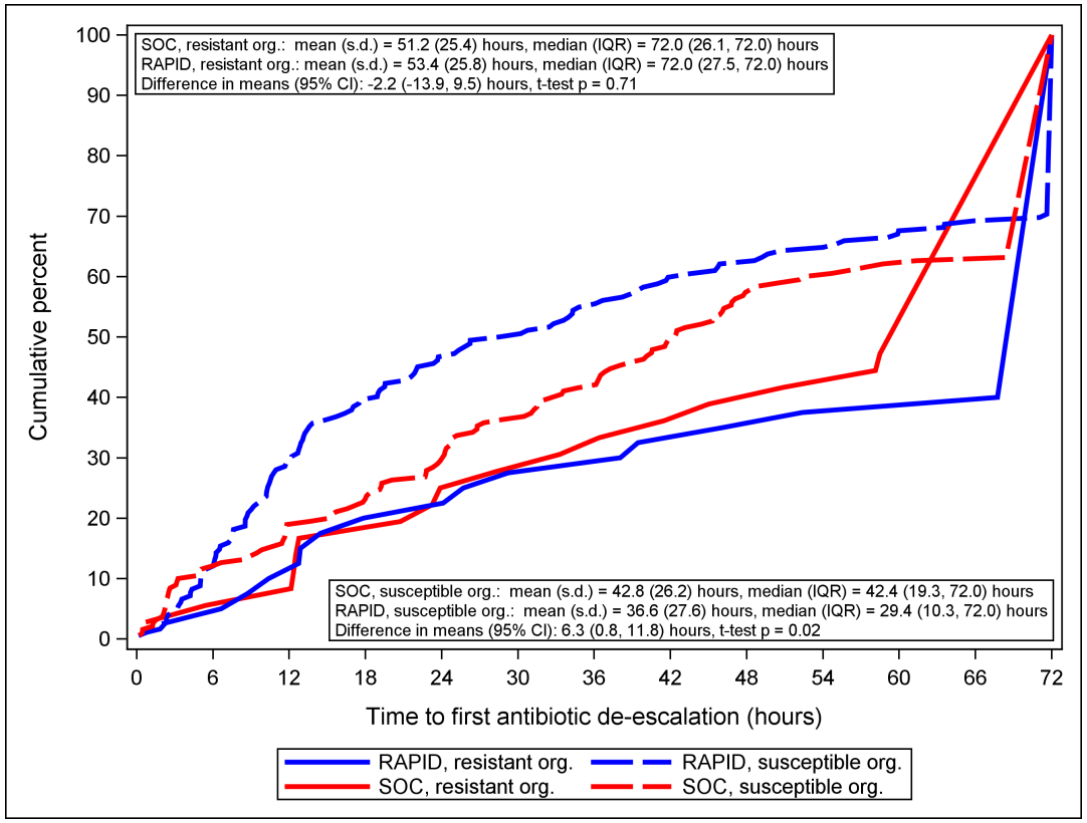
# Randomized Trial Evaluating Clinical Impact of RAPid IDentification and Susceptibility Testing for Gram-negative Bacteremia: RAPIDS-GN

## Time to antibiotic escalation




## Time to antibiotic de-escalation

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

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# PCR-Based Detection PLUS Resistance Profiling

- **Xpert (Cepheid)**
  - **MRSA/SA Nasal Complete Assay, SSTI Assay**
  - **MTB/RIF Assay**
- SpeeDx
  - *ResistancePlus*® GC - *Neisseria gonorrhoeae* and ciprofloxacin susceptibility markers
  - *ResistancePlus*® MG *Mycoplasma genitalium* and macrolide resistance markers

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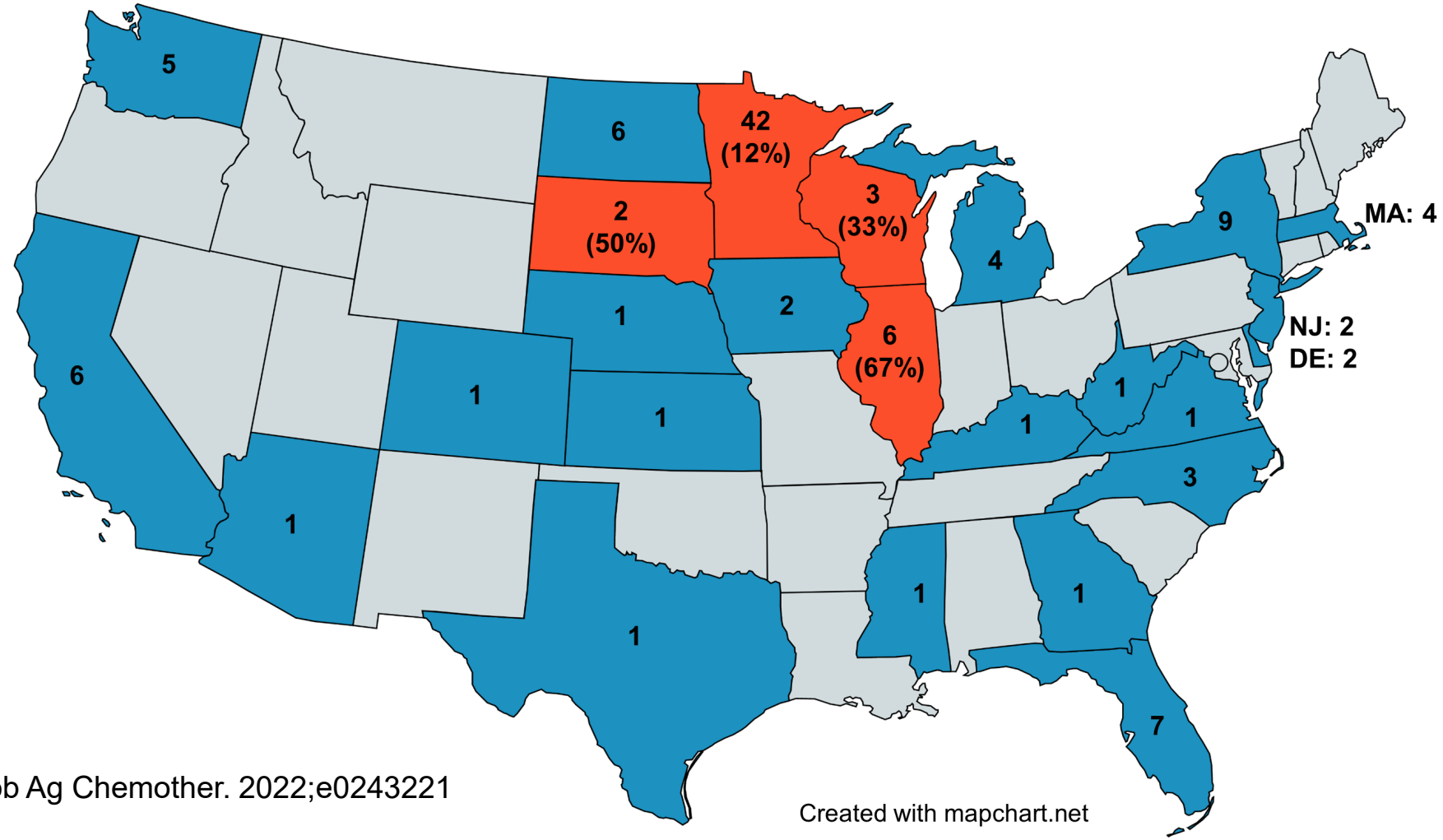
# *Mycoplasma pneumoniae* PCR Assay Targeting Common Macrolide Resistance Mutations (23S rRNA gene A2063G/A2064G)




