Molecular Diagnostics and it's Role in Infection Prevention

Sanchita Das, M.D. D(ABMM)
Clinical Assistant Professor
Department of Pathology and Laboratory Medicine
NorthShore University Health System

Hosted by Paul Webber
paul@webbertraining.com

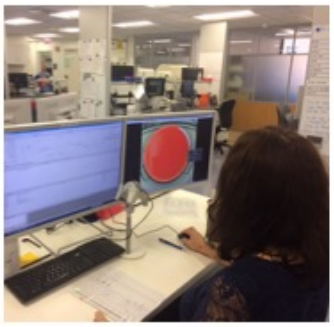
A Teaching Affiliate of THE UNIVERSITY OF CHICAGO PRITZKER SCHOOL OF MEDICINE

NorthShore University HealthSystem

The Changing Laboratory

Late 1800's

Early 2000's



2013 ⟶

Theparee et al. 2018. Total Laboratory Automation and MALDI-TOF Improve Turn-Around-Times in the Clinical Microbiology Laboratory: A Retrospective Analysis. J. Clin. Microbiol. 56:e01242-17.

NorthShore University HealthSystem

3

The Challenging Laboratory

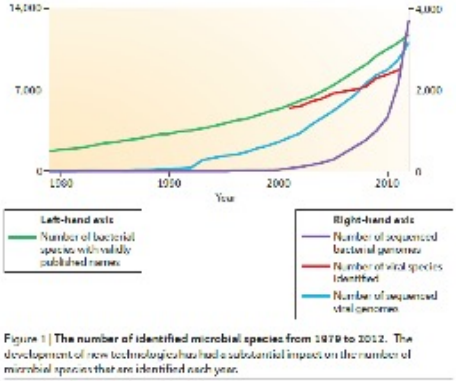
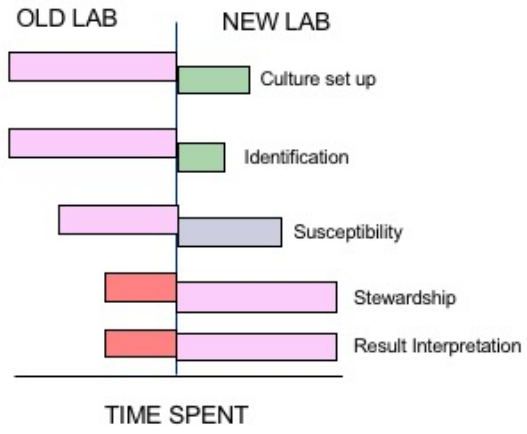


Figure 1 | **The number of identified microbial species from 1979 to 2012.** The development of new technologies has had a substantial impact on the number of microbial species that are identified each year.

Fournier et al. 2013. Nature Reviews Microbiology



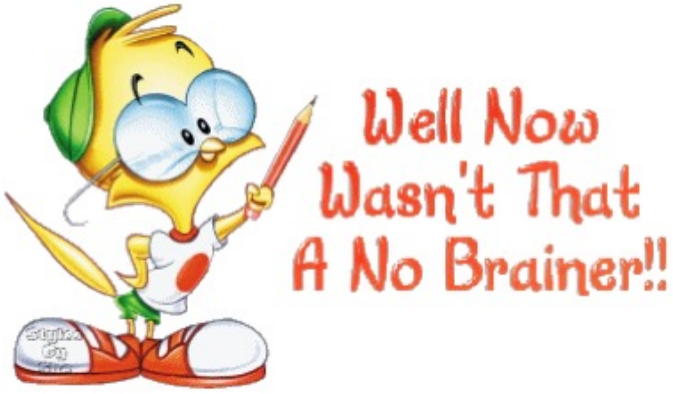
4

Use of Molecular Assays in Infection Prevention

- Early diagnosis that can impact management
- Antimicrobial stewardship
- Screening and surveillance for Infection Prevention
- Choice of testing algorithms and platforms that improve infection control
- Implementation of new technologies (NGS): are we there yet?

5

Early Diagnosis?



NorthShore University HealthSystem

6

Case

Lung Abscess in a Young Adult

- 37YO female former smoker presents on 11/17 with hemoptysis for 1 month
- Undergoing outpatient management of lung abscess
- Initially started on augmentin, later switched to clindamycin
- Bilateral chest pains worse with deep inspiration
- Rare coughing
- Generalized malaise, weakness and occasional nausea
- Underwent bronchoscopy on 10/27

NorthShore University HealthSystem

7

Bronchoscopy Lab Results (10/27)

Culture Results:
Streptococcus mitis group
1+ rare
Streptococcus salivarius group
1+ rare
Rothia mucilaginosa
1+ rare

Susceptibility Testing:

	R. mucilaginosa	S. mitis	S. salivarius
Ceftriaxone	S	S	R
Clindamycin	R	S	S
Erythromycin	S	R	R
Penicillin G	S	I	R
Tetracycline	S		
Vancomycin	S		
Trimeth/Sulfa	S	S	S

Cytopathology

FINAL DIAGNOSIS:

Lung, right lower lobe (superior segment), transbronchial biopsy
- Non-necrotizing granulomatous inflammation.
- Acute and chronic inflammation.
- No dysplasia or malignancy present.

NorthShore University HealthSystem

8

Admission and Subsequent Management

- Admitted, started to vancomycin and piperacillin/tazobactam
- ID and Pulmonology consulted
- Chest Xray findings:
  - Large right perihilar mass similar in size to previous study. Left apical consolidation that may be due to pneumonia is smaller but still present. Heart and pulmonary vessels are normal in size. No pleural effusion.

NorthShore University HealthSystem

9

Other Lab Results and Clinical Impression

- Fungal and vasculitis studies negative
- TB qunatiferon gold negative
- Non-TB mycobacteria possible
- Vancomycin intolerant
- Changed to linezolid and ertapenem

Lung abscess not responding to antibiotics
Recommend surgery

NorthShore University HealthSystem

10

VATS Laboratory Results (11/22)

DIRECT SMEAR

Smear Result: Positive Acid fast bacilli 3+(10-90/field)

HISTOPATHOLOGY

Right lower lobe of lung, wedge resection:
- Necrotizing granulomatous inflammation. See comment and laboratory data.

COMMENT:

Sections taken from the right lower lobe of lung confirm the presence of the necrotizing granulomatous inflammatory process. An AFB stain is positive for numerous acid fast bacteria. Cultures were obtained from the lung parenchyma and AFB stains on that tissue are also positive. A Silver methenamine stain is negative for fungal elements.

TBPCR POSITIVE

NorthShore University HealthSystem

11

Culture and Susceptibility (12/28)

Culture Results:
M. tuberculosis complex

Susceptibility Testing:

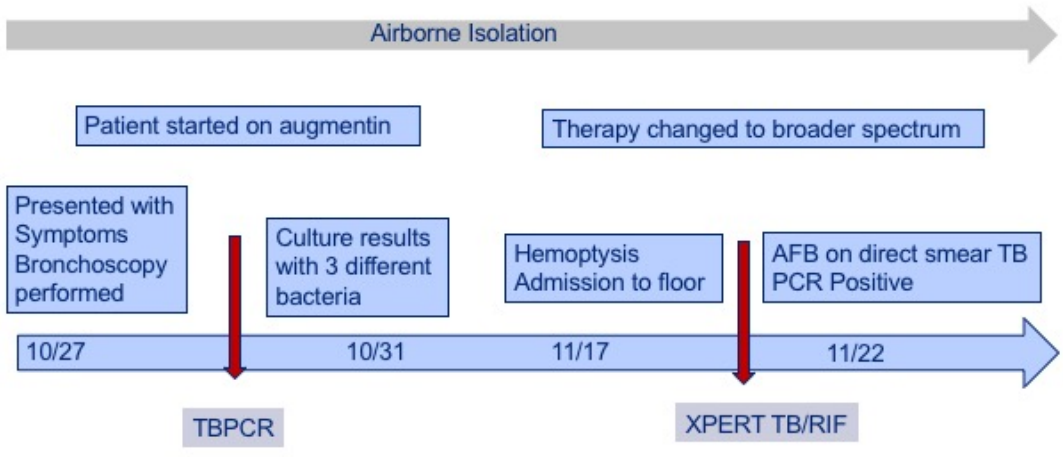
	M. tuberculosis
Capreomycin	S
Ciprofloxacin	S
Ethambutol	R
INH (L&H)	R
Kanamycin	S
Ofloxacin	S
Pyrazinamide	S
Rifampin	R
Streptomycin	R
Ethionamide	R

