ashp MIDYEAR 2017
Clinical Meeting & Exhibition

Identifying Bacteria, Viruses, and Fungi by Their Genes:
Infectious Diseases Diagnostic Testing

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Disclosure

All planners, presenters, and reviewers of this session report no financial relationships relevant to this activity.

Session Objectives

By the end of the session, the participants should be able to:

- Describe the mechanisms used in gene based infectious diseases diagnostic testing to identify various bacteria, viruses, and fungi.
- Compare and contrast the various genetic testing methods used in gene based infectious diseases diagnostic testing.
- Evaluate the role of positive and negative test results from gene based infectious diseases diagnostic testing in identifying multi-drug resistant organisms.

Session Objectives

- Demonstrate the significance of gene based infectious diseases diagnostic testing on patient care and its role as part of an antimicrobial stewardship program.
- Discuss the significance of gene based infectious diseases diagnostic testing as a new technological advance which can improve patient care in an institution.

Case Introduction

- A 67 year-old black male presents to the ED with a chief-complaint of shortness of breath, severe fatigue, and presence of fever for the past 24 hours
- PMH: Diabetes Mellitus (uncontrolled), cocaine abuse, and COPD, and chronic renal disease (CRD)
- The patient was previously admitted to the hospital for MRSA-bacteremia in 2014 which was treated successfully with vancomycin

Mortality Risk
Infection Variables

Co-Infection/Source site	Hospital Mortality	30 day mortality
No focus	11-45%	22-48%
SSTI	10-19%	15-17%
Pneumonia	42-62%	39-67%
Bone/Joint	0-14%	0-29%
CNS	25-56%	11%
Endocarditis	22-66%	25-60%
UTI	9.7%	10%

Van Hal SJ. Clin Microbiol Rev 2012;25(2):362-86

POLL QUESTION

- True/False: MRSA bacteremia is associated with a high mortality rate than a CRE infection
  - True
  - False

Mortality Risk
Infection Variables

- Organisms:
  - MRSA vs. MSSA 30 day mortality rate
    - OR 1.88-2.12; P<0.001 [1-3]
  - ESBL (E. Coli ST 131)
    - OR 1.33; P< 0.05 [4]

[1]Cosgrove SE. Clin Infect Dis 2003;36:53-9
[2]Hurley JC. Clin infect Dis 2003;37:866-8
[3]Hurley JC. Med J Aust 2002;176:188-9
[4]Joo EJ. Eur J Clin Microbiol Infect Dis 2017:epub DOI 10.1007/s10096-017-3031-7 (ahead of print)

Mortality Risk
Infection Variables

- Organisms:
  - CRE and KPC
    - KPCs account for 9.7% of BSI in the US (8.1-11.6%) and as high as 17.2% in some medical centers
    - CR-E. coli and Enterobacteriaceae spp accounted for 0.1 and 2.2% of all BSIs
    - 14-day mortality 34%
      - ST258 clade II(49%), and KPC-3-Kp (44%)
    - 30-day and in-hospital mortality was 50%
      - ST258 clade II (63%), and KPC-3Kp (58%)

Satlin MJ. Antimicrob Agents Chemother 2017;61(4):e02349-16. Ahead of Print

Mortality Risk
Infection Variables

- Organisms:
  - CRE and KPC
    - Median 10-days between hospitalization and death
    - Average delay of 2 days before effective therapy was initiated
    - 30-day mortality rates:
      - Use of a single agent (30-50% mortality)
      - Use of combination therapy (33-62% mortality)
      - Regimens with carbapenem (55%) vs no carbapenem (37%)
    - Resistance rates:
      - Piperacillin-tazobactam 97%
      - Aztreonam 87%
      - Colistin 17%

Satlin MJ. Antimicrob Agents Chemother 2017;61(4):e02349-16. Ahead of Print

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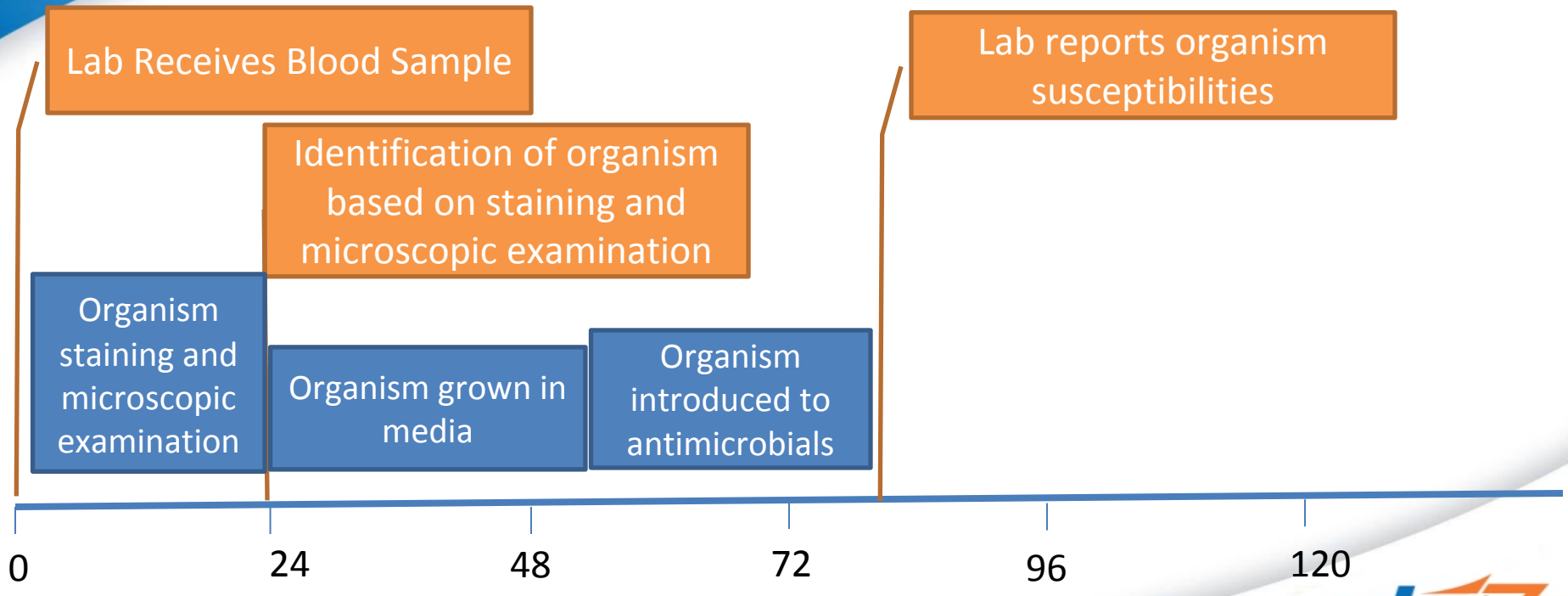
Case Continued

- The patient is started on empiric therapy with vancomycin and piperacillin-tazobactam
- The intensivist orders blood cultures, sputum cultures, and a check x-ray
- Additional standard labs are ordered at the same time

Culture & Susceptibility



Culture and Susceptibility

- Different automated microdilution systems such as Vitek II™, Microscan™, and Phoenix™ vary in organism identification and MIC determination
  - Example: Vancomycin MIC with MRSA (bacteremia)
    - MIC of ≥ 1.5mg/dL is associated with:
      - 2.4-fold increase in clinical failure rates (36.4% vs 15.4%; P=0.045)[1]
      - 1.5-fold increase in clinical failure rates with AUC:MIC < 421 (61.2% vs 48.6%; P=0.038)[2]
      - Infection Related Mortality (24% vs 10%; P=0.16)[3]

[1]Lidose TP. Antimicrob Agents Chemother. 2008;52(9):3315–3320
[2]Kullar R. Clin Infect Dis 2011;52:975-81
[3]Hidayat LK. Arch Intern Med 2006;166:2138-44

Culture and Susceptibility

- Disadvantages:
  - Delay in susceptibility and/or organisms identification information
  - Sample contamination
    - Normal flora
    - Antimicrobials
  - Sample misrepresentation of infection site
  - Sample size
  - Inconsistent results with automated systems