Rotstein et al. Antimicrob Ag Chemother. 2022;e0243221

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# FDA-Approved/Cleared Multiplex Panels for Gastrointestinal Pathogens in Stool

| | Verigene EP | Luminex GPP | BioFire GIP |
|---|---|---|---|
| Number of targets | 8 | 14 | 22 |
| *Campylobacter* species | ✓ | ✓ | ✓ |
| *Salmonella* species | ✓ | ✓ | ✓ |
| *Shigella* species/Enteroinvasive *E. coli* | ✓ | ✓ | ✓ |
| *Vibrio* species | ✓ | ✓ | ✓ |
| *Yersinia enterocolitica* | ✓ | ✓ | ✓ |
| *Escherichia coli* 0157 | | ✓ | ✓ |
| Enterotoxigenic *E. coli* | | ✓ | ✓ |
| Enteropathogenic *E. coli* | | | ✓ |
| Enteroaggregative *E. coli* | | | ✓ |
| *Plesiomonas shigelloides* | | | ✓ |
| Shiga toxin-producing *E. coli* | ✓ | ✓ | ✓ |
| *Clostridioides difficile* | | ✓ | ✓ |
| Norovirus | ✓ | ✓ | ✓ |
| Rotavirus A | ✓ | ✓ | ✓ |
| Astrovirus | | | ✓ |
| Adenovirus 40/41 | | ✓ | ✓ |
| Sapovirus | | | ✓ |
| *Cryptosporidium* species | | ✓ | ✓ |
| *Entamoeba histolytica* | | ✓ | ✓ |
| *Giardia lamblia* | | ✓ | ✓ |
| *Cyclospora cayetanensis* | | | ✓ |

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# BIOFIRE FilmArray Meningitis/Encephalitis Panel

| Viruses | Bacteria | Fungi |
|---|---|---|
| Cytomegalovirus | *Escherichia coli* K1 | *Cryptococcus neoformans/gattii* |
| Enterovirus | *Haemophilus influenzae* | |
| Herpes simplex virus 1 | *Listeria monocytogenes* | |
| Herpes simplex virus 2 | *Neisseria meningitidis* | |
| Human herpes virus 6 | *Streptococcus agalactiae* | |
| Human parechovirus | *Streptococcus pneumoniae* | |
| Varicella zoster virus | | |

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# Lower Respiratory Tract Panels

| | Curetis Unyvero | BioFire |
|---|---|---|
| **Bacteria** | | |
| *Acinetobacter* spp. | ✓ | |
| *Acinetobacter calcoaceticus-baumannii* complex | | ✓ |
| *Chlamydia pneumoniae* | ✓ | ✓ |
| *Citrobacter freundii* | ✓ | |
| *Klebsiella aerogenes* | | ✓ |
| *Enterobacter cloacae* complex | ✓ | ✓ |
| *Escherichia coli* | ✓ | ✓ |
| *Haemophilus influenzae* | ✓ | ✓ |
| *Klebsiella oxytoca* | ✓ | ✓ |
| *Klebsiella pneumoniae* | ✓ | |
| *Klebsiella pneumoniae* group | | ✓ |
| *Klebsiella variicola* | ✓ | |
| *Legionella pneumophila* | ✓ | ✓ |
| *Moraxella catarrhalis* | ✓ | ✓ |
| *Morganella morganii* | ✓ | |
| *Mycoplasma pneumoniae* | ✓ | ✓ |
| *Proteus* spp. | ✓ | ✓ |
| *Pseudomonas aeruginosa* | ✓ | ✓ |
| *Serratia marcescens* | ✓ | ✓ |
| *Staphylococcus aureus* | ✓ | ✓ |
| *Stenotrophomonas maltophilia* | ✓ | |
| *Streptococcus agalactiae* | | ✓ |
| *Streptococcus pneumoniae* | ✓ | ✓ |
| *Streptococcus pyogenes* | | ✓ |

| | Curetis Unyvero | BioFire |
|---|---|---|
| **Viruses** | | |
| Influenza A | | ✓ |
| Influenza B | | ✓ |
| Respiratory Syncytial Virus | | ✓ |
| Human Rhinovirus/Enterovirus | | ✓ |
| Human Metapneumovirus | | ✓ |
| Parainfluenza virus | | ✓ |
| Adenovirus | | ✓ |
| Coronavirus (non-SARS-CoV) | | ✓ |
| SARS-CoV-2 | | ✓ |
| **Fungi** | | |
| *Pneumocystis jirovecii* | ✓ | |
| **Resistance genes** | | |
| $bla_{KPC}$ | ✓ | ✓ |
| $bla_{NDM}$ | ✓ | ✓ |
| $bla_{IMP}$ | | ✓ |
| $bla_{OXA-23}$ | ✓ | |
| $bla_{OXA-24}$ | ✓ | |
| $bla_{OXA-48}$ | ✓ | |
| $bla_{OXA-58}$ | | |
| $bla_{OXA-48-like}$ | | ✓ |
| $bla_{VIM}$ | ✓ | ✓ |
| $bla_{CTX-M}$ | ✓ | ✓ |
| $bla_{TEM}$ | ✓ | |
| *mecA* | ✓ | |
| *mecA/C* and *MREJ* | | ✓ |

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# T2Direct Diagnostics
# Direct from Blood

- **Multiplex PCR and T2 magnetic resonance, average turnaround time 4.3 hours**
- **T2Candida Panel**
  - ***Candida albicans***
  - ***Candida tropicalis***
  - ***Pichia kudriavzevii***
  - ***Nakaseomyces glabrata***
  - ***Candida parapsilosis***
- **T2Bacteria Panel**
  - ***Enterococcus faecium***
  - ***Staphylococcus aureus***
  - ***Klebsiella pneumoniae***
  - ***Pseudomonas aeruginosa***
  - ***Escherichia coli***

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# The 16S ribosomal RNA gene is found in:

a. Bacteria
b. Fungi
c. DNA viruses
d. RNA viruses

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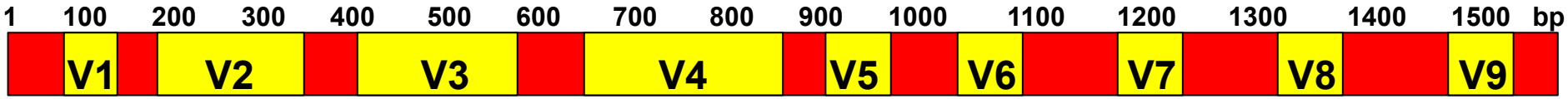
# 16S Ribosomal RNA Gene



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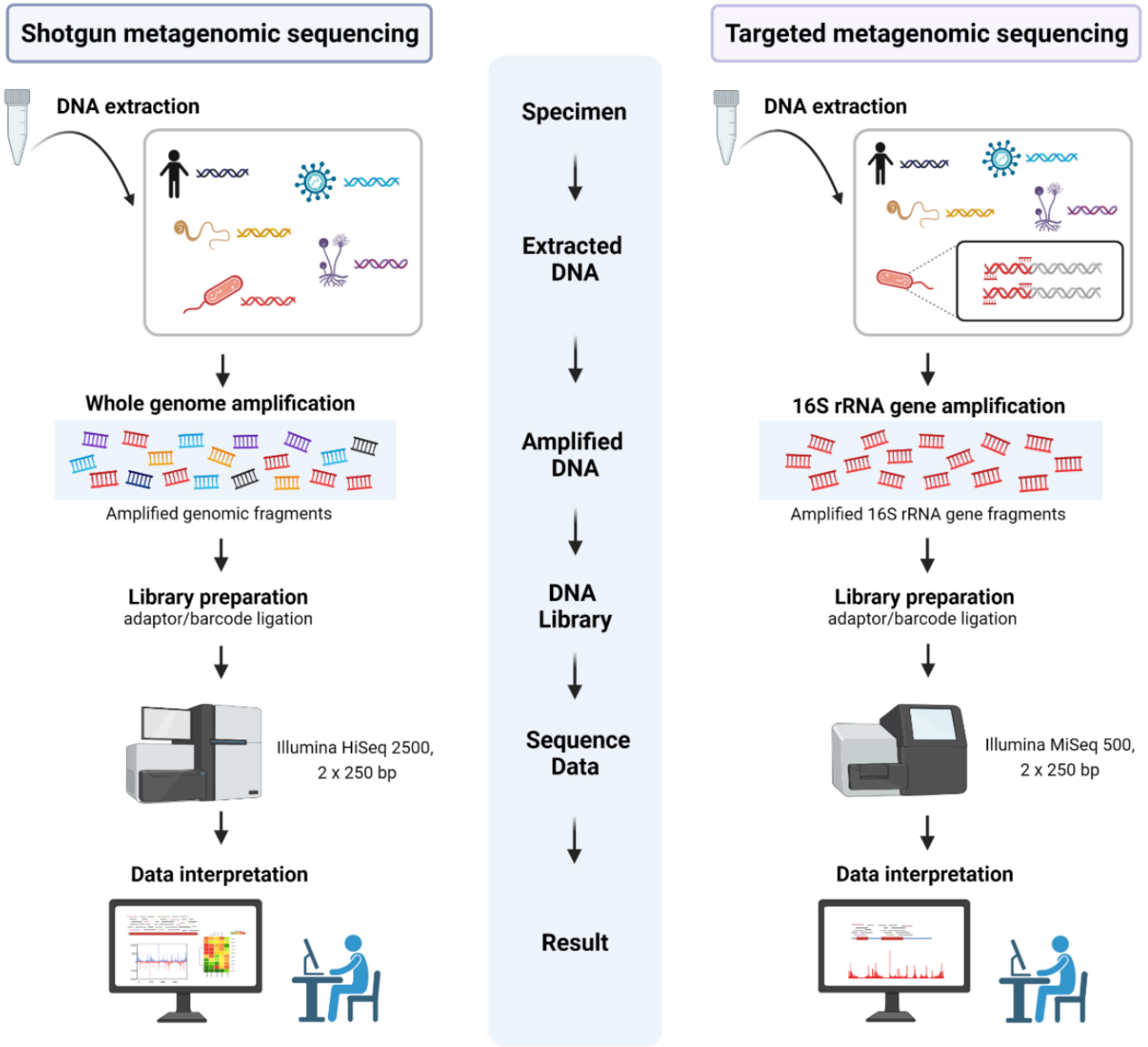