MDRTB!
Total time to Identification and Susceptibility
~2 months

NorthShore University HealthSystem

12

Timeline of Diagnosis and Management



NorthShore University HealthSystem

13

WHO-RECOMMENDED DIAGNOSTIC TOOLS

RECOMMENDED FOR USE (detailed policy guidance: http://www.who.int/tb/areas-of-work/laboratory/policy_statements/en)

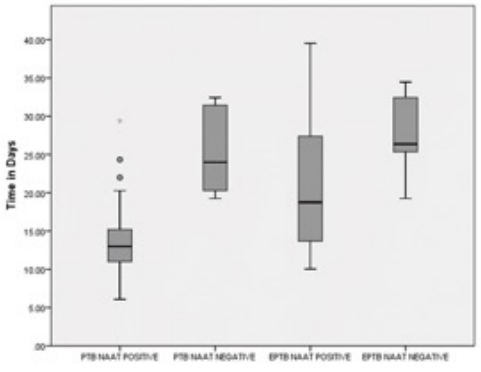
- **LED microscopy:** For use at all laboratory levels as replacement of conventional fluorochrome and light microscopy.
- **Commercial liquid culture and DST systems**: For use at central/regional reference laboratory level, as current reference standard.
- **Rapid speciation strip technology**: For use with conventional culture and DST at central/regional reference laboratory level, to identify *Mycobacterium tuberculosis*.
- **Automated real-time nucleic acid amplification - Xpert MTB/RIF system**: For rapid detection of pulmonary and extrapulmonary TB and rifampicin resistance in both adults and children at decentralised laboratory and health care centres.
- **Lateral flow urine lipoarabinomannan (LF-LAM) assay** may be used to assist in the diagnosis of TB in HIV positive patients with signs and symptoms of TB (pulmonary and/or extrapulmonary) who have a CD4 cell count less than or equal to 100 cells/μL, or HIV positive patients who are seriously ill regardless of CD4 count or with unknown CD4 count.
- **Loop-mediated isothermal amplification test kit for TB (TB-LAMP) -** manual molecular assay to replace microscopy to diagnose TB in settings where automated molecular tests cannot be used.
- **Line probe assay (LPA)** as a rapid diagnostic test for detection of rifampicin and isoniazid resistance. The WHO recommended commercially available tests include GenoType MTBDRplus VER 1 and 2 (Hain Lifescience, Germany), Nipro NTM+MDRTB detection kit 2 (Nipro, Japan). Suitable for use on smear-positive specimens or culture isolates.
- **Second-line line probe assay (SL-LPA)** as a rapid diagnostic test in patients with confirmed rifampicin-resistant TB or MDR-TB to detect resistance to fluoroquinolones and the second-line injectable drugs.

From: www.who.int/tb Factsheet TB diagnosis

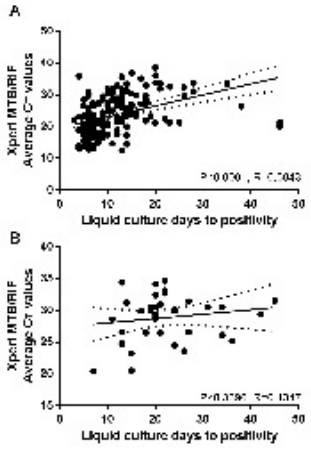
NorthShore University HealthSystem

14

Advantages of Molecular Diagnosis: TB



Das, S. Abstract ID week 2017



Theron, G. Scientific Reports 2014

NorthShore University HealthSystem

15

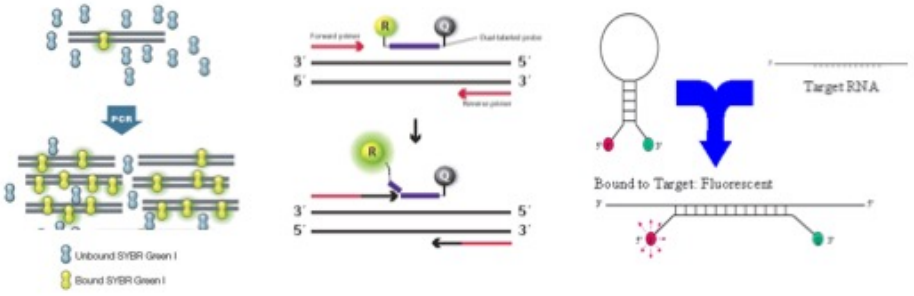
Molecular Diagnosis of TB in Resource Limited Areas

- Advantages:
  - Rapidity of result delivery
  - Standardization of assay techniques
  - Potential for high throughput
  - Reduced requirements for biosafety
  - Sensitivity as high as 95% for some platforms
- Disadvantages:
  - Calibration
  - Instrumentation
  - Constant electric supply

NorthShore University HealthSystem

16

Real-Time PCR Assay Technologies: RECAP



Advantages

- Minimizes cross-contamination
- Automated, low-complexity
- Quantitative
- High sensitivity

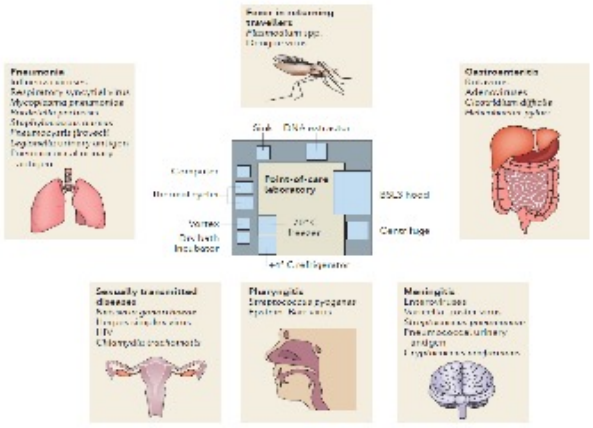
Disadvantages

- Known pathogens only
- Only 2-6 plex
- Primer design: challenging for melt-curve
- Fluorescent-labeled primers: expensive

NorthShore University HealthSystem

17

Syndromic Approach to Diagnostics



Fournier et al. Modern Clinical Microbiology Nature Reviews Microbiology 2013. 11:574.

18

The GI Panels

TABLE 1 Targets included on three commercial, FDA-cleared, multiplex assays for the detection of gastrointestinal pathogens

	Multiplex panel[a]		
Target[a]	Verigene EP	FilmArray GI	xTAG GPP
Aeromonas		IUO[c]	
Campylobacter	✓	✓	✓
Clostridium difficile (toxin A/B)		✓	✓
Plesiomonas shigelloides		✓	
Salmonella	✓	✓	✓
Yersinia enterocolitica	✓	✓	IUO[c]
Vibrio spp.	✓	✓	✓
EAEC		✓	
EPEC		✓	
ETEC		✓	✓
STEC (stx_1 and stx_2)	✓[c]	✓	✓
E. coli O157		✓	✓
EIEC/*Shigella*	✓	✓	✓
Cryptosporidium		✓	✓
Cyclospora cayetanensis		✓	
Entamoeba histolytica		✓	✓
Giardia lamblia		✓	✓
Adenovirus 40/41		✓	✓
Norovirus GI/GII	✓	✓	✓
Rotavirus A	✓	✓	✓
Sapovirus		✓	
Astrovirus		✓	

TABLE 2 Comparison of features between three commercial, FDA-cleared, multiplex platforms for the detection of gastrointestinal pathogens

	Platform[a]		
Feature	Verigene EP	FilmArray GI	xTAG GPP
No. of FDA-cleared targets	9	22	14
Processing (hands-on) time per run, min	<5	2	45
Separate extraction required?	No	No	Yes (~45 min)
Time/run, h	~2	~1	~5
Throughput, specimens/run	1[b]	1[b]	96
Technology	PCR + gold nanoparticle hybridization	Nested PCR + melting curve	PCR + xTag (fluorescent bead-based detection)
Open or closed system	Closed	Closed	Open
Footprint	Small to moderate	Small	Moderate
List price per instrument, $	40,000[c]	39,500	37,000
List price reagent cost per specimen, $	80[d]	155[d]	80–90[d]

Binnicker et al. J. Clin. Microbiol. December 2015

19

The Problem with Diarrhea

- *Issues with lab diagnosis*
  - Wide range of pathogens causing different types of diarrhea
  - Childhood diarrhea different from adult
  - A multitude of techniques needed for diagnosis
    - » ELISA for toxins
    - » Fluorescent microscopy for parasites
  - Global problem with varying etiology
- *Solution?*
  - Antimicrobial susceptibility and public health impact?
  - False positives with *Vibrio and Entamoeba*
  - Reimbursement: Do all patients need to get all 17 targets?
  - What will we miss if we move solely to panel based testing? (*Aeromonas)*

NorthShore University HealthSystem

20

The Problem with Gold Standards: STI

- PCR found to be 20-50% more sensitive for diagnosis of chlamydial infections than traditional culture methods
- Comparing results to an imperfect method produces biased sensitivity and specificity
- Prevalence being constant, sensitivity of older assays will be overestimated if standard tests are suboptimal
- Comparison with comparable technology
- Caveats?