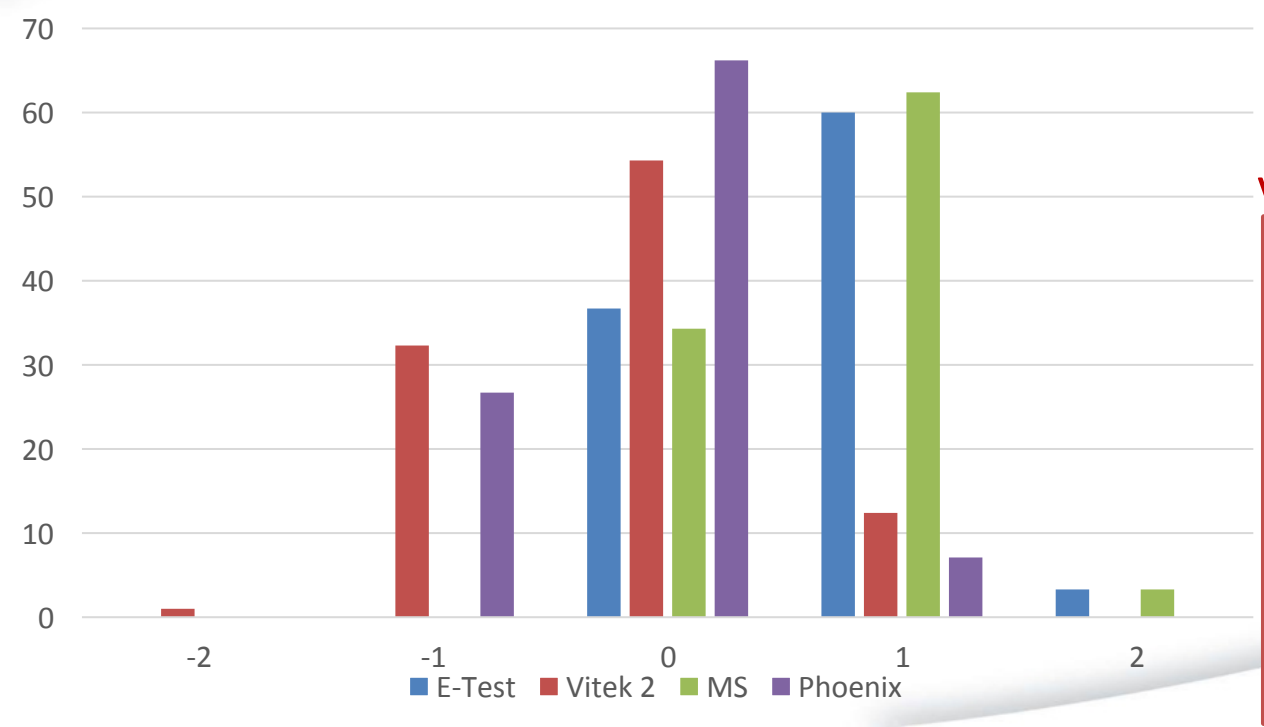
Poll

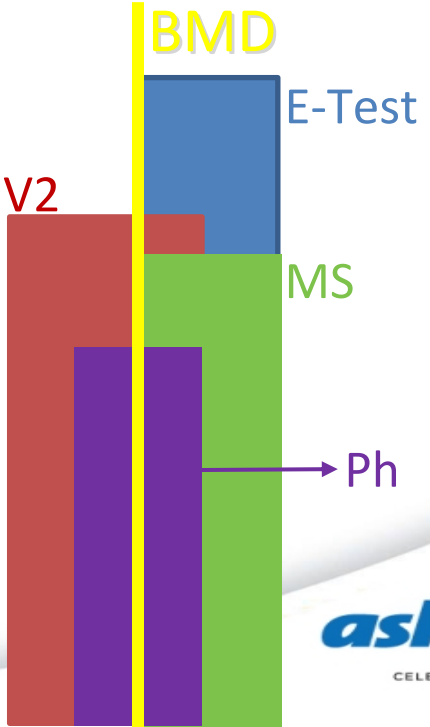
- Which method of MIC determination best correlates with the gold standard (broth microdilution)?
  - A. E-test
  - B. Phoenix
  - C. Vitek 2
  - D. Microscan

Rybak M. J. Clin Micro 2013;51(7):2077-81

Culture and Susceptibility

- Variations in vancomycin-MRSA MIC based on system/method:





Case Continued

- The intensivist would like to consider a molecular-based system for the hospital to help identify blood-borne pathogens more quickly than the traditional C&S system.

Poll

- Which molecular/genetics based test would be the most appropriate to help identify pathogens in patients with bacteremia/fungemia/viremia directly from blood samples?

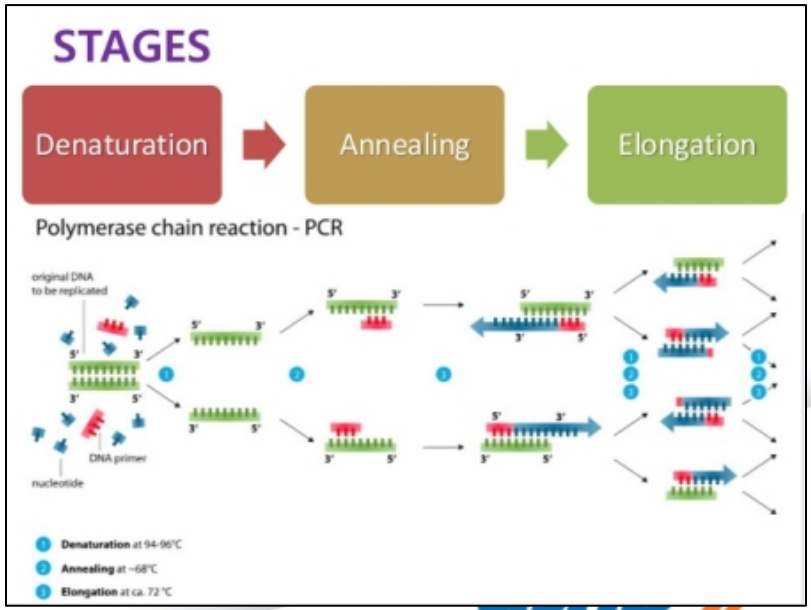
A. MALDI-TOF (Vitek MS, MALDI-Biotyper)
B. Multiplex PCR (Xpert, Filmarray)
C. LAMP (Illumigene)
D. Nanosphere Probe/NAE-PCR (Verigene)

Genetic/Molecular Testing

TYPES OF TEST PLATFORMS

Qualitative vs. Quantitative PCR

- Polymerase Chain Reaction (PCR)
  - Amplification of target nucleic acid sequences on the genome
  - Target is amplified through probes



https://image.slidesharecdn.com/editedpcrdystrophingene-160403103303/95/pcr-amp-dystrophin-gene-4-638.jpg?cb=1459679733

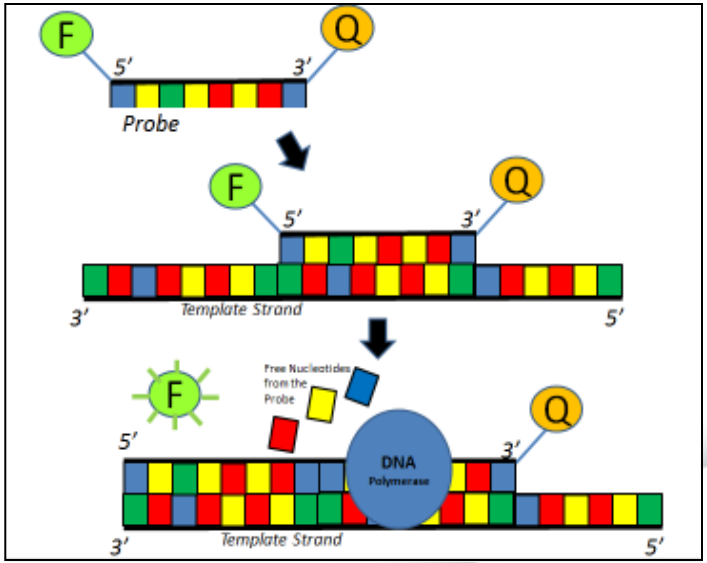
Qualitative vs. Quantitative PCR

Real-time PCR Instruments (Thermocyclers) track the signal of the amplification through the use of a fluorescent dye or probe in the reaction.

- Qualitative PCR is the identification of a marker during the amplification process.
- Quantitative PCR (qPCR) is the measuring of the sample with the use of amplification.

Qualitative PCR

- TaqMan probes
  - Attaches to markers of interest
  - Signal occurs when the enzyme comes in contact with the probe



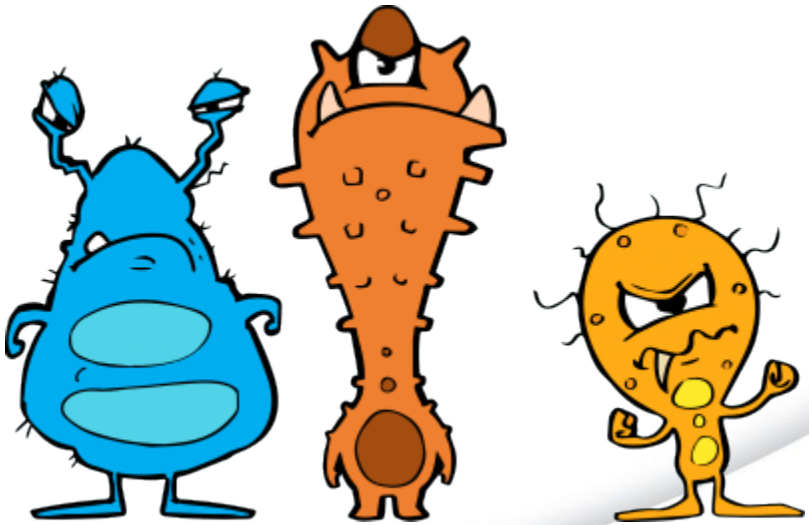
Qualitative PCR

- Examples of TaqMan probes
  - TaqMan Staphylococcus aureus Detection Kit (Thermo Fisher Scientific)
  - Microbial DNA qPCRAssay Kits (Qiagen)

Qualitative PCR

Multiplex PCR

- Identification of multiple organisms in one reaction.
- Example:
  - Biofire FilmArray (Meningitis,/Encephalitis, GI, Respiratory, & Blood Culture)
  - Roche –LightCycler SeptiFast Test (Blood)



http://www.biofiredx.com/products/the-filmarray-panels/

PCR/Hybridization

- Extraction of the nucleic acids and PCR
- Nucleic acids are incubated on a plate
- Plate is read by a scanner
- Example: Verigene System (Luminex) – Bloodstream Infections, GI Infections, and Respiratory Tract Infections

https://www.luminexcorp.com/clinical/instruments/the-verigene-system/

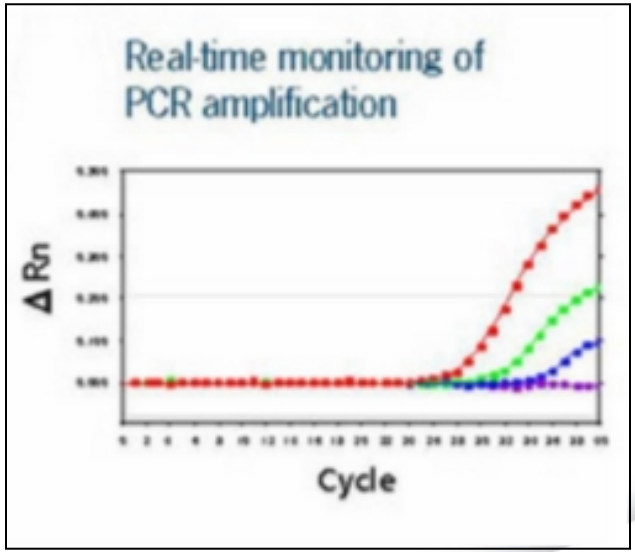
Qualitative PCR/Hybridization

Advantages of Qualitative PCR/Hybridization

- Process Time is 1-3 hrs.
- Medium to High Throughput
- Medium Cost
- Focused Variant Software