Hong et al. Clin Infect Dis. In Press

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# 16S rRNA Gene PCR/Sanger Sequencing in Clinical Practice

- Retrospective analysis specimens clinically analyzed by 16S rRNA PCR/Sanger sequencing, Mayo Clinic (MN) 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
  - 5% patients → impacted clinical care
  - 22% positive specimens (21/97), 16S rRNA gene detected but bacterium not identified by Sanger sequencing (mixtures)
  - +ve Gram stain: 12X ↑'d odds positivity (CI OR, 5.2–31.4)
  - Cardiovascular ↑'d positivity (37%) *vs* other specimen-types

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

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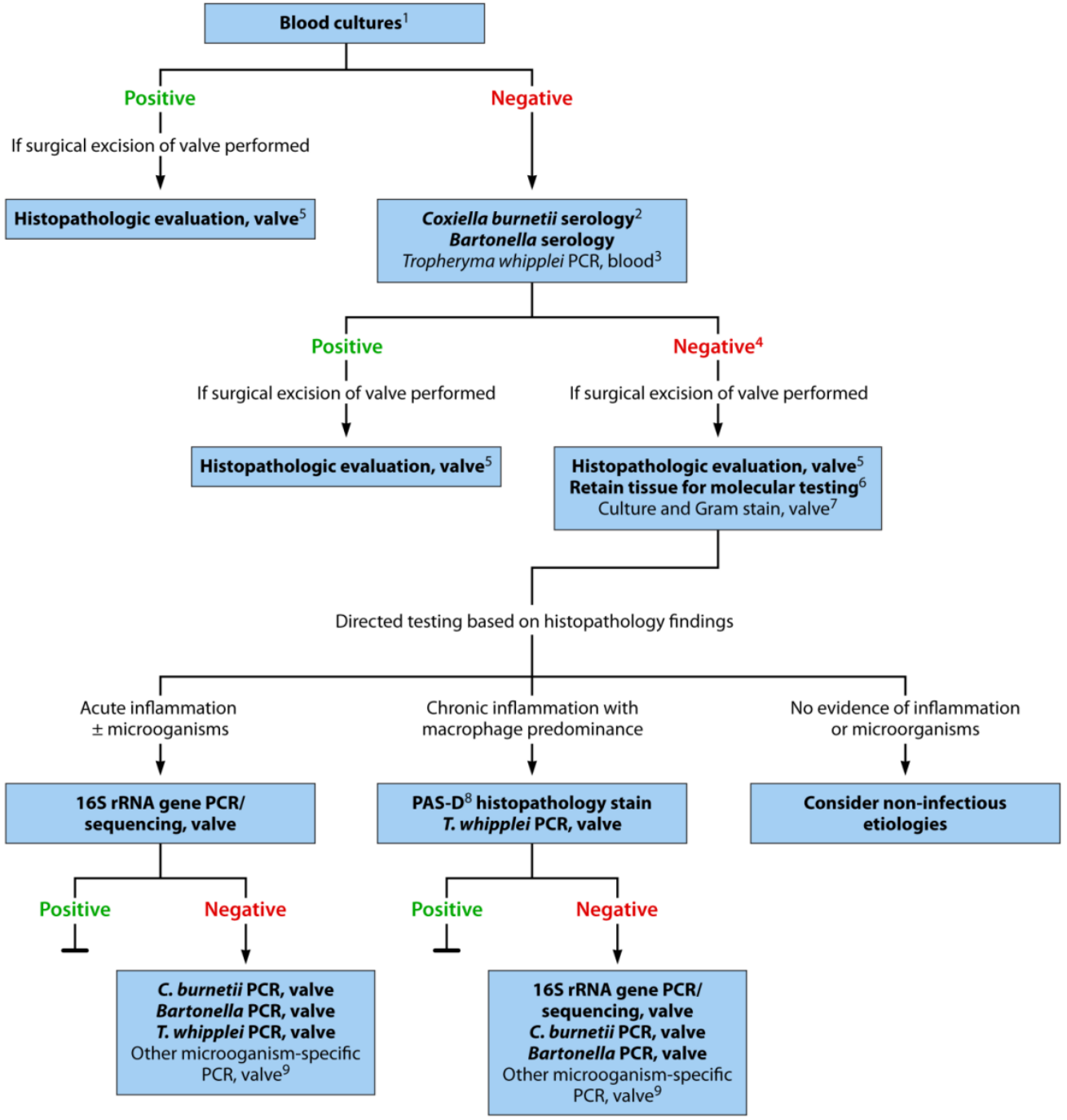
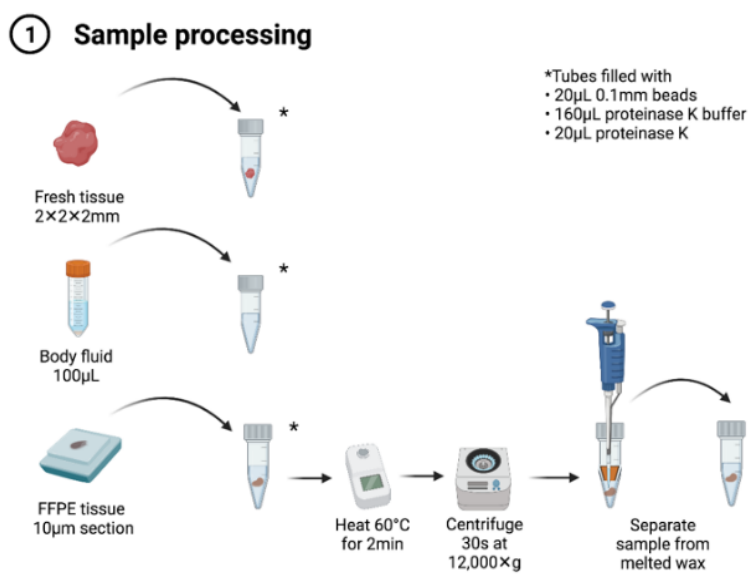
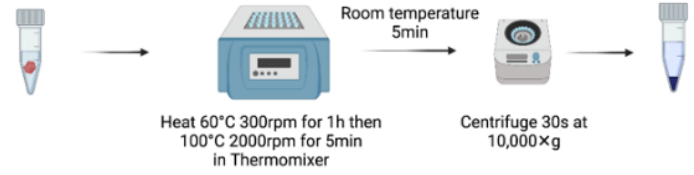
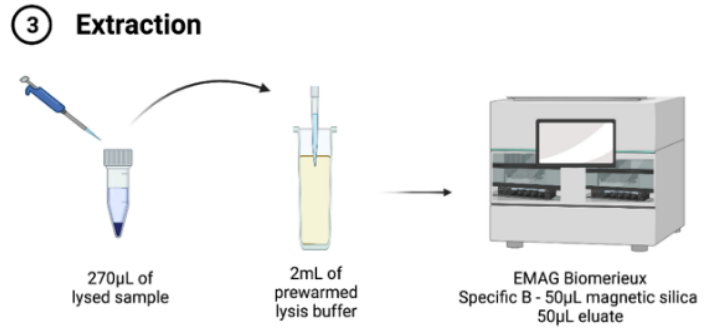
# Microbiologic and Pathological Diagnostic Algorithm For Endocarditis