NorthShore University HealthSystem

21

Impact of Commensal Flora

Assay Name	Assay format	Stability of specimen at 2-30°C	Nucleic acid target	Comments
Abbott RealTime CT/NG (Abbott Laboratories, Abbott Park, IL)	Molecular beacon probe	14 days	Specific sequence within the C. trachomatis cryptic plasmid opa gene of N. gonorrhoeae.	Does not detect plasmid free C. trachomatis Commensal Neisseria species does not cause false positive reaction
Xpert CT/NG assay (Cepheid, Sunnyvale, CA)	Multiplexed Taqman assay	Variable depending on specimen type (8 hours to 45 days)		Detects plasmid free C. trachomatis Commensal Neisseria species does not cause false positive reaction
cobas CT/NG test (Roche Diagnostics, Indianapolis, IN)	Dual probe Taqman assay	Variable depending on specimen type (up to 1 year)	Two distinct genes one within the chromosome and other in the cryptic plasmid A highly conserved direct repeat region of N. gonorrhoeae called DR-9	Detects plasmid free C. trachomatis Commensal Neisseria species does not cause false positive reaction*
Aptima COMBO 2 assay Aptima CT assay Aptima GC assay (Hologic/Gen-Probe Inc., San Diego, CA	Multiplexed Taqman assay	24 hours, 30-60 days if using transport cup	23S rRNA and 16S rRNA gene of C. trachomatis 16S rRNA gene of N. gonorrhoeae	Detects plasmid free and new variant C. trachomatis Commensal Neisseria species does not cause false positive reaction
BD ProbeTec ET CT/GC Amplified DNA assay (Becton Dickinson and Company, Sparks, MD)	Transcription- Mediated Amplification, and Dual Kinetic Assay	30 hours If urine transport cup used up to 30 days		Does not detect plasmid free C. trachomatis N. cinerea and N. lactamica might cause false-positive test results

- Extra-genital *Chlamydia* and *Neisseria:* Impact the detection in pharyngeal rectal specimens impacts MSM, minors where detection in extra genital sites of importance

NorthShore University HealthSystem

22

Respiratory Viral Panels

Assay name	Assay format/CLIA waived	Specimen	Extraction of nucleic acids required	Sensitivity/specificity	Pathogens detected
ProFAST®+Assay (Prodesse®, Gen-Probe/Hologic)	Multiplex real-time reverse transcription PCR	NP swabs	Yes	94-99%	Depends on the panel used
FilmArray (BioFire)	Real-time PCR with melt curve analysis	NP swabs, respiratory secretions	No	84.5-100%	AdV; CoV HKU1, NL63; influenza virus A/B with typing; hMPV; PIV1, -2, -3, -4; RSV; RhV/EV, CoV HKU1 and NL63, Bordetella pertussis, Chlamydophila pneumoniae, and Mycoplasma pneumoniae
Xpert flu (Cepheid)	Multiplex real-time reverse transcription PCR assay CLIA waived (Y)	NP swabs/Nasal aspirate	No	98-100%	Influenza A (H1), Influenza A (2009 H1), Influenza B
Simplexa Flu A/B & RSV kit (Diasorin)	Multiplex real-time reverse transcription PCR	NP swabs	Yes	95%/99%	Influenza A/B and RSV
xTAG/xTAG FAST® (Luminex Corp.)	PCR, hybridization to fluorescent bead based liquid array	NP swabs,	Yes	97-100%	AdV; influenza virus A (H1, H3); influenza virus B; MPV; PIV1, -2, -3; RSV (A/B); RhV/EV
eSensor® Respiratory Viral Panel (GenMark)	PCR followed by hybridization and electrochemical detection	NP swabs, respiratory secretions	Yes	98-99%	Influenza A(H1, 2009 H1, H3), Influenza B, Parainfluenza, RSV, human metapneumovirus, Rhinovirus and adenovirus
Verigene RV Plus (Nanosphere)	Multiplex reverse transcription PCR followed by gold nanoparticle hybridization assay	NP swabs	No	96-100%	Influenza A(H1, 2009 H1, H3), Influenza B RSV,
Verigene RV flex (Nanosphere)		NP swabs	No	NA	Influenza virus A/B with typing; hMPV; PIV1, -2, -3, -4; RSV; RhV, , Adenovirus
Alere (Abbott)	isothermal PCR assay	NP and nasal swabs	No	88-100%	Influenza A/B
LIAT (ROCHE)	Multiplex real-time reverse transcription PCR assay CLIA waived (Y)	NP Swabs	No	98-100%	Influenza A/B and RSV

23

Elderly Gentleman with Pneumonia?

- 85YO male in *clinic* on **13th January** with moderate productive cough for 1 day, fever up to a 100˚F
- Chest X-ray ordered, patient prescribed cefpodoxime and advised to come back if worse
- *Admitted* on 14th of January
  - Fever of 101.5, congestion, generalized weakness
  - Concern for pneumonia
  - Antibiotics: vancomycin and zosyn continued
  - Blood culture and NP swab for respiratory virus were sent

NorthShore University HealthSystem

24

Acute Exacerbation of Asthma?

- 65YO female with asthmatic bronchitis presents *to ED* on **25th January** with complaints of 5 days of cough and shortness of breath
- Associated wheezing, nausea, vomiting, diarrhea and body ache
- Started on nebulizer and IV steroids-- minimal improvement; transferred to *ICU* for further management
- NP swab sent for respiratory viruses

NorthShore University HealthSystem

25

Fever in an Infant

- Otherwise healthy 12 month old female presents *to Urgent Care Center* on **February 6th** with fever since waking this morning
- No other symptoms
- Does not attend day care, no sick contacts at home
- Vaccinations up-to-date
- NP swab sent for respiratory viruses

NorthShore University HealthSystem

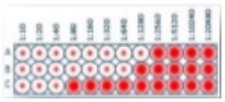
26

Laboratory Testing Viruses: The Past

Specimen

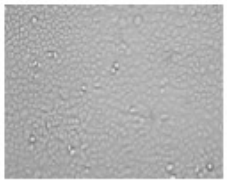
Chorioallantoic Membrane & Allantoic cavity

Harvest from infected egg ~1 week

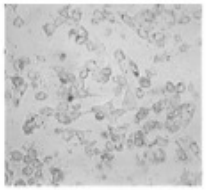


Hemagglutination/Hemagglutination inhibition

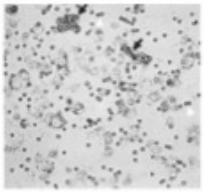
Viral Cell Culture Flask



Uninfected Cell Line



Viral Cytopathic Effect (~1 week)