Disadvantages of Qualitative PCR/Hybridization

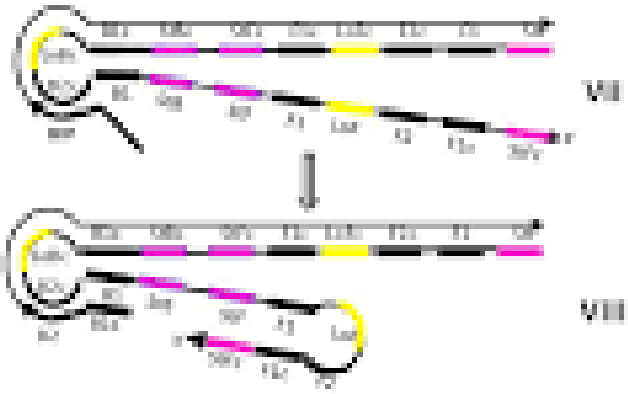
- Limitations to the technologies
- Probes may be expensive



https://www.intechopen.com/source/html/49685/media/fig1.png

Loop Mediated Isothermal Amplification

- Two step amplification process
- Increases specificity for genetic material
- Further procedures can enhance the identification of the sample
- Example: Illumigene (Meridian)
    - C. difficile
    - Streptococcus
    - H.pylori
    - Respiratory Panel



http://www.meridianbioscience.eu/media/catalog/product/cache/3/image/9df78eab33525d08d6e5fb8d27136e95/i/l/illumigene_kitbox_malaria_modified_1__2.jpg

Loop Mediated Isothermal Amplification

Advantages of LAMP

- Process Time is 1-2 hrs.
- Medium to High Throughput
- Medium Cost
- Simple Process

Disadvantages of LAMP

- Limitations to the technologies and types of markers

http://www.meridianbioscience.eu/media/catalog/product/cache/3/image/9df78eab33525d08d6e5fb8d27136e95/i/l/illumigene_kitbox_malaria_modified_1__2.jpg

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Peptide Nucleic Acid Fluorescence *In Situ* Hybridization (PNA-FISH)

- Prepare w/ smear by fix it to the slide
- Hybridize with adding probe
- Wash the slide
- View under the microscope
- Example – AdvanDx (Opgen)
  - S. aureus
  - Gram-Negative

http://www.opgen.com/pathogenid/

Peptide Nucleic Acid Fluorescence *In Situ* Hybridization (PNA-FISH)

Advantages of PNA-FISH

- Process Time is 1-2 hrs.
- Medium to High Throughput
- Medium Cost
- FDA Approved

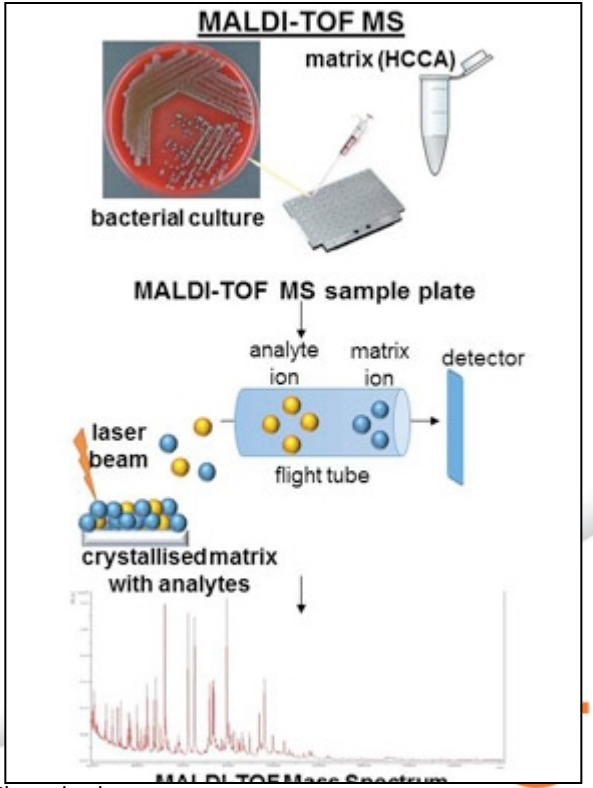
Disadvantages of PNA-FISH

- Trained Individual
- Limit in the types of kits

MALDI -TOF

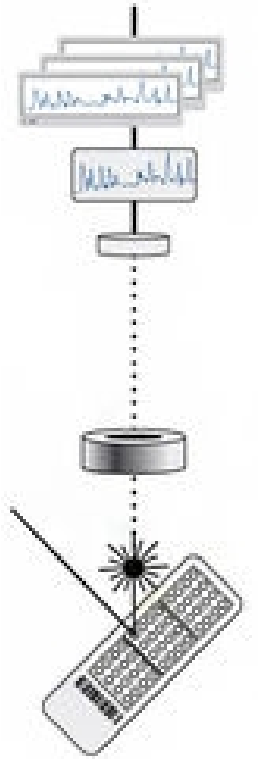
Matrix-assisted laser desorption/ionization – time of flight mass spectrometer (MALDI-TOF)

- Culture Samples
- Proteins are ionized and detected by MS
- Proteins are measured for bacteria signature



http://www.jbmethods.org/public/journals/1/cover_article_125_en_US.jpg

MALDI -TOF



Examples of MALDI-TOF

- Vitek MS: Fungal infections, GI infections Respiratory Tract Infections, Sepsis, Sexually Transmitted Diseases, Urinary Tract infections
- MALDI Biotyper

http://www.biomerieux-diagnostics.com/vitek-ms

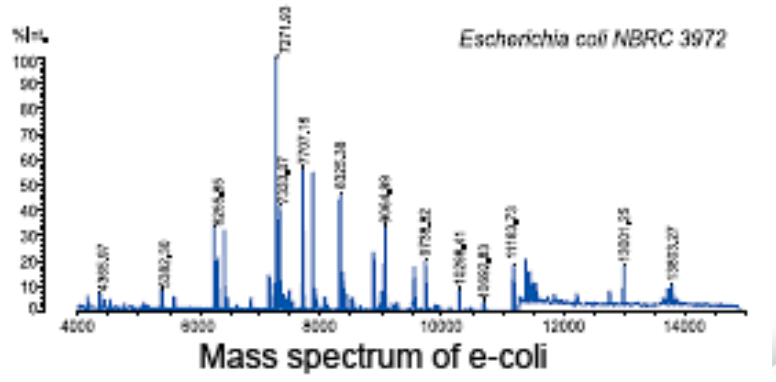
MALDI -TOF

Advantages of MALDI-TOF

- Process Time varies
- Medium to High Throughput
- High Sensitivity
- Focused Variant Software
- Multiplexing Capabilities

Disadvantages of MALDI-TOF

- Higher Equipment Cost
- Technician Expertise
- Labor Intensive



Mass spectrum of e-coli

https://www.shimadzu.co.uk/sites/default/files/sites/files/mis4.gif

Single PCR

- Polymerase Chain Reaction (PCR) based platforms refers to the use of 2-4 primers to identify 1-2 unique DNA fragments
- Considered the most basic molecular/genetic test type
- Used to determine the presence/absence of a specific predetermined organism
- Used most commonly for epidemiological tracking of infections/colonization
- Available for specific high-risk/prevalence organisms

Single PCR

- Polymerase Chain Reaction (PCR) utilizing 2 probes
  - Identification of MRSA through *mecA* detection
    - Blood
    - Skin
  - Identification of Clostridium difficile (C. difficile) through toxin-B gene (*tcdB*) detection
    - Stool
  - Identification of Vancomycin Resistant Enteroccocus (VRE) through VanA/VanB detection
    - Stool

Food and Drug Administration. Nucleic Acid Based Tests. https://www.fda.gov/MedicalDevices. Last updated 10/4/2017

Types of Platforms
Single PCR

- Polymerase Chain Reaction (PCR) utilizing 2 probes
  - Identification of Group-B Streptococcus (S. agalactiae) through *cfb* detection
    - Vaginal secretions
  - Use outside the U.S. (not cleared by the FDA)
    - Detection of carbapenemases
    - Detection of Extended-Spectrum Beta-Lactamases

Food and Drug Administration. Nucleic Acid Based Tests. https://www.fda.gov/MedicalDevices. Last updated 10/4/2017

Single PCR

- Advantages
  - Rapid results (1-2 hours)
  - No culturing required to identify organism
  - Sample processed directly from blood/feces/vaginal secretions
  - Low cost compared to other platforms
  - High sensitivity (>93% for all tests)
  - High specificity (>92% for MRSA, GBS, and VRE)
  - Batching

Emmadi R. J Molec Diag 2011;13(6):583-604
Food and Drug Administration. Nucleic Acid Based Tests. https://www.fda.gov/MedicalDevices. Last updated 10/4/2017