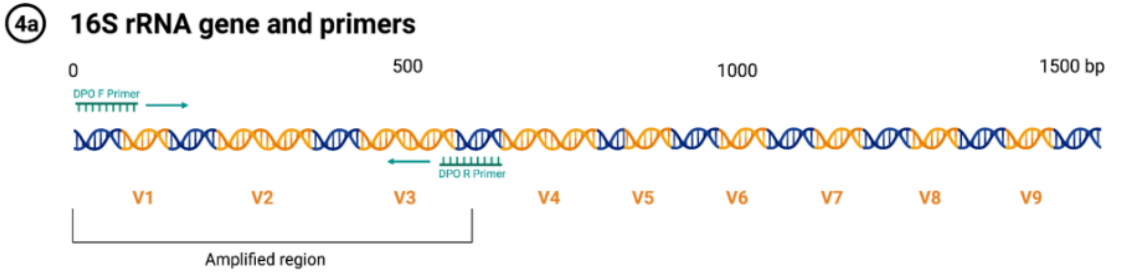


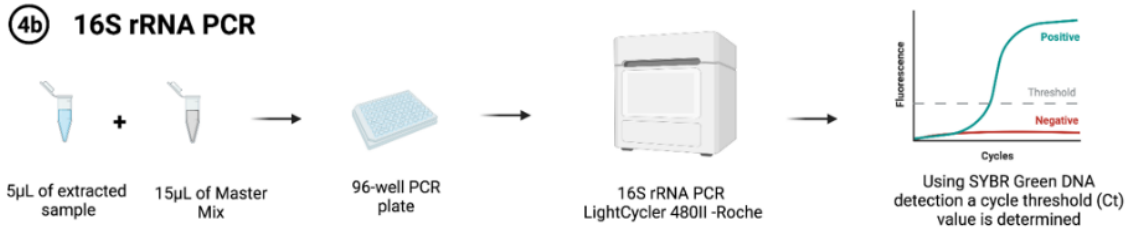

Liesman et al. J Clin Microbiol 2017:55:2599-608

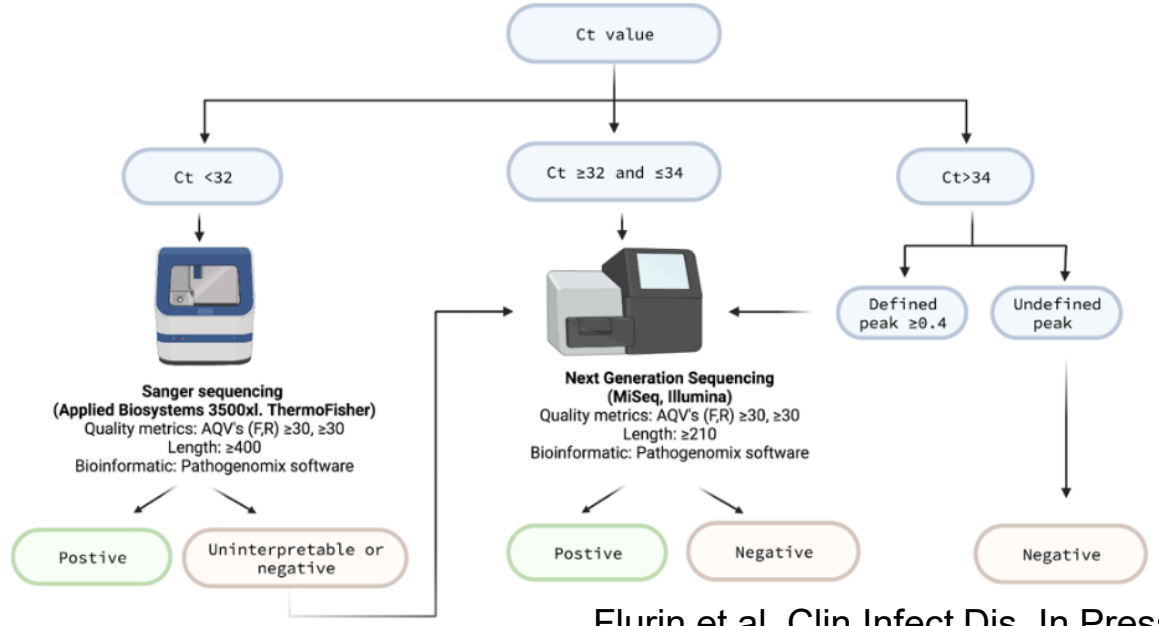