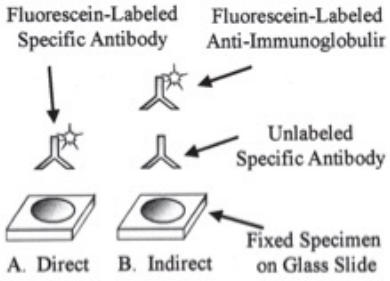


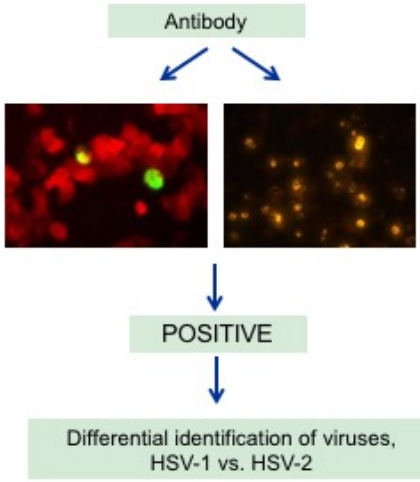
Hemadsorption to RBC for identification

NorthShore University HealthSystem

27

Direct Fluorescent Antibody

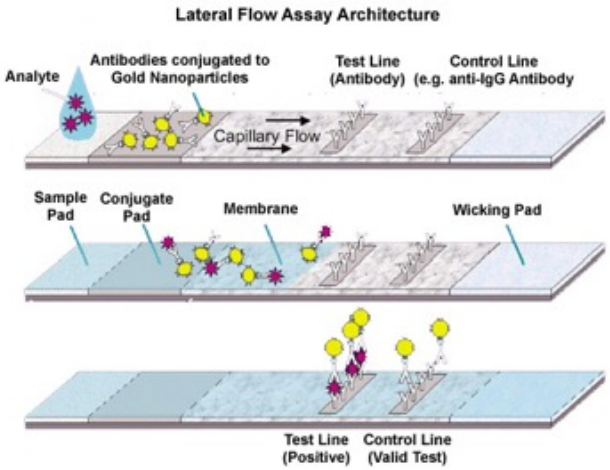


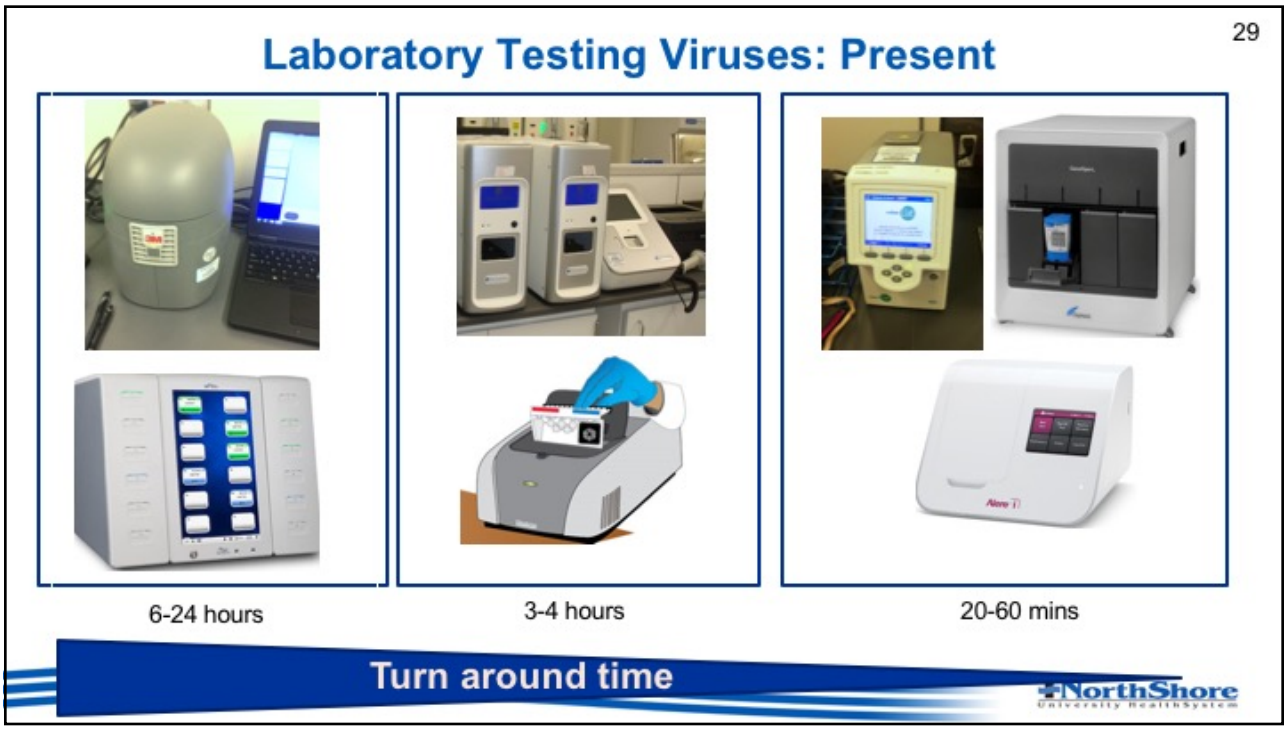


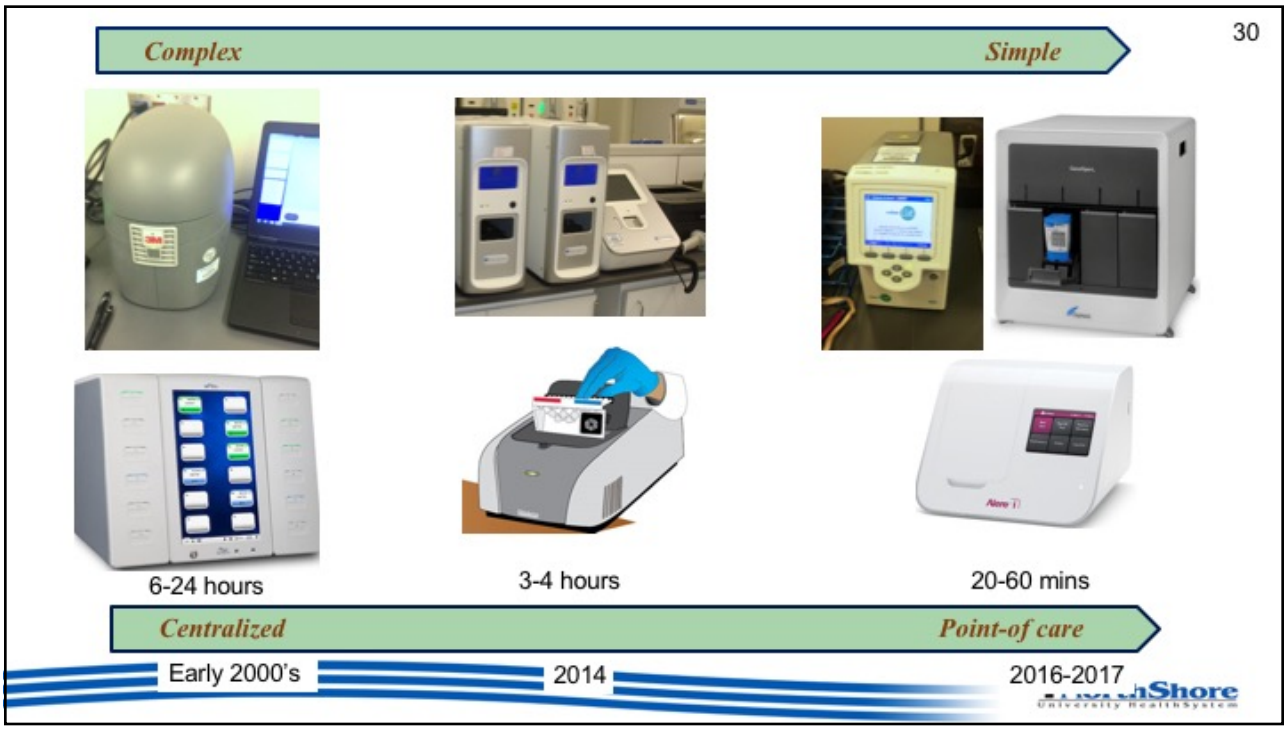
28

Rapid Antigen Immunoassay

Influenza A and RSV
Performance inferior to PCR




29
Laboratory Testing Viruses: Present
6-24 hours
3-4 hours
20-60 mins
Turn around time
NorthShore


30
Complex
Simple
6-24 hours
3-4 hours
20-60 mins
Centralized
Point-of care
Early 2000's
2014
2016-2017
NorthShore

31

Our Patients' Lab Results

Case 2

Elderly inpatient with CAP

Positive for Influenza A by PCR
Negative for Influenza B by PCR
Negative for RSV by PCR