Single PCR

- Disadvantages
  - One test one organism
  - Subculturing of positive blood cultures is necessary to recover organisms for definitive identification and susceptibility testing
  - FDA cleared for epidemiological purposes and not clinical use*
  - Relatively low specificity with C. difficile (>80)
  - False negative results due to high level of bodily fluids/secretions (c. difficile and GBS)
  - False positive MRSA results with MecA as CoNS can test positive

Emmadi R. J Molec Diag 2011;13(6):583-604
Food and Drug Administration. Nucleic Acid Based Tests. https://www.fda.gov/MedicalDevices. Last updated 10/4/2017

Multiplex PCR

- Similar to PCR tests except for the use of 4 or more primers instead of 2
- Subculturing of positive blood cultures is necessary to recover organisms for definitive identification and susceptibility testing
- More extensively used in the clinical setting:
  - Ability to screen for multiple organisms and/or resistance genes at one time
  - Ability to screen for multiple genes to decrease false-negative/positive results

Multiplex PCR

- Use of Xpert MRSA/SA vs conventional methods in patients with suspected MRSA BSI
  - Decrease in antibiotic therapy with Xpert MRSA/SA (76% vs 55%; P<0.01)[1]
  - 44.6 hour reduction in mean time to appropriate therapy[2]
  - Mean time to switch from empiric therapy to nafcillin/cefazolin decreased by 1.7 days (P=0.02)[2]
  - Mean hospital cost were $21,387 less per patient (P=0.02)[2]

[1]Parta M. Infect Control Hosp Epidemiol 2010;31:1043-8
[2]Bauer K. Clin Infect Dis 2010;51:1074-80

MULTIPLEX PCR

- Xpert MRSA/SA BC and intervention in patients with CoNS
  - Anti-staphylococcus antibiotics stopped 32 hours sooner ($P<0.05$)
  - Total antibiotic exposure decreased by 43.5% ($P<0.01$)
  - Infection related length of stay decreased by a mean of 4.5 days ($P<0.018$)

Wong J. Ann Pharmacother 2012;46:1484-90

MULTIPLEX PCR

- Multiplex PCR Systems – Organism Specific
  - MRSA, MSSA, and ± CoNS* (*depending on platform brand)
    - In addition to the detection of mecA gene, multiplex systems target the MREJ types 2,3, and 7 (MREJ has at least 7 known types)
  - C. difficile
    - Identification of the tcdB gene (different sequence)
  - Adenovirus serotypes 1-51 through the detection of Hexon gene
- FDA cleared platform(s): BioFire® FilmArray™
  - Discussed later in slides

Multiplex - MicroArray

- Similar to Multiplex PCR testing
- Difference in detection of primers (through fluorescence)
- Multiple concurrent reactions each within a well
- Ability to identify multiple organisms/resistance
- Subculturing of positive blood cultures is necessary to recover organisms for definitive identification and susceptibility testing

Multiplex vs MicroArray

FilmArray (Multiplex PCR)

- Respiratory Infections
- Gastrointestinal Infections
- Bloodstream Infections
- CNS Infections

Verigene (Multiplex Microarray)

- Respiratory Pathogens Flex Test
- Enteric Pathogen Test
- Bloodstream Infections
  - Verigene Gram-Positive Blood Culture Test
  - Verigene Gram-Negative Blood Culture Test

POC	FilmArray BCID	Verigene GP BCID	Verigene GN BCID
Gram Positive	Staphylococcus, S. aureus, Streptococcus, S. agalactiae, S. pyogenes, S. pneumoniae, L. monocytogenes, Enterococcus	Staphylococcus, S. aureus, S. epidermidis, S. lugdunensis, Streptococcus, S. anginosus Group, S. agalactiae, S. pneumoniae, S. pyogenes, E. faecalis, E. faecium, Listeria	
Gram Negative	E. coli, K. pneumoniae and oxytoca, S. marcescens, E. cloacae complex, Proteus, P. aeruginosa, A. baumannii, N. meningitidis, H. influenzae		E. coli, K. pneumoniae and oxytoca, P. aeruginosa, S. marcescens, Acinetobacter, Citrobacter, Enterobacter, Proteus
Resistance	mecA (MRSA), vanA/B (VRE), and KPC (Carbapenemase)	mecA (MRSA), VanA/B (VRE)	CTX-M (ESBL) IMP, KPC, NDM, VIM, OXA (carbapenemases)
Fungi	C. albicans/ tropicalis/ parapsilosis/ krusei/ glabrata		

Multiplex vs MicroArray

FilmArray BCID

- 1.07-1.5 hours from sample arrival
- Detected 87.2% of important organisms
- 1 (0.6%) false-negative results
- 30 (16.7%) false-positive results
- 9 FN and 5 FP for GN-resistance genes

Verigene

- 2.3-2.5 hours from gram-stain to results
- Detected 90.6% of important organisms
- 7 (3.9%) false-negative results
- 6 (3.3%) false-positive/misidentification
- 2 FN and 3 FP for GN-resistance genes

Both systems identified all MecA

Bhatt MM. J Clin Microbiol 2014;52(9):3433-36
Ward C. Eur J Clin MicroBiol Infect Dis 2015;34:487-96

Multiplex vs MicroArray

POC	FilmArray[1]	Verigene[2,3]
Sensitivity (%)	GP (96.5-100), GN (92.2-100), Fungi (96.7-100), mecA (80), vanA/B (100)	GP (93.1-100), GN (99.4-100), mecA (92-97.5), vanA/B (94.2-100), ESBL (98.7), CRE (95.3-100)
Specificity (%)	GP (99.1-100), GN (99.6-100), Fungi (99.8-100), mecA (85.7), vanA/B (100)	GP (98.9-100), GN (99.6-100), mecA (81.5-98.8), vanA/B (99.8-100), ESBL (99.9), CRE (99.9-100)
Time to Results	1 hour	2.5 hours

[1]BioMerieux FilmAray BCID Panel. Accessed http://www.biomerieux-diagnostics.com/filmarrayr-bcid-panel. Las accessed on 9/18/2017

[2]Lumniex Verigene Gram-Negative Blood Culture Nucleic Acid Test (BC-GN) IVD. Accessed https://www.luminexcorp.com/clinical/infectious-disease/bloodstream-infection-tests/gram-negative-blood-culture/ Last Accessed 9/20/17

[3]Luminex Verigiene Gram-Positive Blood Culture Nucleic Acid Test (BC-GN) IVD. Accessed https://www.luminexcorp.com/clinical/infectious-disease/bloodstream-infection-tests/gram-positive-blood-culture/ Last Accessed 9/2017

Poll

You meet with the microbiologist to discuss possible genetic platforms to use in combination with conventional C&S testing. The microbiologists asks that you help select a platform which does not require gram-staining as to decrease the workload in the lab. Which of the following two platforms would you choose?

A. FilmArray (multiplex PCR)

B. Verigene (multiplex – Microarray)

Multiplex vs MicroArray

FilmArray BCID

- No staining needed as both GP and GN in one test
- Inclusion of fungal organisms
- Detection of the Enterobacteriaceae family members
- Detection of S. lugdunensis (pathogenic similar to S. aureus)
- False-positive results when sample obtained from BioMerieux BacT anaerobic blood bottle for P. aeruginosa and Enterococcus

Verigene

- Requires accurate gram-staining to determine which test to use (GP vs GN)
- Larger spectrum of organisms identified if both GP and GN tests used vs FilmArray
- Inability to detect P. aeruginosa resistance to carbapenems

Bhatt MM. J Clin Microbiol 2014;52(9):3433-36
Ward C. Eur J Clin MicroBiol Infect Dis 2015;34:487-96

Multiplex vs MicroArray

FilmArray

BCID Panel Result	Cross-Reactive Organism(s)/Isolate(s)/Gene
Gram-positive Bacteria	
Enterococcus	Some coagulase-negative staphylococci[a]
Gram-negative Bacteria	
Acinetobacter baumannii	*Acinetobacter calcoaceticus-baumannii* (ACB) complex species: *Acinetobacter calcoaceticus* (ssp. *anitratus*)[b] *Acinetobacter pittii* (formerly *genomospecies* 3)[b]
Escherichia coli/ *Enterobacteriaceae*	*Shigella* species: *Shigella boydii* *Shigella dysenteriae* *Shigella flexneri* *Shigella sonnei* *Escherichia fergusonnii*
Klebsiella pneumoniae/ *Enterobacteriaceae*	*Klebsiella variicola* (aka *Klebsiella pneumoniae* variant 342) *Enterobacter aerogenes* *Raoultella ornithinolytica*[c]
Serratia marcescens/ *Enterobacteriaceae*	*Serratia* species (*S. entomophila*[e], *S. ficaria*, *S. odorifera*[d], and *S. rubidaea*[d]) *Raoultella ornithinolytica*[c] *Pseudomonas aeruginosa* (ATCC 25619)[f] *Pseudomonas putida*[e]
Haemophilus influenzae	*Haemophilus haemolyticus*[g]
Yeast	
Candida parapsilosis	*Candida orthopsilosis* (Group III *Candida parapsilosis*)[h]
Antimicrobial Resistance Genes	
vanA/B	*vanM*[i]

FDA. Nucleic Acid Based Tests. 501(k) Post Market Notification K130914

Verigene GN

BC-GN Test Target for Which Cross Reactivity Observed	*Cross Reactive Organism*
Citrobacter spp.	*Buttiauxella gaviniae*
	Enteric group 137
Enterobacter spp.	*Klebsiella variicola*
	Leclercia adecarboxylata
Escherichia coli	*Escherichia albertii*
	S. dysenteriae
	S. flexneri
	S. boydii
	S. sonnei
Klebsiella oxytoca	*Kluyvera ascorbata*
	Raoultella ornithinolytica
	Raoultella planticola
	Cedecea davisae
CTX-M	*Kluyvera georgiana**
	Leminorella grimontii
	Enterococcus raffinosus
	Candida parapsilosis
	blaKLUA
	blaKLUG
	blaKLUY