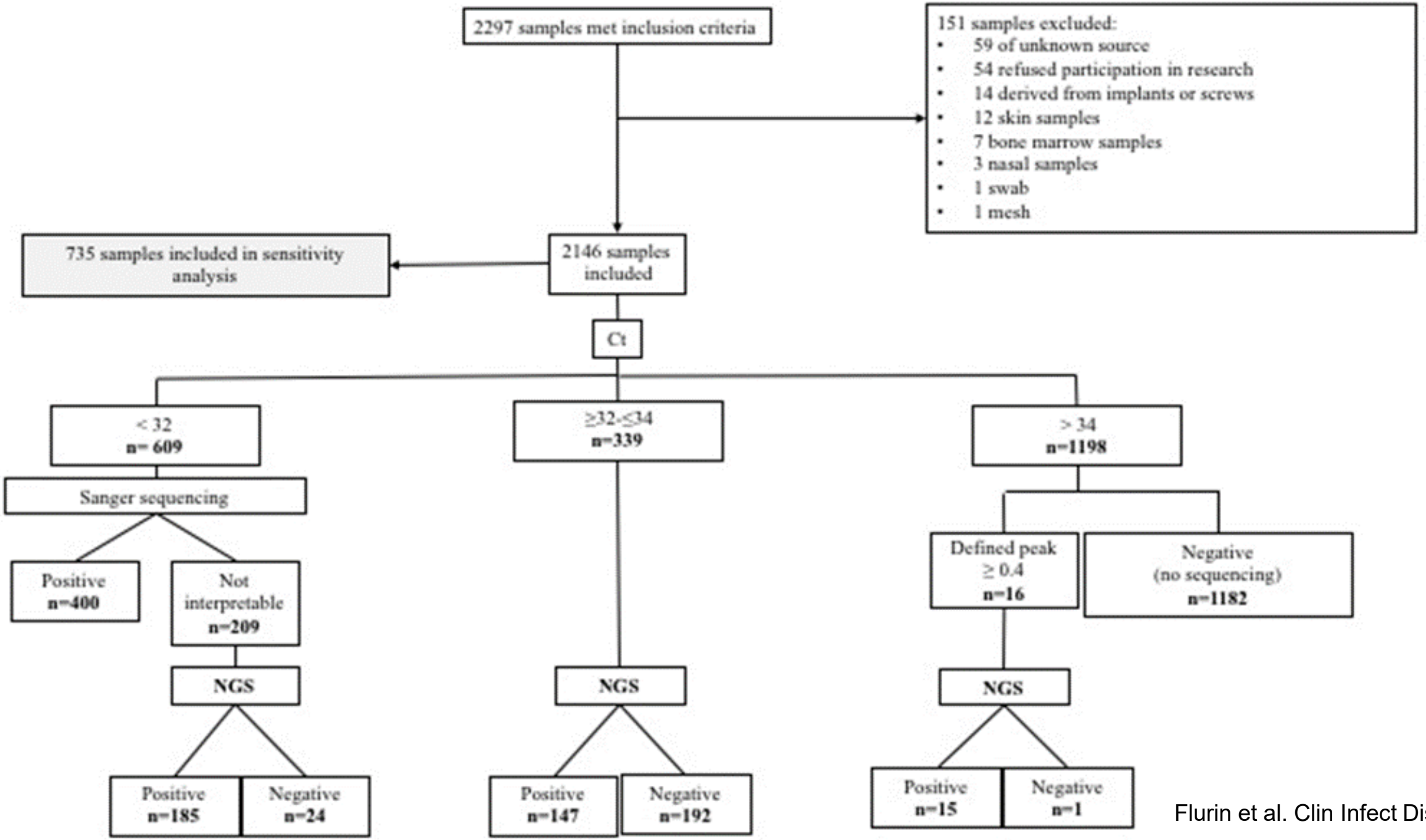



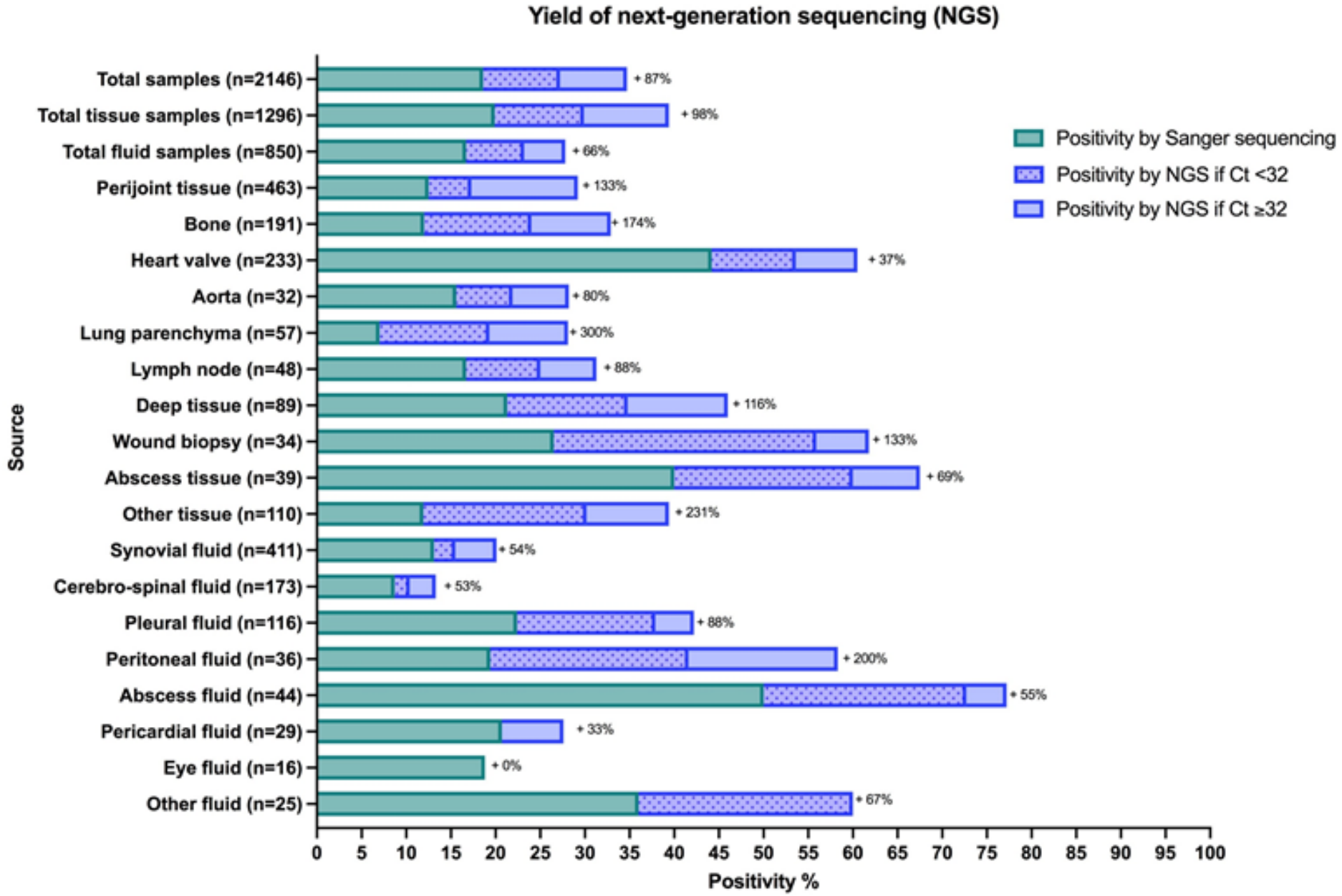




Flurin et al. Clin Infect Dis. In Press

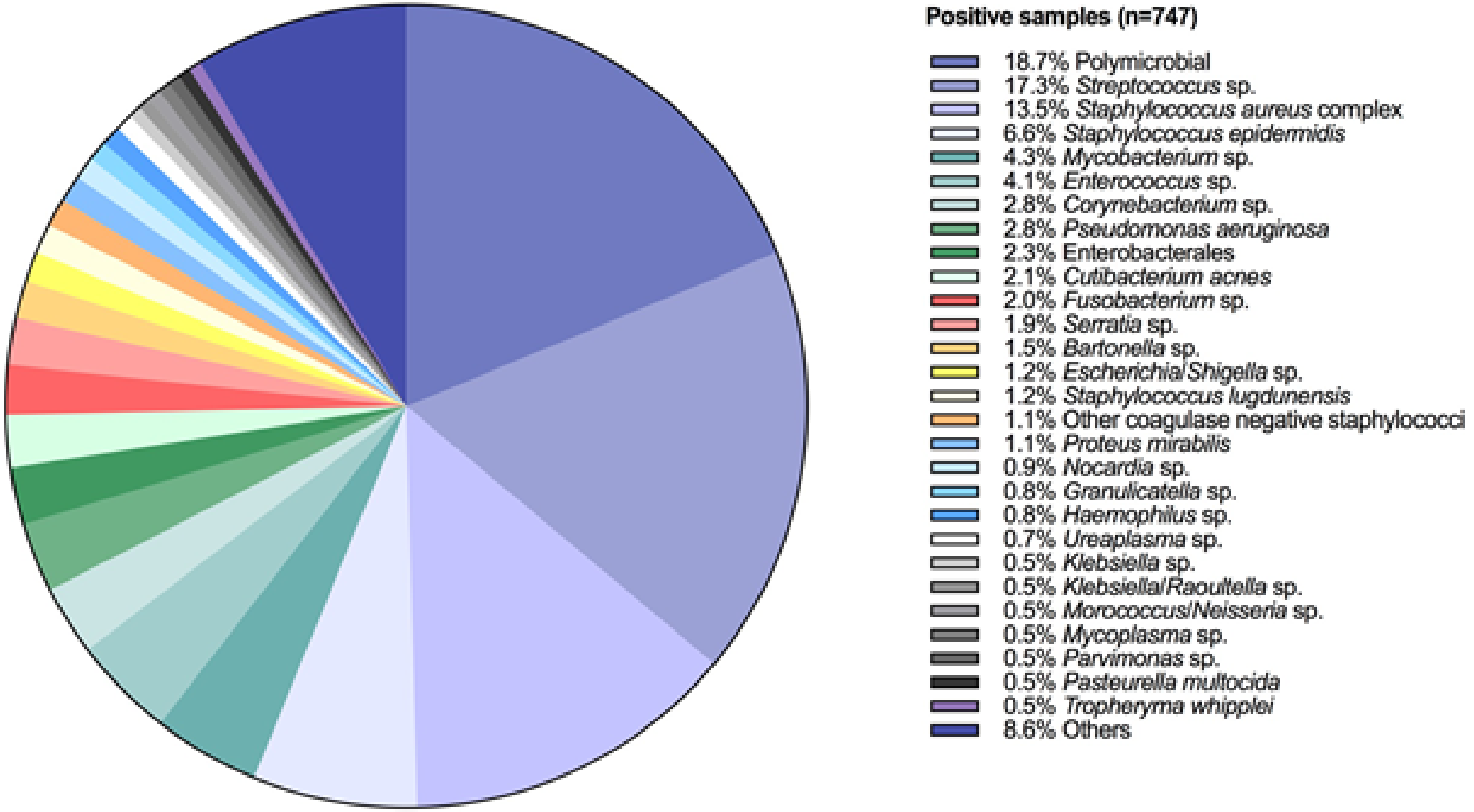



Flurin et al. Clin Infect Dis. In Press