Case 3

Asthmatic in ICU

Influenza A	Positive
Influenza A H1	Not Detected
Influenza A H3	Positive
Influenza B	Not Detected
Respiratory Syncytial virus (RSV) A	Not Detected
Respiratory Syncytial virus (RSV) B	Not Detected
Parainfluenza (PIV) 1	Not Detected
Parainfluenza (PIV) 2	Not Detected
Parainfluenza (PIV) 3	Not Detected
Parainfluenza (PIV) 4	Not Detected
Human Metapneumovirus (hMPV)	Not Detected
Human Rhinovirus (HRV)	Not Detected
Adenovirus	Not Detected

Case 4

Infant in ACC

Positive Influenza A detected
Negative Influenza B not detected

NorthShore University HealthSystem

32

Our Patients' Lab Results

Case 2

Elderly inpatient with CAP

Turn around time up to ~6h
$

Positive for Influenza A by PCR
Negative for Influenza B by PCR
Negative for RSV by PCR

Case 3

Asthmatic in ICU

Turn around time ~3-4h
$$$

Influenza A	Positive
Influenza A H1	Not Detected
Influenza A H3	Positive
Influenza B	Not Detected
Respiratory Syncytial virus (RSV) A	Not Detected
Respiratory Syncytial virus (RSV) B	Not Detected
Parainfluenza (PIV) 1	Not Detected
Parainfluenza (PIV) 2	Not Detected
Parainfluenza (PIV) 3	Not Detected
Parainfluenza (PIV) 4	Not Detected
Human Metapneumovirus (hMPV)	Not Detected
Human Rhinovirus (HRV)	Not Detected
Adenovirus	Not Detected

Case 4

Infant in ACC

Turn around time<1h
$$

Positive Influenza A detected
Negative Influenza B not detected

NorthShore University HealthSystem

33

Impact on Clinical Decision

Table 3
Impact of Rapid Influenza testing on clinical decision making in the ED for suspected Influenza Patients (n = 143)

Clinical Touchpoint	Total cases impacted (%) (+/-95% CI) (95/136)	% reduction in utilization/change in discharge	% increase in utilization or admission
Antimicrobial* prescribing total	56% (83/142)	10% (14/142) ⬇	15% (21/142) ⬆
Antibiotic prescribing	55.9% (78/139)	9% (13/144) ⬇	33.6% (48/143) ⬆
Antiviral prescribing	39.2% (56/143)	24.5% (35/142) ⬇	14.7% (21/143) ⬆
Medical Procedure/Imaging	15.4% (22/142)	2.1% (3/143) ⬇	13.2% (19/143) ⬆
Laboratory studies	14% (20/143)	3.5% (5/144) ⬇	11.1% (16/144) ⬆
Hospital Admission/Discharge	18% (26/142)	10.5% (15/142) ⬇	7.7% (11/143) ⬆

Based on total number of ED patients meeting all inclusion criteria (N = 143).
*P = .001, binomial one sample test where the null hypothesis was Proportion = 0.
† Antimicrobial prescribing refers to sum of antibiotic and antiviral prescribing.

Hansen GT. et al. 2018.Clinical decision making in the emergency department setting using rapid PCR: Results of the CLADE study group. J. Clin Virology; 102:42-49

NorthShore University HealthSystem

34

Impact on Clinical Decision: Outpatient Study

Test Result	Number	Prescribed Medications				*p*-value
		Antiviral*	Antibiotic**	None	Both	
LIAT (POC-PCR) – Site A						
Negative	128	3	57	68	0	*<0.0001
Positive (FluA)	63	47	2	9	5	
Positive (FluB)	51	38	3	6	9	
RADT (POC-Antigen) – Site B						
Negative	108	32	38	48	10	
Positive (FluA)	82	69	3	12	2	
Positive (FluB)	51	33	6	16	4	

Significant difference in antiviral prescription for patients with negative LIAT

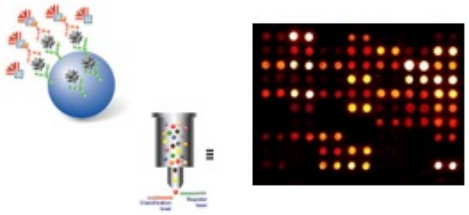
Benirschke et al.; Abstract: Clinical Virology Symposium 2018

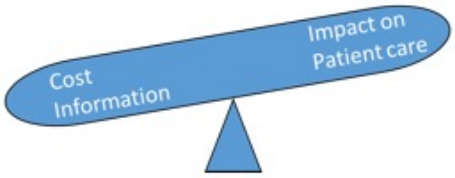
NorthShore University HealthSystem

35

Multiplexed PCR Assays: Syndromic approach

- Concept of one test for one diagnosis:
  - Pneumonia panel
  - Sepsis panel
  - Diarrhea panel
  - Meningitis panel
- Advanced technology:
  - Appeal of complete automated sample handling
  - Easy read-out and software for analysis
- Strategic combination of targets:
  - Short time for broad range of pathogens
  - Provides some anti-infective information





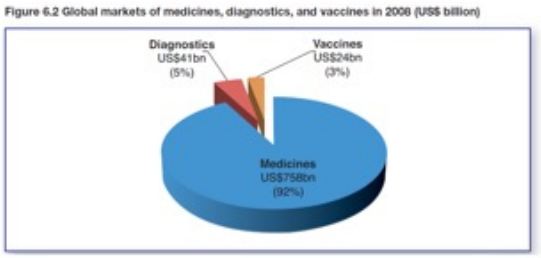
NorthShore University HealthSystem

36

Antimicrobial Stewardship: A lost Cause?

The Economist, March 31st, 2011

"There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria cannot react in some way acquiring 'fastness' [resistance]" ---- Alexander Fleming, 1946



WHO, April 2014

NorthShore University HealthSystem

37

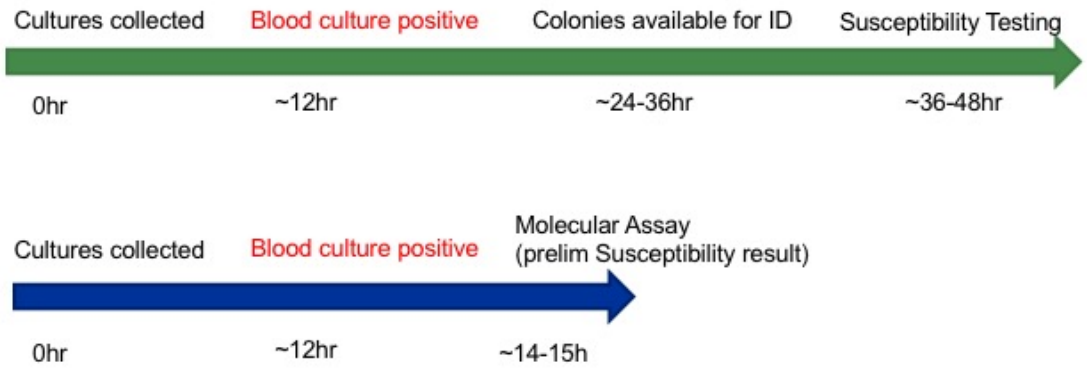
Elderly Gentleman with Sepsis

- 76 year old male from nursing home, Parkinson's disease
- On chronic Foley catheter, and failure to thrive
- Brought to ED for fever, low blood pressure
- Alert and oriented but hypotensive
- Laboratory Results
  - Serum lactate level: 6.3
  - WBC count: 30,000/ul
- Working Diagnosis: Sepsis, blood cultures sent and started on 3rd generation cephalosporin

NorthShore University HealthSystem

38

Use of Molecular Assays on Blood Culture



NorthShore University HealthSystem

39

Molecular Assays for Antimicrobial Stewardship

- Elderly sepsis patient
  - E. coli by molecular assay (14h after blood culture collection)
  - Prelim result CTXM positive
  - 3rd generation cephalosporin changed to carbapenem
    - » De-escalated to beta-lactamase inhibitor combination after full susceptibility

NorthShore University HealthSystem

40

Clinical Decision Support

Molecular Assay vs. Conventional Susceptibility:

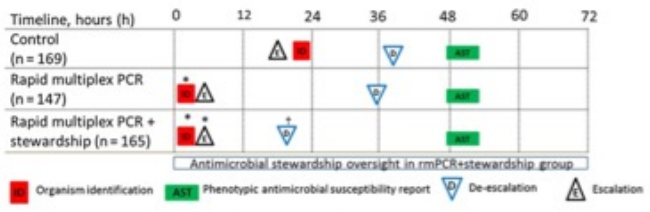
Negative Predictive Values for ceftriaxone susceptibility in *Escherichia coli* and *Klebsiella pneumoniae* in the absence of either CTX-M or a carbapenemase gene were 98% and 93 to 94%, respectively.