FDA. Nucleic Acid Based Tests. 501(k) Post Market Notification K122514 (GP) and K132843 (GN)

Multiplex vs MicroArray

POC	FilmArray Respiratory	Verigene Respiratory Flex Test
Bacteria	Bordetella pertussis, Chlamydophila pneumoniae, Mycoplasma pneumoniae	*Bordetella pertussis, Bordetella parapertussis/B. bronchiseptica, Bordetella holmesii*
Viruses	Adenovirus, Coronavirus HKU1, Coronavirus NL63, Coronavirus 229E, Coronavirus OC43, Human Metapneumovirus, Human Rhinovirus, Enterovirus, Influenza A, Influenza A/H1, Influenza A/H3, Influenza A/H1-2009, Influenza B, Parainfluenza Virus 1-4, RSV	Adenovirus, Human Metapneumovirus, Influenza A, Influenza A (subtype H1), Influenza A (subtype H3), Influenza B, Parainfluenza 1-4, Rhinovirus, RSV A, RSV B

Multiplex vs MicroArray

POC	FilmArray Gastrointestinal	Verigene Gastrointestinal
Bacteria	Clostridium difficile (Toxin A/B), Campylobacter (jejuni, coli and upsaliensis), Plesiomonas shigelloides, Salmonella spp, Yersinia enterocolitica, Vibrio (parahaemolyticus, vulnificus and cholerae), *Diarrheagenic E.coli/Shigella,* EAEC, EPEC, ETEC) *lt/st,* STEC *stx1/stx2, E. coli* O157, *Shigella/Enteroinvasive E. coli* (EIEC),	*Campylobacter* Group, *Salmonella* spp., *Shigella* spp., *Vibrio* Group, *Yersinia enterocolitica,*
Toxins		Shiga Toxin 1 (stx1), Shiga Toxin 2 (stx2)
Viruses	Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotovirus A, Sapovirus (I, II, IV, and V)	Norovirus, Rotavirus

Multiplex vs MicroArray

Film Array ME Panel	
Bacteria	*E. coli K1*,*H. influenzae*, *L. monocytogenes* *N. meningitidis*, *S. agalactiae*, *S. pneumoniae*
Viruses	CMV, Enterovirus, HSV-1, HSV-2, HHV-6, Human parechovirus, VZV
Fungi	*Cryptococcus neoformans/gattii*

Multiplex - ASP

- Three arm study with FilmArray
  - Conventional BCx, FilmArray with charting, FilmArray with ASP in patients with bacteremia
- Results:
  - Time to identification of organism(s) with FilmArray was 1.3 vs 22.3 hours with Conventional BCx (P<0.001)
  - Decrease in duration of broad spectrum antibiotics in both FilmArray Arms (44 and 45 hours) vs 56 hours with BCx (P<0.001)
  - Time to appropriate antibiotics was decreased to 21 hours with FilmArray and ASP compared to 34 and 38 hours with FilmArray alone and conventional BCx (P<0.001)
  - No difference in LOS, morbidity, and cost

Banerjee R. Clin Infect Dis 2015;61(7):1071-80

MICROARRAY - ASP

- Patients with suspected enterococcal bacteremia and use of Verigene BC-GP
  - Intervention included an infectious diseases pharmacist/physician communicating the results and recommendations
  - Results:
    - Mean time to appropriate antibiotics decreased by 23.4 hours (P=0.005)
    - Decrease hospital length of stay of 21.7 days (P=0.048)
    - Decrease mean hospital cost by $60,729 (P=0.02)

Sango A. Clin Infect Dis 2007;44:190-6

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

&

PEPTIDE NUCLEIC ACID FLUORESCENCE IN SITU HYBRIDIZATION (PNA-FISH)

Loop Mediated Isothermal Amplification (LAMP)

- Amplification of target DNA (cytotoxin specific sequence)
- Amplification results in the formation of Magnesium pyrophosphate → precipitates
- Similar to PCR tests, LAMP tests are used to determine the presence/absence of one organism
- Samples obtained directly from patient
- Subculturing of positive blood cultures is necessary to recover organisms for definitive identification and susceptibility testing

Loop Mediated Isothermal Amplification (LAMP)

- Organisms which can be tested for by LAMP platforms (FDA cleared):
  - C. difficile
  - GAS
  - GBS
  - M. pneumoniae
- The only current FDA-cleared LAMP based platform is the Illumigene™ System created by Meridian®

Loop Mediated Isothermal Amplification (LAMP)

- C. difficile test
  - Intended for clinical use in hospitals
  - Targets the pathogenicity locus (PaLoc) of toxigenic C. difficile
    - Differs than PCR tests by Targeting both **tcd-A** and tcd-B
  - Sensitivity 95.2% (90.0-100% based on age) and specificity of 96.3% (94.3-100% based on age)
  - Time to results 1-1.5 hours

FDA. Nucleic Acid Based Tests. 501(k) Post Market Notification K100818 and K110012

Loop Mediated Isothermal Amplification (LAMP)

Illuminex Panel	Sensitivity	Specificity	Time to Results
GAS (throat swabs)	98%	97.7%	< 60 minutes
GBS (Vaginal Swabs)	98.6%	93.2%	60 minutes following 18-24 hours of enriching the culture
M. pneumoniae (throat/ nasopharyngeal swabs)	100% (51-100%) from either source	100% (91.8-100) from either source)	60 minutes

PNA-FISH

- Fluorescence in situ Hybridization (FISH) using protein nucleic acid (PNA) probes from blood samples
- Qualitative nucleic acid hybridization assay
- identification of specific organisms on smears from positive blood cultures
- Subculturing of positive blood cultures is necessary to recover organisms for definitive identification and susceptibility testing

PNA-FISH

- The only FDA-cleared PNA-FISH platform is the PNA-FISH™ by AdanceDx®
  - 1-2 organism identification:
    - E. faecalis
    - E. coli
    - GBS
    - S. aureus
    - C. albicans and C. glabrata
    - E. coli and P. aeruginosa

PNA-FISH

- The only FDA-cleared PNA-FISH platform is the PNA-FISH™ by AdanceDx®
  - Multiple organism identification:
    - PNA-FISH Gram-Negative Traffic Light
      - E. coli, K. pneumoniae , and P. aeruginosa
    - PNA-FISH Yeast Traffic Light
      - C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, and C. krusei

PNA-FISH

POC	Organism	Sensitivity (%)	Specificity (%)	Time (min)
GN Traffic Light	E. coli	100 (97.8-100)	97.5 (92.8-99.5)	30-90
	K. pneumoniae	98.7 (93.1-100)		
	P. aeruginosa	96.9 (89.2-99.6)		
Yeast Traffic Light	C. Albicans C. Parapsilosis	100 (95.1-100)	100 (86.7-100)	30
	C. Tropicals	100 (90.8-100)		
	C. Glabrata C. krusei	97.9 (88.7-99.6)		

PNA-FISH

- Important notes
  - Gram-negative Traffic Light
    - Escherichia (non-coli) cross-react and can give a green or yellow signal
    - Acinetobacter can cross-react and give a red signal
  - Yeast Traffic Light
    - C. albicans and tropicals are not differentiated as both given green signal
    - C. nivariensis, C. bracarensis, and Kluyveromyces delphensis cross-reacted and produce a red signal
    - *C. orthopsilosis* (3/3) *and C. metapsilosis* cross-reacted to create a green signal
    - Candida sojae cross-reacted and produce a yellow signal

PNA-FISH - ASP

- PNA-FISH in Enterococcal bacteremia
  - 200 patients with enterococcal bacteremia
  - Inclusion of ASP intervention with communicating the results and treatment recommendations
  - Results
    - PNA-FISH identified E. faecalis and E. faecium 3 and 2.3 days earlier than conventional methods respectively (P<0.001)
    - More rapid appropriate initiation of therapy for E. faecium (1.3 vs 3.1 days; P<0.001)
    - Decrease 30-day mortality (26 vs 45%; P=0.04)

Forrest G. Antimicrob Agents Chemoth 2008;52:3558-63

MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME OF FLIGHT MASS SPECTROMETRY (MALDI-TOF MS)

MALDI-TOF MS

- 1975 - Mass Spectrometry was first used to identify organisms
- 1985 – Koichi Tanaka first described soft desorption ionization
- 2002 – Dr. Tanaka was awarded the Nobel Prize in Chemistry
- 2005 – Development of the first MALDI-TOF MS commercial system by Brunker®
- 2017 – Two FDA-cleared MALDI-TOF MS Systems are cleared by the FDA
  - MALDI Biotyper CS™ by Brunker®
  - Vitek MS Plus™ by BioMerieux®

MALDI-TOF MS

- Requires removal of a organism colony from growth media
- Organism colony is placed on matrix tray and energized by the laser beam (ionization)
- Time of flight of proteins is dependent on mass to charge ratio