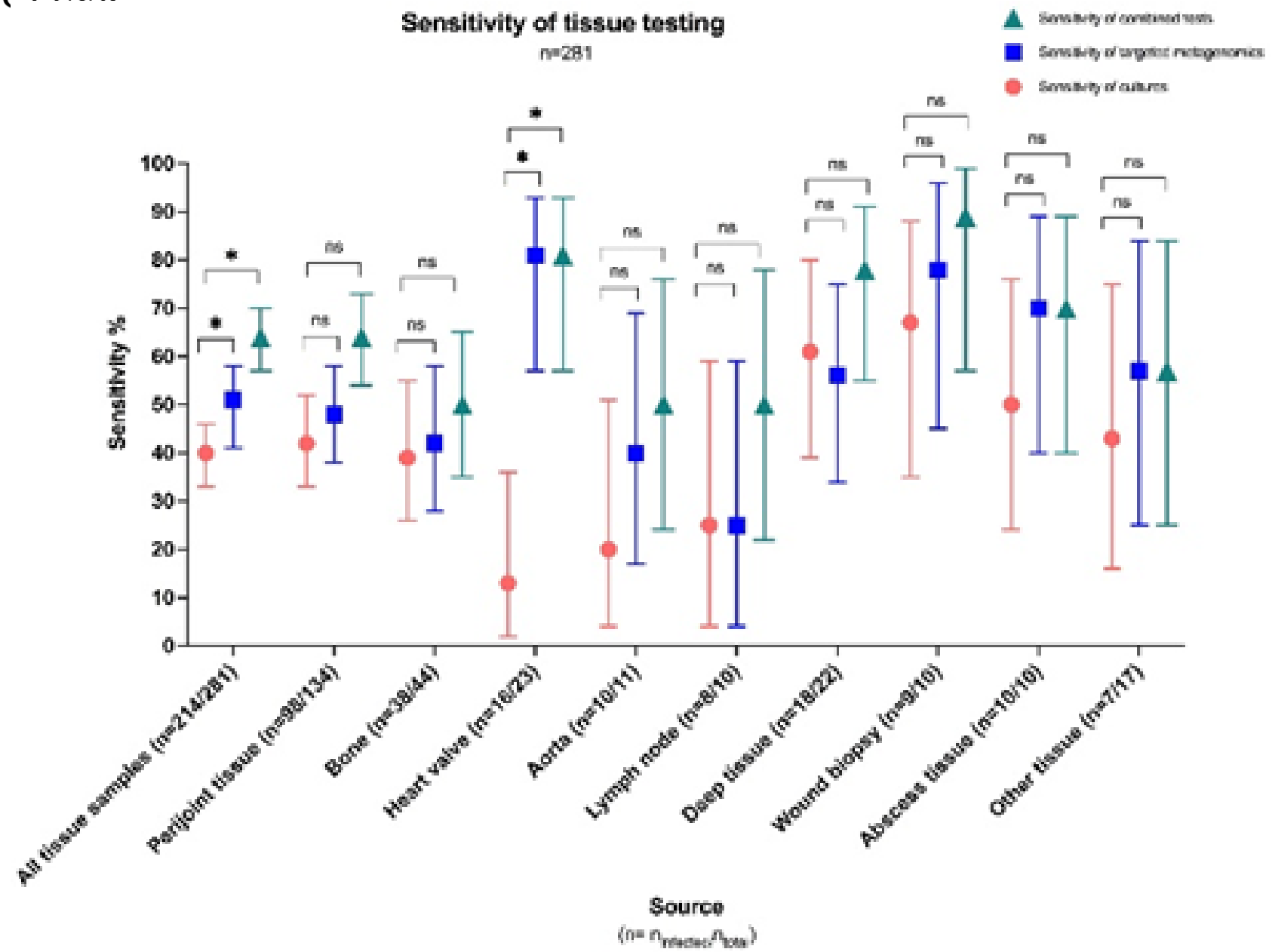



Flurin et al. Clin Infect Dis. In Press

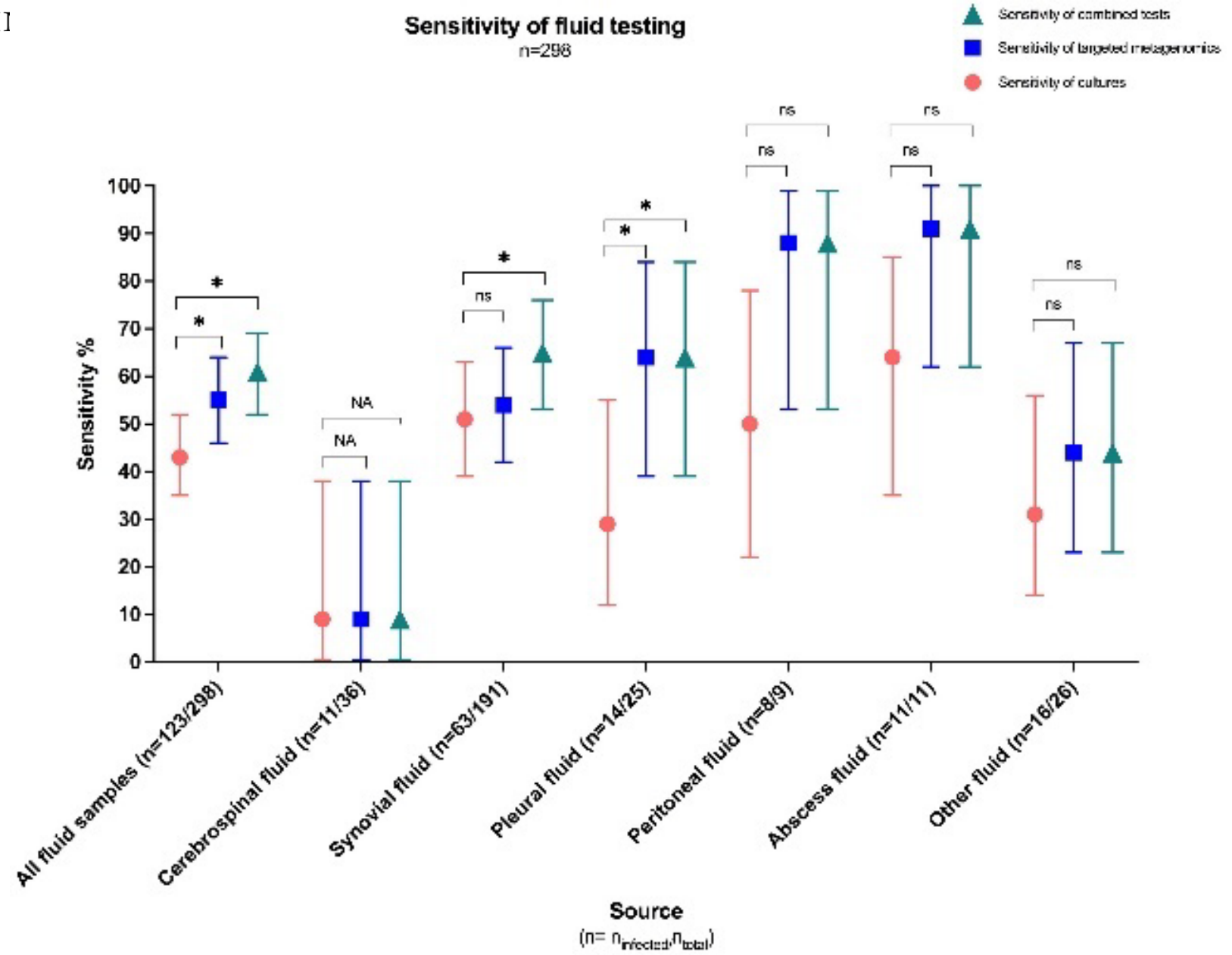
# Identification by targeted metagenomics