Similar results were seen with other target bug-drug scenarios, with NPVs of 94 to 100%

with the exception of *P. aeruginosa*, for which NPVs were poor, likely due to the more complex nature of resistance in this pathogen.

Pogue JM et al. Antimicrob Agents Chemother. 2018 Apr 26;62(5)

Molecular Assay vs. Conventional Susceptibility

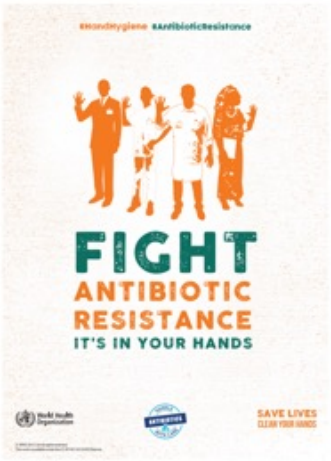


Banerjee R, et al. Clin Infect Dis. 2015 Oct 1; 61(7): 1071–1080.

NorthShore University HealthSystem

41

Infection Control Efforts and Surveillance



NorthShore University HealthSystem

42

MRSA: The Success Story

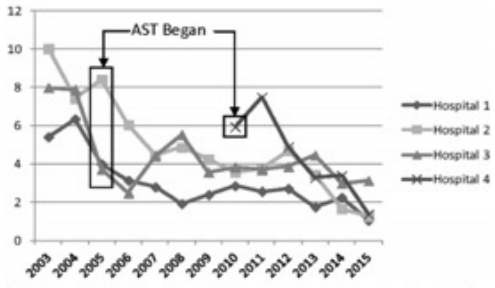


FIG 1 Rate of MRSA nosocomial infection (per 10,000 patient days). When calculated from baseline for each hospital, the aggregate *P* value was <0.001.

Peterson L, J. Clin. Microbiol. November 2016 Volume 54

Impact of active screening and decolonization in ICU

- MRSA infection decreased from 3.58 to 0.42% (*p<0.05)*
- Interruption of active surveillance and decolonization led to resurgence of infection rates up to 2.21%
- Reintroduction of intervention reduced in-hospital MRSA infection rates to 0.18%
- Decolonization and active surveillance saved $22 for each $1 spent on the intervention

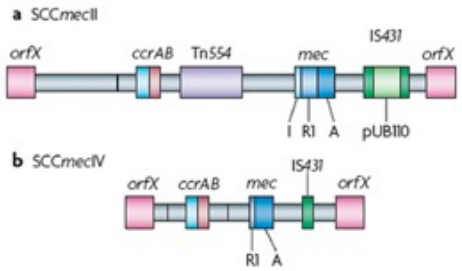
Lee et al. Critical Care (2015) 19:143

NorthShore University HealthSystem

43

MRSA: The Success Story

- What qualities ensure success of MRSA screening test?
  - PCR test with quick result makes intervention simple and impact significant
  - Nasal swabs simple to collect and easy to test (not messy)
  - Site specific insertion of resistance gene, easy PCR design
  - Buy in from hospital and public health authorities



NorthShore University HealthSystem

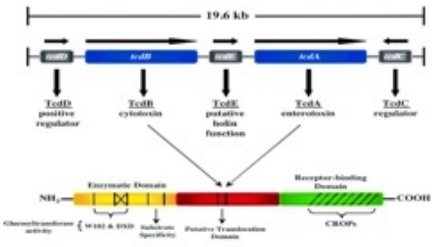
44

C. difficile: The failure

Published in final edited form as:
JAMA Intern Med. 2015 November ; 175(11): 1792–1801. doi:10.1001/jamainternmed.2015.4114.

Overdiagnosis of *Clostridium difficile* Infection in the Molecular Test Era

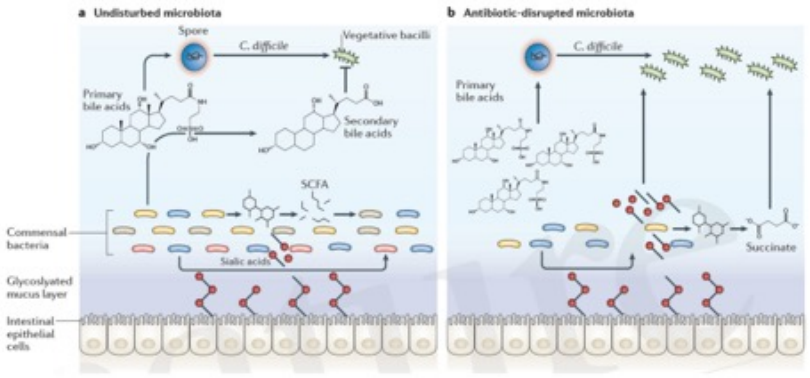
Christopher R. Polage, MD, MAS, Clare E. Gyorke, BS, Michael A. Kennedy, BS, Jhansi L. Leslie, BS, David L. Chin, PhD, Susan Wang, BS, Hien H. Nguyen, MD, MAS, Bin Huang, MD, PhD, Yi-Wei Tang, MD, PhD, Lenora W. Lee, MD, Kyoungmi Kim, PhD, Sandra Taylor,



NorthShore University HealthSystem

45

The difference is in the disease!



Abt MC, et al. *Nature reviews Microbiology*. 2016;14(10):609-620. doi:10.1038/nrmicro.2016.108.

NorthShore University HealthSystem

46

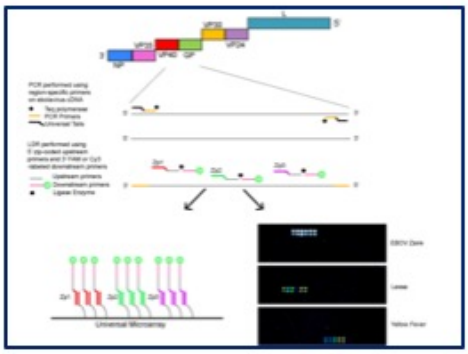
The Art of Assay Design: *Un*limited?

- Ideal Molecular Assay: Key Challenges
  - Rapid with relatively low technical complexity
  - **Minimal handling of samples**
  - **High throughput low turnaround time**
  - **A strategic combination of molecular targets based on specimen type**
  - Multiplexing: a panel for a syndrome
  - Guide to anti-infective therapy
  - Cost effective
  - Easy readout, software for analysis

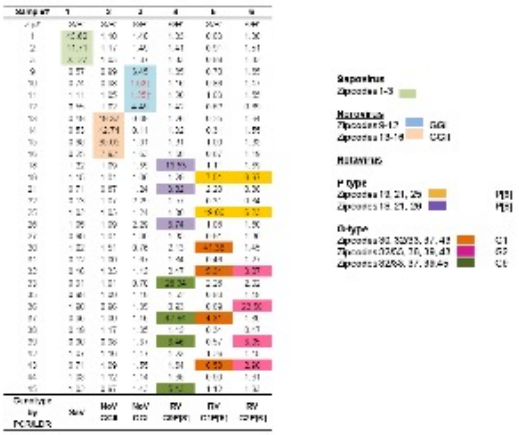
NorthShore University HealthSystem

47

The Art of Assay Design: *Un*limited?



Das. S, et al.A Multiplex PCR/LDR Assay for the Simultaneous Identification of Category A Infectious Pathogens: Agents of Viral Hemorrhagic Fever and Variola Virus. PLoS One, 10(9):e0138484



Mirza A., Das. S, et al. A Multiplex PCR/LDR Assay for Viral Agents of Diarrhea with the Capacity to Genotype Rotavirus. *In Press*, Scientific Reports (Nature), Accepted July 2018

NorthShore University HealthSystem

48

Fever Spikes….Again?!!!