MALDI-TOF MS

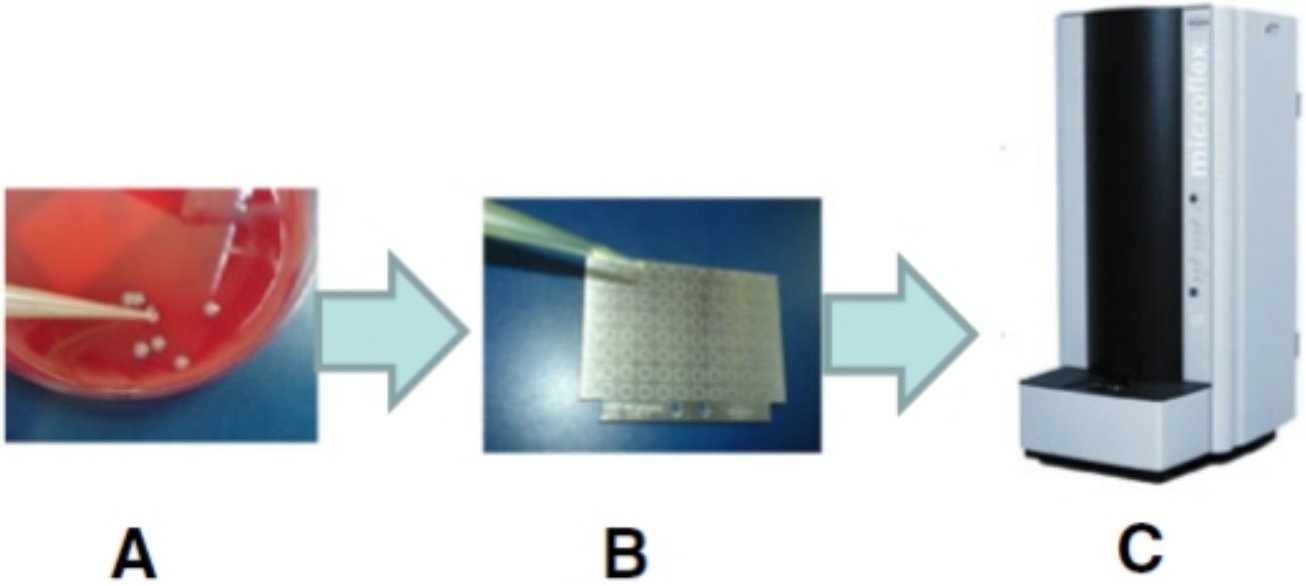


Image Source: Weiser A. Appl Microbiol Biotechnol 2012;93:965-74

MALDI-TOF MS

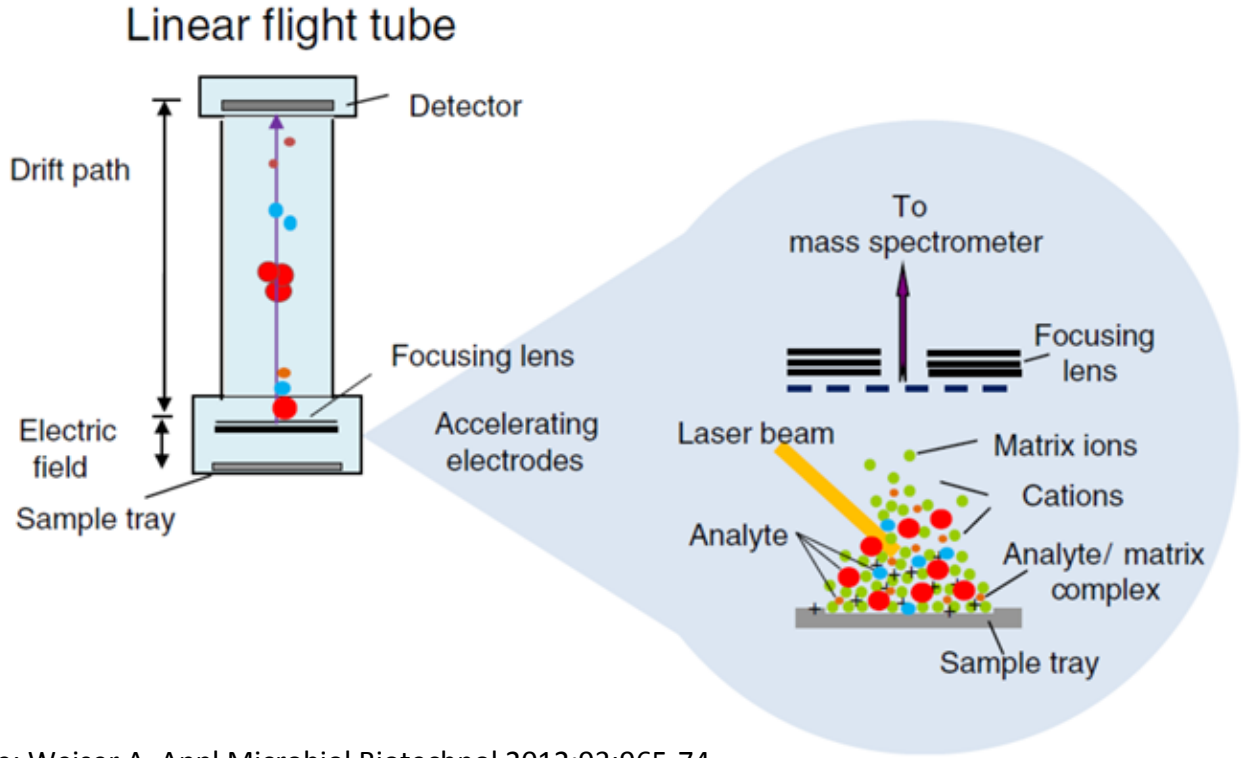


Image Source: Weiser A. Appl Microbiol Biotechnol 2012;93:965-74

MALDI-TOF MS

- Time of flight is compared to a commercial and sometimes site-specific database
- Identification based on signature of peaks/troughs (mass to charge ratio profile)

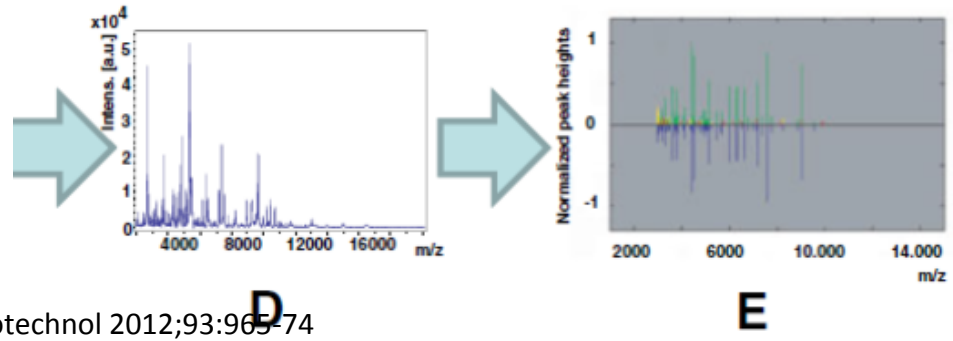
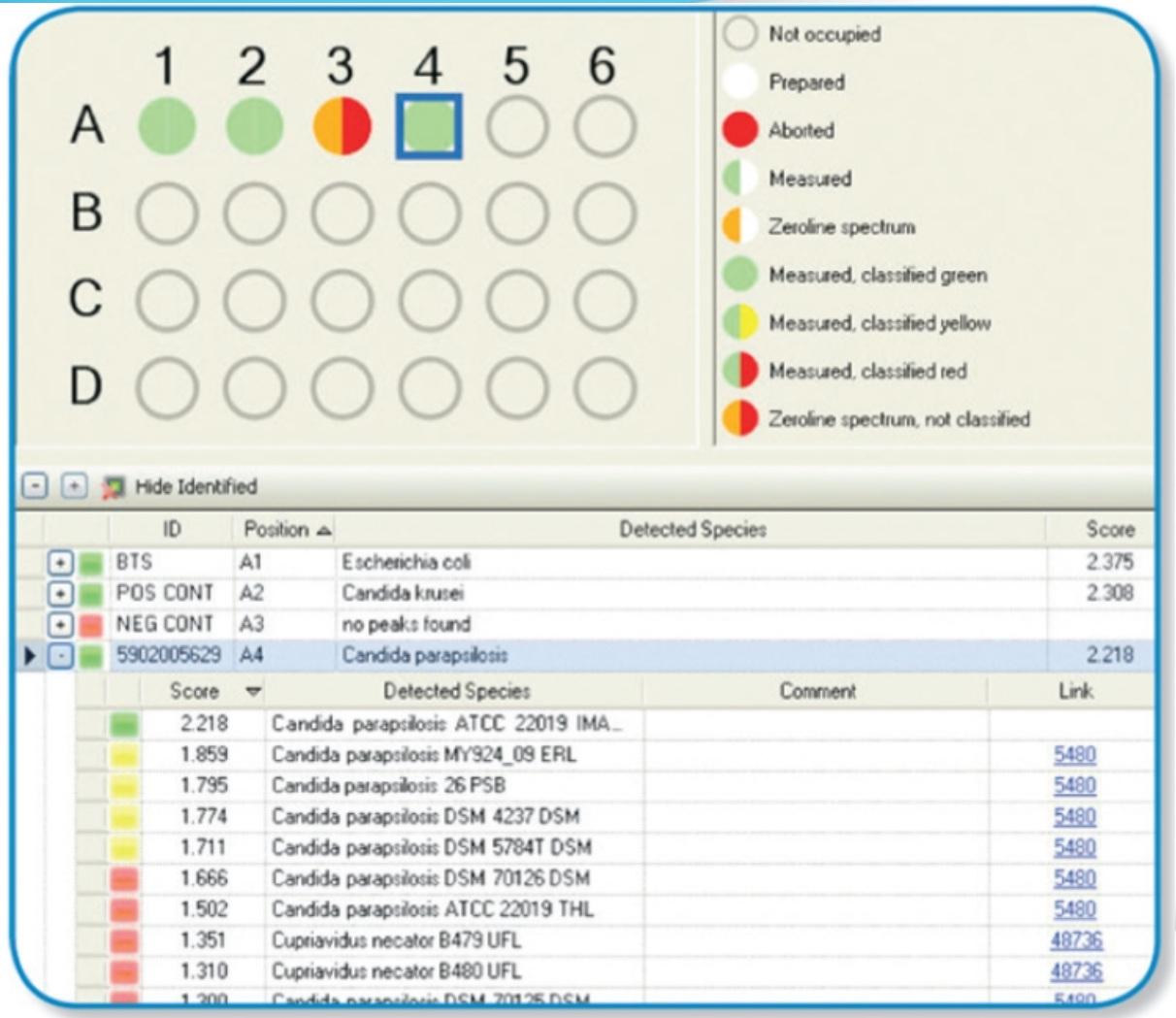