Flurin et al. Clin Infect Dis. In Press

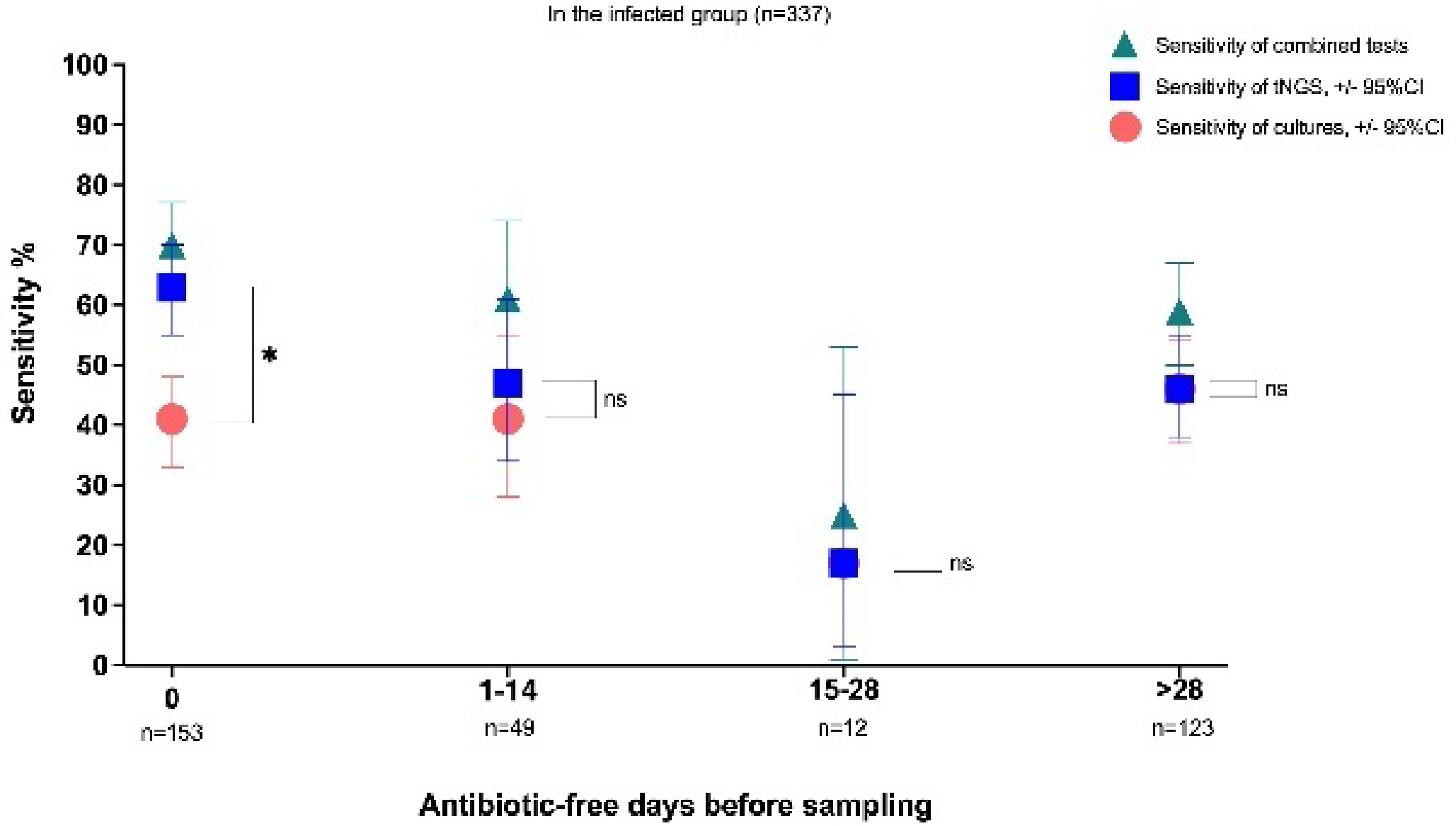



Flurin et al. Clin Infect Dis. In Press




Flurin et al. Clin Infect Dis. In Press

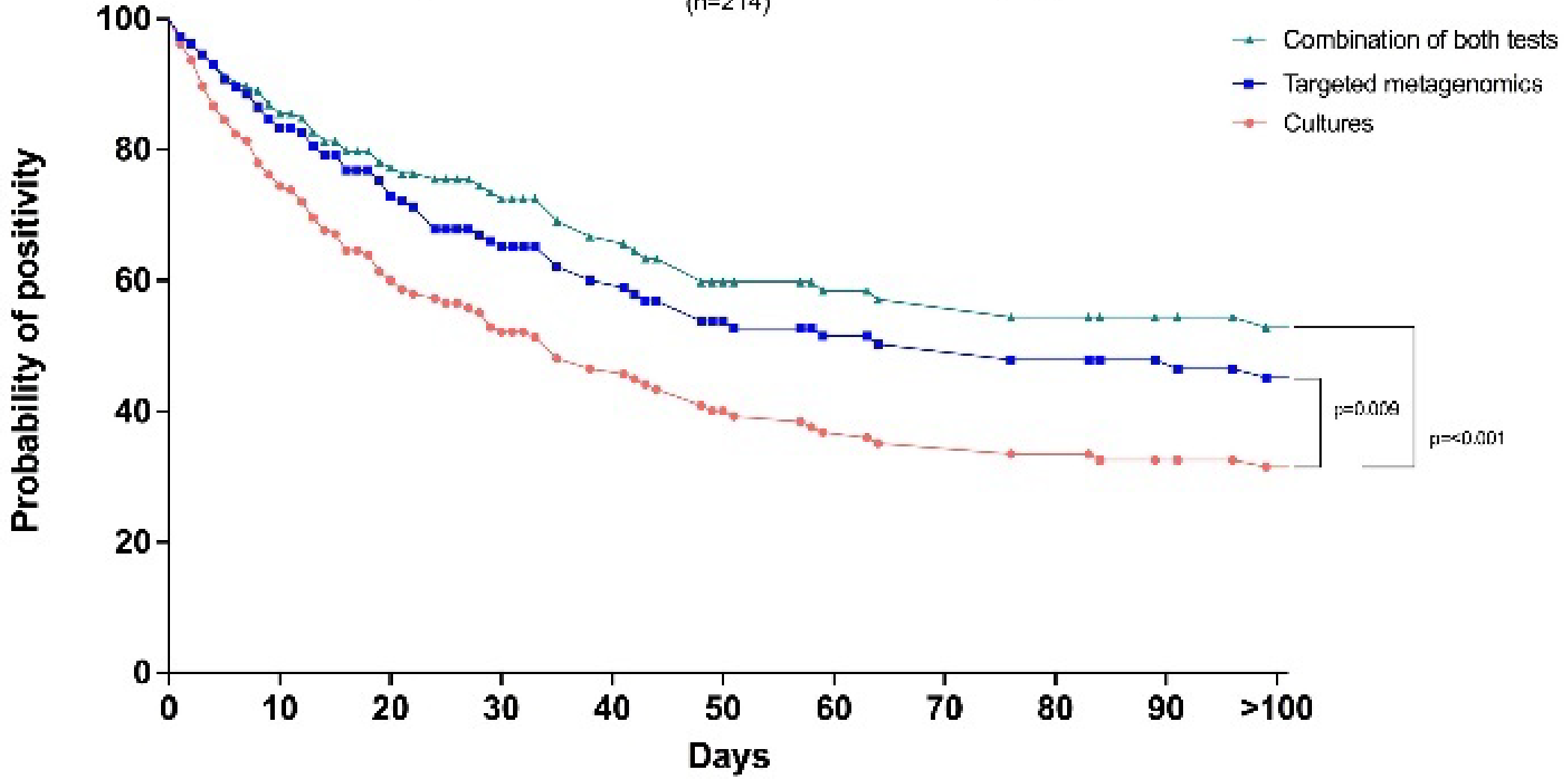
# Sensitivity of tests depending on antimicrobial exposure




Flurin et al. Clin Infect Dis. In Press

# Probability of a positive test depending on duration of antimicrobial therapy

In patients that received antibiotics <4 weeks before sampling
(n=214)




Flurin et al. Clin Infect Dis. In Press

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# 16S rRNA PCR/Sequencing

- Compared to Sanger sequencing alone, test positivity increased by 87% with added NGS
- Sensitivity 53%, higher than culture (42%, $p<0.001$)
- Impact on clinical decision-making in 14% of infected cases
- Sensitivity in subgroup that received antibiotics until the day of sampling
  - 41% for cultures
  - 63% for tNGS
  - 70% for both tests combined