Case

Present Illness
- 48y/o female presents with pyelonephritis
- Nephrostomy tubes placed
- Drained fluid sent for culture
- Cultures negative
- Sent home on p/o antibiotics

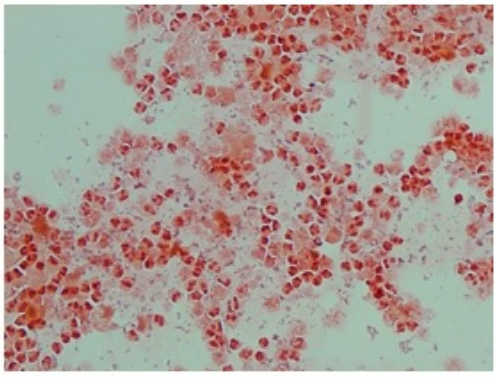
Disease Course
- Comes back to ED in a few hours for fever and pain
- High WBC count 16000
- Fever up to a 103
- Blood, urine and drain fluid sent for culture
- Rocephin and Vanco started
- All cultures negative

Management
- Continues to be febrile to 102.6
- Pain at site of nephrostomy and back
- Nephrostomy tube draining well
- WBC 15.2
- CRP 256

NorthShore University HealthSystem

49

Gram Stain and Culture of Nephrostomy Drainage



4+ WBCs (PMNs)
No organisms seen

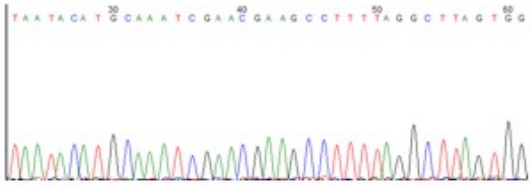
Culture Results:
Aerobic and anaerobic culture

No organism isolated

NorthShore University HealthSystem

50

The Panbacterial Target:16SrRNA Gene



Culture Results:
Aerobic and anaerobic culture

No organism isolated
Ureaplasma species. Isolate Comment: Detected by 16S rRNA gene sequencing. Results discussed with ID physician

Description	Max score	Total score	Query cover	E value	Ident	Accession
Ureaplasma urealyticum serovar 8 strain ATCC 27618 16S ribosomal RNA gene, partial sequence	898	898	98%	0.0	99%	NR_041710.1
Ureaplasma parvum strain ATCC 27815 16S ribosomal RNA, complete sequence	863	863	98%	0.0	98%	NR_074762.2

NorthShore University HealthSystem

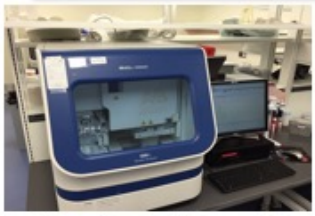
51

The Future: New Technology?

NorthShore University HealthSystem

52

Next Generation Sequencing: The Future?



Genetic analyzer: Sanger Sequencing

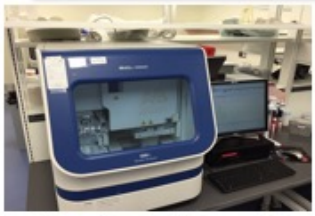
Palm top whole genome sequencer

- Ribosomal RNA gene sequence is used for ID
- Sequence information of whole genome
- Known pathogens and novel organisms
- Identify mutations for virulence and resistance
- Trace spread of pathogens

NorthShore University HealthSystem

53

Next Generation Sequencing: The Future?



Genetic analyzer: Sanger Sequencing

Palm top whole genome sequencer

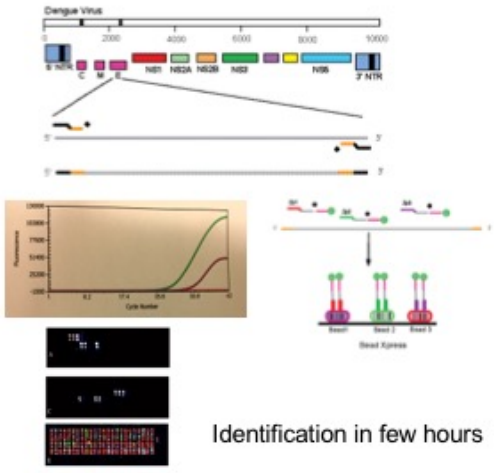
- Ribosomal RNA gene sequence is used for ID
- Sequence information of whole genome
- Known pathogens and novel organisms
- Identify mutations for virulence and resistance
- Trace spread of pathogens

- Reduction in running cost
- Clinical interpretation of data
- Streamlining bioinformatics and data analysis

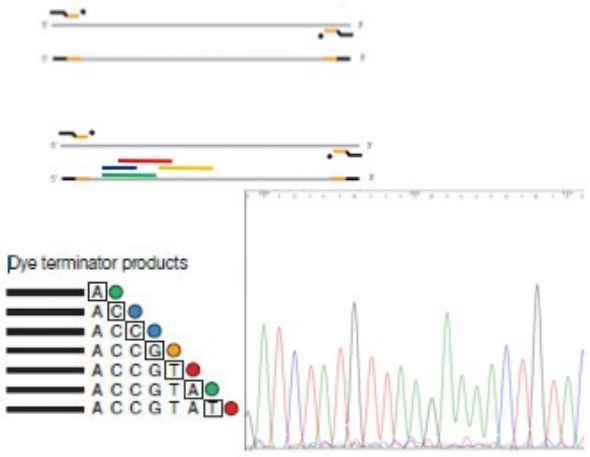
NorthShore University HealthSystem

54

Targeted PCR vs. Sanger Sequencing



Identification in few hours



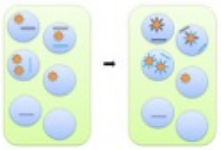
NorthShore University HealthSystem

55

Next Generation Sequencing: Workflow

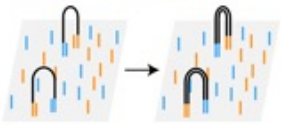
- Template Preparation
  - Randomly break into fragments
  - Templates need to be amplified

EmPCR



100-200 million beads

Bridge PCR



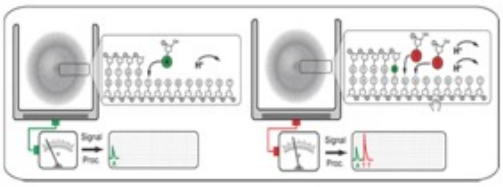
Billion primed single molecule target

- Sequencing and Imaging
  - Reversible termination with fluorescent dyes
  - Ligation with fluorescent dyes
  - Semiconductor that measures pH when base added
  - Nanopore measures changes in electrical impulse
- Data Analysis
  - Align sequence to a reference database OR assemble *de novo*

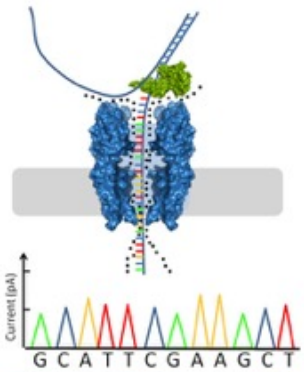
NorthShore University HealthSystem

56

Next Generation Sequencing: Workflow



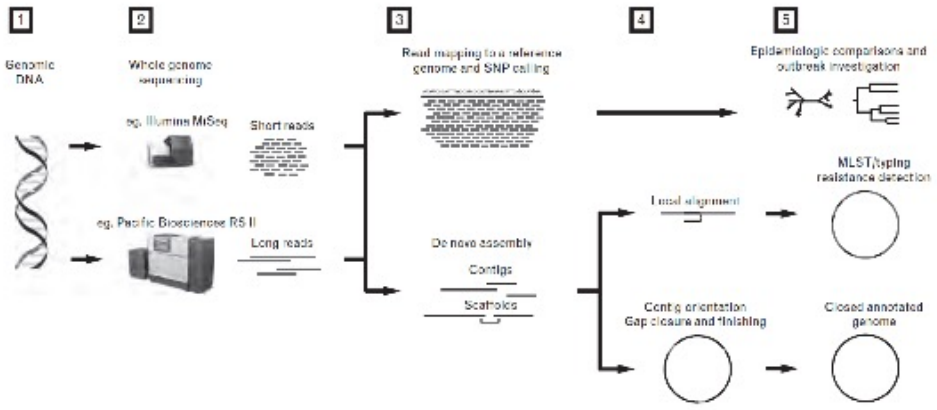
OXFORD NANOPORE TECHNOLOGY



NorthShore University HealthSystem

57

Next Generation Sequencing: WGS



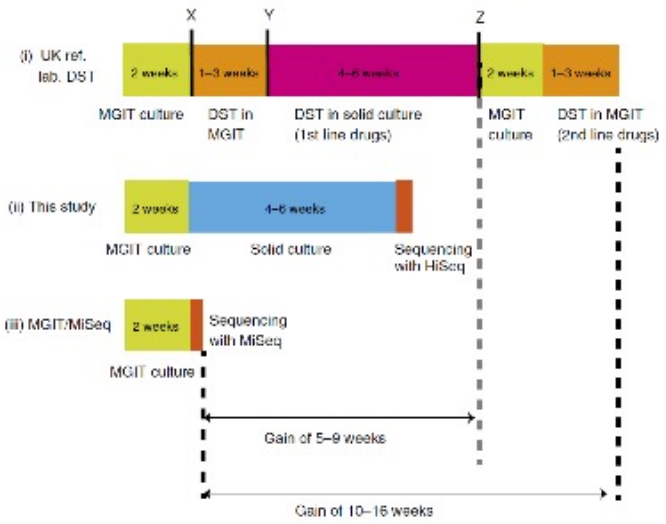
58

Genotype: Phenotype Correlation Simplified?