Image Source: Weiser A. Appl Microbiol Biotechnol 2012;93:965-74

MALDI-TOF MS

- The mass to charge profile is compared to a proprietary database specific for each brand of MALDI-TOF MS system
  - Vitek MS: database of 193 species (bacterial and fungal)
  - MALDI Biotyper: database of 210 species (bacterial and fungal)
- A score of 0-3 is provided based on the degree of match between tested organism profile and profiles in the database


1 2 3 4 5 6
A
B
C
D
Not occupied
Prepared
Aborted
Measured
Zeroline spectrum
Measured, classified green
Measured, classified yellow
Measured, classified red
Zeroline spectrum, not classified
Hide Identified
ID Position Detected Species Score
BTS A1 Escherichia coli 2.375
POS CONT A2 Candida krusei 2.308
NEG CONT A3 no peaks found
5902005629 A4 Candida parapsilosis 2.218
Score Detected Species Comment Link
2.218 Candida parapsilosis ATCC 22019 IMA...
1.859 Candida parapsilosis MY924_09 ERL 5480
1.795 Candida parapsilosis 26 PSB 5480
1.774 Candida parapsilosis DSM 4237 DSM 5480
1.711 Candida parapsilosis DSM 5784T DSM 5480
1.666 Candida parapsilosis DSM 70126 DSM 5480
1.502 Candida parapsilosis ATCC 22019 THL 5480
1.351 Cupriavidus necator B479 UFL 48736
1.310 Cupriavidus necator B480 UFL 48736

MALDI-TOF MS

- Scores are not interchangeable between the two MALDI-TOF MS systems
- Scores:
  - 0-1.7 → Unable to identification
  - 1.7-1.999 → Identification on genus level (e.g. Staphylococcus spp)
  - 2.0-3.0 → Identification based on species level (e.g. S. aureus)
- Of note, several studies have used a cut-off of ≥ 1.7 to identify organism on a species level

MALDI-TOF MS

- Scores can be affected by:
  - Density of organisms placed on plate
  - Cell wall structure of organism
  - Presence of mucus or other fluids (mycobacterium and molds)
  - Presence of reference in database
  - Use of formic acid to lyse organisms prior to testing
    - Increases the likelihood of a score ≥ 1.7

Poll

- Which of the following types of organisms is most unlikely to be detected and/or correctly identified by MALDI-TOF?
  - a. Clostridium spp
  - b. P. aeruginosa
  - c. Shigella spp
  - d. C. glabrata

POC with Vitek 2	MALDI BioTyper[1]	Vitek MS[2]
Overall Identification	90.4-93%	96.9%
Bacterial Identification (%)	91.4%	97.7
Gram-Positive bacteria	82-85%[3]	95
Gram-Negative Bacteria	94-100%[4]	78-98.1[4]
Anaerobes	89-100%[5]	91-100%[5]
Mycobacterium	42.8[6]	42.3
Yeast		92.8
Mold		80

[1]Febbraro F .Curr Microbiol 2016;73:843-850
[2]Wattal C. Eur J Clin Microbiol Infect Dis 2017;36:807-812
[3]Schulthess B. J Clin Microbiol 2014;52:1089-97
[4]Karpanoja P. Eur J Clin Microbiol Infect Dis 2014;33:779-88
[5]Barreau M. Anaerob 2013;22:123-5
[6]Balada-Llasat J. J Clin Microbiol 2013;51:2875-9

Comparison of MALDI-TOF MS Systems

POC	MALDI BioTyper[1]	Vitek MS[1]
Overall Identification Species level Genus level No Identification Misidentification	86.4 75.1 20.6 10% 3.6	92.3 88.1 9.1 5.1 2.6
Fungi (overall)	50.7	15.5
Fungi (when in database)	76.5	50
Yeast (overall)	70.7	70.7
Yeast (when in database)	93.3	82.9
Time to Results	5-7 minutes per sample and 1 hour for a plate (~90 samples)[2]	

[1]Levesque S. PLOS ONE 2015;10(12):e0144878

[2]Wieser A. Appl Microbiol Biotechnol 2012;93:965-74

MALDI-TOF MS

- MALDI-TOF MS Important Limitations:[1,2]
  - Both MALDI-TOF MS Systems can not distinguish E. coli spp from Shigella spp
  - Difficulty distinguishing MSSA from MRSA without labor intensive extra steps
    - Clades can be differentiated by not resistance patterns
  - Detection of resistance (especially ESBL and KPC) is difficult without extra steps[1-3]
    - Exposure of bacteria to antibiotic then measurement via MALDI-TOF MS
  - Minor colonies may go undetected

[1]Van Belkum A. Ann Lab Med 2017;37:475-83
[2]Weiser A. Appl Microbiol Biotechnol 2012;93:965-974
[3]Patel R. Clin Chem 2015;61(1):100-111

MALDI-TOF MS - ASP

- MALDI-TOF MS along with real-time notification and infectious diseases pharmacist intervention
  - Results:
    - 46 hour reduction in time to antibiotic optimization (P<0.004)
    - Time to active treatment decreased by 36.7 hours in patients with inactive empiric therapy (P<0.001)
    - Decrease in length of stay by a mean of 2.6 days (P=0.01)
    - Reduction in hospital costs from $45,709 to $26,126 (P=.009)

Perez K. Arch Pathol Lab Med 2013;137:1247-54

MALDI-TOF MS - ASP

- Use of MALDI-TOF MS in addition to ASP notification followed by intervention
  - Indication of bacteremia/candidemia
  - Results
    - 43 hour reduction in time to antibiotic optimization (P<0.001)
    - 9.7 hour reduction in time to active therapy (P=0.021)
    - Reduced mortality rate from 20.3% to 14.5% (P=0.02)

Huang A. Clin Infect Dis 2013;57:1237-45

MALDI-TOF MS - ASP

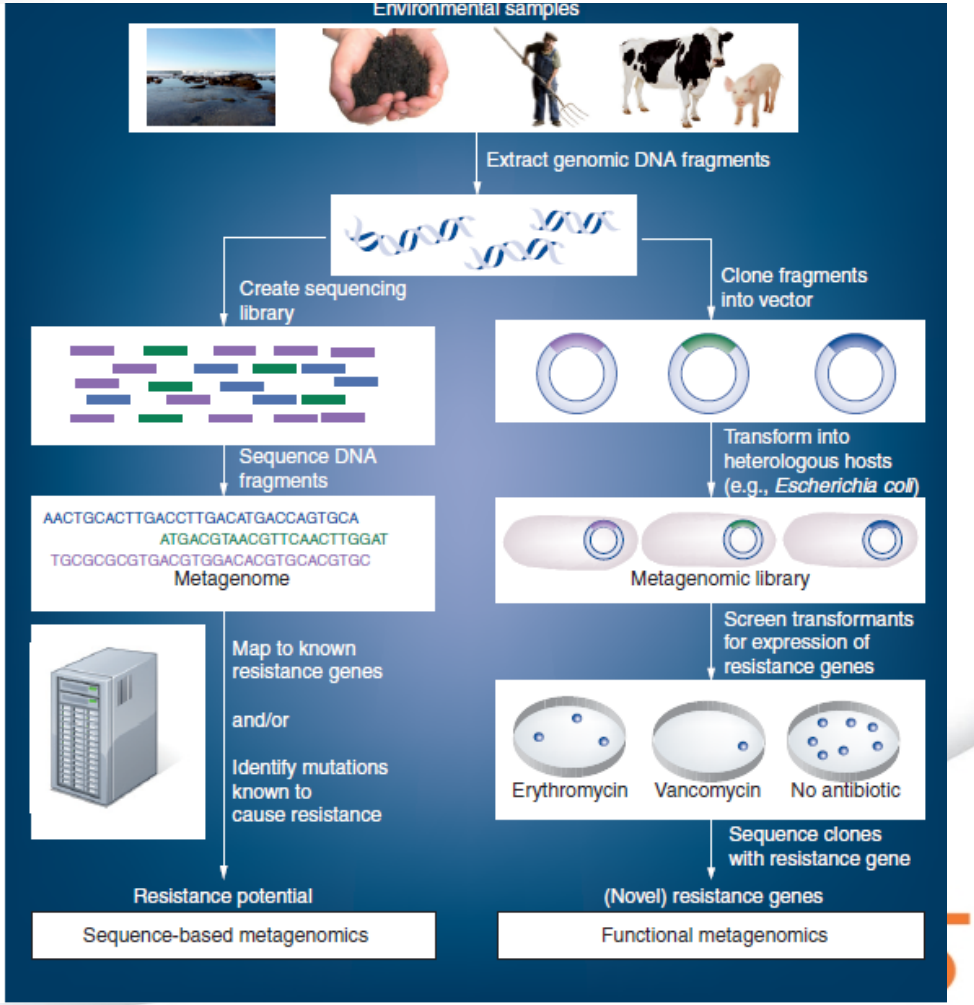
- MALDI-TOF MS vs conventional therapy (other studies)
  - Better antibiotic choice by ID physicians in patients with bacteremia (35.1 vs 20.8%)[1]
  - Time to optimal antibiotic therapy in patients with BSI from 80.9 hours to 23 hours (P<0.001) and decreased mortality rate from 21 to 8.9% (P=0.01)[2]
  - A. baumannii bacteremia/pneumonia: decrease time to appropriate antibiotic therapy from 77.7 hours to 36.6 hours (P=0.016) and increase in cure rate from 15% to 34% (P=0.016)[3]

[1]Clerc O. Clin Infect Dis 2013;56:1101-7
[2]Perez K. J Infect 2014;69:215-25
[3]Wenzler E. EECCMID Meeting Barcelona Spain 10-13 May 2014;eP484

Next-Generation Sequencing

NGS is able to read the genomic sequence of the organism.