Flurin et al. Clin Infect Dis. In Press

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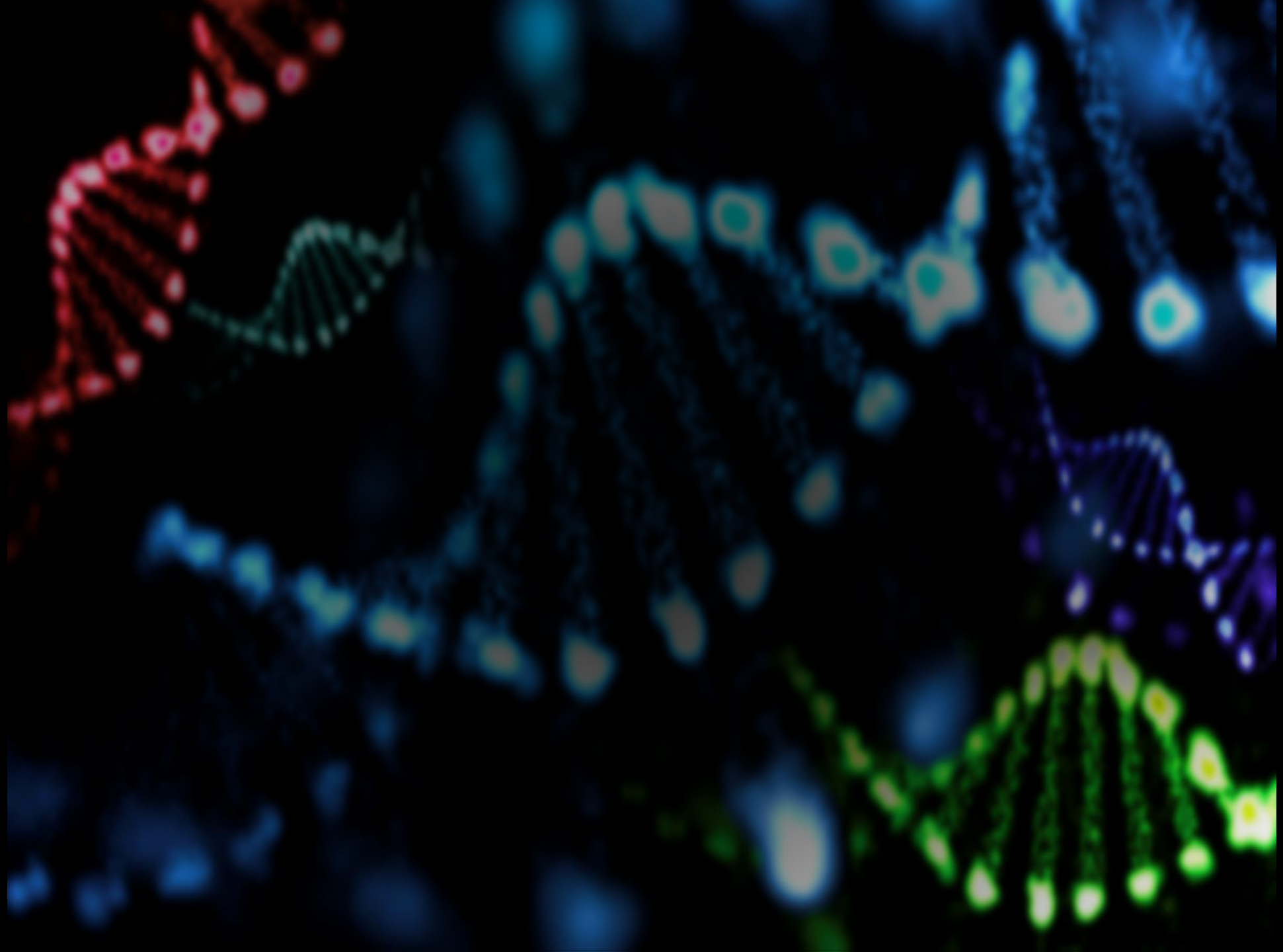
# Metagenomic shotgun whole genome sequencing detects which of the following?

a. Viral, bacterial, fungal, and parasitic volatile molecules
b. Viral, bacterial, fungal, and parasitic carbohydrates
c. Viral, bacterial, fungal, and parasitic proteins
d. Viral, bacterial, fungal, and parasitic nucleic acids



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# Orthopedic Implant Processing - Mayo Clinic

Prosthesis Placed in Container (Operating Room)

400 ml Ringer's Solution Added

Vortex 30 sec

Sonicate 5 min

Vortex 30 sec

Centrifuge 5 min

Aspiration

Plating

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# Shotgun Metagenomic Sequencing *versus* Culture - SONICATE FLUID

| | Samples | Identical Findings | Organisms Not Identified by Shotgun Metagenomic Sequencing | New Organisms Detected by Shotgun Metagenomic Sequencing |
|---|---|---|---|---|
| Non-Infectious Arthroplasty Failure | 195 | 188 (96.4%) | N/A | 7 (3.6%) |
| Culture-Positive PJI | 115 | 99 (86.1%) | 6 (5.2%) | 11 (9.6%) |
| Culture-Negative PJI | 98 | 55 (56.1%) | N/A | 43 (43.9%) |

Thoendel et al. Clin Infect Dis. 2018:67:1333

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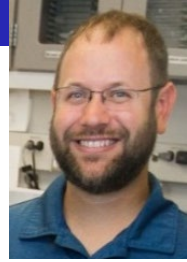

# New or Missed Identifications by SHOTGUN Metagenomic SEQUENCING vs. Culture – SONICATE FLUID

| | | | |
|---|---|---|---|
| **PJI Organisms Not Detected by Metagenomics** | *Bacillus* species<br>*Candida albicans* | *Mycobacterium abscessus*<br>*Porphyromonas* species | *Pseudomonas aeruginosa* (2) |
| **New Organisms Detected in Aseptic Failures** | *Cutibacterium acnes* (2) | *Staphylococcus aureus* (3) | *Streptococcus sanguinis* (2) |
| **New Organisms Detected in Culture-Positive PJIs** | *Anaerococcus obesiensis*<br>*Clostridium* species<br>*Cutibacterium acnes*<br>*Enterobacter cloacae** | *Finegoldia magna* (3)*<br>*Peptoniphilus harei*<br>*Prevotella nanciensis*<br>*Staphylococcus aureus* | *Staphylococcus epidermidis* (6)<br>*Staphylococcus lugdunensis* (2)<br>*Varibaculum cambriense* |
| **New Organisms Detected in Culture-Negative PJIs** | *Anaerococcus urinae*<br>*Candida albicans* (2)*<br>*Candida parapsilosis**<br>*Clostridium perfringens*<br>*Corynebacterium pseudogenitalium*<br>*Cutibacterium acnes*<br>*Enterococcus faecalis* (3)* | *Enterobacter cloacae* (2)*<br>*Facklamia languida*<br>*Granulicatella adiacens* (2)*<br>*Mycobacterium bovis* BCG*<br>*Metamycoplasma salivarium*<br>*Peptoniphilus* species<br>*Pasteurella multocida** | *Staphylococcus aureus* (10)*<br>*Staphylococcus epidermidis* (5)*<br>*Staphylococcus haemolyticus* (2)*<br>*Staphylococcus lugdunensis*<br>*Streptococcus agalactiae* (4)<br>*Streptococcus dysgalactiae* (4)*<br>*Streptococcus oralis** |

* indicates organism identified in cultures other than sonicate fluid

Thoendel et al. Clin Infect Dis. 2018:67:1333

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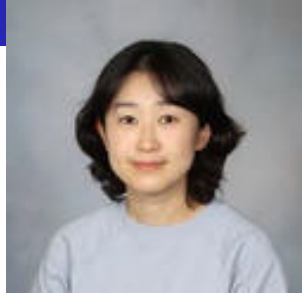

# Shotgun *versus* Targeted Metagenomic Sequencing – Sonicate Fluid

- 395 sonicate fluids
  - 208 PJI
  - 187 non-infectious failure
- Targeted metagenomic sequencing detected potential pathogens in 48% culture-negative PJIs
- No difference between positive percent agreement of targeted (72%) and shotgun (73%) metagenomic sequencing (P = 0.83)

Hong et al. Clin Infect Dis. In Press.

ARLG
Antibacterial Resistance Leadership Group

NATIONAL INSTITUTES OF HEALTH

R01 AR056647
R01 AI91594
R21 AI125870
UM1 AI104681

Slide 1 photo courtesy of Raalen Brown