- How often does genotype predict resistance clinically?
- Can population genome graphs be used for identification and susceptibility?
- Is it better or rapid compared to existing methods?
- Algorithmic approach could work for some pathogens?

59

M. tuberculosis AST: Game changer?



Bradley P. et al. Nature Communications 6,: 10063 (2015)

NorthShore University HealthSystem

60

M. tuberculosis is Unique

- AST is performed by proportion method (subjective and slow)
- Slow growing pathogen, time to result can be weeks
- XDR and MDR TB is on the rise and rapid identification is key to control

- All drug resistance is mediated through chromosomal mutations
- Mutations known and annotated
- Mutation database publicly available

NorthShore University HealthSystem

61

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

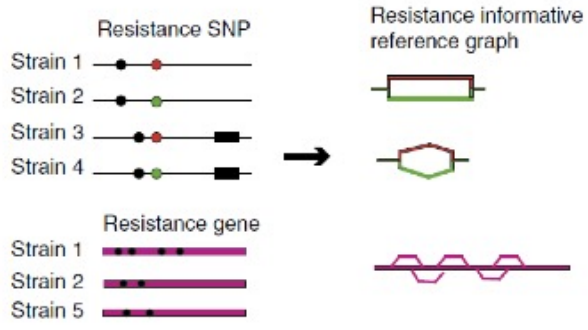
Review

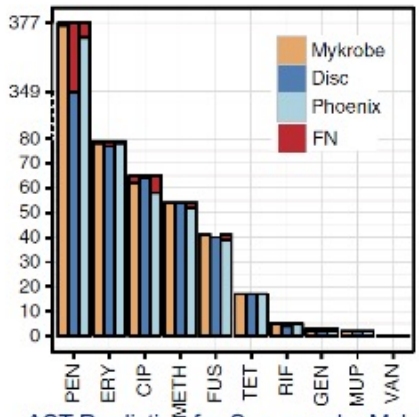
The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee

M.J. Ellington [1,†], O. Ekelund [2,†], F.M. Aarestrup [3], R. Canton [4], M. Doumith [1], C. Giske [5], H. Grundman [6], H. Hasman [7], M.T.G. Holden [8], K.L. Hopkins [1], J. Iredell [9], G. Kahlmeter [2], C.U. Köser [10], A. MacGowan [11], D. Mevius [12,13], M. Mulvey [14], T. Naas [15], T. Peto [16], J.-M. Rolain [17], Ø. Samuelsen [18], N. Woodford [1,*]

62

Rapid Antibiotic Resistance Predictions





AST Prediction for *S. aureus* by Mykrobe

Build a graph of loci of interest
Mark R loci (red) and S loci (green), predict by statistical algorithm

Bradley P. et al. Nature Communications 6,: 10063 (2015)

63

Single nucleotide changes do not reflect plasmid mediated resistance

Sci Transl Med 2014 September 17; 6(254): 254ra126. doi:10.1126/scitranslmed.3009845

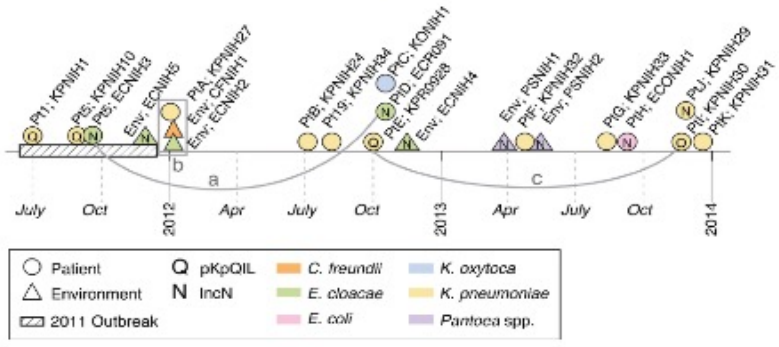
Single molecule sequencing to track plasmid diversity of hospital-associated carbapenemase-producing Enterobacteriaceae

Sean Conlan[1], Pamela J. Thomas[2], Clayton Deming[1], Morgan Park[2], Anna F. Lau[3], John P. Dekker[3], Evan S. Snitkin[1], Tyson A. Clark[4], Khai Luong[4], Yi Song[4], Yu-Chih Tsai[4], Matthew Boitano[1], Jyoti Gupta[2], Shelise Y. Brooks[2], Brian Schmidt[2], Alice C. Young[2], James W. Thomas[2], Gerard G. Bouffard[2], Robert W. Blakesley[2], NISC Comparative Sequencing Program[2], James C. Mullikin[2], Jonas Korlach[4], David K. Henderson[3], Karen M. Frank[3,*], Tara N. Palmore[3,*], and Julia A. Segre[1,*]

[1]National Human Genome Research Institute, Bethesda, MD

64

Tracking a plasmid during outbreak of KPC+ isolates at NIH



- Wide array of plasmids with carbapenem resistance genes found in several *Enterobacteriaceae* spp.
- Horizontal transfer of plasmids in the hospital environment
- Difficult to pin down person to person transmission

65

Roadblocks

- Cost and Time
- Bioinformatics consideration: making sense of the data
- Genotype: phenotype correlation (expression)
- Significance of a genome within a specimen: clinical correlation
- Quality Assurance: Curating and maintaining reliable database
- Regulatory considerations
- Patient outcomes

NorthShore University HealthSystem

66

Molecular Assays: Making the Difference?

- Summary
  - New technology can provide accurate diagnosis in a shorter time
  - Consultative microbiology could enhance understanding of the "lab report"
  - Consultation with clinical pathologist help in choice of platform
  - With novel pathogens, antimicrobial resistance and technology laboratory stewardship becomes an integral part of patient care
  - Applications of NGS in clinical microbiology is promising but need careful cost benefit evaluation depending on the application

NorthShore University HealthSystem

67

Thank You!

NorthShore University HealthSystem

www.webbertraining.com/schedulep1.php	
September 20, 2018	THE SILENT TSUNAMI OF AZOLE-RESISTANCE IN THE OPPORTUNISTIC FUNGUS *ASPERGILLUS FUMIGATUS* Speaker: **Prof. Paul E. Verweij**, Radboud University Center of Expertise in Mycology, The Netherlands
September 27, 2018	CHLORHEXIDINE USE AND BACTERIAL RESISTANCE Speaker: **Prof. Jean Yves Maillard**, Cardiff University, Wales
September 30, 2018	*(FREE European Teleclass - Broadcast live from the 2018 IPS conference)* ***Cottrell Lecture ...*** SURVEILLANCE BY OBJECTIVES: USING MEASUREMENT IN THE PREVENTION OF HEALTHCARE ASSOCIATED INFECTIONS Speaker: **Prof. Jennie Wilson**, University of West London
October 2, 2018	*(FREE European Teleclass - Broadcast live from the 2018 IPS conference)* ***Ayliffe Lecture ...*** (TO BE POSTED) Speaker: **Prof. Shaheen Mehtar**, Stellenbosch University, Cape Town, South Africa
October 11, 2018	*(FREE CBIC Teleclass)* INFECTION CONTROL CHAMPIONS ARE MADE, NOT BORN


Thanks to Teleclass Education
PATRON SPONSORS
Diversey
www.diversey.com
VIROX
www.virox.com
GOJO
www.gojo.com
World Health Organization
Infection Prevention and Control Global Unit
www.who.int/gpsc/en