- Increased sensitivity by identifying subpopulations
- Duration can occur within 48hrs



Next-Generation Sequencing

Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study

Simon R Harris*, Edward J P Cartwright*, M Estée Török, Matthew T G Holden, Nicholas M Brown, Amanda L Ogilvy-Stuart, Matthew J Ellington, Michael A Quail, Stephen D Bentley, Julian Parkhill†, Sharon J Peacock†

Lancet Infect Dis 2013; 13: 130–36
Published Online November 14, 2012
http://dx.doi.org/10.1016/S1473-3099(12)70268-2
See Comment page 99

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Correspondence to: Prof Sharon J Peacock, Department of Medicine,

Summary

Background The emergence of meticillin-resistant *Staphylococcus aureus* (MRSA) that can persist in the community and replace existing hospital-adapted lineages of MRSA means that it is necessary to understand transmission dynamics in terms of hospitals and the community as one entity. We assessed the use of whole-genome sequencing to enhance detection of MRSA transmission between these settings.

Methods We studied a putative MRSA outbreak on a special care baby unit (SCBU) at a National Health Service Foundation Trust in Cambridge, UK. We used whole-genome sequencing to validate and expand findings from an infection-control team who assessed the outbreak through conventional analysis of epidemiological data and antibiogram profiles. We sequenced isolates from all colonised patients in the SCBU, and sequenced MRSA isolates from patients in the hospital or community with the same antibiotic susceptibility profile as the outbreak strain.

Findings The hospital infection-control team identified 12 infants colonised with MRSA in a 6 month period in 2011, who were suspected of being linked, but a persistent outbreak could not be confirmed with conventional methods. With whole-genome sequencing, we identified 26 related cases of MRSA carriage, and showed transmission occurred within the SCBU, between mothers on a postnatal ward, and in the community. The outbreak MRSA type was a new sequence type (ST) 2371, which is closely related to ST22, but contains genes encoding Panton-Valentine leucocidin. Whole-genome sequencing data were used to propose and confirm that MRSA carriage by a staff member had allowed the outbreak to persist during periods without known infection on the SCBU and after a deep clean.

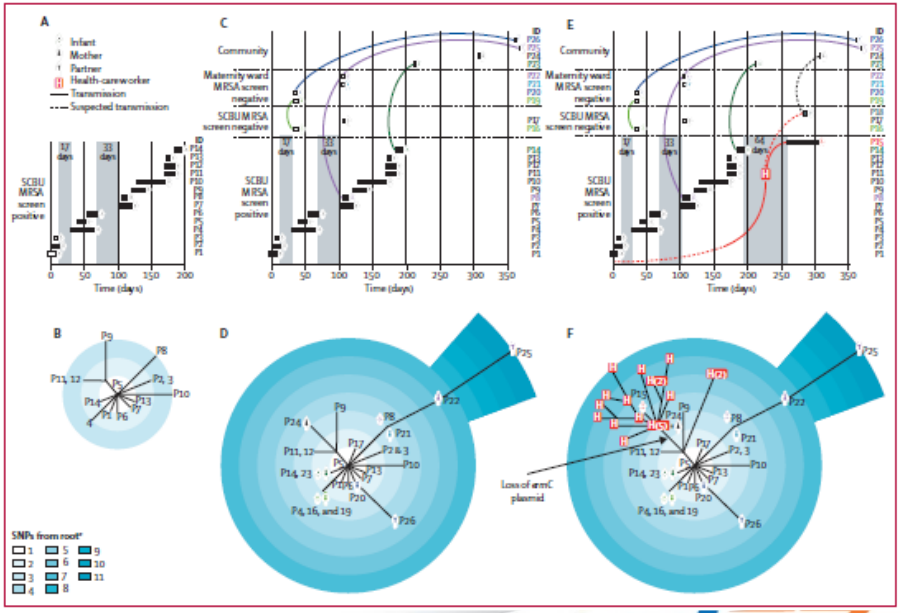
Interpretation Whole-genome sequencing holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

Funding UK Clinical Research Collaboration Translational Infection Research Initiative, Wellcome Trust, Health Protection Agency, and the National Institute for Health Research Cambridge Biomedical Research Centre.

Introduction

Successful prevention of health-care-associated meticillin-resistant *Staphylococcus aureus* (MRSA) depends on effective programmes of infection control, including detection of transmission events and outbreaks regarded as separate entities. This distinction is problematic, because effective linkage between the two settings is challenging for present strategies used for hospital-based infection control.

One approach to tracking of MRSA transmission



Harris T. Lancet Infect Dis 2017;13(2): 130 - 136

ashp CELEBRATING 15 YEARS

Next-Generation Sequencing

- Illumina (Solexa), Ion Torrent semiconducting sequencing (Thermo), Pacific Biosciences (PacBio)
  - Massive parallel through the use of beads or flow cell
  - pH Technology for sequencing
  - SMRT (Single molecule real time sequencing)- Long reads (over a1000bps); Using uninterrupted template-directed synthesis
- Instead of 96 or 384 reactions, NGS can have 500,000 to **over 1,000,000 reads**
- **Advantages:** Increased depth of coverage, high sensitivity, low cost (per read), specialized sequencing software, and high throughput
- **Disadvantages**: Supplies can be expensive, high equipment acquisition cost, homopolymers (artifacts from the system), and highly-trained technician.

Next-Generation Sequencing

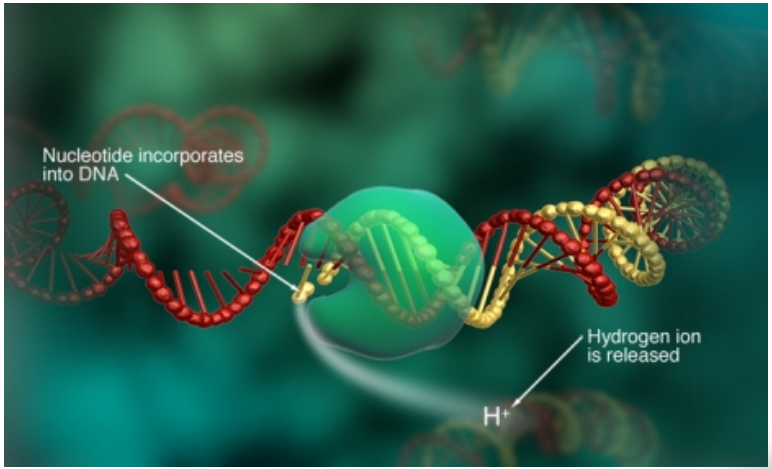
Illumina (Solexa)

- 30-300 bps
- Longer time to process (~2 days)
- Most common NGS instrument
- Very “hands off”
- Cheap for amount of information; a few cents for each nucleotide
- Requires a large amount of DNA

Next-Generation Sequencing

When a nucleotide is incorporated into the strand, a hydrogen ion is released.

- 300-500 bps
- Homopolymers artifacts
- Technician needs to be involved in the process (labor intensive)
- Equipment is less expensive ($50,000 for the instrument)
- Cheaper for supplies (large amount of data for a smaller cost)



https://i.ytimg.com/vi/ZL7DXFPz8rU/hqdefault.jpg

Next Generation Sequencing

- Monitoring patients who are infected with HIV
  - Sentosa® SQ HIV-1 Genotyping Assay
- Process time - 27 hrs.
- Multiple samples at a time
- Higher detection than Sanger sequencing.
  - Overall mutation detection rates aggregated were 98.74% for the NGS assay and 79.5% for the TruGene kit

End of Session Assessment

- Which of the following types of organisms is most unlikely to be detected and/or correctly identified by MALDI-TOF?
  - a. Clostridium spp
  - b. P. aeruginosa
  - c. Shigella spp
  - d. C. glabrata

End of Session Assessment

- Which of the following is a common pitfall of all genetic/molecular testing platforms?
  - A. Lack of identification of an organism on a species level
  - B. Lack of identification of an organism on a genus level
  - C. Lack of identification of resistance pattern of an organism
  - D. Long processing time resulting in delay in appropriate therapy

End of Session Assessment

- Which of the following platforms is most likely to identify an unknown organism or an organism with a genetic shift?
  - A. Multiplex PCR
  - B. PNA-FISH
  - C. LAMP
  - D. MALDI TOF

End of Session Assessment

- Which of the following is the most important aspect of incorporating a genetic molecular test into practice to ensure its success
  - A. Provider education on how to order the test
  - B. Rapid entering of the results into the patient record
  - C. Intervention of ASP on results and communicating recommendations to providers
  - D. Restricting access to such tests to limit costs

Key Takeaways

- Key Takeaway #1
  - All molecular tests save time. It is the responsibility of the ASP to communicate results and recommendations in a timely manner
- Key Takeaway #2
  - No genetic/molecular test is perfect. Study the test your hospital uses
- Key Takeaway #3
  - Each molecular test have its benefits but all still require the use of conventional C&S testing

THANK YOU FOR YOUR ATTENTION AND PARTICIPATION

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